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Kinetic analysis of the multi-sited enzyme systems

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PREFACE

At the beginning of the last century the kinetic theory for single-sited enzyme system was formulated and the technique of their full kinetic analysis was refined. Its essence consisted in the transformation of hyperbolic function into a rectilinear dependency, that is characteristic of only single-sited systems. Later on, however, it was found that many enzymes because of their multi-site feature are characterized by curve-linear kinetic dependency.

This necessitated to formulate principles for kinetic analysis of such curves and to work out new kinetic parameters. In spite of multiple attempts made in this direction this goal has not been achieved. The monograph is concerned with theoretical basis for deciphering the molecular mechanism for the multi-sited enzyme systems, and relying on experimental data, statistically valid methods for the determination of fundamental kinetic parameters is set forth.

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Chapter 1. INTRODUCTION

1.1. A notion of enzyme

Enzymes, catalysts of chemical reactions play a special role in the functioning of biological systems. Life is dependent on enzyme stipulated chemical reactions any change of which exerts a crucial influence on the normal functioning of these systems. Accordingly, study of the molecular mechanisms of the enzyme activity has a great importance. To solve these problems is the subject of study of enzyme kinetics.

The term "ferment" has been in use in the Russian literature, while in the English literature the term "enzyme" has been used. The term "ferment" is more common in Georgia, while the science concerned with the study of ferments is commonly called enzymology.

Enzymes are biological catalysts of protein nature. They take part in thousands of chemical reactions occurring in the cell. Enzymes are the essential components virtually of all biological machinery. Enzymes as catalysts act on the chemical reactions rate and have no effect on the equilibrium (i.e accelerate with equal degree both the direct and reverse reactions).

Enzymes are characterized by a high specificity in respect to both the reactions catalyzed by them and to substrate. Each enzyme accelerates one or several related chemical reactions. As a rule, the degree of specificity is very high, sometimes even absolute. Enzymes usually work in co-operation. Often, the product of one enzyme reaction is a substrate for the subsequent one.

Some enzymes, for instance pepsin, consists of only protein molecules, while others are composed of two components, of which one is of protein nature (apoenzyme), while the other is, as a rule, of non-protein nature (cofactor). The role of a cofactor can be performed by metal ions, or a complex organic compound which are called co-enzymes. Cofactors are thermostable compounds, whereas the majority of enzymes are inactivated at high temperature. Frequently, their isolation from the enzyme is possible by way of dialysis, though occasionally they are closely bound with apoenzyme and in that case, they are referred to as a prosthetic group. Neither apoenzyme nor coenzyme individually possess any catalyzing activity and they have enzyme activity only while acting in harmony. It is remarkable that a cofactor may be considered as a necessary activator, for it is virtually impossible to find experimentally any difference between them.

In the case of most enzymes, only a small part of the enzyme molecule, the so-called active centre, interacts with the substrate. It contains amino acid residue, which are directly concerned with the formation of chemical links with the substrate, or with its cleavage. The active centre is a three dimensional structure in the form of a narrow concave or cleft, which by its form precisely fits

the substrate. However, the active centres of some enzymes do not represent a rigid structure and modification of its form occurs at substrate linkage.

The active centre of similar enzymes renders complementary to the substrate only after linkage with it. The active centre constituent component is an "active catalytic " region, which directly interacts with the substrate and contact region which provides specific affinity and formation of enzyme-substrate complex.

It is necessary for enzyme catalyzing activity that its molecule possesses specific spatial conformation which considerably changes in the course of catalyzing cycle and at the end of the process assumes the same conformation as it had at the start of the cycle.

Thus, the enzyme is the most important protein compound without which it is impossible for thousands of chemical reactions to occur in the cell and, accordingly, existence of live organisms.

1.2. Enzyme classification

A specific feature in which each enzyme varies from others is the chemical reaction which it catalyzes. Therefore, logically, this feature underlies the enzyme classification and nomenclature. Enzymes by the type of catalyzed reaction fall into groups and subgroups which more precisely characterize this reaction.

The name of enzymes is made up of: a) name of a substrate and b) the word ending in 'ase' and defines the type of reaction catalyzed by all enzymes of the group, to which this enzyme belongs. It is also often given trivial names not involving chemical information. These are, for example, pepsin, trypsin, catalase, etc.

In accordance with the classification worked out by the Nomenclature Committee of the International Union of Biochemistry enzymes fall into six main classes. These are:

1. Oxidoreductases participating in the oxidation-reduction reactions.
2. Transferases which transfer functional groups.
3. Hydrolases responsible for the reaction of hydrolysis.
4. Lyases carry out cleavage from or attachment of certain groups to the substrate through non-hydrolytic way by formation of double links.
5. Isomerases responsible for isomerization reactions.
6. Ligases joining two molecules at the expense of ATP.

It is worked out a system of enzyme numeration. Every class falls into subclasses and sub-subclasses. Code of every enzyme contains four numerals which are divided from each other by points. The first numeral indicates to which class the given enzyme belongs; the second indicates

the subclass; the third indicates the sub-subclass, while the fourth is the order number of the enzyme in the sub-subclass.

Thus, every enzyme has an identification number (code) and systematic name. However, many systematic names are long and that's why trivial names are often applied. Thus, for instance, the trivial name of Na^+ and K^+ activated, Mg^{++}-stimulated adenosin-triphosphatase (EC 3.6.1.3) is NaK-ATPase.

1.3. Study of enzyme action

Enzymology as a discipline concerned with a thorough study of enzymes is distinguished by diversity and complexity, due to the diversity and complexity of the aims and methods. This will be evident from the text below that is a quotation from the well-known book by Dixon and Webb entitled "Enzymes" where the topics related to studies of a number of enzyme features are considered.

1. Biological properties implying the importance of participation of enzymes in the metabolism and conjugation of interaction of different enzymes; enzyme distribution in a variety of living organisms and tissues; intracellular localization; enzyme synthesis and genetics; effect of genetic mutations; existence of isoenzymes; effect of enzyme deficiency on the organism; a biological effect of enzyme selective "poisoning"; antienzymes.

2. Protein properties: homogeneity, number of isoenzymes, sedimentation and diffusion coefficients, molecular shape (central axes ratio), titration curve, isoelectric point, electrophoretic motility, hydration rate, stability to heating and irradiation, dissociation into subunits, absorption spectrum, etc.

3. The structure that implies: the amino acid content; sequence and number of amino acids; chain "wrapping" and tertiary structure of molecules; specific groups and metal atoms; number and nature of active groups within the enzyme molecule; number of SH-groups and their influence on enzyme activity; action of chemical reagents.

4. Enzyme properties, namely, nature of the reaction catalyzed by it; in the case of coenzyme involvement, type of its nature and action; substrate specificity, substrate chemical structure peculiarities required for its binding to the enzyme and reaction performance; stoichiometries specificity; action specificity with inhibitors.

5. Characterization of the active centres that implies: number of active centres in a molecule; their chemical structure, its effect on the substrate when linked to the active centre; reaction mechanism; character of active groups, in the case of their availability.

6. Thermodynamic properties, namely the enzyme reaction reversibility and equilibrium constant; temperature coefficient; thermal effects; free energy and entropy values of enzyme-

substrate linkage, enzyme-substrate complex activation, its conversion into enzyme-product and into free enzyme of this complex and dissociation product; enzyme affinity to substrate; Michaelis constant; pH effect on the enzyme affinity to a substrate; enzyme affinity to inhibitors; inhibitors' concurrence with the substrate.

7. Kinetic features. Namely, specific activity; molecular activity; absolute activity calculated per one active centre; velocity constant of enzyme linkage to a substrate; substrate and product dissociation constants; cumulative reaction rate constants; effect of activators; allosteric effects; reaction sequence; pH effect; reaction rate analytical equation.

It should be noted that a small number of enzymes have been studied considering all the above features. Especially it concerns the enzymes kinetic analysis, establishment of their molecular mechanism.

1.4. Enzyme rate estimating parameters

The enzyme rate is dependent on many a factor and physical-chemical parameters. Among them the basic one is the enzyme amount, concentrations of substrate, cofactor and modifiers. In addition, the enzyme rate is much affected by a reaction medium: its ionic content, ionic power, pH, temperature and other thermodynamic parameters. Therefore, in the case of relative analysis of enzyme rate, it is necessary that the optimal and steady state reaction medium be maintained (in standard conditions). One must be aware of the range of ionic content, pH and temperature at which the enzyme is stable and maintains maximal activity. While concentration of substrate and cofactors should be within the saturation concentration ranges to ensure that only the enzyme concentration remained a determinant factor for reaction rate and not be dependent upon substrate and coractors' concentration. Standard conditions imply optimal content of reaction medium and 30° C temperature as recommended by the International Biochemical Association. Although it is remarkable that in the case of warm-blooded animals, the enzyme rate is often measured at 36-37°C.

The basic characterizing parameter for enzyme is its activity. It is determined as the amount of substance converted by one or a definite amount of enzyme in time unit. The main problem while measuring the rate is to ascertain the enzyme amount. Even in the conditions of exhaustive information accurate measurement of its amount is impossible. The enzyme rate is, therefore, reflected by means of three different units: molecular activity, number of rotations, and specific activity.

If it is possible to determine the number of enzyme molecules in an incubation solution, then we can use a single molecular activity. Molecular activity implies the amount of substrate converted by one enzyme molecule in a time unit or that of product (mol/ min).

If it is possible to determine the number of enzyme active centers, the term rotation number is used. Rotation number is the amount of substrate converted by one active center in a time unit or that of the product (mol/ min).

Suppose it is impossible to determine the amount of an enzyme or the number of its active centers. Let's consider that the enzyme is evenly distributed in protein, i.e. the enzyme amount is directly proportional to that of the protein, it is then possible to determine enzyme rate as specific activity. Specific activity is the amount of substrate converted per time unit (min) or the amount of the end product (mol) per one unit of protein amount (mg). One may use different units (mol, mmol, μmol, etc) reflecting the amount of a substance. As well as different units of mass (kg, mg, μg, ng, etc) and of time (hour, min, sec, etc). For instance, μmol Pm/hour 1 mg protein is frequently used as a unit of NaK-ATPase activity.

As has been already mentioned, enzyme amount is determined by means of rate of the reaction catalyzed by it. Therefore, in enzyme exploit methods accurate measurement of reaction rate has been of utmost importance. One requires a simple and facile analytical method which allows us to determine the rate of substrate conversion or end product and statistical treatment of results. This problem will be discussed in details later on. This problem is also coupled with the necessity of initial rate measurement, as the course of a reaction is attended by reduction of substrate concentration, i.e. stability of reaction medium is disturbed. Therefore, measurement of approximate rate to the origin is satisfactory. Satisfactory is regarded the situation when the substrate concentration does not alter by more than 10%. Exception makes oxidation-reduction processes whose substrate (or product) has, as a rule, a narrow absorption spectrum, a distinct maximum. It is then possible to use double beam spectrophotometer accurate measurement of original rate (substrate amount by means of tangent plotting at zero point on time graph).

1.5. Reaction order

Simple and complex reactions are distinguished in the chemical kinetics. Simple reactions take one stage and mainly involve unidirectional acting. To complex reactions are attributed: 1) reversible reactions which proceed in both directions; 2) parallel reactions when several reactions occur simultaneously in different directions and each yield a different product; 3) consecutive reactions taking place concomitantly and performed in several stages. The end-product of the first stage is an intermediate substance and starts the second stage, etc. 4) conjugate reactions; 5) parallel-consecutive ones; 6) consecutive-branching reactions and 7) cyclic ones.

Chemical reactions can be characterized by molecules or order. Molecularity is determined by number of molecules converted in the reaction. Thus, for example, reaction $A \rightarrow P$ is unimolecular or monomolecular; $A+B \rightarrow P$ is bimolecular, while $A+B+C \rightarrow P$ is trimolecular.

Reaction order is, in a reaction equation, the number of concentration members multiplied by each other. According to how, in the given circumstances, reaction rate is dependent upon reacting substance concentration reactions of the first, second, third and zero order are distinguished.

The reaction whose rate is proportional to one reacting substance concentration are attributed to the first order.

Second order reactions are those whose rate is the product of two reacting substance concentrations, or is proportional to the quadrate of one of the substances concentration. It should be pointed out that reaction $A+B \rightarrow P$ does not always proceed as the second order reaction. In some cases, it may occur as the first order one. Thus, for instance, if concentration of A substance is much greater than that of B substance, this reaction then would be of the first order in respect to B substance, as its rate would be proportional to the concentration of only one reacting substance (in this case of B substance). Such reactions are referred to as pseudo-first order.

The rate of third order reactions is proportional to the product of three reacting substances concentrations.

Reactions whose rate is not dependent upon reacting substances concentration are called zero order ones. In this case, reaction rate depends on the concentration of catalyst or on any other factor, but not the reacting substance concentration.

For simple reactions which take only one stage, or for a separate stage of complex reactions, the reaction order usually coincides with molecularity. For several consecutive unimolecular and bimolecular stages occurring complex reactions it is not necessary that full reaction molecularity be coincident with its order. It should be noted that reversed reaction molecularity and order does not frequently coincide with direct reaction molecularity and order. The reactions whose molecularity is more than two are very common. While the reactions whose order is more than two are quite rare. Thus, for example, trimolecular reactions do not, as a rule, proceed in one stage, they and consist of two or more elementary stages. If one of the stages is far slower than others, then the full reaction rate would be equal to the slow (so-called limiting) stage rate. If the limiting stage is not distinct, then the reaction rate equality, as a rule, has a complex form and is characterized by a non-stable order.

The graph, that reflects dependence of reaction rate log on reacting substance concentration log, is a circle whose slope would be equal to the common slope. Reaction order is, as

a rule, determined in respect to each reagent, reaction order is defined individually. For this, concentration of the given reagent is altered at a constant concentration of other reagents.

1.6. Effect of temperature on reaction rate

As mentioned above, reaction rate strongly depends on temperature. Thus, for example, as temperature increases by every 10°C reaction rate increases approximately twice. Therefore, in order to get significant results in kinetic experiments, strict control over temperature is necessary. Besides, temperature dependence can be used for the purpose of gaining information concerning reaction mechanism.

All contemporary theories which explain dependence of velocity constants on temperature are underlined by Van't Hoff and Arrhenius exploits. They have compared the known properties of equilibrium constant with kinetic data and made an attempt to ascertain this dependence. At absolute temperature (T) variation the equilibrium constant (K) alters in terms of Van't Hoff equation:

$$\frac{d\ln K}{dT} = \frac{\Delta H^0}{RT^2} \qquad (1.1)$$

where, R is gas constant, ΔH^0 is a standard enthalpy change in the course of the reaction. In an analogous way, Arrhenius formulated an equation reflecting velocity constant alteration at temperature change

$$\frac{d\ln k}{dT} = \frac{E_a}{RT} \qquad (1.2)$$

where, E_α is activation energy. By integration of T in this equation we get:

$$\ln k = \ln A - \frac{E_a}{RT} \qquad (1.3)$$

where, lnA is an integration constant.

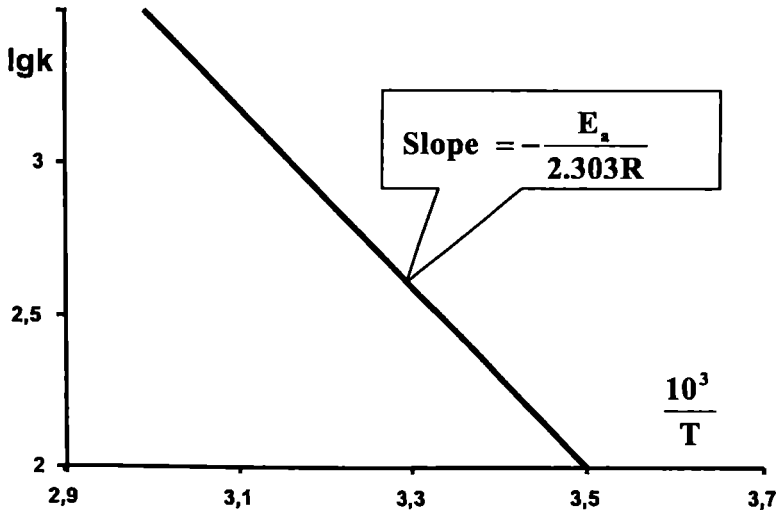


Fig 1.1 Arrhenius graph

This form of Arrhenius equation is convenient for plotting graphs of results, as $\ln k = f(1/T)$ dependence denoting graph is a line whose slope is equal to $-E_\alpha/R$ (in practice a circle $\lg k = f(1/T)$ with a slope $(-E_\alpha/2.303\ R)$. This graph is known as Arrhenius graph and by means of it E_α-value (Fig. 2.1) can be readily determined.

To explain physical essence of activation energy let's write the equality (2.3) as follows: $k = A\exp(-E_\alpha/RT)$. The exponential member $\exp(-E_\alpha/RT)$ is often called Boltzmann multiplier, as in accordance with Boltzmann's theory, in the mixture the number of the molecules whose energy is more than E_α is proportional to $\exp(-E_\alpha/RT)$ value. Accordingly, the Arrhenius equality can be interpreted in the following way: "Molecules can participate in the reaction only when their energy exceeds the definite threshold value – activation energy". According to this interpretation, constant A must be equal to molecules strike frequency (z). In gas phase, for some reactions, the coefficient A is really equal to z. Though in a general case it is necessary to introduce additional multiplier (P) $\{k = Pz\exp(-E_\alpha/RT)\}$ and assume that for a chemical reaction to occur, molecules should have not only sufficient energy, but they should be definitely oriented to each other. In this case P quantity would be probability of straight disposition of molecules to each other.

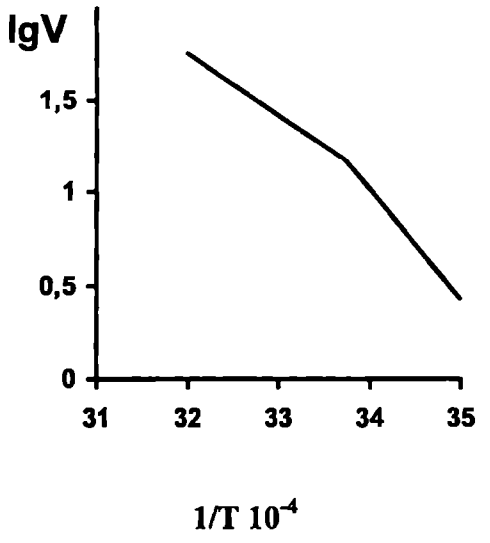


Fig. 1.2. NaK-ATPase activity log (IgV) vs. inverse value of absolute temperature (the brain microsomes). Critical temperature 23.5^0C; activation energies 24 Kcal/mol ($15\text{-}20^0$C and 13 Kcal ($25\text{-}40^0$C)L. Tsakadze, Z. Kometiani. Bull. Georgian Acad. Sci., 1970, 60, N 2, 449-452.

Dependence of the reaction rate on the inverse value of absolute temperature is not always rectilinear. Frequently, the slope of a graph changes (Fig. 1.2) and assumes a broken form. This indicates that there occurs activation energy variation. There may several reasons: 1) change of a solution phase; 2) Existence of two parallel reactions having different temperature coefficients; 3) Existence of two successive reactions having different temperature coefficients; 4) Existence of an enzyme in two different forms of activity; 5) Reversible enzyme inactivation; 6) Variation of slope (activation energy) occurs only on the direct reaction graph.

1.7. Transient state theory

Any chemical reaction, say A→P, proceeds because some part of A substance molecules in any given moment possesses more energy than other parts of molecules and this energy is sufficient for passing to the active state. Activation energy is the energy that is necessary for all molecules of one mol substance be turned into an active state at a given temperature.

When the reacting system "moves" across the imaginary "reaction coordinate", it passes through different energy states on a continuous spectrum (Fig. 1.3). Thus, all chemical reactions at a definite stage passes through this state which is characterized by maximal energy and is called the transient state. The transient state theory associates chemical reaction rate with thermodynamic properties of the molecules being in the transient state.

Chemical reaction may be fancied as follows: $A + B \leftrightarrow X^{\#} \rightarrow P + Q$, where, $X^{\#}$ is a transient state. It is considered that $X^{\#}$ concentration measurement is governed by the thermodynamic laws, therefore $[X^{\#}=K^{\#}[A][B]$. For $K^{\#}$ constant the following thermodynamic equation being appropriate: $\Delta G^{\#} = -RT \ln K^{\#} = \Delta H^{\#} - T\Delta S^{\#}$

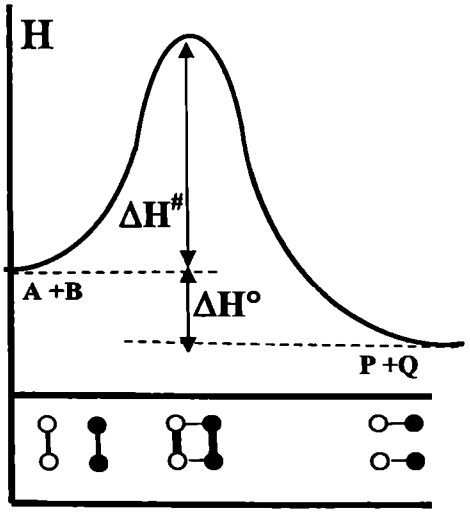


Fig. 1.3. Reaction profile in terms of transient theory. Reaction co-ordinates are expressed schematically on the axis of abscissa.

where, $\Delta^{\#}$, $\Delta H^{\#}$ and $\Delta^{\#}$ denote respectively, the transient state forming free energy, enthalpy and entropy. The activation enthalpy and entropy quantity provides information about the nature of the transient state and accordingly, about the reaction mechanism. High value of activation enthalpy indicates that for the formation of transient state strong state, distortion and sometimes even cleavage of chemical bonds are necessary. Activation entropy characterizes reality of transient state existence without taking into account energy processes. If $\Delta S^{\#}$ value is high and negative, then to form the transient state the reacting molecules should assume a strictly determined conformation and should approach each other at a definite angle. It is remarkable that a catalyst increases the reaction rate through $\Delta H^{\#}$ or $\Delta S^{\#}$, or reduction of both values.

Chapter 2. CLASSICAL KINETICS OF ENZYME SYSTEMS

2.1. Measurement of enzyme reaction rate

A typical curve reflecting enzyme reactions is represented in Fig. 2.1. When reaction rate decreases, this can be due to different causes, for instance: a) formation of reaction products; b) substrate concentration reduction in the course of reaction; c) temperature or pH instability; d) in incubation solution because of admixtures an enzyme (or coenzyme may undergo inactivation, etc). In some cases, several causes listed above may act simultaneously. Because of this, enzyme reaction curves are not, as a rule, described in terms of ordinary equalities used for homogeneous chemical reactions, and it is rather a hard task to achieve equalities for experimental curves.

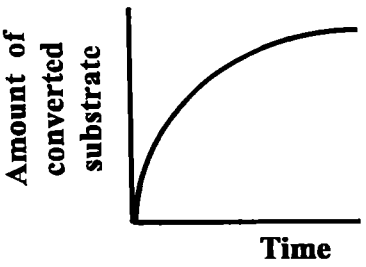


Fig.2.1 . A typical curve of reaction course in the case of a single-sited enzyme (A multi-sited enzyme may have a complex geometrical shape)

To avoid this complication, other approaches are applied to enzyme reaction studies. Namely, original reaction rate is determined, as in the initial period the mentioned factors fail to reveal their activity. Therefore, while working at enzymes it is accepted, at constancy of other factors to make observation on reaction initial rate at variable rate determining any one of the factors.

To the main determinant factors for enzyme reaction initial rate are attributed: enzyme and substrate concentration, pH, temperature and activators, or availability of inhibitors.

2.2. Effect of enzyme concentration on reaction rate

The enzyme reaction rate is, as a rule, proportional to enzyme concentration (Fig. 2.2. B), certainly if dissociation-association of enzyme complex is ruled out. However, in some cases it is still noted a deviation from linearity within small and large concentration ranges of an enzyme, this is often due to imperfectness of activity determination system. Occasionally, such a deviation is a result of properties of enzyme preparation or incubation solution.

linearity follows from enzyme activity measurement, therefore it is required for correct information that experiment be carried out within linearity ranges.

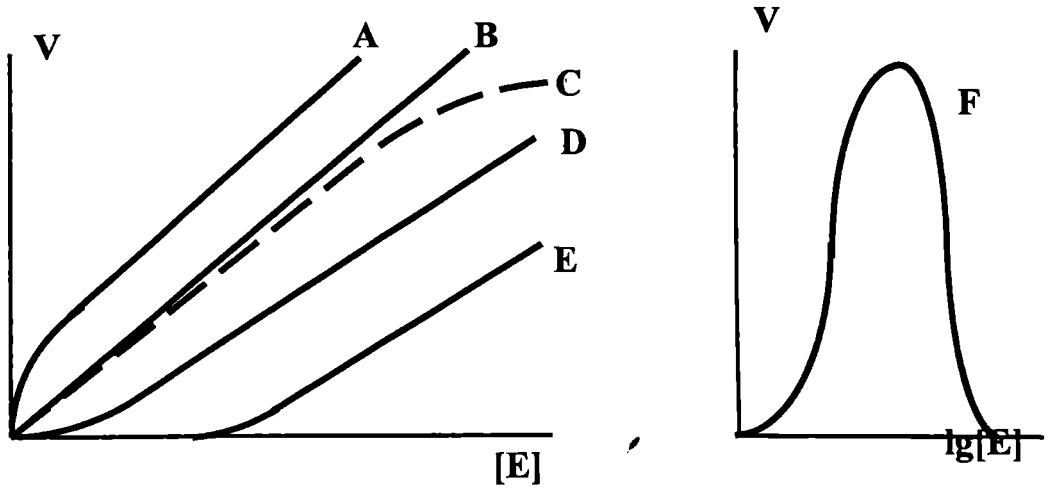


Fig. 2.2. Amount of the substrate converted per one time unit vs. enzyme amount (E).

1. Convex curves. Convexity of V=f(E) dependence is due to two main causes:

a) Existence of high toxic admixture in minute amount in the reaction medium and not in enzyme preparation itself. This admixture "poisons" the first portion of added enzyme. Afterwards, when the amount of added enzyme (or other protein available in enzyme preparation) will exceed the amount required for inhibition of toxic admixtures, enzyme activity will completely recover and a normal rectilinear dependence is achieved. The rectilinear section on the origin of coordinates is shifted to some distance, that is proportional to the amount of toxic admixture (Fig. 2.2 E).

b) Availability of dissociable activator or coenzyme in the enzyme preparation. At this time active complex EA(E+A=EA) is formed. Respectively, the share of the enzyme being in active form increases in the incubation medium as increases the enzyme concentration. In the given case activator enters into system together with enzyme preparation and that is why, as enzyme concentration increases its still more part would be in active form and V=f(E) dependence curve would be concave. At simple stoichiometries 1:1 for which equality E+A=EA is responsible, reaction rate dependence on protein concentration must be of second degree, but at high concentration when the enzyme is completely saturated with an activator, the rate will be proportional to protein concentration (Fig. 2.2 D).

Extremely interesting is the case when the enzyme is a set of subunits of which each one separately is inactive. It may be considered that the given subunit becomes activated at interaction with

another subunit. Concentration increase conditions aggregation of subunits, therefore the graph of rate dependence on enzyme concentration will have a convex curve form.

2. Concave curves. The curve of reaction rate dependence on enzyme concentration in this case turns to plateau, i.e., attains imaginary threshold velocity (Fig. 2.2. C). Such a deviation from linearity occurs more frequently than the cases described above and can be accounted for by several reasons:

a) Imperfectness of measurement method and actual reduction of inactivity. When determination of the given enzyme activity requires addition of another enzyme, the second enzyme activity may become a reaction rate limiting factor as a result of which a curve similar to that given in Fig. 2.2. C can be obtained. At the increase of amount of the second enzyme the rate will also increase and a circle is obtained (Fig. 2.2. B). A similar pattern will be also in the case when in this system amount of one enzyme changes while that of others remains constant. Until the amount of the given enzyme is relatively small, general velocity of the process will be proportional to the concentration of this enzyme. When concentration of this enzyme will become sufficiently high, reaction rate limiting will become concentration of another enzyme.

b) If a pure enzyme can be available to the investigator, then it becomes possible to add it in a very large amount to the explored system. If at this time the enzyme possesses rather high affinity to any component of this system, say to coenzyme, then the whole coenzyme will be bound with this enzyme and the coenzyme will be inaccessible for other enzymes of the system. In this case, as enzyme concentration increases, complex enzyme reaction rate will be reduced (Fig. 2.2. F).

c) The curves similar to the one in Fig. 2.2. C are achieved also in the case when was measured not the initial velocity, but the change resulting from the reaction in a definite time section. A similar result is caused by substrate depletion and does not indicate the non proportional dependence between the initial velocity and enzyme concentration.

d) If enzyme preparation contains a reversible acting inhibitor which when linked with the enzyme forms an inactive complex, then on increasing in the mixture of inhibitor's concentration the share of the enzyme being in inactive state will also increase. As at this time inhibitor is added together with an enzyme, therefore occurs a concomitant rise of enzyme and inhibitor concentration, due to which activity at high concentration of the enzyme will be far smaller than it would be expected from the initial rectilinear dependence, resulting in achievement of a monotonously concave curve.

The concave form of enzyme concentration depending reaction rate curve might also be due to polymerization of the enzyme in question, if its dissociated form is active.

In spite of the above named reasons, in most cases a direct proportional dependence between enzyme concentration and the reaction initial velocity is maintained.

Specific activity of an enzyme (v) is direct proportional to enzyme velocity (V) and enzyme amount (e_o). Equality $v=Ve_o$ is achieved. Therefore, further on in the case of theoretical studies we shall use V (rate) value.

2.3. Effect of substrate concentration on enzyme reaction rate

One of the important determinant factors of enzyme reaction rate is substrate concentration. Enzyme reactions in contrast to non-enzyme ones are characterized by substrate saturation. For simple enzyme reactions ($A \rightarrow P$), at a low substrate concentration, reaction rate increases in proportion to substrate concentration, i.e. reaction is of the first-order in respect with substrate. At substrate concentration increase reaction rate increases gradually slower and proportional relation gets disturbed (in this concentrate region reaction is of a mixed-order). At further rise in substrate concentration reaction rate will be constant and will not depend on substrate concentration (reaction in respect with substrate will be of zero-order), will occur enzyme saturation with substrate. In these circumstances the reaction rate limiting factor will be enzyme concentration. Saturation effect is characteristic of all enzymes, it differs for different enzymes and is highly distinguishable from each other.

Studies of enzyme with substrate saturation effect Michaelis and Menten (1913) led to the formulation of general theory of enzyme kinetics. According to Michaelis and Menten, the following generalized mechanism governs the enzyme reaction:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} ES \xrightarrow{k_{+2}} E + P \qquad (2.1)$$

Michaelis and Menten made an assumption that the reversible stage is rapid and concentration of intermediate ES complex concentration is expressed as follows: $[ES]=x=es/K_s$, where K_s is the equilibrium constant $K_s=K_{-1}/K_{+1}$ and e – enzyme concentration. It is remarkable that direct measurement of enzyme (e) and substrate (s) instant concentration is impossible, therefore it is measured by a convenient sum concentration: $e_o=e+x$ and $s_o=s+x$. As it follows from the first equality, x cannot exceed e_o. So, if s_o is much more than e_o, then it would be far greater than x and can be considered that $s= s_o$, then

$$v = k_{+2}x = \frac{e_0 k_{+2}}{1+(K_s/s)} = \frac{e_0 k_{+2} s}{s + K_s} \qquad (2.2)$$

Similar results have been obtained by Van Slyck and Cullen. They considered the first stage of enzyme reaction as an irreversible step: $E + S \xrightarrow{k_{+1}} ES \xrightarrow{k_{+2}} E + P$. Certainly, in this case x is reflected through the equilibrium constant and we get: $\frac{dx}{dt} = k_{-1}(e_0 - x)s - k_{+2}x$

Van Slyck and Cullen regarded the intermediate compound concentration as being constant, i.e. dx/dt=0; therefore, $x = \frac{k_{+1}e_0 s}{k_{+2} + k_{+1}s}$. In this case too, the reaction rate equation is identical to equality 2.2 and to find the difference between them experimentally is not feasible.

Briggs and Haldane offered a generalized mechanism which involved the both particular cases described above:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} E + P$$

wherefrom, $dx/dt = k_{+1}(e_0 - x)s - k_{-1}x - k_{+2}x$

If we consider that in the course of reaction a stationary equilibrium is achieved in which the intermediate compound concentration is constant (i.e. dx/dt=0) then reaction rate assumes the following pattern: $v = k_{+2}x = \frac{e_0 k_{+2} s}{\frac{k_{-1} + k_{+2}}{k_{+1}} + s} = \frac{V_{max} \cdot s}{K_m + s}$ (2.3)

where, K_m is Michaelis constant which is determined by the following ratio of constants: $(K_{-1}+K_{+2})/K_{+1}$, while V_{max} is maximal velocity and is determined by the product: $K_{+2}.e_0$. Equality (2.3) is a fundamental equality in enzyme kinetics and is called Michaelis-Menten equation. This equality is performed for more complex mechanisms as well, although in this case images for K_m and K_{max} have a more complex view. V_{max} is not a fundamental characteristic of the enzyme, since it depends on enzyme concentration, when constant K_m is equal to substrate concentration then the reaction rate is a half of maximal velocity.

From the practical point of view it would be interesting to assume that K_m is the measure of dissociation constant (K_s) (i.e. $K_{+2} \ll K_{-1}$), but K_m actually is not even upper limit of K_s value. Naturally there arises a question: what is the use of K_m measurement if it cannot be used as a measure of substrate binding rigidity. In fact, knowledge of K_m quantity is needed for a number of reasons: 1) during analysis of complex mechanisms it is required to reflect complex kinetic effect with maximally simple values which describe in full the enzyme system. For this reason, under variable experimental conditions, change of basic kinetic parameters (K_m, V_{max}, V_{max}/K_m) are explored. 2) While determining enzyme activity K_m enables correct carrying out of the experiment. For this it is desirable that the

velocity to be measured be dependent only on enzyme concentration and did not react to small deviations. This is achieved in the case of saturation with substrate. Practically to attain this it is sufficient that [S]=10 K_m. 3) If we consider K_m as the cumulation of data on dissociation constants of substrate analogs (which act as inhibitors), occasionally it can be regarded as K_s analog.

K_m and V_{max}, as already mentioned, are important kinetic parameters, as they completely determine the dependence of the given enzyme reaction rate on substrate. Operation with these parameters yields information about the molecular mechanism of enzyme systems. v=f(s) is a hyperbola, while V_{max} is the asymptotic value and its precise measurement is impossible. Therefore, for the purpose of K_m and V_{max} graphic plotting such methods of variables' conversion are commonly applied as would enable linerization of original hyperbolic dependence.

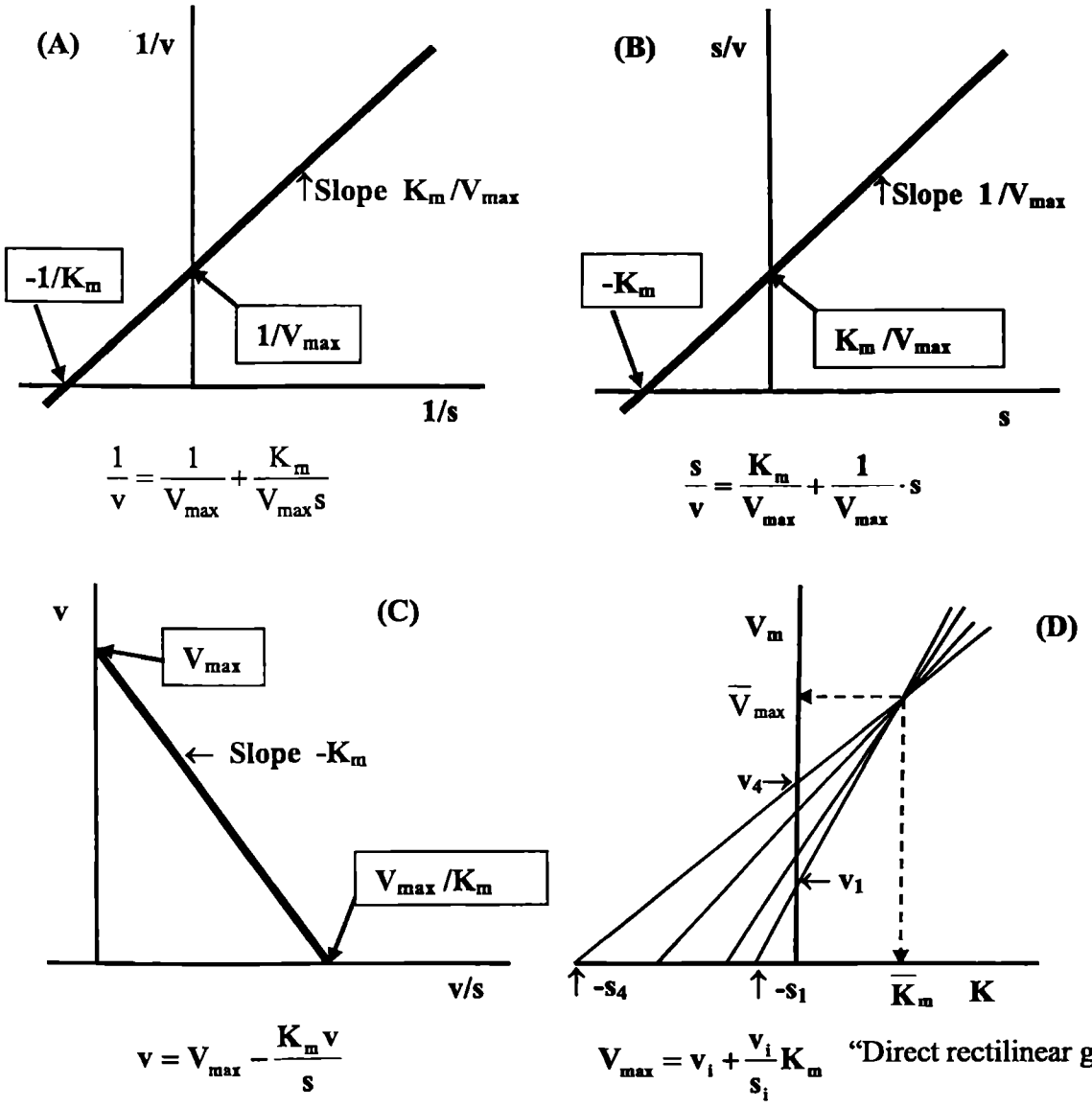


Fig. 2.3. Methods used for graphical determination of K_m and V_{max}

These are traditional transformations: 1/v=f(1/s), (Fig. 2.4, A); s/v=f(s), (Fig. 2.4, B); v=f(v/s), (Fig. 2.4, C) and the so-called "direct rectilinear graph". In the case of this latter, each velocity is measured on the ordinate, while the intersection point coordinates of the lines drawn on them would be K_m and V_{max} values (Fig. 2.4, D).

Illustration of these methods in a summarized way is presented in Fig. 2.3.

2.4. Steady-State principle

The steady-state principle has been introduced by Bodenstein. As Van Slyke and Cullen, so Briggs and Haldan considered that in the course of enzyme reaction a stationary state is achieved, during which the concentration of intermediate compound is constant, but none of them provides proof for meeting steady-state conditions. Unfortunately they regarded that the steady-state principle is self-evident and is always fulfilled. However, this principle is not actually fulfilled for some A+B↔C→D type non-catalytic reactions, which at one glance resemble the Briggs and Haldan scheme and respectively there must be steady-state.

The methods for formulating the majority of velocity equations are based on the assumption (dx/dt=P, t is time) about a stationary course of the reaction. Thus, for example, in the case of Michaelis-Menten mechanism it is very easy to come to this assumption. But if we assume that the reaction proceeds steady-state, then to solve (2.4) differential equality its integration is required, as a result the following equality (2.5) is obtained:

$$\int \frac{dx}{k_{+1}e_0 s - (k_{+1}s + k_{-1} + k_{+2})x} = \int dt \qquad (2.4)$$

$$v = \frac{V_{max}s\{1 - \exp[-(k_{+1}s + k_{-1} + k_{+2})t]\}}{K_m + s} \qquad (2.5)$$

where V_{max} and K_m are determined so, as earlier. At t's high quantity, when exponential term will get infinitely small, 2.5 equality will assume a form of 2.3 equality.

2.5 equality type of equation, as a particular case, was formulated by Laidler (1955). He considered the case when the reaction proceeded for a long time resulting in such a reduction of substrate concentration that it would not have been assumed that it was equal to the initial concentration. However, in this case too the system attains the stationary state, wherein

$$x = \frac{k_{+1}e_0(s_0 - p)}{k_{-1} + k_{+2} + k_{+1}(s_0 - p)}$$

where, s_o is substrate initial concentration and p is product concentration. Respectively, we get:

$$v = \frac{k_{+1}k_{+2}e_0(s_0 - p)}{k_{-1} + k_{+2} + k_{+1}(s_0 - p)} = \frac{V_{max}(s_o - p)}{K_m + (s_o - p)} \qquad (2.6)$$

2.6 equality is identical to 2.3 equation, but here s value is altered by (s_o-p) value. At first glance it is illogical to speak of the stationary state, where x is denoted by time depending p value. This paradox can be readily explained if we bear in mind the fact that the time dependence of x value described by 2.6 equality yields a far less alteration of this value than in the transient phase (before reaching the stationary state). Briggs and Haldan's mechanism would alter to a less extent if we substitute dx/dt=0 assumption by assumption that dx/dt value is very small.

Taking into account the above-said, it can be said that the kinetic curve of enzyme reaction consists of three sections (Fig. 2.4): 1) a transient section, that is described by 2.5 equation, 2) the initial velocity section, described by 2.3 equation and 3) the section for the main course of reaction. Here substrate and product concentrations substantially change and the process velocity drops to zero. In this section reaction velocity is described by 2.6 equation.

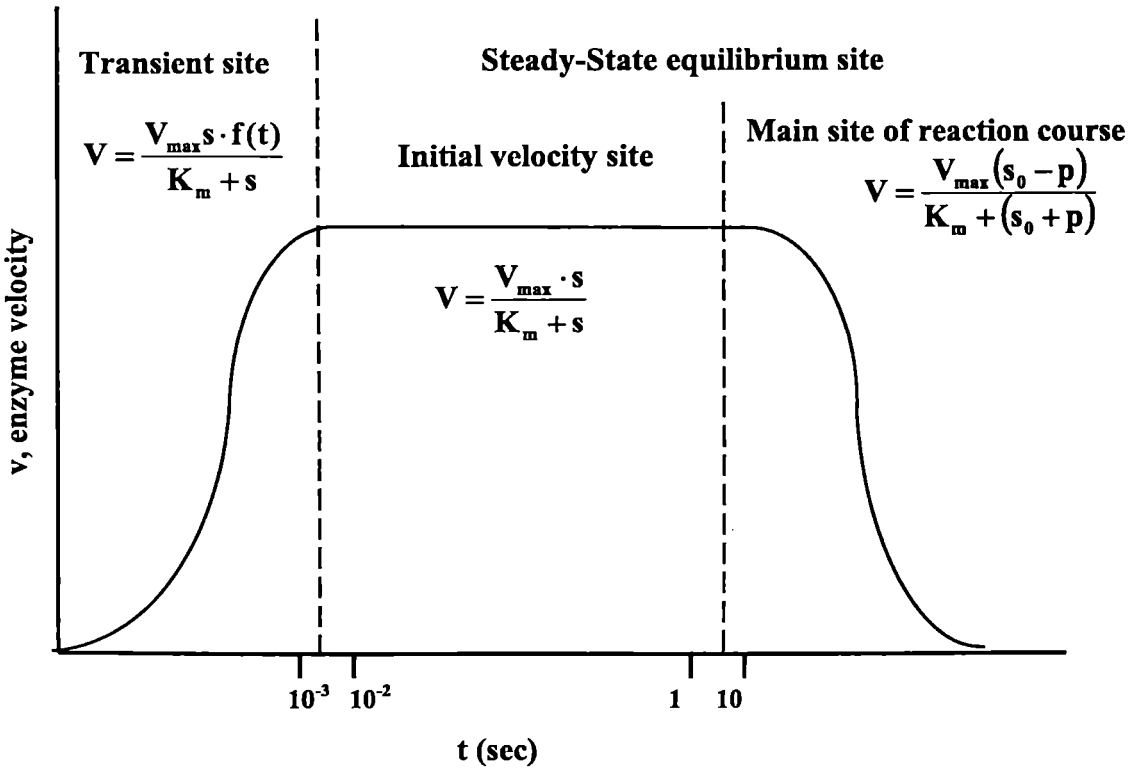


Fig. 2.4. Reaction course in time. The reaction is governed by Michaelis-Menten mechanism. The transient site is often referred to as the prestationary equilibrium site. $f(t) = \exp[-(k_{+1}s+k_{-1}+k_{+2})t]$. (A. Cornish-Bouden. Basis of enzyme kinetics)

The methods for solving most of velocity equations are underlaid by the assumption on the reaction stationary state. Therefore, while studying enzyme reaction one should be certain that the reaction proceeds under stationary conditions. At this time, reaction rate is constant and there is a proportional dependence between activity and time at stationary course of the reaction. The curve of the dependence of activity on time is represented in Fig. 2.5, where at a certain time section (Δt) curve is rectilinear, then deviates, indicating accumulation of the product and respectively, reduction of activity. The value of time at which there occurs inflexion of the rectilinear curve is the value as a result of which there is no longer a stationary course of the reaction. As has been already stated, stationary course of the reaction occurs in the initial velocity section. Therefore, in order to be sure that the reaction proceeds steadily, one must ascertain that the enzyme system operates in the initial velocity domain.

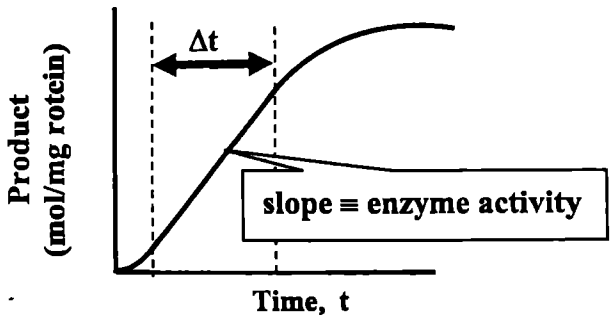


Fig. 2.5. V=f(t)

2.5. Michaelis-Menten equation and action of modifiers

In this chapter we are discussing single-sited enzyme systems, i.e. an enzyme has one substrate and one modifier site (permissible is one site per each of different modifiers). Modifiers are called the substances addition of which to the reaction medium results in a change of enzyme reaction state. Modifiers, according to the kind of their action on enzyme reaction rate are divided into activators and inhibitors. Activators have an increasing effect on reaction rate, while inhibitors reduce it. It is clear that classification of modifiers must be based on their effect on the reaction basic parameters.

As stated above, in the case of substrate low concentration ($S<<K_m$) reaction rate, $v=(V_m/K_s).S=e_oK_sS$, is determined by K_s constant, while in the case of high substrate concentration ($S>>K_m$), reaction rate $c=e_oK_c$, is determined by K_c constant. On the grounds of the effect on these constants the type of modifiers are distinguished. (This principle has been recommended by the

International Biochemical Society). That is, we can have specific, catalytic and mixed types of modifiers.

A general kinetic scheme of modifiers' action on the enzyme system was proposed by Botts and Morales in the 50s of the last century (Fig. 2.6).

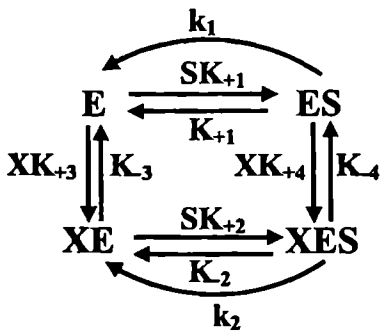


Fig. 2.6. A general kinetic scheme of modifiers' activity (after Botts and Morales)
E – enzyme; S – substrate; X – modifier

In the case of inhibitors, reversible and irreversible inhibitors are considered individually. It is accepted that if inhibitor treated enzyme recovers its activity during dialysis in the solution devoid of inhibitor, inhibition is reversible, if not, it is irreversible. Reversible inhibition is characterized by achievable equilibrium between enzyme and inhibitor. At this time, equilibrium constant is the measure of their affinity. Efficiency of inhibitors' action is expressed by the inhibition constant K_i, which represents inverse value (dissociation constant) of enzyme affinity to an inhibitor. Exactly the same can be said about activators whose action is characterized by activation constant K_A.

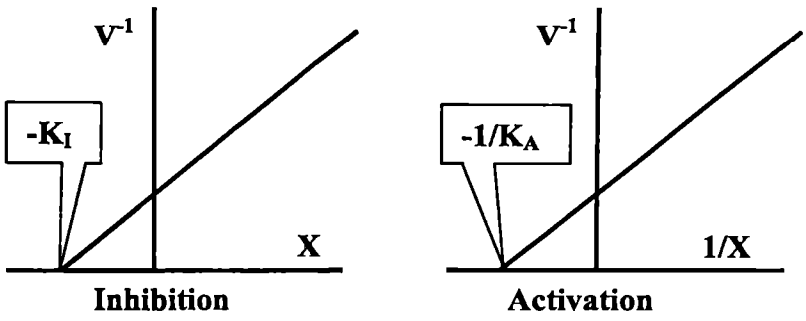


Fig. 2.7. Effect of modifiers on the enzyme velocity (single-sited system, rectilinear dependence). $V^{-1}=f(x)$ – inhibition; $V^{-1}=f(1/x)$ – activation; It is implied that [S]=const.

In Fig. 2.7, $V^{-1}=f(X)$ dependence is rectilinear. In this case the term rectilinear inhibition is used. It may well be that $V^{-1}=f(X)$ function is not a straight line, then the term non-linear inhibition is employed. Occasionally the rectilinear inhibition refers to complete inhibition, while in the case of non-linear inhibition the term partial inhibition is common. This question will be dealt with in more details below, while discussing the multi-sited systems.

It is often impossible to make an absolute delineation between reversible and irreversible inhibitors, as some inhibitors are firmly associated with the enzyme system and its removal is rather a difficult process. That is why, reversible inhibitors which can hardly be distinguished from irreversible ones are called rigidly linked inhibitors.

Let us consider Michaelis-Menten equation in inverse values without modifiers and in the case of their influence:

$$\frac{1}{V}=\frac{1}{V_m}+\frac{K_m}{V_m S}\Rightarrow\frac{e_0}{V}=\frac{1}{k_C}+\frac{1}{k_S S},\ ([X]=0)\quad\text{and}\quad\frac{e_0}{V}=\frac{1}{k_C^{app}}+\frac{1}{k_S^{app}\cdot S},\ ([X]\neq 0);\qquad(2.7)$$

where k_C^{app} and k_S^{app} are imaginary catalysts and specific constants.

If an inhibitor (I) decays the specific constant and does not affect the catalytic constant, then it is named a competitive inhibitor, while inhibition constant is called competitive inhibition constant K_{IC}).

$$\frac{1}{k_S^{app}}=\frac{1}{k_S}\left(1+\frac{X}{K_{IC}}\right),\qquad(2.8)$$

While in the case when an inhibitor acts only on the catalytic constant it is named a uncompetitive inhibitor, and inhibition constant, non-competitive inhibition constant (K_{JU}).

$$\frac{1}{k_C^{app}}=\frac{1}{k_C}\left(1+\frac{X}{K_{IU}}\right)\qquad(2.9)$$

If the inhibitor affects the both constants, it is then named mixed. Instead of mixed the term uncompetitive inhibitor is often used. The mixed inhibitors may be divided into separate subgroups. According to the way of dependence between K_{IC} and K_{IU}) constants.

If $K_{IC}<K_{IU}$, then the term competitive inhibition is preferential, if $K_{IC}=K_{IU}$, then we may use the term pure uncompetitive inhibition, while in the case if $K_{IC}>K_{IU}$, then uncompetitive inhibition is likely to occur.

By analogy, if an activator increases the catalytic constant it is named a catalytic activator and if it increases the specific constant it is named a specific activator, when the activator by its action has an effect on both constants, then it is referred to as mixed. Proceeding from the physical essence of the action of a specific activator, the process is referred to as a specific activation and the use of competitive activation is devoid of any sense, as under this term competence between the activator and substrate is meant. Proceeding from this it is also unreasonable to use in respect to an activator. If the reaction does not proceed without an activator, such a modifier is called the necessary activator.

Finally it should be stated that for single-sited substrate enzyme systems it is possible to ascertain type of a modifier in inverse co-ordinate system (Fig. 2.7) by analysis of function inflexion

(providing information about the specific constant) and the cut section on the ordinate axis (information concerning the catalytic constant).

2.6. An alternative principle of classification of modifiers

Let us consider a single-sited enzyme system which has one site for substrate and one for a modifier (Fig. 2.8).

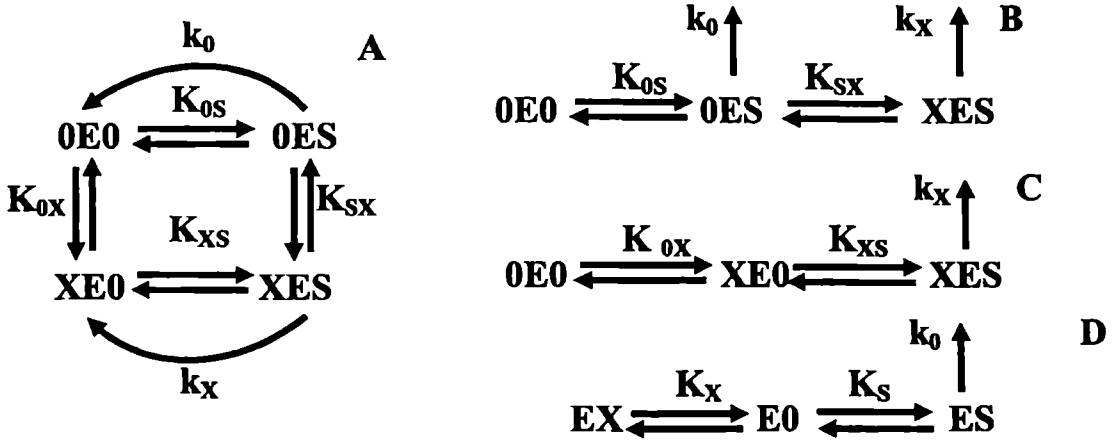


Fig. 2.8. A single-sited enzyme system K_{os}, K_{ox}, K_{sx} and K_{xs} are respective dissociation

A - [XE0]≠0, [0ES]≠0, B - [XE0 constants]=0, [0ES]≠0, C - [XE0]≠0, [0ES]=0:

D – one site for substrate and modifiers

Let us assume that the system is in rapid equilibrium, interaction between the sites is permissible and $\lambda = k_X/k_0$, then we'll have:

$$[0ES]=\frac{S}{K_{0S}}[0E0];\quad [0EX]=\frac{X}{K_{0X}}[0E0];\quad [XES]=\frac{SX}{K_{0S}K_{SX}}[0E0]=\frac{SX}{K_{0X}K_{XS}}[0E0];$$

$\gamma=\frac{K_{SX}}{K_{0X}}=\frac{K_{XS}}{K_{0S}}$; ([XE0]≠0; [0ES]≠0, γ – is the interaction coefficient).

$V=k_0[0ES]+k_X[XES];\qquad e_0=[0E0]+[0ES]+[XE0]+[XES]$

Proceeding from this, in accordance with A, B, C, D schemes (Fig. 2.8) we'll obtain velocity equation:

$$(A)\ U=a+bt=\frac{\left(1+\frac{X}{\gamma K_X}\right)}{k_0\left(1+\frac{\lambda X}{\gamma K_X}\right)}+\frac{K_S\left(1+\frac{X}{K_X}\right)}{k_0\left(1+\frac{\lambda X}{\gamma K_X}\right)}\cdot t;\quad (B)\ U=a+bt=\frac{\left(1+\frac{X}{K_X}\right)}{k_0\left(1+\frac{\lambda X}{K_X}\right)}+\frac{K_S\cdot t}{k_0\left(1+\frac{\lambda X}{K_X}\right)};$$

$$(C)\ U=a+bt=\frac{1}{k_X}+\left(1+\frac{K_X}{X}\right)\frac{K_S}{k_X}t;\qquad (D)\ U=a+bt=\frac{1}{k_0}+\left(1+\frac{X}{K_X}\right)\frac{K_S}{k_0}t \qquad (2.10)$$

where $U = e_0/V$, $t = 1/S$, $a = 1/k_C^{app} > 0$ and $b = 1/k_S^{app} > 0$ „ while the abscissa is intersected in t_o=-a/b point. In the case if X=0 then 2.10 equations will be usually transformed into the Michaelis-Menten equations – $e_o/V=1/K_o (1+K_s/S)$, and if $X \to \infty$ we'll have $e_o/V=1/K_x (1+\gamma K_s/S)$.

Let us suppose that to two diverse concentrations (X1 and X2) corresponds two equations U=a1+b1t and U=a2+b2t the co-ordinates of their intersection points are U*and t*. Let us determine the dependence of U* and t* signs on numerical meanings of λ and γ coefficients.

$$t^* = -\frac{a_2 - a_1}{b_2 - b_1} \text{ and } U^* = b_1 b_2 \frac{t_{02} - t_{01}}{b_2 - b_1}, \text{ where } t_{01} = -\frac{a_1}{b_1} \text{ da } t_{02} = -\frac{a_2}{b_2}$$

As a>0 and b>0, we can easily guess that

$$\text{sign}(t^*) = -\frac{\text{sign}(da/dX)}{\text{sign}(db/dX)} \text{ and } \text{sign}(U^*) = \frac{\text{sign}(dt_0/dX)}{\text{sign}(db/dX)}. \quad (2.11)$$

where , $\frac{da}{dX} = \frac{(1-\lambda)}{k_0 \gamma K_X \left(1 + \frac{\lambda X}{\gamma K_X}\right)^2}$, $\frac{db}{dX} = \frac{K_S(\gamma - \lambda)}{k_0 \gamma K_X \left(1 + \frac{\lambda X}{\gamma K_X}\right)^2}$ and $\frac{dt_0}{dX} = \frac{(\gamma - 1)}{\gamma K_X K_S \left(1 + \frac{X}{K_X}\right)^2}$.

From this it is derived that

$$\text{sign}(t^*) \equiv \frac{\text{sign}(\lambda - 1)}{\text{sign}(\gamma - \lambda)} \text{ and } \text{sign}(U^*) \equiv \frac{\text{sign}(\gamma - 1)}{\text{sign}(\gamma - \lambda)}. \quad (2.12).$$

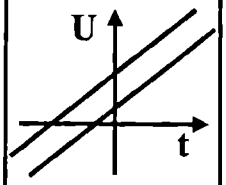
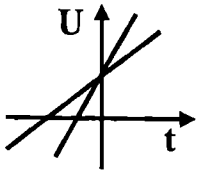
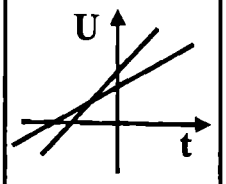
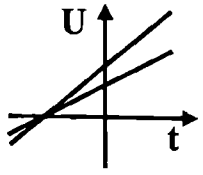
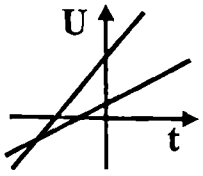
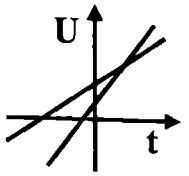
The location of intersection point in the co-ordinate plane is determined by their sign and as is seen from the above analysis, as well as by numerical value of λ and γ coefficients. Since λ and γ numerical value is the basis of modifiers' classification, the location of the intersection point may also become the principle of modifiers' classification, i.e. according to the location of intersection point a modifier's type can be ascertained. This is testified by the examples given Table 2.1.

Modifiers' classification, brought up above, exhaustively describes every possible mechanism of their action on enzyme rate for a single-sited systems. This principle of classification is in full harmony with the recommendations of the International Biochemical Society Nomenclature Committee (NC-IUB).

For single-sited enzyme systems, the intersection point may be located in the I, II and III quadrants of the co-ordinate system, its location in the negative section of ordinate and in the IV quadrants is excluded (a>0, b>0 i.e. t_o<0). In order to determine the position of the intersection point suffice it to ascertain experimentally two dependencies {$U=a(X_1)+b(X_1)t$ and $U=a(X_1)+b(X_1)t$}. While for a full deciphering of the molecular mechanism it is additionally required to establish V=f(X) function form, since one kind of activator & inhibitors rectilinear (L) and non-rectilinear action do not affect the position of intersection point.

Table 2.1

Modifiers' classification according to the intersection point[L] is a rectilinear modifier, UI is a competetive modifier, UI is an uncompetitive inhibitor, NCI is competetive modifier, UI is an uncompetitive inhibitor, NCI is noncompetetive inhibitor. AA is an essential activator (K_o=0) (see Fig. 2,8, schemes A, B, C, D)

№	Graph	sign U^*, t^*	Schemes and conditions		Modifier's type	
			Activator	Inhibitor		
1		$U^* \to \infty$ $t^* \to \infty$	(A) $1 < \gamma = \lambda$	(A) $1 > \gamma = \lambda$	Catalytic activator and inhibitor	
				(B) [XE0]=0, k_X=0; (UI), [L].		
			$dt_0/dX > 0$	$dt_0/dX < 0$		
2		$U^* > 0$ $t^* = 0$	(A) $\gamma < \lambda = 1$	(A ϱ∂ B) $\gamma > \lambda = 1$	Specific activator and inhibitor	
			(C) [0ES)=0, k_0=0; (AA), [L].	(D) k_X=0, (CI), [L].		
			$dt_0/dX < 0$	$dt_0/dX > 0$		
3		$U^* > 0$ $t^* < 0$	(A) $\gamma < 1 < \lambda$	(A) $\gamma > 1 > \lambda$	Preferentially specific	Mixed activator and inhibitor
			$\gamma < 1$, k_0=0; (AA), [L].	$\gamma > 1 > \lambda$=0, [L].		
			$dt_0/dX < 0$	$dt_0/dX > 0$		
4		$U^* = 0$ $t^* < 0$	(A) $1 = \gamma < \lambda$	(A) $1 = \gamma > \lambda$	Affinity constant (to substrate)	
			$1 = \gamma$, k_0=0; (AA), [L]	$1 = \gamma > \lambda$=0, (NCI), [L]		
			$dt_0/dX = 0$	$dt_0/dX = 0$		
5		$U^* < 0$ $t^* < 0$	(A) $1 < \gamma < \lambda$	(A) $1 > \gamma > \lambda$	Preferentially catalytic	
			$1 < \gamma$, k_0=0; (AA), [L].	$1 > \gamma > \lambda$= 0, [L].		
			$dt_0/dX > 0$	$dt_0/dX < 0$		
6		$U^* > 0$ $t^* > 0$	(A) $\gamma < \lambda < 1$	(A) $\gamma > \lambda > 1$	Simultaneously activator and inhibitor	
			$dt_0/dX < 0$	$dt_0/dX > 0$		

Chapter 3. MULTI-SITED ENZYME SYSTEMS

3.1. Basic equation of kinetics

As mentioned above, enzyme reaction rate is a multi-variable function. By convention, they may fall into two groups: 1) reaction medium parameters (temperature, pH, ionic force, solution content (composition), etc); and 2) ligands which represent the enzyme substrate and modifiers. The basic principle of kinetic analysis is to bring the enzyme reaction rate to one-variable function. Proceeding from our goals let the reaction medium (first group parameters) be optimal and constant, while ligand concentration, except one (x) be also constant. In this case we obtain one-variable function and it will be possible to formulate the principal theorem of kinetics:

Theorem. Given constant reaction medium and variability of only one ligand concentration, then the velocity equations of stable enzyme (polymerization, dissociation and association of the enzyme being ruled out), under both rapid equilibrium and steady-state, will have form of ratio of polynomials:

$$\frac{V}{\epsilon_0} = \frac{x^n \sum_{i=0}^{P} \alpha_i x^i}{\sum_{i=0}^{S} \beta_i x^i}; \qquad n + m + p = s \tag{3.1}$$

where, e_o is the enzyme overall concentration, x is concentration of a variable ligand; n, m, p and s are power parameters. n, m and p parameters may assume zero quantity, but not simultaneously. The maximal degree of a nominator is not to exceed that of a denominator $n+p \leq s$, i.e. $m \geq o$. α_1 and β_2 are constant coefficients and are the sums of products of different constants of velocity, therefore $\alpha_1 \geq 0$ and $\beta_2 \geq 0$.

Despite resemblance of analytical form of velocity equation (3.1), derived under rapid equilibrium and steady-state conditions, there is a principal difference between them, what is expressed in the difference of physical essence between equation coefficients and degree parameters.

Under rapid equilibrium conditions, rate equation was derived and analyzed by Wong. Let us define physical meaning of degree parameters obtained in these conditions. Let's denote by ε the sum of the number of sites to be bound by ligand, and the number of ligand occupied sites by i, then for ε site of enzyme systems, each form can be expressed through the free enzyme concentration, ligand concentration and dissociation constant:

$$[Ex_i]=\frac{[x]}{K_i}[Ex_{(i-1)}]=\frac{x^i}{\prod_{j=1}^{i}K_j}[E0] \tag{3.2}$$

where, [E0] is the concentration of a free enzyme, [x] is a variable ligand concentration and K_i and K_j are the dissociation constants.

Suppose, a catalyzing ability is possessed by the form of an enzyme in which n site is occupied, further on when h quantity of sites is occupied the reaction course is not interrupted (only increases or decreases the enzyme reaction rate), while at the occupation of the remaining m site the process gets entirely inhibited. As the reaction rate is a circular combination of the forms having catalyzing ability, in the nominator of velocity equation there will be product, forming able n,.....(n+h) forms, multiplied by relevant rate constants (k), while in the denominator are all parameters, including m ligand-bound form and the velocity equation will look like:

$$\frac{V}{e_0}=\frac{k_n[Ex_n]+\cdots+k_{n+h}[Ex_{n+h}]}{[E0]+\cdots+[Ex_\varepsilon]}=\frac{k_n\frac{x^n}{K_n}+\cdots+k_{n+h}\frac{x^{n+h}}{K_n\cdots K_{n+h}}}{1+\sum\frac{x}{K_i}+\cdots+\frac{x^\varepsilon}{K_1\cdots K_\varepsilon}}=\frac{x^n\sum_{i=n}^{n+h}\alpha_i x^{i-n}}{\sum_{i=0}^{\varepsilon}\beta_i x^i} \tag{3.3}$$

Thus, both the nominator and denominator in the rate equation are polynomials, with respective maximal ([n+h] & ε) and minimal power (n & 0), α_i and β_i of rate constants and combinations of ligand concentration members. If we draw a parallel with basic velocity equations (3.1), it will be clear that p=h and s=ε. From this, clarification of the physical essence of qualitative parameter is easy. If ligand is a modifier, then n is the number (N type sites ligand formation of which is necessary for the enzyme activity to be manifested) of sites necessary for activators; p is the number of sites for the partial effect modifiers (activator or inhibitor) (H type sites which does not require ligand formation for revealing the enzyme activity). m is the number of sites for true inhibitors (M type – during ligand formation of which the enzyme possesses no catalytic activity), and s is the sum of site number. If ligand is a substrate, then, as a rule, n=1, although it is possible that n>1. In this case, theoretically n is the sum of site number for substrate and essential activators.

For multi-sited enzyme systems, under steady-state conditions, to solve the rate equation is a more complex task than under rapid equilibrium conditions. For this it is required to determine velocity of all intermediate forms of enzyme concentration changes, to make it equal to zero and to work the obtained sets of equations. This approach is rather laborious and therefore, a graphical method proposed by King-Altman is, as a rule, used. In the case one ligand being in the stationary equilibrium and the other in the rapid one the method of Cha is applied, this greatly facilitates solving of the equation.

According to King-Altman method, to each enzyme form corresponds a graph node and its relevant determinant (D_{ij}), index i designates number of liganded sites, while j is the numerical number of similar forms. If the total number of sites is ε, then the number of forms with i amount of ligand formed sites would be $\nu_i = \varepsilon!/i!(\varepsilon - i)!$ and total number of forms $\nu_\Sigma = \sum_{i=0}^{\varepsilon} \nu_i = 2^\varepsilon$. By the King-Altman method, the node determinant in general looks like:

$$D_i = \left(G_i^{(0)} x^i + \cdots + G_i^{(\tau)} x^{i+\tau} + \cdots + G_i^{(q_i)} x^{i+q_i}\right),$$

while the sum of determinant forms of sites with uniform i amount of ligands will have the following pattern:

$$\sum D_i = \sum_{j=1}^{\nu_i} D_{ij} = \left[\sum_{j=1}^{\nu_i} G_{ij}^{(0)} x^i + \cdots + \sum_{j=1}^{\nu_i} G_{ij}^{(\tau)} x^{i+\tau} + \cdots + \sum_{j=1}^{\nu_i} G_{ij}^{(q_i)} x^{i+q_i}\right] \tag{3.4}$$

where $\left(G_{ij}^{(\tau)} x^{i+\tau}\right)$ represent the trees oriented to i node, which in turn, is the product of concentrations of all ligands implicated in the velocity constants and transitions. In the most general case q_i may vary for different i and j, but if there exists all transitions in enzyme forms, then for all D_{ij} the maximal degree q_i is constant. It is remarkable that during $D_i \rightarrow D_{j+1}$ transition the minimal and maximal degree of a determinant increases by one, given q_i=constant.

Thus, if the enzyme system has ε site and q_i=const, then we'll have the following sequence of determinants:

$$i=0 \qquad D_0 = \sum_{j=1}^{\nu_0} D_{0j} = \left[\sum_{j=1}^{\nu_0} G_{0j}^{(0)} + \cdots + \sum_{j=1}^{\nu_0} G_{0j}^{(\tau)} x^\tau + \cdots + \sum_{j=1}^{\nu_0} G_{0j}^{(q)} x^q\right]$$

$$i=1 \qquad D_1 = \sum_{j=1}^{\nu_1} D_{1j} = x^1\left[\sum_{j=1}^{\nu_1} G_{1j}^{(0)} + \cdots + \sum_{j=1}^{\nu_1} G_{ij}^{(\tau)} x^\tau + \cdots + \sum_{j=1}^{\nu_1} G_{1j}^{(q)} x^q\right]$$

$$i=n \qquad D_n = \sum_{j=1}^{\nu_n} D_{nj} = x^n\left[\sum_{j=1}^{\nu_n} G_{nj}^{(0)} + \cdots + \sum_{j=1}^{\nu_n} G_{nj}^{(\tau)} x^\tau + \cdots + \sum_{j=1}^{\nu_n} G_{nj}^{(q)} x^q\right]$$

$$i=n+h \qquad D_{n+h} = \sum_{j=1}^{\nu_i} D_{(n+h)j} = x^{n+h}\left[\sum_{j=1}^{\nu_i} G_{(n+h)j}^{(0)} + \cdots + \sum_{j=1}^{\nu(n+h)} G_{(n+h)j}^{(q)} x^q\right]$$

$$i=\varepsilon \qquad D_\varepsilon = \sum_{j=1}^{\nu_\varepsilon} D_{\varepsilon j} = x^\varepsilon\left[\sum_{j=1}^{\nu_\varepsilon} G_{\varepsilon j}^{(0)} + \cdots + \sum_{j=1}^{\nu_\varepsilon} G_{\varepsilon j}^{(\tau)} x^\tau + \cdots + \sum_{j=1}^{\nu_\varepsilon} G_{\varepsilon j}^{(q)} x^q\right]$$

By summing we'll get a polynomial whose coefficients are

$$\beta_i = \sum_\tau \left[\sum_{j=1}^{\nu_i} G_{\tau j}^{(i)}\right]: \qquad \sum D_i = \beta_0 + \beta_1 x + \cdots + \beta_i x^i + \cdots + \beta_S x^S, \text{ where } s=\varepsilon+q \tag{3.5}$$

Suppose the enzyme system for essential activators possesses n site (N type) and additionally h site whose liganding results in an increase or decrease (H type) of the reaction rate. The relevant nodes of this type ligand sites show catalytic activity and the product of their determinants by the catalytic constant will be:

$$i=n \qquad (kD)_n = k_n x^n \left(G_n^{(0)} + \cdots + G_n^{(\tau)} x^{\tau} + \cdots + G_n^{(q)} x^q\right)$$

$$n<i<n+h \qquad (kD)_i = \sum_{j=1}^{\nu_i} k_{ij} D_{ij} = x^i \left[\sum_{j=1}^{\nu_i} k_{ij} G_{ij}^{(0)} + \cdots + \sum_{j=1}^{\nu_i} k_{ij} G_{ij}^{(\tau)} x^{\tau} + \cdots + \sum_{j=1}^{\nu_i} k_{ij} G_{ij}^{(q)} x^q\right]$$

$$i=n+h \qquad (kD)_{n+h} = k_{(n+h)} x^{n+h} \left(G_{(n+h)}^{(0)} + \cdots + G_{(n+h)}^{(\tau)} x^{\tau} + \cdots + G_{(n+h)}^{(q)} x^q\right)$$

By summing we also get a polynomial, where p=h+q

$$\sum_i (kD)_i = x^n \left(\alpha_0 + \cdots + \alpha_i x^i + \cdots + \alpha_p x^p\right) \tag{3.6}$$

Under steady-state the enzyme reaction rate is the ratio of the sum of product of catalyseable node determinant and respective catalytic constants to the sum of all node determinants:

$$\frac{V}{e_0} = \frac{\sum_{i=n}^{n+h} (kD)_i}{\sum_{i=0}^{\varepsilon} D_i} \Rightarrow \frac{x^n \sum_{i=0}^{p} \alpha_i x^i}{\sum_{i=0}^{s} \beta_i x^i}; \quad m = s - n - p \tag{3.7}$$

From the analysis of the above given sequence it is evident that the physical essence of n parameter is the same as in rapid equilibrium, but alters the physical meaning of p parameter, which is the q sum of partial effect modifiers (h) and of a new power parameter. Accordingly $s=\varepsilon+q$. q – that reflects complexity of the structure of the enzyme system molecular mechanism, let's refer to it as a complexity parameter. At a strict sequence of ligand binding (when first N type sites are liganded, then H type and finally M type) both in stationary and rapid equilibrium q=0. If in a functional enzyme unit ligand binding reduces to zero the site affinity to ligand, q would not have one meaning for all D_{ij} determinants. Accordingly, D_i will have various maximal degrees. In the basic velocity equation (n+p) is a maximal degree of $K_{n+n}D_{n+n}$ expression, while s is the maximal degree of D_ε determinant, although they may have a diverse meaning of q_i. Respectively, we'll have $q_{(n+n)}$ and q_ε. In the case of rapid equilibrium we had ε-n-h=s-n-p=m. Let's determine s-(n+p) in stationary equilibrium:

$$\left.\begin{array}{l} s = \varepsilon + q_\varepsilon \\ p = h + q_{(n+h)} \end{array}\right\} \Rightarrow \left.\begin{array}{l} s \geq p;\ m \neq 0;\ q_\varepsilon > q_{n+h}; \\ q_m = (q_\varepsilon - q_{n+h}) \end{array}\right\} \Rightarrow \quad s-(n+p) = \varepsilon - (n+h) + (q_\varepsilon - q_{n+h}) = m + q_m$$

i.e. nominally nothing changes, although the degree parameters s, p, m and h gain a new physical meaning (n physical meaning remains unaltered) at increased number of sites. But, as already mentioned, the analytical form of the enzyme reaction rate equation is complicated (3.1). This occurs particularly in stationary equilibrium. This is verified by the interaction between the site number and the degree parameters, represented in Table 3.1

Table 3.1

Number of sites	Number of enzyme forms	Maximal degree of meaning, s		q=const
		Rapid equilibrium	Stationary equilibrium	
1	2	1	1	0
2	4	2	3	1
3	8	3	7	4
4	16	4	15	12
7	128	7	127	120

The sites (M type) forming dead-end branching falls into two groups: to the first group (M_1 type, amount m_1) belong the sites having a capacity of binding a ligand in the case of ligand formation in other sites. This means that they are able to form a dead-end branching in the case of ligand formation in N and H type of all (n+h) sites and M_1 type (m_1-1) site. To the second group (type M_2, amount m_2) belong the sites which are capable of ligand binding only when the definite r_j sites are filled with a ligand, while at ligand binding of (r_j+1) site, because of a negative co-operativity, no ligand filling occurs ($K_d \rightarrow \infty$). If the number of such sites (m_{2j}+r_j) is more than n+h+m_1, then they can cause an increase in the maximal degree of the node determinant (D_ε) only by Δm_j value (m_j=m_{2j}=r_j-n-h-m_1). An imitation is created that m_j sites are attributed to M_1 type of sites. The rest (m_{2j}-m_j) sites do not affect maximally the degree parameter (s) and their manifestation on the basis of equation 3.1 is impossible.

$$m = [m_j + \Sigma(m_j)] = s\text{-}n\text{-}p$$

m designates the number of sites for full inhibition and the rest – non-exhibited sites (m_o). If a ligand is a substrate, then such kind of (non-exhibited) sites are referred to as non-productive ones.

m=s-(n+p) is a maximal number of ligand occupied passive sites (the sites forming dead-end ramifications capable of enzyme inactivation. Such a definition offered by Z. Kometiani differs from that of Bardsley's who under s-(n+p) expression meant the number of all the sites ligand binding to which results in a dead-end branching. Thus, the sites forming dead-end branching are to be divided into the sites that can be manifested (m) and those which cannot be revealed (m_o).

Similar situation may occur in the rapid equilibrium conditions as well. Therefore, the physical essence of degree parameters requires correction.

Conclusion. Thus, the qualitative parameters of the main velocity equation (3.1) have the following physical meaning under rapid and stationary equilibrium:

n is the number of sites for essential activators;

h is the number of sites for partial effect activators;

m=[$\mathbf{m_1}$+$\Sigma(\mathbf{m_j})$ is the number of sites for the full inhibitors;

$\mathbf{m_0}$ is the number of sites for inhibitors, whose filling with the ligand results in dead-end branching, but has no effect on the maximal degree of the denominator;

q represents the degree parameter reflecting the complexity of the enzyme molecular mechanism.

In the case of rapid equilibrium **(q=0)**, $\mathbf{p=h+q_{n+n}}$, $\mathbf{s=\varepsilon+q_\varepsilon}$; (if $\mathbf{q_i=const}$ $\mathbf{q_{(n+h)}=q_k}$)

3.2. ω function

The clarification of the molecular mechanism of multi-sited enzyme systems is primarily associated with the determination of digital meaning of qualitative parameters in the main velocity equation (3.1), this being rather a complex task. As regards the qualitative indices, they are associated with the reaction order (see 1.5). Let us consider a simple reaction

$$E + nX \rightarrow EX_n \quad \Rightarrow \quad V = d[EX_n]/dt = k[E]X^n \quad \Rightarrow \quad lnV = lnk + ln[E] + nlnX$$

where, V is the enzyme reaction rate, X is a variable ligand concentration, while the rectilinear InV=f(InX) function slope is a reaction order, n=dInC/dInX. For the multi-sited enzyme systems, as a rule, the logarithmic function is of far more complex pattern and it is impossible to calculate its genuine order. Therefore, a kinetic parameter apparent order of the reaction ω=dInV/dInX has been introduced.

In complex reactions, as distinct from the elementary ones, the apparent order may not be an integer, or a negative value may be obtained. Proceeding from this, the direct physical essence of ω function seems to be abolished. Let us reflect the first derivative of V=f(x) function through ω function.

$$V'_x = \frac{dV}{dx} = \frac{V}{x}\frac{d\ln V}{d\ln x} = \frac{V}{x}\omega \qquad \Rightarrow \qquad \omega = \frac{V'_x}{V/x}$$

V/x is nominally the first order velocity (V=Kx) constant (slope); while V_x represents the slope of tangent of the genuine velocity V=f(x) function in V_1x point. ω is the ratio of these two slopes, i.e. represents a measure of the difference of genuine velocity and the first order velocity.

There may be another interpretation too: ω function is specific acceleration of V=f(x) function, expressed in the units of the first-order velocity gradient. These definitions clearly display the physical essence of ω function.

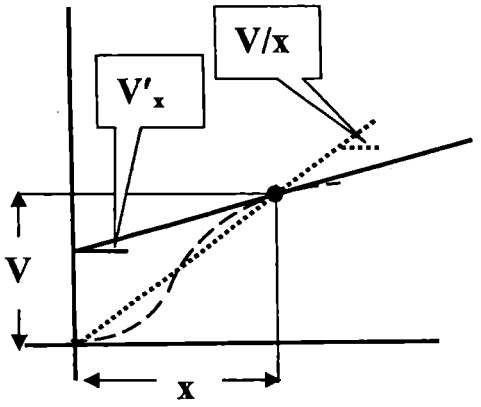


Fig. 3.1. Physical essence of ω

V'_x – a slope of a tangent of V=f(x) function in x point (V,x).

V/x – a slope of the first-order velocity (V=kx)

In addition to all this, ω function has a paramount importance for the thorough theoretical kinetic analysis of the multi-sited enzyme systems. It is, therefore, reasonable to study in more details the properties of ω function.

1. ω and ω' are continuous functions and they, as τ–variable (τ=Int) functions have the following analytical pattern:

$$\omega = \frac{n\alpha_0\beta_0 + \sum_{k=1}^{s+p-1} \Psi_k e^{-k\tau} - m\alpha_p\beta_s e^{-(s+p)\tau}}{\alpha_0\beta_0 + \sum_{k=1}^{s+p-1} B_k + \alpha_p\beta_s e^{-(s+p)\tau}}; \qquad \frac{d\omega}{d\tau} = \frac{\sum_{q=0}^{2(s+p)} W_q e^{-q\tau}}{\left(\sum_{k=0}^{s+p} B_k e^{-k\tau}\right)^2} \tag{3.10}$$

where

$$\begin{cases} 0 \le k \le p \Rightarrow \Psi_k = \sum_{i=0}^{k} (n-k+2i)\alpha_i\beta_{k-i}; \quad B_k = \sum_{i=0}^{k} \alpha_i\beta_{k-i} \\ p \le k \le s \Rightarrow \Psi_k = \sum_{i=0}^{p} (n-k+2i)\alpha_i\beta_{k-i}; \quad B_k = \sum_{i=0}^{p} \alpha_i\beta_{k-i} \\ s \le k \le (s+p) \Rightarrow \Psi_k = \sum_{i=k-s}^{p} (n-k+2i)\alpha_i\beta_{k-i}; \quad B_k = \sum_{i=k-s}^{p} \alpha_i\beta_{k-i} \\ 0 \le q \le (s+p) \Rightarrow W_q = \sum_{k=0}^{q} (q-2k)\Psi_k B_{q-k} \\ (s+p) \le q \le 2(s+p) \Rightarrow W_q = \sum_{k=q-s-p}^{s+p} (q-2k)\Psi_k B_{q-k} \end{cases} \tag{3.11}$$

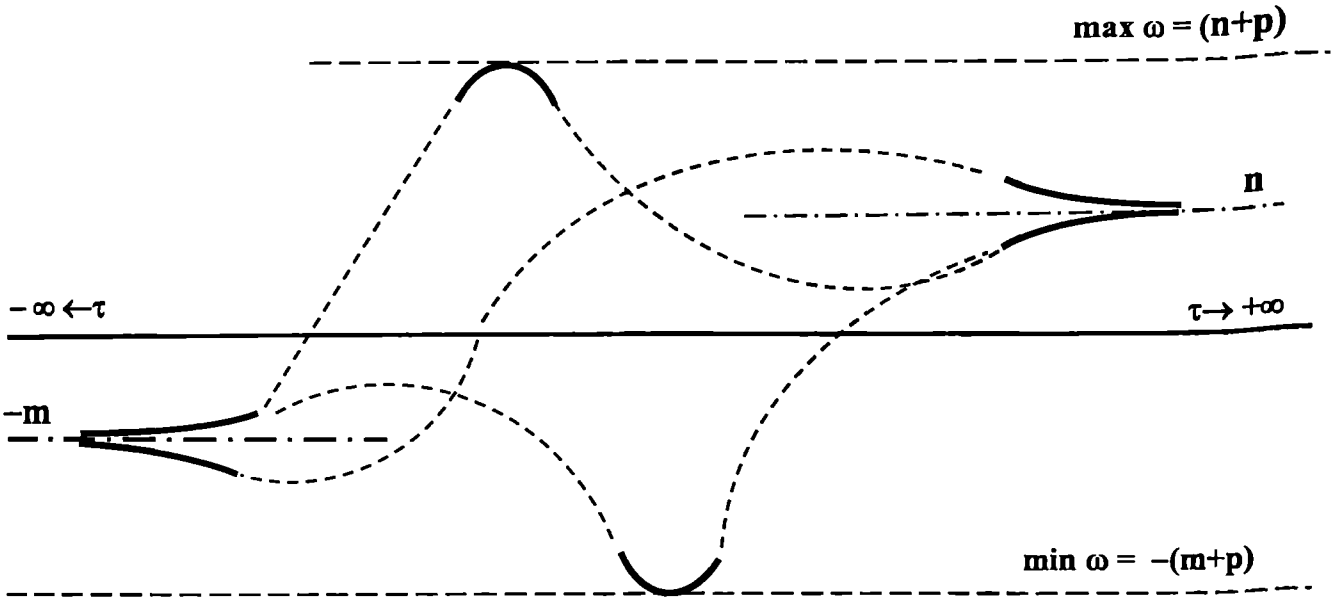


Fig. 3.2 $\omega = f(t)$. (see the text)

2. $$\omega = \frac{xV'_x}{V} = \frac{d\ln V}{d\ln x} = \frac{d\ln(1/V)}{d\ln(1/x)} = \frac{tU'_t}{U}; \qquad (U = 1/V,\ t = 1/x). \qquad (3.8)$$

$$\frac{d^2\ln V}{d\ln x^2} = -\frac{d^2\ln U}{d\ln t^2} = -\frac{d^2\ln U}{d\ln x^2}; \qquad \omega' = \frac{d\omega}{d\tau};\ (\tau=\ln t) \qquad (3.9)$$

In the case of reversed variables ω does not alter, while its derivative changes the sign.

3. ω function is restricted, **$-(m+p) \le \omega \le (n+p)$**

4. ω function has horizontal asymptotes: $\lim_{\tau\to\infty}\omega = n$, $\lim_{\tau\to\infty}\omega' = \pm 0$; $\lim_{\tau\to-\infty}\omega' = -m$, $\lim_{\tau\to-\infty}\omega' = \pm 0$

5. $\lim_{\tau\to\infty}\omega'$– sign is determined by the sign of $D_{-1}=(_0\beta_1-\alpha_1\beta_o)$ expression , if $D_{01}=0$, then sign of $D_{02}=(\alpha_0\beta_2-\alpha_2\beta_0)$ and etc. $\lim_{\tau\to-\infty}\omega'$–sign is determined by the sign of expression $H_{01}=(\alpha_p\beta_{s-t}-\alpha_{p-1}\beta_s)$, if $H_{01}=0$, then $H_{02}=(\alpha_p\beta_{S-2}-\alpha_{p-2}\beta_S)$ expression sign and etc.

6. The number of turning points of $V=f(x)$ and $U=f(t)$ functions is dependent on p degree parameter, the sign domain of ω function and the meaning of n & m degree parameters. The number of turning points is defined by the root number v, in $\omega=0$ equation.

	n>1, m≥0	n=1, m≥0	n=0, m≥0
sign $\lim_{\tau\to\infty}\omega \neq$ sign $\lim_{\tau\to-\infty}\omega$	$1 \le \mu \le (2p-1)$	$0 \le \mu \le (2p-2)$	– – –
sign $\lim_{\tau\to\infty}\omega =$ sign $\lim_{\tau\to-\infty}\omega$	– – –	$1 \le \mu \le (2p-1)$	$0 \le \mu \le (2p-2)$

7. During analysis of kinetic curves the tangents passing through the origin of coordinates is used. Their number, for $V=f(x)$ and $U=f(t)$ functions, is determined by the root number μ in $\omega=1$

equation, which also depends on p degree parameter, the signs in ω function domain and on the meaning of n & m degree parameters.

	n≠0, m≠0	n≠0, m=0	n=0, m≠0	n=m=0
$\operatorname{sign}\lim_{\tau\to\infty}\omega \neq \operatorname{sign}\lim_{\tau\to-\infty}\omega$	$1\le\nu\le(2p-1)$			$1\le\nu\le(2p-3)$
$\operatorname{sign}\lim_{\tau\to\infty}\omega = \operatorname{sign}\lim_{\tau\to-\infty}\omega$	– –	$0\le\nu\le(2p-2)$		

8. lnU=f(lnt), lnU=f(lnx) და lnV=f(lnx) functions have sloped asymptotes

$(\ln x\to-\infty)\Rightarrow \ln V=\ln(\alpha_0/\beta_0)+n\ln x$ $\quad$ $(\ln x\to+\infty)\Rightarrow \ln V=\ln(\alpha_p/\beta_s)-n\ln x$

$(\ln t\to-\infty)\Rightarrow \ln U=\ln(\beta_s/\alpha_p)-m\ln t$ $\quad$ $(\ln t\to+\infty)\Rightarrow \ln U=\ln(\beta_0/\alpha_0)+n\ln t$

$(\ln x\to-\infty)\Rightarrow \ln U=\ln(\beta_0/\alpha_0)-n\ln x$ $\quad$ $(\ln x\to+\infty)\Rightarrow \ln U=\ln(\beta_s/\alpha_p)+m\ln x$

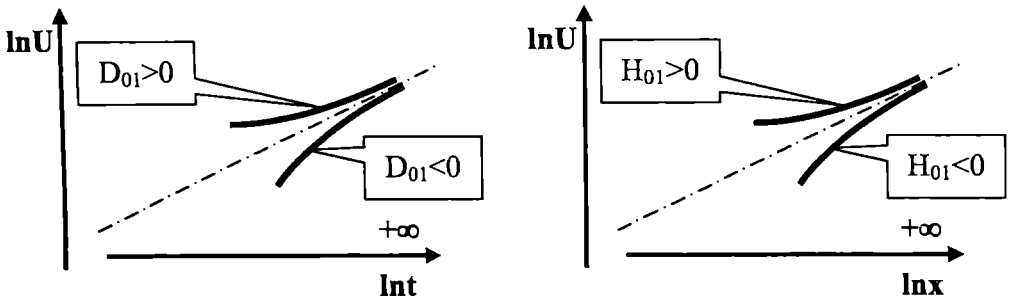


Fig. 3.3 lnU=f(lnt) და lnU=f(lnx) function asymptotes and the shape of curves in the limit $(\to\infty)$ $D_{01}=\alpha_0\beta_1-\alpha_1\beta_0$; $H_{01}=\alpha_p\beta_{s-1}-\alpha_{p-1}\beta_s$.

9. Through ω and ω' it is possible to reflect the derivative of some important fur ctions (see Table 3.2; The following designations are used: U=1/V, t=1/x, τ=lnt)

Table 3.2

F=f(G)	F'_G	$F-G\cdot F'_G$	F''_{GG}
lnU=f(lnt)	$\frac{d\ln U}{d\ln t}=\omega$	lnU–ωlnt	$\frac{d\omega}{d\tau}=\omega'_\tau$
V=f(x)	$\frac{V\omega}{x}$	$\frac{V}{x}(1-\omega)$	$\frac{V}{x^2}[-\omega'_\tau+\omega(\omega-1)]$
$y(r,t)=\sqrt[r]{U}=f(t)$	$\frac{\sqrt[r]{U}}{rt}\omega$	$\frac{\sqrt[r]{U}}{rt}(r-\omega)$	$\frac{\sqrt[r]{U}}{r^2t^2}[r\omega'_\tau+\omega(\omega-r)]$
$y(r,x)=\sqrt[r]{U}=f(x)$	$-\frac{\sqrt[r]{U}}{rx}\omega$	$\frac{\sqrt[r]{U}}{r}(r+\omega)$	$\frac{\sqrt[r]{U}}{r^2x^2}[r\omega'_\tau+\omega(\omega+r)]$
$z(r,t)=\frac{U}{t^r}=f(t)$	$\frac{U(\omega-r)}{t^{r+1}}$	$\frac{U}{t^r}(r+1-\omega)$	$\frac{U}{t^{r+2}}[\omega'_\tau+(\omega-r)(\omega-r-1)]$
$z(r,x)=\frac{U}{x^r}=f(x)$	$\frac{U(\omega-r)}{t^{r+1}}$	$\frac{U}{x^r}(r+1+\omega)$	$\frac{U}{x^{r+2}}[\omega'_\tau+(\omega+r)(\omega+r+1)]$
$\ln U=f(t)$	$\frac{\omega}{t}$	$\ln U-\omega$	$\frac{\omega'_\tau-\omega}{t^2}$

The methods for transformation of variables have been commonly employed in kinetic studies. Their purpose was a linearization of the initial hyperbolic function V=(f(x) via transformation of variables and by means of them to determine the basic kinetic parameters V_{max} and K_m. Analysis of the main equation (3.1) poses certain difficulties. On the one hand, the equation coefficients are complex combination of kinetic constants and because of this, the information yielded by analysis of a slope and intersection of coordinate axis is rather restricted. And on the other hand, the equation (3.1) contains the terms being in a high degree of concentration and measurement of degree parameters by experimental curves is a complex task. Solution of this problem requires a detailed analysis of geometric forms of the functions resulted from the transformation of variables. From this viewpoint, it is preferential to reflect the geometric shape features via any one function and to use ω function for this reason (Table 3.2).

The presented transition formula enables to follow the intertransformation of the geometric shape determinant parameters of the given functions (the turning point, inflexion point, the tangent passing in the origin of co-ordinates.

Thus, via ω function it is possible to ascertain the geometric shape determinant parameters for other functions and to make a relative analysis of their shapes.

3.3. The principles of kinetic curves' analysis for the multi-sited enzyme system

Deciphering of the molecular mechanism for single-sited enzyme systems is associated with the measurement of V_{max} and K_m , graphical plotting of which is possible with the traditional methods used for variables' transformation, once they provide linearization of the initial hyperbolic dependencies. In the case of multi-sited enzyme systems it is obtained a new kinetic parameter, degree index of which is associated with the type and number of ligand binding sites. Therefore, while making analysis of the main velocity equation, that is a fractional-rational function, definite difficulties are encountered. On the one hand, the equation coefficients are complex combinations of kinetic constants, that's why the information furnished by analysis of slope and intersection of the co-ordinate axis is very limited. So, for the multi-sited enzyme systems clarification of kinetic parameters for each individual site is difficult and it became necessary to find such parameters that would change V_{max} and K_m. On the other hand, the equation contains a high degree of concentration members and determination of degree parameters of experimental curves is an important task. A single way for solving this problem is the analysis of geometrical shape of the curve. Any kind, including fractional-rational analysis of function implies, above all, determination of arrangement and number of extremum and inflexion points (Fig. 3.4).

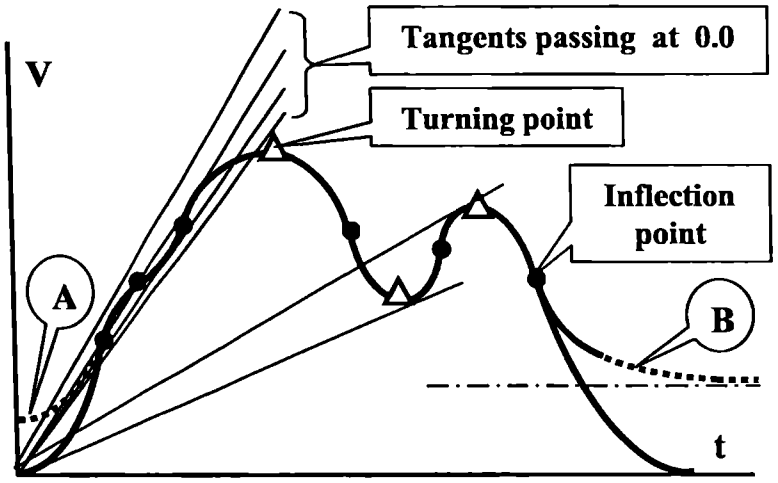


Fig.3.4. Graphs of V=f(x) function

A – (n=0), B – (m=0).

$$\frac{V}{e_0} = \frac{x^n \sum_{i=0}^{P} \alpha_i x^i}{\sum_{i=0}^{S} \beta_i x^i}, \quad s=n+m+p$$

A stepwise increase of velocity determines existence of more than one tangent passing at 0.0

The points of extremum is determined by $V' = 0$ equation and the inflexion point by $V'' = 0$ equation. The point of horizontal inflexion, existence of which requires fulfillment of two conditions, $V' = 0$ and $V'' = 0$, is important in the kinetic curve studies. Apart from the equality to zero of the first and second derivatives of the function, determination of their signs is of importance. In the first case it provides information about an increase or decrease of the function, while in the other case, about its convexity and concavity. While classifying kinetic curves by their shape it is important to determine the curve point on which passes the tangent in the origin of co-ordinate. As regards the infinitely high and infinitely low concentrations of the argument, analysis of the function in this site requires assessment of the above parameters with constrained meanings of the argument ($x \rightarrow 0$ and $x \rightarrow \infty$). It is also important to ascertain the arrangement of a function graph against asymptote. The parameters discussed above enable analysis of the curves of geometrical shape.

Bardsley and co-workers were the first make an attempt to decipher the molecular mechanism of the multi-sited enzyme systems through the analysis of geometrical shape of a curve. They have studied a fractional-rational function 3.1 and analyzed the possible shapes of kinetic curves. Study of local graphical features of a curve makes it possible to discuss the degree parameters of the velocity equation. Thus, for example, if at infinitely great value of the argument V=f(x) the graph asymptotically approaches zero, this means that the maximal degree (power) of a nominator is more than that of a denominator S>n+p; if at infinitely great value of an argument V=f(x) graph tends to a horizontal asymptote, then on S=n+p, V=f(x) graph availability of a sigmoid inflexion point testifies to a minimum 2:2 degree and justification of $\alpha_2\beta_o > \alpha_1\beta_1$ inequality. Minimum 2:2 degree is plausible also in the case, if in 1/V=f(1/x) co-ordinate system the graph is not rectilinear or on V=f(x) graph of a function there is a single maximum point. On V=f(x) curve

existence of a minimum point implies the least 3:3 degree. If on V=f(x) graph there are multiple inflexion points and on 1/V=f(1/x) there is one inflexion point, this still testifies to a minimum 2:3 degree, while V=f(x) graph with a horizontal asymptote and a horizontal plateau implies a minimum 3:3 degree. Similar rules are numerous, but it should be noted that for an accurate estimation of the degree of velocity equation the use of other co-ordinate systems alongside with V=f(x) and 1/V=f(x) graphs is beneficial. The curves having a definite geometrical feature are characteristic of each fractional-rational function. In spite of the fact that the high-degree equations yield all curves specific for low degrees, there are still certain features which are characteristic of only high degrees, therefore, to discover such specific features the use of different co-ordinate system is required. The transition formula allows for the transfer of one co-ordinate system to another (Table 3.2).

The given formula enables to follow the transformation of the turning and inflexion points while shifting from one co-ordinate system to another. It is clear that the turning points available on the initial function V=f(x) graph will be maintained on all the rest of function graphs. It is remarkable that the first inflexion point (n>1) on V=f(x) graph is not reflected on 1/V=f(1/x) graph. It should be noted that the shape of multi-sited enzyme kinetic curve, except for the degree parameters, is affected by α and β coefficients. The degree parameters of a fractional-rational function determine the upper limit of curve complexity, while the coefficients, the lower limit.

It must be borne in mind that a complex analysis of complex geometrical shape curves for the multi-sited enzyme systems is impossible because of a sparse experimental material, therefore, for the sake of facility Bardsley has separated three sites to which corresponded the sites with extremely low, medium and extremely high ligand concentrations, the site where the curve shape is affected, apart from the degree parameters (n, m, p), by α and β coefficients. It must be noted that with extremely low ligand concentrations which involves the section to the first turning point of V=f(x) function, the curve shape is influenced chiefly by n parameter.

At extremely high concentrations, which involves the section from the last point of turning of V=f(x) function to the infinitely high quantity of argument, the curve shape is affected by m parameter, while the middle concentration range is located between the given sites and here curve shape is influenced by p parameter. Bardsley considered a particular case, when argument of the function represents substrate (n=1), while m parameter equals to zero, therefore he did not have to determine such parameters of rate equation as the least power index (n) of a nominator and difference between the maximal power of denominator and nominator (m). This problem is topical, when a modifier is considered as argument of a function. Thus, in the present case determination of degree parameters of the (n=1, m=0) velocity equation is restricted by p parameter. Analysis of the

geometrical shape of the curve has shown that in the site of medium concentrations of a ligand estimation of p parameter is possible on the basis of analysis of extremum and inflexion points.

It must be noted that α and β coefficients of fractional-rational function as representing a combination of velocity constants depend on a concrete mechanism of reaction. This property makes it possible to distinguish the individual mechanisms, or in other words, it is possible to relate probability with the definite curve shape and calculate its meaning. Bardsley has proposed a method for the calculation of the given probabilities and a probability for a definite curve shape appeared to correspond to each molecular mechanism. Different shape probabilities were determined for 2:2 and 3:3 fractional-rational functions, as well as for concrete mechanisms generating the equation in the given degree. Deciphering of a molecular mechanism for enzyme systems is possible using the approximation method of rectilinear regression for kinetic curves.

Thus, it should be said that both in calculation of geometrical shape probabilities of kinetic curves and in the case of approximation via curved linear regression of the given curves, fitting procedure was used, on the basis of which different molecular mechanisms were distinguished and the highest powers of a nominator and denominator of the fractional-rational function were determined. From the viewpoint of deciphering the molecular mechanism, more universal than this approach is to establish at the first stage n, m and p parameters and on the basis of it determine a «minimal model» that actually represents regulation principle for the molecular mechanism. Only afterwards it is reasonable to use the rectilinear regression conventional approaches for the calculation of numerical quantities of the coefficients, that would enable extention of the scheme and final deciphering of the molecular mechanism. From this viewpoint, none of the traditional methods for variable transformation in the extreme ligand concentration site allows for n and m to be determined. Therefore, for the purpose of solving the tasks related to the molecular mechanism's deciphering for the multi-sited enzyme systems, it was necessary to introduce a new co-ordinate system. Naturally, the use of geometric functions could not solve the question of n and m determinations. Of logarithmic functions, study of ω function made it evident that n and m degree parameters could be determined through it.

From the viewpoint of n and m measurement, analysis of degree functions is of interest. In particular, to study the function resulting from the universed power transformation (when a function and argument simultaneously undergo all the possible power alterations: root taking, raising to a power, multiplication and division. Let us designate the transformed new function and argument respectively by F and G, $F=V^{\rho}x^{\lambda}$ and $G=V^{\mu}x^{\nu}$, where ρ, λ, μ and v are real numbers. The traditional graphs, except the logarithmic one, are a particular case of the given transformation. The geometric shape determining main parameters allow for the analysis of the given F/G function. It appeared

that in order to determine them it is convenient to reflect them by means of a ω function, or Ω value, which is formally analogy to ω. Thus, for example:

$$\Omega=\frac{d\ln F}{d\ln G}=\frac{\rho\omega+\lambda}{\mu\omega+\nu};\qquad \Omega'=\frac{d^2\ln F}{d\ln G^2}=\frac{\omega'(\rho\nu-\mu\lambda)}{(\mu\omega+\nu)^2} \tag{3.12}$$

The geometric shape of F=f(G) function is determined by its first (F'_Ω) and second ($F''_{\Omega\Omega}$) derivatives and by way of the ordinate section cut by a tangent, when reflected by means of ($F-GF'_\Omega$), it will look like:

$$\frac{dF}{dG}=\frac{F}{G}\Omega=\frac{V^{\rho+\mu}[\rho\omega+\lambda]}{x^{\nu-\lambda}(\mu\omega+\nu)};$$

$$\frac{d^2F}{dG^2}=\frac{F}{G^2}[\Omega'+\Omega(\Omega-1)]=\frac{V^{(\rho-\mu)}\{(\rho\nu-\mu\lambda)\omega'+[(\rho\omega+\lambda)-(\mu\omega+\nu)](\rho\omega+\lambda)(\mu\omega+\nu)\}}{x^{(2\nu-\lambda)}(\mu\omega+\nu)^2}; \tag{3.13}$$

$$F-GF'_G=F(1-\Omega)=V^\rho x^2\frac{(\mu\omega+\nu)-(\rho\omega+\lambda)}{\mu\omega+\nu}$$

The given formulae make it possible to study a curve of any shape obtained as a result of power transformation.

Analysis of curve shape in the medium site of ligand concentrations implies to establish the extremum and inflexion points and to explore the function's first and second derivatives' sign transformation as dependent on the degree parameters. In the ligand medial concentrations site it is impossible to determine the numerical meaning of n and m degree parameters. This site enables to delineate the regularities that exist between the extremum, inflexion point and the tangents passing in the origin of coordinates.

The extremum points are defined by the equation F'=0. If λ=0, then the extremum points of all the rest degree transformation are maintained and for this one requires to meet ω=0 equality. Thus, if the initial functions extremum lies in X_i point, then F=f(G) function as well will have it in the respective points of argument, for any values of p, μ and v. Maximal number of positive roots of ω=0 equation is (2p-1). When λ≠0, the number of extremum points is determined by the roots of pω+λ=0 equation, given μω+v≠0. In this case the points of extremum do not persist and their maximal number again equals to (2p-1). The inflexion points are determined by the roots of F''=0 equation.

In a general case, during power transformation the inflexion points are subject to transformation, though there are exceptions in the case if F=f(G) function undergoes the following power transformations:

$$\begin{aligned}
&F=f(G) \Rightarrow 1/F=f(1/G) \quad (\rho=\nu=-1,\ \lambda=\mu=0)\\
&F=f(G) \Rightarrow F=f(F/G) \quad (\rho=\mu=1,\ \lambda=0,\ \nu=-1)\\
&F=f(G) \Rightarrow G/F=f(G) \quad (\lambda=\nu=1,\ \mu=0,\ \rho=-1)
\end{aligned} \tag{3.14}$$

then, the second derivatives of these functions will respectively look like:

$$\frac{d^2(1/F)}{d(1/G)^2}=\frac{G^2}{F}[-\Omega'+\Omega(\Omega-1)];$$
$$\frac{d^2F}{D(F/G)^2}=\frac{G^2}{F}\frac{[-\Omega'+\Omega(\Omega-1)]}{(\Omega-1)^2}; \qquad (3.15)$$
$$\frac{d^2(G/F)}{d(G)^2}=\frac{1}{FG}[-\Omega'+\Omega(\Omega-1)].$$

In the case of the very function, the inflexion points will be maintained as a result of transformation and will be determined by $[\Omega(\Omega\text{-}1)-\Omega']=0$ equation.

Both the first and second derivatives in F=f(G) function are discontinuous functions when $\mu=0$. Function break occurs only in the case when $\mu\neq0$ and the respective points are determined by $(\mu\omega+v)=0$ equation. When $\lambda=0$, at all other power transformations the horizontal inflexion point maintains and represents the roots of $\omega=0$ $\omega'=0$ equation system.

As mentioned earlier, for the analysis of geometric shape of curves important is the point at which the tangent passes in the origin of coordinates. This point is defined by &!=1 equation.

In the medium site of ligand concentrations, relying on analysis of the curve geometric shape, the certain regularities were found to exist between the points of extremum, inflexion and those at which lies the tangent passing in the origin of coordinates. This is expressed in the following rules:

1) Between the two adjacent extremum and horizontal inflexion points there surely is at least one inflexion point. Their number is always odd.

2) Between two points at which lies the tangent passing in the coordinates origin there is sure to be at least one inflexion point. Their number is always odd.

3) Between two inflexion points there may not be the points of extremum, of horizontal inflexion and the points at which lies the tangent passing in the coordinate origin.

Hence, analysis of the kinetic curve geometric shape implies calculation and determination of turning points, inflexion points, the signs of the first and second derivatives, the tangents passing in the origin of coordinates and asymptotes.

Particularly important is the analysis of the curve geometric shape in the ranges of extremely small and large values of argument, as within these ranges $\lim_{\tau\to\infty}\omega=n$ and $\lim_{\tau\to-\infty}\omega'=-m$.

The geometric shape of a curve is chiefly determined by degree parameters (n, m, p) and the coefficients (α_i, β_i), but as a result of power transformation of variables also alters the geometric shape of the curve and respectively the degree parameters. There is an impression that the degree parameters (n, m, p) can be defined on the basis of analysis of the curve geometric shape. These

questions have been studied in details. It emerged that there is no sense in the transformation of variables by means of trigonometric and exponential functions. And of various versions of variable transformations ($F=V^{\rho}x^{\lambda}$ and $G=V^{\mu}x^{\nu}$) only four can be used:

1) $\mu=0$, $\lambda=0$, $\nu=-1$ and $\rho=-\frac{1}{r}$

2) $\mu=0$, $\lambda=0$, $\nu=+1$ and $\rho=-\frac{1}{r}$;

3) $\mu=0$, $\rho=-1$, $\mu=0$ and $\lambda=-r$;

4) $\mu=0$, $\rho=-1$, $\mu=0$ and $\lambda=+r$;

where r is a variable parameter. Thus, if we add the logarithmic functions, there would emerge six of them by means of which there is a theoretical possibility for determining the degree parameter (n&m):

1) $\ln V=f(\ln x)$, $\ln V = n\delta + \ln\sum_{i=0}^{p}\alpha_i e^{i\delta} - \ln\sum_{i=0}^{s}\beta_i e^{i\delta}$; where $\delta=\ln x$ (3.16)

2) $\ln U=f(\ln t)$, $\ln U = n\tau + \ln\sum_{i=0}^{s}\beta_i e^{-i\tau} - \ln\sum_{i=0}^{p}\alpha_i e^{-i\tau}$; where $\tau=\ln x$ (3.17)

3) $z(r,t)=U/t^r$, $z(r,t)=\dfrac{t^{n-r}\sum_{i=0}^{s}\beta_i t^{-i}}{\sum_{i=0}^{p}\alpha_i t^{-i}}$; (3.18)

4) $z(r,x)=U/x^r$, $z(r,x)=\dfrac{x^{m-r}\sum_{i=0}^{s}\beta_{s-i} t^{-i}}{\sum_{i=0}^{p}\alpha_{p-i} t^{-i}}$; (3.19)

5) $y(r,t)=[U(t)]^{-1/r}$, $y(r,t)=\left[\dfrac{t^{n}\sum_{i=0}^{s}\beta_i t^{-i}}{\sum_{i=0}^{p}\alpha_i t^{-i}}\right]^{1/r}$; (3.20)

6) $y(r,x)=[U(x)]^{-1/r}$, $y(r,x)=\left[\dfrac{x^{m}\sum_{i=0}^{s}\beta_{s-i} t^{-i}}{\sum_{i=0}^{p}\alpha_{p-i} t^{-i}}\right]^{1/r}$ (3.21)

Chapter 4. EXPERIMENTAL DETERMINATION OF n AND m POWER PARAMETERS

4.1. n parameter determining transformations

Based on the curve shape analysis and by means of transformation of variables there are three theoretical means of determination of n parameter. These are: $\ln U = f(\ln t)$, $z(r,t)=U/t^r=f(t)$ and $y(r,t)=\sqrt[r]{U}=f(t)$ transformations. In the limit $(t\to\infty)$ the geometric shape of the three functions is dependent on numerical value of n that constitutes the basis for its determination. Before starting a detailed consideration of this principle, for simplicity, let us introduce the following designations: $D_{ij}=(\alpha_i\beta_j-\alpha_j\beta_i)$ and $H_{ij}=(\alpha_{p-i}\beta_{s-j}-\alpha_{p-j}\beta_{s-i})$.

1. In the case of **$\ln U=f(\tau)$ function** to set up n's numerical value is possible by determining the slope of an asymptote, since it is known that:

$\ln U=f(\ln t)$, $\ln U = n\tau + \ln\sum_{i=0}^{S}\beta_i e^{-i\tau} - \ln\sum_{i=0}^{P}\alpha_i e^{-i\tau}$; where $\tau=\ln x$, $U=1/V$ and $t=1/x$.

$$\lim_{\tau\to\infty}\frac{d\ln U}{d\tau}=n,\quad \lim_{\tau\to\infty}[U-tU']=\ln(\beta_0/\alpha_0)\quad\text{and}\quad \lim_{\tau\to\infty}\frac{d^2\ln U}{d\tau^2}\to\pm 0,$$

$$\ln U=\ln(\beta_0/\alpha_0)+n\tau,\quad\text{(asymptote)}\qquad (4.1)$$

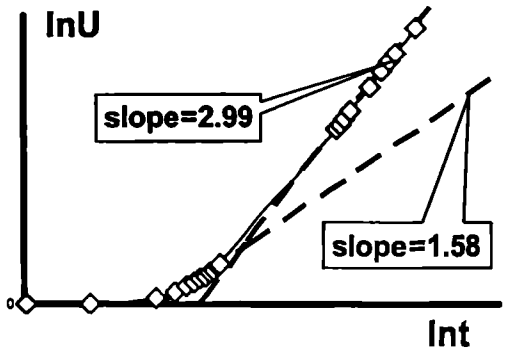


Fig. 4.1. $\ln U=f(\tau)$ function, $(\tau=\ln t)$

Broken lines represent regression lines, drawn on two different groups of points, whose slopes vary markedly from each other. Therefore, correct choice of operating interval is particularly important.

Hence, if the working range has been correctly selected and we draw a regression line on these points (Fig. 4.1), then through the calculation of circle slope n-parameter can be determined. But the problem lies in finding whether the range is correct and accordingly the results obtained are valid.

2. In the case of **$z=f(r,t)$ function**, at extremely high values of argument, the limiting values of the first and second derivatives of a function and of the ordinate axis intersection by tangent will assume the following pattern:

$$\lim_{t\to\infty} z(r,t) = \lim_{t\to\infty}\left(\frac{t^n\sum_{i=0}^{s}\beta_i t^{-i}}{t^r\sum_{i=0}^{p}\alpha_i t^{-i}}\right) = \frac{\beta_0}{\alpha_0}\lim_{t\to\infty} t^{n-r}; \tag{4.2}$$

$$\lim_{t\to\infty}\frac{dz}{dt} = \frac{\beta_0}{\alpha_0}\left(\lim_{t\to\infty} t^{n-r-1}\right)\left[(n-r)+\frac{D_{10}+(n-r)B_1}{\alpha_0\beta_0}\lim_{t\to\infty} t^{-1}+\cdots\right]; \tag{4.3}$$

$$\lim_{t\to\infty}(z-t\cdot z_t')) = \frac{\beta_0}{\alpha_0}\left(\lim_{t\to\infty} t^{n-r}\right)\left[(n-r)+\frac{D_{10}+(n-r)B_1}{\alpha_0\beta_0}\lim_{t\to\infty} t^{-1}+\cdots\right]; \tag{4.4}$$

$$\lim_{t\to\infty} z'' = \frac{\beta_0}{\alpha_0}\left(\lim_{t\to\infty} t^{n-r-2}\right)\left[\begin{array}{l}(n-r)(n-r-1)+\dfrac{2(n-r-1)(D_{10}+(n-r)B_1)}{\alpha_0\beta_0}\lim\limits_{t\to\infty} t^{-1}+\\ +\left\{\begin{array}{l}[D_{10}+(n-r)B_1][D_{10}+(n-r-1)B_1]+\\ +2(n-r-1)(2D_{20}+(n-r)B_2)\alpha_0\beta_0-2\alpha_0\beta_0 D_{20}\end{array}\right\}\dfrac{\lim\limits_{t\to\infty} t^{-2}}{(\alpha_0\beta_0)^2}+\cdot\end{array}\right]. \tag{4.5}$$

Analysis of these formulae may lead to an unequivocal conclusion that as r-dependent, the curve shape determinant parameters in the limit adopt various values that is represented in Table 4.1.

Table 4.1

Values of geometrical shape determinant parameters for $z=f(r,t)$ function at infinite quantities of argument

$\lim\limits_{t\to\infty} \Rightarrow$	z_t'	$z-tz_t'$	z_{tt}''		Curve shape
r<(n-2)	$+\infty$	$-\infty$	>0	$+\infty$	Convex curve
r=(n-2)				$2\beta_0/\alpha_0$	
r<(n-1)				$+\infty$	
r=(n-1)	β_0/α_0	$\pm(D_{10}/\alpha_0^2)$	± 0		Inclined curve $Z_{n-1}=\left(\frac{D_{10}}{\alpha_0^2}\right)+\left(\frac{\beta_0}{\alpha_0}\right)t$
r=n	± 0	β_0/α_0	± 0		Horizontal asymptote $Z_n=\beta_0/\alpha_0$
r>n	-0	$+0$	$+0$		Concave curve

The table shows that when r(n-1), $z=f(r,t)$ function is increasing and concave, when r=(n-1), z=f(r, t) function has an inclined asymptote, which crosses the coordinate origin (if $D_{01}=0$), up (if $D_{01}>0$) or down (if $D_{01}<0$); $z=f(r,t)$ function may be concave and may approach asymptote from above (if $z''<0$) and approach asymptote from below; if r=n, then $z=f(r,t)$ function has a horizontal asymptote, which $z=f(r,t)$ function may approach downward (if $D_{01}>0$) or upward (if $D_{01}<0$); when r>n $z=f(r,t)$ function is decreasing and convex.

Thus, analysis of geometrical shape of $z=f(r,t)$ function makes it clear that during transformation of $z=f(r,t)$ there is an open interval $[t_1, +\infty]$, where r numerical value unequivocally determines the curve shape. According to the curve shape numerical value of n-parameter can be established. If $z=f(r,t)$ function is increasing and convex, then $r<n-1$. If $z=f(r,t)$ function has an inclined asymptote, then $r=n$. And when $z=f(r,t)$ function is decreasing and convex, then $r>n$. Hence, in the case of $z=f(r,t)$ function attention must be paid to the availability of asymptote and the limit sign of the function's first derivative, for r-dependent alteration of only these parameters allows for unequivocal determination of n. But here too, as earlier, the main problem is a correct choice of the operating range. The correct interval should be sufficiently approached to infinity, to exclude availability of turning and inflexion points on this interval. More precisely, an extremely small operating range should be followed by the turning and inflexion points.

3. In the case of $y=f(r,t)$ function, let us first examine the geometrical shape determinant parameters of a function at infinite values of argument. Constraints of geometrical shape determinant parameters for $z=f(r,t)$ function are sufficiently many, therefore we'll restrict ourselves only by the elements participating in finding the limiting values:

$$\lim_{t\to\infty} y(r,t) = \lim_{t\to\infty}\left[t^n \sum_{i=0}^{s}\beta_i t^{-i} \Big/ \sum_{i=0}^{p}\alpha_i t^{-i}\right]^{1/r} = \sqrt[r]{\frac{\beta_0}{\alpha_0}}\left(\lim_{t\to\infty}\sqrt[r]{t^n}\right) \tag{4.6}$$

$$\lim_{t\to\infty} y'_t = \sqrt[r]{\frac{\beta_0}{\alpha_0}}\left[\frac{t^{\left(\frac{n}{r}-1\right)}}{r}\right]\left(\frac{n\alpha_0\beta_0 + \Psi_1 \lim\limits_{t\to\infty} t^{-1} + \cdots}{\alpha_0\beta_0 + B_1 \lim\limits_{t\to\infty} t^{-1} + \cdots}\right) \tag{4.7}$$

$$\lim_{t\to\infty}(y - t\cdot y') = \sqrt[r]{\frac{\beta_0}{\alpha_0}}\left[\frac{t^{\left(\frac{n}{r}\right)}}{r}\right]\left(\frac{(r-n)\alpha_0\beta_0 + [D_{01} + (r-n)B_1]\lim\limits_{t\to\infty} t^{-1} + \cdots}{\alpha_0\beta_0 + B_1 \lim\limits_{t\to\infty} t^{-1} + \cdots}\right) \tag{4.8}$$

$$\lim_{t\to\infty} y''_{tt} = \sqrt[r]{\frac{\beta_0}{\alpha_0}}\left[\frac{t^{\left(\frac{n}{r}-2\right)}}{r^2}\right]\left\{ n(n-r) + \frac{2(n-r)[D_{10} + nB_1]\lim\limits_{t\to\infty} t^{-1}}{\alpha_0\beta_0} + \left((D_{10} + nB_1)\left[\begin{matrix}(D_{10} + nB_1)(D_{10} + (n-r)B_1) + \\ 2\alpha_0\beta_0(2n-3r)D_{20} + 2n(n-r)\alpha_0\beta_0 B_2\end{matrix}\right]\right)\frac{\lim\limits_{t\to\infty} t^{-2}}{(\alpha_0\beta_0)^2}\right\} \tag{4.9}$$

Depending on r parameter, the given expressions assume discrete values. The results of analysis are given in Table 4.2.

It is evident from the table that when $n>2r>r$, then $y=f(r,t)$ function is increasing and convex; when $r=n$, $y=f(r,t)$ function has an inclined asymptote, which may pass in the origin of coordinates ($D_{01}=0$), upwardly ($D_{01}>0$) or downwardly ($D_{01}<0$); $y=f(r,t)$ function approaches the

asymptote from below $\left(\lim_{t\to\infty} y''_{tt}\right)<0$ or from above $\left(\lim_{t\to\infty} y''_{tt}\right)>0$. If r>n y=f(r,t) function is increasing and concave, $\left(\lim_{t\to\infty} y''_{tt}\right)<0$.

Table 4.2

Values of geometrical shape determinant parameters for y=f(r,t) function at infinite quantities of argument, (t→∞).

$\lim_{t\to\infty}$	y'_t	$y-ty'_t$	y''_{tt}		Curve shape
n>2r>r	+∞	−∞	>0	+∞	Convex curve
n=2r>r				$+2(\beta_0/\alpha_0)^{2/n}$	
2r>n>r				+0	
r=n	$\sqrt[n]{\frac{\beta_0}{\alpha_0}}$	$\pm\left(\frac{D_{01}}{n\alpha_0\beta_0}\sqrt[n]{\frac{\beta_0}{\alpha_0}}\right)$	±0		Inclined asymptote $Y_n=\sqrt[n]{\frac{\beta_0}{\alpha_0}}\left(\frac{D_{01}}{n\alpha_0\beta_0}+t\right)$
r>n	+0	+∞	<0		Concave curve

Thus, relying on analysis of geometrical shape in the limit y=f(r,t) function, the following conclusion can be drawn:

1) the limit sign of the first derivative of y=f(r,t) function does not depend on r parameter; at any value of the latter it is positive $\left(\lim_{t\to\infty} y'_t\right)>0$.

2) The sign of ordinate axis intersection by a tangent of y=f(r,t) function depends on r parameter and changes from minus to plus.

3) The limit sign of y=f(r,t) function's second derivative depends on r parameter. If n<r $\left(\lim_{t\to\infty} y''_{tt}\right)>0$, if n>r $\left(\lim_{t\to\infty} y''_{tt}\right)<0$, while when n=r, then $\left(\lim_{t\to\infty} y''_{tt}\right)=0$.

4) The function has an inclined asymptote, when n=r, if n>r – function is convex, if r>n it is concave. So, in the case of y=f(r,t) function attention should be paid to the sign of second derivative and asymptote, since dependence of other parameters on n (e.g. the tangent passing in the origin of coordinates, the first derivative) does not allow for unequivocal determination of n.

The existence of asymptote can be experimentally verified by means of drawing a regression line on the operating range points and by estimating the experimental curve's approximation to linearity. Since the second derivative of linearity dependency equals to zero, in an ideal case, within the range of errors, the second derivative of an experimental curve is to

approximate zero. $z(r,t)=U/t^r=f(t)$ and the second derivatives of $y(r,t)=\sqrt[r]{U}=f(t)$ functions will look as follows (Table 3.2):

$$z''_{tt}=\frac{U}{t^{r+2}}\left[\omega'_\tau+(\omega-r)(\omega-r-1)\right]=\frac{U}{t^{r+2}}\left\{t^2(\ln U)''+r+\left[t(\ln U)'-r\right]^2\right\}, \tag{4.10}$$

$$y''_{tt}=\frac{\sqrt[r]{U}}{r^2t^2}\left[r\omega'_\tau+\omega(\omega-r)\right]=\frac{\sqrt[r]{U}}{r^2}\left\{r(\ln U)''+\left[(\ln U')\right]^2\right\} \tag{4.11}$$

where $(\ln U)''=d^2\ln U/dt^2$ and $(\ln U)'=d\ln U/dt$. Since by determining r>0, then for performance of $z''_{tt}=0$ and $y''_{tt}=0$ inequalities in the operating range availability of $(\ln U)''<0$ inequality is an insufficient, but indispensable condition (4.10 and 4.11). The first and second derivatives of $\ln U=f(t)$ function have the following form (see 3.10 and 3.11).

$$(\ln U)'=\frac{\omega}{t}=\frac{\sum_{k=0}^{s+p}\Psi_k t^{-k}}{t\sum_{k=0}^{s+p}B_k t^{-k}}=\frac{n}{t}+\frac{D_{10}}{\alpha_0\beta_0 t^2}+\cdots \tag{4.12; 4.13}$$

$$(\ln U)''=\frac{\omega'_\tau-\omega}{t^2}=\frac{\sum_{q=0}^{s+p}t^{-q}\sum_{k=0}^{q}(2k+1-q)\Psi_k B_{q-k}+\sum_{q=s+p+1}^{2(s+p)}t^{-q}\sum_{k=q-k}^{s+p}(2k+1-q)\Psi_k B_{q-k}}{t^2\left[\sum_{k=0}^{s+p}B_k t^{-k}\right]^2}=-\frac{n}{t^2}-\frac{2D_{10}}{\alpha_0\beta_0 t3}+\cdots$$

Thus, $\ln U=f(t)$ function in the limit ($t\to\infty$) has an increasing and concave curve shape and certainly, one can always find extremely great interval of argument, where the inequalities $\lim_{t\to\infty}(\ln U)'>0$ and $\lim_{t\to\infty}(\ln U)''<0$ can be performed (Fig. 4.2). At any rate, the check-up of this condition can be readily accomplished by way of transformation of $V_i=f(x_i)$ curve into $\ln U_i=f(t_i)$. It is remarkable that the last turning point (if it exists) of $\ln U=f(t)$ function lies before the inflexion point.

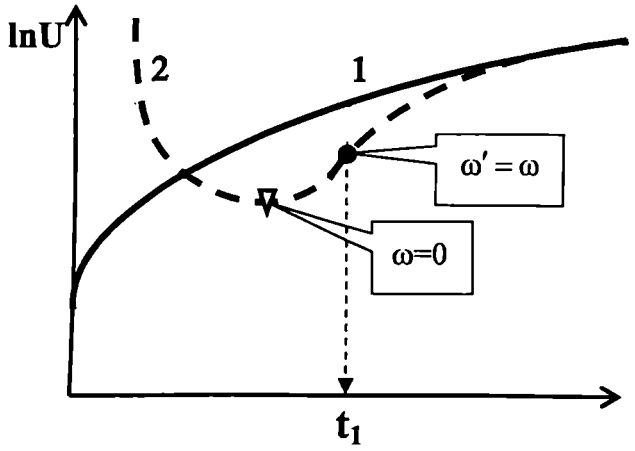


Fig. 4.2 Examples of $\ln U=f(t)$

1. $\ln U=-\ln V_m+\ln\prod_{i=1}^{n}(1+K_i t)$

   Working interval $0\le t<+\infty$

2. $\ln U=f(t)$; Working interval $t_1\le t<+\infty$

For an authentic determination of n parameter through the asymptotes $(\ln U)'' < 0$ inequality is an indispensable condition, but insufficient. There exist the so-called "hazardous points", existence of which in the operating range is not permissible, either. This problem will be dealt with in the next section.

4.2. Relative analysis of n-parameter determinant transformation s

Study of geometrical shapes of $\ln U = f(\tau)$, $y = f(r,t)$, $z = f(r,t)$ functions has made it clear that by way of them it is theoretically possible to determine n degree parameter. Of particular importance have r-dependent $y = f(r,t)$ and $z = f(r,t)$ transformations. As has been shown in the previous section, n-parameter can be defined by means of asymptote of these functions:

$$\ln U = f(\tau) \Rightarrow \ln U = \ln(\beta_0/\alpha_0) + n\tau \tag{4.10}$$

$$z = f(r,t) \Rightarrow (r=n-1) \Rightarrow Z_{n-1} = \left(\frac{D_{10}}{\alpha_0^2}\right) + \left(\frac{\beta_0}{\alpha_0}\right)t \quad \text{and} \quad (r=n) \Rightarrow Z_n = \beta_0/\alpha_0 \tag{4.11}$$

$$y = f(r,t) \Rightarrow (r=n) \Rightarrow Y_n = \sqrt[n]{\frac{\beta_0}{\alpha_0}}\left(\frac{D_{01}}{n\alpha_0\beta_0} + t\right) \tag{4.12}$$

For proving the existence of asymptote a crucial importance is attributed to the position of operating range $[t_1, t_2]$. The regression line built on their experimental points, is, on the one hand, to maximally approximate asymptote (i.e. $[t_1, t_2] \to \infty$), and on the other hand, should be far enough from the extremely low velocity site, where authenticity substantially decreases because of great specific (or absolute) errors.

$\ln U = f(\tau)$ function is directly related with n degree parameter, as inclination of its asymptote is equal to n parameter. In the limit interval, however, finding of numerical value of asymptotic coefficients is associated with a cardinal error. The only criterion for the existence of $\ln U = f(\tau)$ function's asymptote is a rectilinear estimation of experimental points (for instance, correlation coefficient). Therefore, it is practically hard to estimate authenticity of the results obtained, it is likewise complex to establish a correct operating range, since the $\ln U = f(\tau)$ function has multiple pseudo-rectilinear sites. Hence, the use of $\ln U = f(\tau)$ transformation for the determination of n parameter is not reasonable.

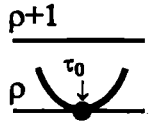
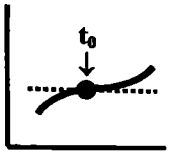
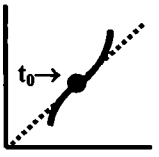
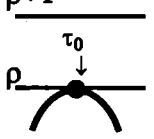
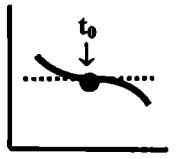
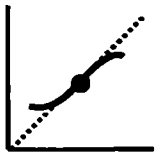
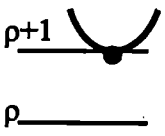
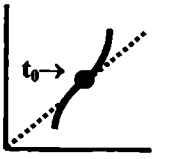
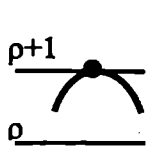
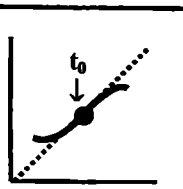
However, there is quite another situation from the viewpoint of measurement of n parameter in the case of $y = f(r,t)$ and $z = f(r,t)$ transformations. For these functions asymptote existence is determined through r-variation and not by determining the digital parameter of asymptote. This makes it possible to determine n and estimate the validity of the result. The chief problem to be solved for $y = f(r,t)$ and $z = f(r,t)$ transformations consists of the correct choice of

operating range, for the curve shape and accurate determination of n parameter depend on the operating range.

The problem is the existence of the so-called "hazardous points". The name "hazardous points" is stipulated by the circumstance that if the operating range involves a narrow site constituting the points in question, this may elicit an imitation of rectilinearity that is likely to become the cause for incorrect measurement of n parameter.

The lnU=f(τ)) function's inflexion points (τ_0=lnt$_0$, $\omega'_\tau(\tau_0)=0$), represent one group of "hazardous points". Their existence results in the complication of r-dependent y=f(r,t) and z=f(r,t) functions' geometric shapes, formation of the turning and inflexion points. The results of analysis made from this viewpoint are presented in Table 4.3, which shows complication of geometric shapes of y=f(r,t) and z=f(r,t) functions, that has been elicited by addition of one pair of turning points (ω=p) and ω= (p+1) of ω=f(r) function. It is seen from the Table that in the presence of "hazardous points", y=f(r,t) and z=f(r,t) functions are the curves of complex geometrical shape. In the vicinity of "hazardous points" the curve is an imitation of linearity. The number of "hazardous points" increases as the number of turning points of ω=f(τ) function increases.

Table 4.3

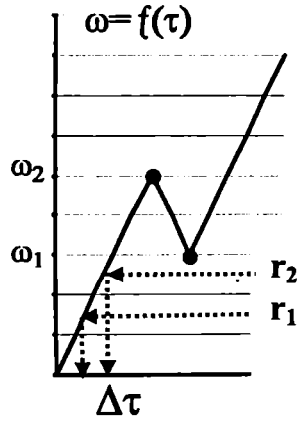
$\omega=f(\tau_0)$		$z=f(r,t_0)$		$y=f(r,t_0)$	
$\omega=r=\rho$ $\omega'=0$ $\omega''>0$		$z'_t=0$ $z-tz'_t=U/t_0^r$ $z''_{tt}=0$ $r=\rho$		$y'_t=\sqrt[r]{U}/t_0$ $y-ty'_t=0$ $y''_{tt}=0$ $r=\rho$	
$\omega=r=\rho$ $\omega'=0$ $\omega''<0$		$z'_t=0$ $z-tz'_t=U/t_0^r$ $z''_{tt}=0$ $r=\rho$		$y'_t=\sqrt[r]{U}/t_0$ $y-ty'_t=0$ $y''_{tt}=0$ $r=\rho$	
$\omega=r=\rho+1$ $\omega'=0$ $\omega''>0$		$z'_t=U/t_0^{r+1}$ $z-tz'_t=0$ $z''_{tt}=0$ $r=(\rho+1)$		In the vicinity of "hazardous points" geometrical shape of $\omega=f(r_0)$, $z=f(r,t)$ and $y=f(t_0)$ functions. Versions are given for ($\tau_0=\ln t_0$, $\omega'=0$) when $\omega=f(r_0)=r$ & $\omega=f(r_0)=r=\rho+1$	
$\omega=r=\rho+1$ $\omega'=0$ $\omega''<0$		$z'_t=U/t_0^{r+1}$ $z-tz'_t=0$ $z''_{tt}=0$ $r=(\rho+1)$			

Another group of "hazardous points" constitutes a pair of inflexion points of $\ln U = f(\tau)$ function (Table 4.2). Let us assume that $\omega = f(\tau)$ function in $\Delta\tau$ interval has a pair of inflexion points ($\omega' = 0$). In the respective Δt interval (with the help of equations in Table 3.2) let us explore as to what effect will have the presence of a pair of inflexion points of $\ln U = f(\tau)$ function on the shape of $z = f(r,t)$ and $y = f(r,t)$ functions. At any r quantity in Δt interval $y = f(r,t)$ function will have only two inflexion points, while $z = f(r,t)$ function is likely to have a far more complex geometrical shape, with multiple "hazardous points". In this case, the curve's geometrical shape at Δt interval is dependent on the numerical value of r-transformation degree parameter. It is remarkable that $y = f(r,t)$ function has an advantage in importance of curve shape complexity, compared to $z = f(r,t)$ function (Table 4.2).

Table 4.2

Shape of $\omega = f(\tau)$, $z = f(r,t)$ and $y = f(r,t)$ functions at $\Delta\tau$ and Δt intervals. ν is the number of turning, λ_H – horizontal inflexion and λ – of inclined inflexion points. r is the power parameter of variable transformation.

Position of $\Delta\tau$ interval		Curve shape at Δt interval					
$r = \omega = f(\tau)$		$z = f(r,t)$			$y = f(r,t)$		
r_1	r_2	ν	λ	λ	ν	λ	λ
$r_1 < r_2 < \omega_1$		0	0	0	0	0	0
$r_1 < \omega_1$	$r_2 = \omega_1$	0	0	2			
$r_1 < \omega_1$	$\omega_1 < r_2 < \omega_2$	0	0	2			
$r_1 < \omega_1$	$r_2 = \omega_2$	0	0	2			
$r_1 < \omega_1$	$r_2 > \omega_2$	0	0	2			
$r_1 = \omega_1$	$\omega_1 < r_2 < \omega_2$	1	1	1			
$r_1 = \omega_1$	$r_2 = \omega_2$	1	1	1	0	0	2
$r_1 = \omega_1$	$r_2 > \omega_2$	1	1	1			
$\omega_1 < r_1 < r_2 < \omega_2$		3	0	2			
$\omega_1 < r_1 < \omega_2$	$r_2 = \omega_2$	3	0	2			
$\omega_1 < r_1 < \omega_2$	$r_2 > \omega_2$	3	0	2			
$r_1 = \omega_2$	$r_2 > \omega_2$	1	1	1			
$\omega_2 < r_1 < r_2$		0	0	0	0	0	0



$r_1 = \omega(\tau_1)$, $r_2 = \omega(\tau_2)$
$\Delta\tau = (\tau_2 - \tau_1) = (\ln t_2 - \ln t_1)$
$\Delta t = (t_2 - t_1)$
$(\omega_1, \omega_2) \Rightarrow \omega' = 0 \Rightarrow (\bullet)$

(See table 3.2)

Let us examine the location and interrelationship of $y = f(n,t)$ and $z = f(n-1,t)$ curves' turning and inflexion points (Fig. 4.3).

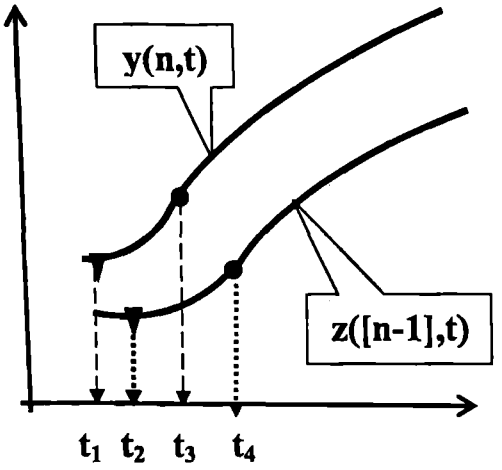


Fig. 4.3. The possible shape of y=f(n, t) and z=f([n-1], t) curves in t→∞ ranges.
$n>1, m\neq 0 \Rightarrow \lim_{t\to\infty} y''_{tt} < 0$; $\lim_{t\to\infty} z''_{tt} < 0$
Turning points:
▼– $y'_t(n,t)=0$; $z'_t([n-1],t)=0$
Inflexion points:
● – $y''_{tt}(n,t)=0$; $z''_{tt}([n-1],t)=0$

In the ranges of high value argument (t→∞), if r=n>1 and t=t_3, then $y''_{tt}(n,t_3)=0$ and $n\omega'+\omega(\omega-n)=0$, i.e. $\omega'(t_3)=-\omega(\omega-n)/n$. At the same time, if r=n-1, then for z=f([n-1],t) function, at t_3 point we'll have:

$$z''_{tt}=\frac{U}{t^{r+2}}[\omega'+(\omega-r)(\omega-r-1)]=\frac{U}{t^{r+2}}\left\{\frac{(\omega-n)^2(n-1)}{n}\right\}>0, \quad \text{g.o.} \quad t_2<t_3.$$

Let us consider another case, when $t^2(\ln U)''_{tt}=(ш'-ш)<0$ and at t_0 point $y''_{tt}(a,t_0)=\frac{\sqrt[r]{U}}{r^2t^2}[a\omega'_\tau+\omega(\omega-a)]=0$, (r=a). In the case, when r=(a+Δ), at t_0 point we'll have $y''_{tt}(a+\Delta,t_0)=\frac{\sqrt[r]{U}}{r^2t^2}[a\omega'_\tau+\omega(\omega-a)+\Delta\omega'-\Delta\omega]=\frac{\sqrt[r]{U}}{r^2t^2}[\omega'-\omega]\Delta$. So, Δ sign determines at t_0 point the curve shape, sign(y″) = sign(Δ). That is, in the case of r increase the inflexion point moves to left.

One more advantage of y=f(r,t) transformation over z=f(r,t) is evidenced by the position of their turning points versus r parameter. It is known that $dy/dt=(\sqrt[r]{U}/rt)\cdot\omega$ and $dz/dt=(U/t^{r+1})\cdot(\omega-r)$. $y'=0$ and $z'=0$. To fulfill $y'=0$ and $z'=0$ condition one requires respectively to fulfill ω=0 and (ωr)=0, i.e. in the first case we have r-independent, and in the other, r-dependent condition. Thus, the position of turning point of y=f(r,t), as distinct from z=f(r,t) does not alter at the variation of r.

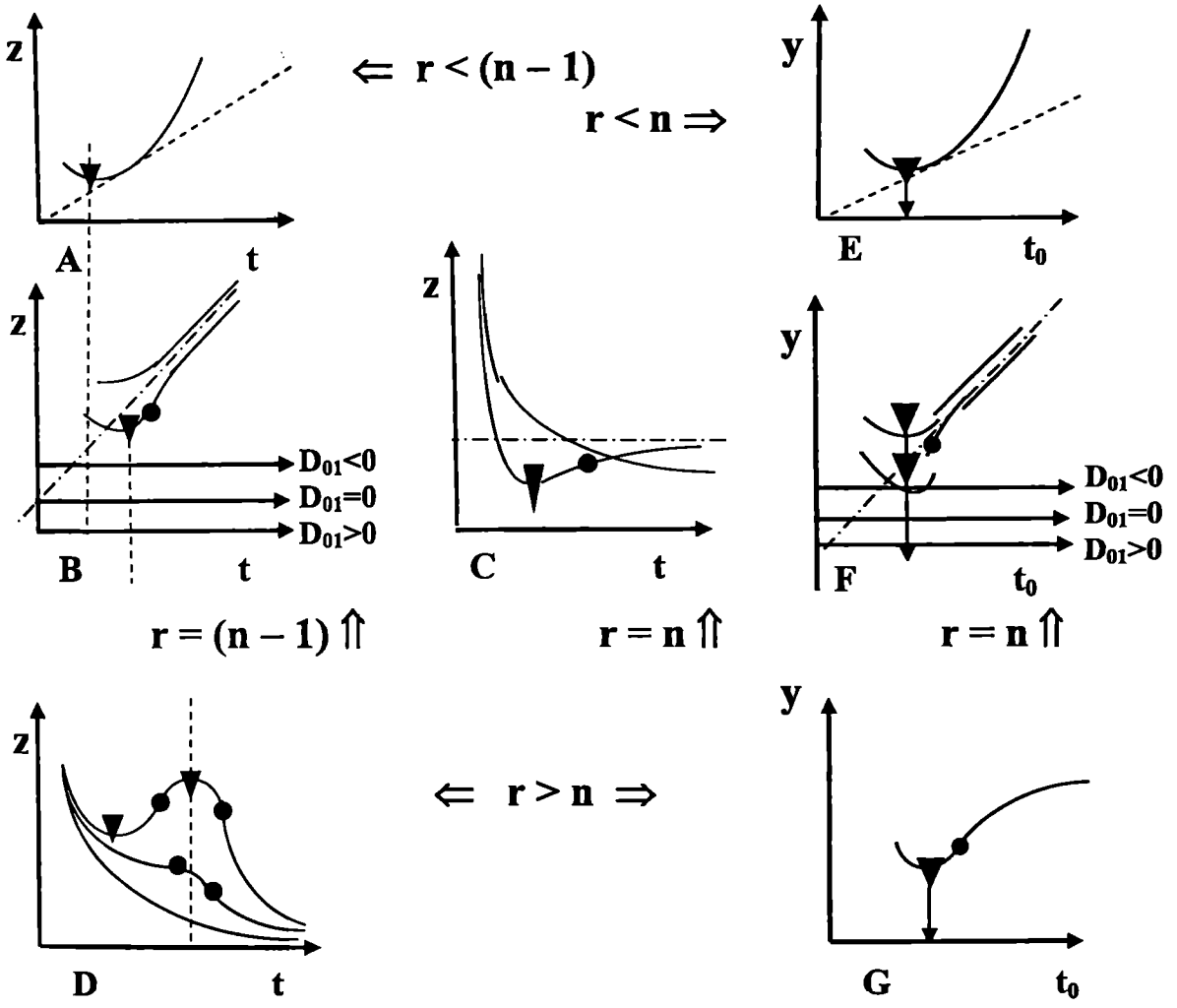


Fig. 4.4. Geometrical shape of $z(r,t) = U(t)/f(t)$ and $y(r,t) = \sqrt[n]{U} = f(t)$ function in the limit ($t \to \infty$) at different r quantities
$A \approx r < (n-1)$; $B \approx r = (n-1)$; $C \approx r = n$; $D \approx r > n$; $E \approx r < n$; $F \approx r = n$; $G \approx r > n$
(▼) turning and (●) inflexion points.
In the case of a stepwise increase in velocity even number of inflexion points are added to the curves (see Fig. 3.3).

Hence, within the ranges of argument's extremely high values at different quantities of r parameter $z=f(r,t)$ and $y=f(r,t)$ curves will have geometrical shapes of various complexity (Fig. 4.4).

Conclusions

1. n parameter can be determined by means of $(\ln U)''_{tt} < 0$, $z=f(r,t)$ and $y=f(r,t)$ transformations. The principle of determination of n parameter relies on the availability of asymptotes. In the case of $z=f(r,t)$ and $y=f(r,t)$ transformations the existence of asymptotes depends on the quantity of power parameter of r transformation.

2. In the correct interval further changes in z=f(r,t) curve shape occur: increasingly concave (r<n); ⇒ inclined asymptote (r=n–1); ⇒ horizontal asymptote (r=n); ⇒ complex geometrical shape decreasingly concave (r>n). In the correct interval there occur further changes in the shape of y=(r, t) curve: increasingly concave (r<n); ⇒ inclined asymptote (r=n); ⇒ increasingly convex (r>n).

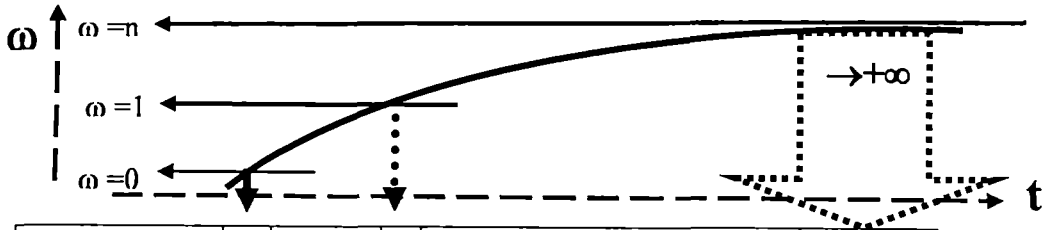
3. A correct operating range implies that: a) at operation interval lnU=f(t) function should by all means be increasingly convex and should not contain the turning and inflexion points, $(\ln U)''_{tt} < 0$ and $(\ln U)'_t \neq 0$; b) at operation interval lnU=f(lnt) function should not contain the turning and inflexion points, ω≠0 and ω'≠0; c) approximation of operating range to infinity is due to the reliability of experimental points, a small specific and absolute error.

4. In lnU=f(lnt) function rectilinearity of a regression line on experimental points does not authentically express the presence of asymptote.

5. z=f(r,t) function, compared to y=f(r,t) transformation, in the domain of extremely great argument, is characterized by a complex geometrical shape, that strongly attenuates significance of the result.

6. From the viewpoint of setting up n parameter, advantage is attributed to y=f(r,t) transformation.

Appendix. Below in Fig. 4.5, an example of the method for a rapid determination of a curve shape, making it possible to solve many a problem. sign(ω & ω') ⇒ sign(U' & U''); $U' = U\omega/t$, $U'' = (U/t)[\omega' + \omega(\omega - 1)]$.



sign ω	0	+ +	+	+	+	+	+	+	+	+	+	+ + +
sign (ω–1)ω	0	– –	0	+	+	+	+	+	+	+	+	+ + +
signω'	+	+ +	+	+	+	+	+	+	+	+	+	0 0 0
sign(U''_{tt})	+	? ± ?	+	+	+	+	+	+	+	+	+	+ + +

Fig. 4.5. An example of a rapid determination of curve shape

4.3. R(t) function

Suppose at $[t_1, t_2]$ interval InU=f(t) function is a continuous and convex curve $(\ln U)''_{tt} < 0$ and lnU= f(lnt) function has no turning and inflexion points, then for any t_i point of the interval $(t_1<t_i<t_2)$ one can always find such r=R_i, that the equation be fulfilled:

$$y''_{tt}(R_i,t_i)=\left(\sqrt[R_i]{U(t_i)}\right)''=\frac{\left(\sqrt[R_i]{U(t_i)}\right)}{(R_i)^2}\left[R_i(\ln U)''+\left[(\ln U')\right]^2\right]=0 \qquad (4.14)$$

At the same time, one can always find such a positive number Δ, that if r=[R_i–Δ], then $y''_{tt}([R_i-\Delta],t)>0$ and if r=[R_i+Δ], then $y''_{tt}([R_i+\Delta],t)>0$, i.e. respectively with r increase the following change will occur in the curve shape [∪] ⇒ [—] ⇒ [∩].

R is a t-dependent value and represents that quantity of r when at t-point $y''_{tt}=0$. As emerging from (4.14) formula we obtain:

$$R(t)=\frac{\omega(t)^2}{\omega(t)-\omega'_t(t)}=\frac{[(\ln U)'_t]^2}{-(\ln U)''_{tt}} \qquad (4.15)$$

$$R(t)=\frac{\sum_{q=0}^{s+p}t^{-q}\left[\sum_{k=0}^{q}\Psi_k\Psi_{q-k}\right]+\sum_{q=s+p+1}^{2(s+p)}t^{-q}\left[\sum_{k=q-s-p}^{s+p}\Psi_k\Psi_{q-k}\right]}{\sum_{q=0}^{s+p}t^{-q}\left[\sum_{k=0}^{q}(2k-q+1)\Psi_kB_{q-k}\right]+\sum_{q=s+p+1}^{2(s+p)}t^{-q}\left[\sum_{k=q-s-p}^{s+p}(2k-q+1)\Psi_kB_{q-k}\right]}, \qquad (4.16)$$

while by dividing the nominator of the equation (4.16) by the dominator we get:

$$R(t)=\sum_i\Theta_it^{-i}=n+\Theta_2t^{-2}+\Theta_3t^{-3}+\cdots;$$

where $\Theta_0=1$, $\Theta_1=0$, $\Theta_2=\left[\frac{D_{12}(D_{12}+nB_1)}{n^2(\alpha_0\beta_0)^2}-\frac{2D_{20}}{n\alpha_0\beta_0}\right]$, etc. (4.17)

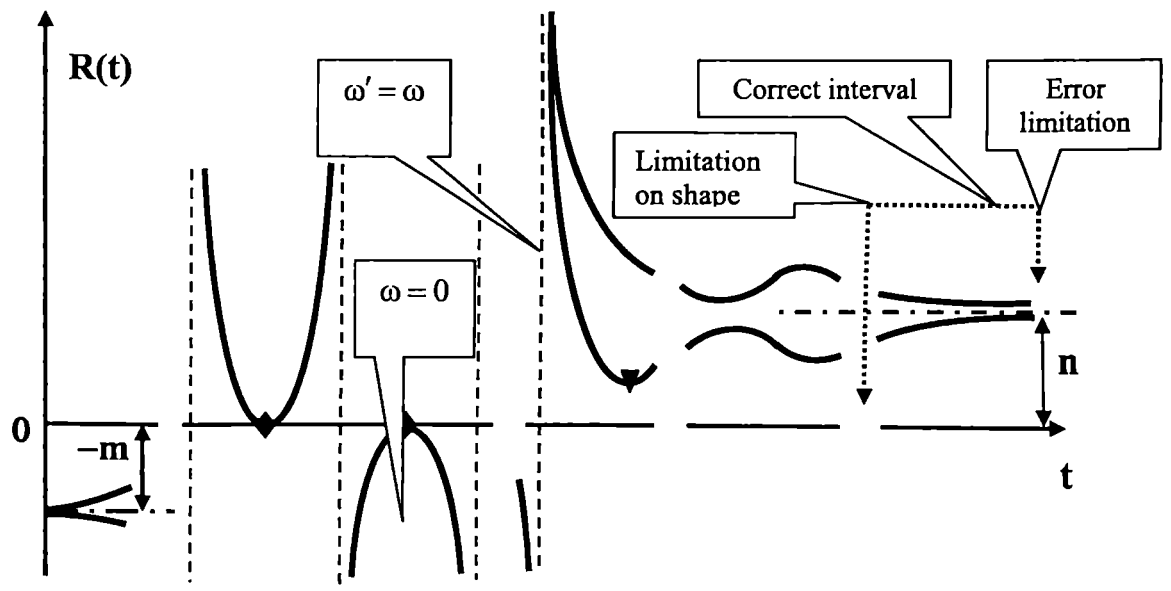
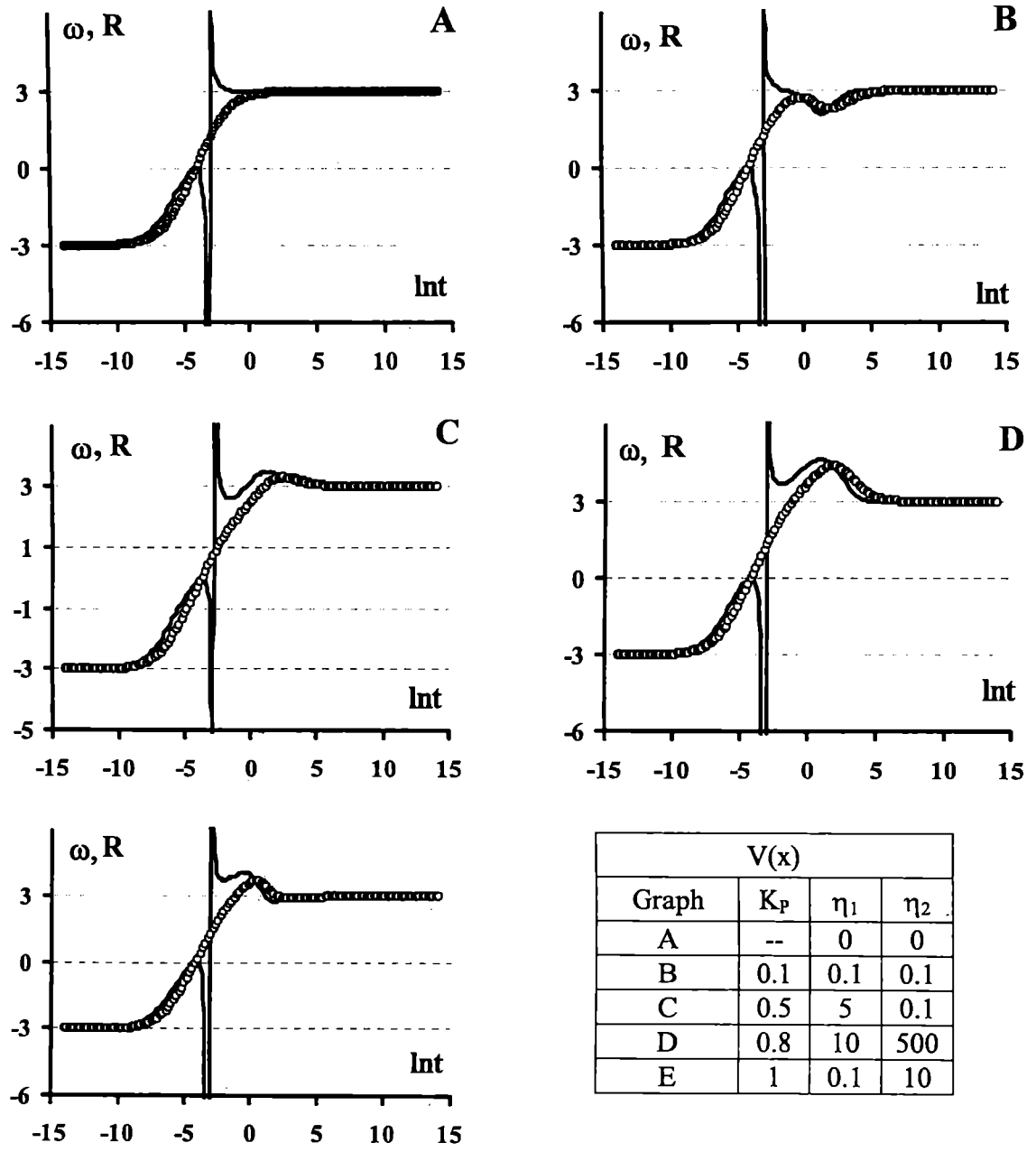


Fig. 4.6. The plausible elements of geometrical shape of R(t) function

R(t) function has a complex geometrical shape (Fig. 4.6), but at a correct interval it assumes a simple shape and approximates a horizontal straight. It is especially remarkable that if we don't take into account a breakpoint, from the geometrical point of view, there is a strong analogy between (ω=ω'), R(t) and ω (r) functions (Fig. 4.7).



V(x)			
Graph	K_P	η_1	η_2
A	--	0	0
B	0.1	0.1	0.1
C	0.5	5	0.1
D	0.8	10	500
E	1	0.1	10

Fig. 4.7. ω=f(τ) and R=f(τ) functions drawn on the basis of V(x) function. τ=In/x), n=m=3, K_n=20, K_m=200.
oooooo – ω=f(τ); ——— – R=f(τ).

$$V(x)=\frac{V}{e_0 k}=\frac{\left(1+\frac{2\eta_1 x}{K_P}+\frac{\eta_2 x^2}{K_P^2}\right)}{\left(1+\frac{K_n}{x}\right)^n\left(1+\frac{x}{K_m}\right)^m\left(1+\frac{x}{K_P}\right)^2}$$

R(t) function has a horizontal asymptote $\lim_{t\to\infty} R(t) = n$, $\lim_{t\to\infty} R'_t = 0$. , when the argument has extremely high quantity the sign of limiting quantity of the first and second derivatives of the function depends on the signs of $\sum_{i=2} \Theta_i t^{-i}$ polynomial's coefficients.

$$R'_t = -2\Theta_2 t^{-3} - 3\Theta_3 t^{-4} - \cdots; \quad R''_{tt} = 6\Theta_2 t^{-4} + 12\Theta_3 t^{-5} + \cdots \qquad (4.18)$$

That is, at extremely large quantity of argument, R(t) function is either decreasing – concave, or increasing – convex. R(t) function undergoes break when $\omega' - \omega = t^2 (\ln U)''_{tt} = 0$ (4.15). When ω=0, then R(t)=0, and when ω'=0, then R(t)= ω. Analysis of R(t) function and its derivatives has demonstrated that at correctly selected operating range, $(\ln U)''_{tt} < 0$, the interval of lnU=f(t) function from the last inclination point to infinity, R(t) function has no turning point and the function is monotonous.

By means of R(t) function, from the point of view of authenticity of the result, based on experimental evidence it is possible to estimate whether the interval is correct. Suppose we have a correct (t_1, t_2) operation interval by ε point, ($t_1 < t_i < t_2$; $1 \le i \le \varepsilon$). Let us introduce some new values.

if $\sum_{i=2} \Theta_i t^{-i} \approx 0$, then $R(t) = n$ $\Rightarrow$ R(t) in null approximation

if $\sum_{i=3} \Theta_i t^{-i} \approx 0$, then $R(t) = n + \Theta_2 t^{-2}$ $\Rightarrow R(t)_I$ in the first approximation (4.19)

if $\sum_{i=4} \Theta_i t^{-i} \approx 0$, then $R(t) = n + \Theta_2 t^{-2} + \Theta_3 t^{-3}$ $\Rightarrow R(t)_{II}$ in the second approximation (4.20)

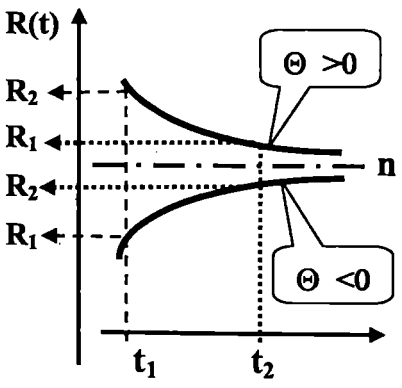


Fig. 4.8 See the text

1. $\Delta Y_i = (Y_i - y_i)$ is the difference between the computed and experimental points. The regression line corresponding point is considered as a computed value (Fig. 4.9)

2. R_1 is that quantity of r, when the first change of ΔY_i sign of points occurs.

3. R_2 is that quantity of r, when there occurs the last change of ΔY_i sign of points. If we do not take into account the experimental error, then at a correct interval we'll have: $\Theta<0 \Rightarrow R_1$ corresponds to t_1 and $R_2 t_2$ or $\Theta>0 \Rightarrow R_1$ corresponds to t_2 and R_2-t_1 (Fig. 4.8).

4. Mean $\overline{R} \Rightarrow \overline{R(t)} = \frac{1}{\varepsilon}\sum_{i=1}^{\varepsilon} R_i \approx \frac{1}{t_2 - t_1}\int_{t_1}^{t_2} R(t)dt = n + \frac{\Theta_2}{t_1 t_2} + \frac{\Theta_3 (t_2 + t_1)}{2 t_1^2 t_2^2} + \cdots$ (4,21)

5. R_0 is that quantity of r, when on a correct interval y(rt) curve maximally approximates rectilinearity.

One can judge correctness of operating range by $(\overline{R}-n)$ value, the smaller $(\overline{R}-n)$, the more correct is the operating range. Let us compute $(\overline{R}-n)$

In the first approximation:

$$(\Theta<0),\ (R_t')>0 \Rightarrow R_1=n+\Theta_2 t_1^2 \text{ and } R_2=n+\Theta_2 t_2^2 \Rightarrow \Theta_2=-\frac{(R_2-R_1)t_1^2t_2^2}{(t_2^2-t_1^2)}$$

$$(\overline{R}-n)=\frac{(R_2-R_1)t_1t_2}{(t_2^2-t_1^2)}<0 \qquad (4.22)$$

$$(\Theta<0),\ (R_t')<0 \Rightarrow R_1=n+\Theta_2 t_2^2 \text{ and } R_2=n+\Theta_2 t_1^2 \Rightarrow \Theta_2=\frac{(R_2-R_1)t_1^2t_2^2}{(t_2^2-t_1^2)}$$

$$(\overline{R}-n)=-\frac{(R_2-R_1)t_1t_2}{(t_2^2-t_1^2)}>0 \qquad (4.23)$$

In the second approximation three sets of equations will be generated

$$(\Theta<0) \Rightarrow \quad \overline{R}=n+\frac{\Theta_2}{t_1t_2}+\frac{(t_2+t_1)\Theta_3}{2t_1^2t_2^2}, \quad R_1=n+\frac{\Theta_2}{t_1^2}+\frac{\Theta_3}{t_1^3}, \quad R_2=n+\frac{\Theta_2}{t_2^2}+\frac{\Theta_3}{t_2^3}$$

$$(\Theta>0) \Rightarrow \quad \overline{R}=n+\frac{\Theta_2}{t_1t_2}+\frac{(t_2+t_1)\Theta_3}{2t_1^2t_2^2}, \quad R_1=n+\frac{\Theta_2}{t_2^2}+\frac{\Theta_3}{t_2^3}, \quad R_2=n+\frac{\Theta_2}{t_1^2}+\frac{\Theta_3}{t_1^3}$$

By solving them we get:

if $(R_t')>0$, (4.24)

$$(\overline{R}-n)_{II}=\frac{2\overline{R}(t_2^3-t_1^3)-(t_2^2-t_1^2)(\overline{R}-R_1+R_2)(t_1+t_2)}{(t_2-t_1)^3}+\frac{(R_2-R_1)(t_1+t_2)t_1t_2-2(R_2t_2^3-t_1^3)}{(t_2-t_1)^3}<0$$

if $(R_t')>0$, (4.25)

$$(\overline{R}-n)_{II}=\frac{2\overline{R}(t_2^3-t_1^3)-(t_2^2-t_1^2)(\overline{R}-R_1+R_2)(t_1+t_2)}{(t_2-t_1)^3}-\frac{(R_2-R_1)(t_1+t_2)t_1t_2-2(R_2t_2^3-t_1^3)}{(t_2-t_1)^3}>0$$

Thus, relying on experimental data, it is possible to compute $\overline{R}$, R_1, R_2, R_0, $(\overline{R}-n)_I$ and $(\overline{R}-n)_{II}$ values, as a result of which we can determine n parameter and estimate validity of the result (how much correct is the operating range).

Appendix 4.2

n determination mainly relies on setting up the curve shape (on the sign of the second derivative). Therefore, great importance is attributed to a simple method which enables to solve this problem.

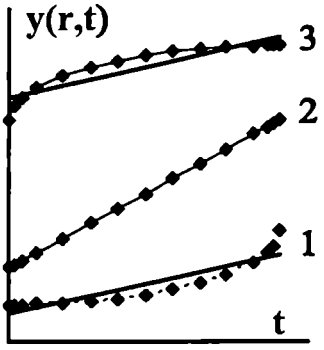


Fig. 4.9. On the points of $Y_r=a_r+b_rt$ function regression lines are drawn, $\Delta Y_i=(Y_i-y_i)$ and the sequence of $\Delta Y_i=(Y_i-y_i)$ value signs are set up.

1. $r<r_0 \Rightarrow \text{sign}(\Delta Y_i)=(-,-,-,+,+,+,-,-,-) \Rightarrow y''_{tt} > 0$
2. $r=r_0 \Rightarrow (\Delta Y_i)=(0,0,0,0,0,0,0,0,0,0,0) \Rightarrow y''_{tt} = 0$
3. $r>r_0 \Rightarrow \text{sign}(\Delta Y_i)=(-,-,-,+,+,+,-,-,-) \Rightarrow y''_{tt} < 0$

0 existence during change of a sign is admissible

$(+,-) \Leftrightarrow (+,0,-)$ or $(-,+) \Leftrightarrow (-,0,+)$

Estimation of the sign of dy/dt and d^2y/dt^2 is possible by means of regression. In regression analysis a regression line plays the same role as a mean arithmetical. Proceeding from this, the regression line is the mean of the function's tangents and meets the following requirements: its slope is the mean of that of functional tangents, intercepts of the ordinate axis is the mean of those of functional tangents and the regression line passes the point whose coordinates are $\overline{y_i}$ and $\overline{t_i}$, regression lines $Y_i = a_i + b_it$ are drawn on experimental points of y(r, t) function and sequence of signs of $\Delta Y_i = (Y_i - y_i)$ value for each point is set up. Distribution of difference (ΔY_i) sign of computed Y_i and experimental y_i values makes an idea of the sign of the second derivative.

The regression line cuts the monotonously concave curve in two points (Fig. 4.9). It can be readily proved that the monotonously concave curve $(y''_{tt} > 0)$ would have the following sequence of signs:

$$(-,-,-,-,-,-,) \Rightarrow (+,+,+,+,+) \Rightarrow (-,-,-,-,-,-,)$$

The regression line cuts the monotonously increasing and convex curve $(y''_{tt} < 0)$ again in two points (Fig. 4.9) and the sign distribution looks like:

$$(+,+,+,+,+) \Rightarrow (-,-,-,-,-,-,) \Rightarrow (+,+,+,+,+)$$

In the case of rectilinear function $(y''_{tt} = 0)$, the rectilinear regression line will coincide the experimental curve in the error ranges.

If one bears in mind that r quantity accounts for $\text{sign}(y''_{tt})$, then r will also account for a change of the sequence of ΔY_i signs.

4.4. Method for n-determination

On the strength of analysis dealt with in the previous sections, a conclusion can be drawn that y=f(r,t) transformation has a clear advantage for n-determination, while determination of the first and second derivatives from the given function allows for finding out whether the asymptote is present and the result is authentic, mainly by the way of regression method (Appendix 2).

The determination method consists of the following stages:

I. Setting up correctness of the operating range

1. Specific error is a determinant of interval's upper limit
2. Lower limit of the interval is determined by "hazardous points".

II. Computation of n-quantity

III. Estimation and correction of reliability of the result.

I. Operating range is located in the extremely low velocity site, since it should maximally approximate infinity ($t_1,t_2)\rightarrow\infty$. Therefore, upper boundary (lower boundary for V=f(x)) may be of the error order. Measurement of extremely small values is bound with great errors. To solve this problem it is required to estimate a specific error - $\varepsilon_i = \sigma_i/V_i$. To achieve an authentic result specific error equal to 0.2 is admissible for some points of interval. But the majority of measurements should satisfy the condition $\varepsilon_i \leq 0.1$, and the number of points should not be more than or equal to 5.

As we have pointed out earlier, the presence of "hazardous points" in the operating range may become the reason for incorrect determination of n. Its lower boundary depends on the nature of molecular mechanism of enzyme system, as the curve originating there may have "hazardous points". Their "elimination" is possible if we explore the interval to the first turning point of V=f(x) function. However, as geometrical shape of y=f(r,t) function is r-dependent, it is clear that "hazardous points" may again be found in this function. Therefore, შეზღუდვა $(\ln U)''_{tt} < 0$ is required, due to which the experimental points available on the interval must yield a convex curve for InU=f(t) transformation. Yet, even this constraint does not rule out the danger of existence of "hazardous points"; therefore, correction of the operating range should be done additionally with InU=f(Int) function. Within the ranges of operation interval it must not possess the turning and inclination points. These constraints extend the permissible lower bound of operating range, but with this we can avoid the danger of the presence of "hazardous points" on the operational interval.

II. n-determination is made via the asymptote of y=f(r, t) function existence of which depends on r parameter (Table 4.2; 4.7-4.9, § 4.2-4.3).

An asymptote can be discovered by a regression line drawn on the points of a correct operation interval and by estimating the approximation to rectilinearity of the curve plotted on $y_i=f(r, t)$ points. Proceeding from the least quadrants, minimalization should be made of the integral $I=\int_{t_1}^{t_2}[y-(a+bt)]^2 dt$, that is equivalent to finding such a, b and r parameters for which I would be minimal. For this, to solve a set of three equations ($I'_a=0$, $I'_b=0$ and $I'_r=0$) is necessary. However, to solve the problem posed the use of the given approach is not reasonable. On the one hand, it is a complex procedure and it is not lucid, on the other. It is necessary that the transformation resulting from r-variation be followed and the needed corrections be introduced in the calculations.

Suppose, we have correctly selected, consisting of k number points, operation interval $[t_1, t_2]$ and r-sequence, $\min(r) \le r_i \le \max(r)$ with a step $\Delta r=[r_{i+1}-r_i]$. For each r_i a regression line $Y_i=a_i+b_i t$ points and the following values are computed:

1. (ΔY_{rj}) signs sequence. Distribution of ΔY_i sign gives an idea of the sign of the function's second derivative. In the case of concave curve distribution of ΔY_i sign has the following pattern $(-,\cdots,-,+,\cdots,+,-,\cdots,-)$, if the curve is convex, then $(+,\cdots,+,-,\cdots,-,+,\cdots+)$ is obtained. And in the case of a straight line distribution of ΔY_i sign is of a chaotic character, induced by an experimental error. It should be noted that the above considered approach is not an accurate method for the measurement of the second derivative, but it provides a certain idea of the curve shape, that was quite sufficient for our purposes.

2. Weighted mean quadratic error: $MY_i=\sum_{j=0}^{k}\left(\frac{Y_{rj}-y_{tj}}{\sigma(y_j)}\right)^2$: $\sigma(y)=\frac{\sigma(V)}{rV\sqrt[r]{V}}$, (4.26)

3. A measure (estimation) of linearity: $\nu_1=\left(\sum_{j=1}^{k}\mu_j\right)-k$ & $\nu_2=k-2$

$$F_i^{exp}=\frac{\sum_{j=1}^{k}\mu_j(Y_{rj}-y_{rj})^2}{(k-2)}\Bigg/\frac{\left[\sum_{j=1}^{k}\mu_j-k\right]}{\sum_{j=1}^{k}\mu_j(\mu_j-1)[\sigma(y_{rj})]^2};\qquad \mu_i=\text{const},\ \nu_1=k(\mu-1),\ \nu_2=k-2$$

$$F_{tab}\Leftarrow\begin{cases}\nu_1=\left(\sum_{j=1}^{k}\mu_j\right)-k & \& \quad \nu_2=k-2\\ \mu_i=\text{const},\ \nu_1=k(\mu-1) & \& \ \nu_2=k-2\end{cases} \qquad (4.27)$$

4. The averaged approximation coefficient, $MV = \frac{1}{k}\sum_{i=1}^{k}\frac{|V_i - v_i|}{\sigma(V_i)}$, (4.28)

5. The correlation coefficient, $CC = \frac{\sum_{i=1}^{k}(Y_{ri} - y_i)(\bar{t} - t_i)}{\left[\sum_{i=1}^{k}(Y_{ri} - y_i)^2 \sum_{i=1}^{k}(\bar{t} - t_i)^2\right]^{1/2}}$ (4.29)

We determine $r=R_0$ for which MY, MV and F_{exp} values are minimal, while CC is a maximal quantity and at the same time there occurs an acute alteration in the signs sequence. As a rule, for a more precise determination of R_0 it might become necessary to reduce Δr step (according to the necessity up to ~0.1) and vary r in a narrower interval.

R_0 optimal is the most approximated value to n, while the regression line drawn for $r= R_0$ is a circle, most approximated to asymptote. It is reasonable to compute the regression coefficients and their errors, as well as to plot the tangential hyperbola (V_n) and their graphical collation $V=f(x)$.

$$Y_R=a_n+b_nt\ ;\ a_n \pm\sigma(a_n)\ ;\ b_n \pm\sigma(b_n)\ ;\quad V_n = \frac{(1/b_n)^n x^n}{[1+(a_n/b_n)x]^n} \qquad (4.30)$$

III. There is a simple way for R_0 estimation. From R_0 determination it emerges that for each t_i point the equation $r_i = \frac{\ln(Y_{R_0}(t_i))}{\ln U(t_i)}$. should be performed. r_i is a mean $\overline{R(t)} = \sum r_i/k$, while its mean arithmetical would be error $\sigma(\overline{R(t)}) = \left[\frac{\sum(\overline{R(t)} - r_i)^2}{k(k-1)}\right]^{1/2}$. From R_0 experimental measurement it follows that R_0 is inaccurately measured mean. At the same time it is obvious that $R_1 \le R_0 \le R_2$ and $R_1 \le R(t) \le R_2$. So, it can be supposed that estimation of $\Delta R = |R_0 - \overline{R(t)}| \le \sigma(\overline{R(t)})$ and R_0 is possible by means of $\sigma(\overline{R(t)})$ value. It should be mentioned that in an ideal case $R_0 = \overline{R(t)}$ and considering $\sigma(\overline{R(t)})$ value may be regarded that $R_0 \approx \overline{R(t)}$.

As has been demonstrated in the previous section, by calculation of $\overline{R}$, R_1 and R_2, it is also possible to determine n-approximation of $(\overline{R} - n)$.

Bearing in mind all the considered, R_0 or $\overline{R(t)}$ gets rounded up to a whole N number and is calculated $\sigma(N) = \left[\frac{\sum(N - N_i)^2}{k(k-1)}\right]^{1/2}$, while $N_i = \frac{\ln(Y_{ni})}{\ln U_i}$.

Hence, the numerical number of n parameter is $N \pm \sigma(N)$. On the basis of the above-stated it is quite possible to implement a computer program for the determination of n-parameter. Fig. 4.10 and Table 2 give a concrete example of determination of n-parameter that has been accomplished by a special computer program.

Determination of the number (n) of sites assigned for essential activators of Na^+

The composition of the reaction medium is: $0 \le [Na^+] \le 22$mM, [MgATP]=2 mM, $[ATP_f]=34\mu M$, $[Mg^{++}]$=5mM, Triss/HCl buffer 20mM, pH=7.7.

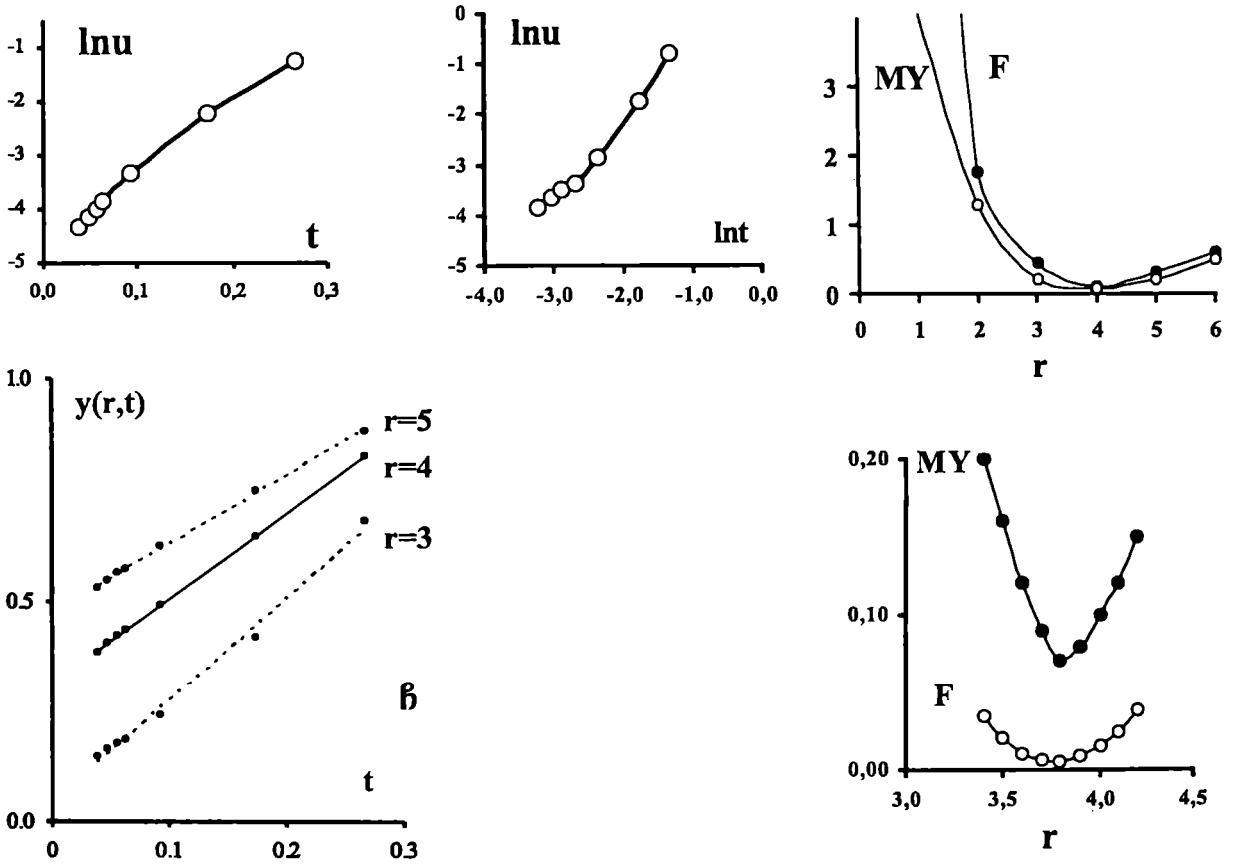


Fig. 4.10. n-determination, graphs are plotted on the correct operation interval
A – lnU= f(t), a convex curve;
B – lnU= f (Int), a curve without turning and inclination points
C – y= f (r, t). r=3 concave, r=4 straight, r=5 convex curves
D and E – MY= f (r) and F=f (r). MY= f (3.8) and F= f (3.8) $\Rightarrow$ min

Table 4.3

r_j	1, 2, 3, 3.5	3.6, 3.7	3.8	3.9, 4, 5, 6, 7
sign(ΔY_j)	− − − + + + − −	− − + − − + − +	− + − + − + − +	+ + + + + − − + +

$r_j = 3.7$		$(\bar{R} - n)_I$	$(\bar{R} - n)_{II}$	$R_0 \pm \sigma(R_0)$	N
min MY	min F				
0.072	0.005	-0.4464	-0.1312	3.8003 ± 0.0362	4

As seen from A and B in Fig. 4.10, lnU=f(t) and lnU=f(lnt) curves have no inclination points, while lnU=f(t) function has a convex shape, i.e. operation interval has been correctly selected. Maximal linearization is achieved when r=4 and weighted mean quadratic error and F test adopt a minimal quantity when r=4 (Fig. 4.10 D). When step is reduced up to 0.1, MY and F test are seen to assume a minimal value at r=3.8 (Fig. 4.10, E). From Table 4.3 it is clear that the distribution of ΔY_0 sign is chaotic when r=3.8. From this it follows that in this case the number of Na^+ as an essential activator equals to 4 (n=4).

4.5. Determination of m-parameter

In the basic velocity equation (3.1) let us substitute argument t=1/x, then we obtain:

$$V(x)=\frac{v}{e_0}=\frac{x^n\sum_{i=0}^{P}\alpha_i x^i}{\sum_{i=0}^{S}\beta_i x^i} \quad \Rightarrow \quad V(t)=\frac{v}{e_0}=\frac{t^m\sum_{i=0}^{p}\alpha_{p-i}t^i}{\sum_{i=0}^{s}\beta_{s-i}t^i} \qquad (4.31)$$

It is clear that V=f(x) and V=f(t) functions have an absolutely identical analytical shape. It must be noted that n parameter is transformed into m parameter $\alpha_i \Rightarrow \alpha_{p-i}$, $\beta_0 \Rightarrow \beta_{s-i}$. Proceeding from this it is obvious that the theory and method used for determination of the number of sites (m) for complete inhibition would be absolutely identical with those applied for essential activators (n). Only instead of U=f(t) and y(r, t) functions U=f(x) and y(r, x) are used.

Appendix 4.3

In the case if n=0, ($V(0)=\alpha_0/\beta_0 \neq 0$), with slight transformation we obtain:

$$\Delta V_0 = V(x)-V(0)=\frac{\sum_{i=0}^{p}\alpha_i x^i}{\sum_{i=0}^{s}\beta_i x^i}-\frac{\alpha_0}{\beta_0}=\frac{\sum_{i=0}^{p}(\alpha_i\beta_0-\alpha_0\beta_i)x^i-\alpha_0\sum_{i=0}^{s}\beta_i x^i}{\beta_0\sum_{i=0}^{s}\beta_i x^i},$$

whose exploration is possible with the application of the above methods. The same applies to the case, given by: m=0, ($V(\infty)=\frac{\alpha_p}{\beta_s}\neq 0$)

$$\Delta V_\infty = V(t)-V(\infty)=\frac{\sum_{i=0}^{p}\alpha_{p-1}t^i}{\sum_{i=0}^{s}\beta_{s-1}t^i}-\frac{\alpha_p}{\beta_s}=\frac{\sum_{i=1}^{p}(\alpha_{p-i}\beta_s-\alpha_p\beta_{s-i})t^i-\alpha_p\sum_{i=p+1}^{s}\beta_{s-i}t^i}{\beta_s\sum_{i=0}^{s}\beta_{s-i}t^i}$$

Chapter 5. ESTIMATION OF p PARAMETER

5.1. Geometrical shape determinant parameter of U=f(t) curve

For deciphering of the molecular mechanism of multi-sited enzyme systems, as pointed out earlier, one of the principal tasks is determination of the degree parameters in the basic velocity equation. The only way to solve this problem is the analysis of the geometric shape of a curve. It implies analysis of the first and second derivatives of the respective function (determination of their signs) and accordingly, setting up the number of turning (ν) and inflection (λ) points. In addition, it is reasonable to establish the number (μ) of the points at which the tangents would cross the origin of coordinates (Fig. 5.1).

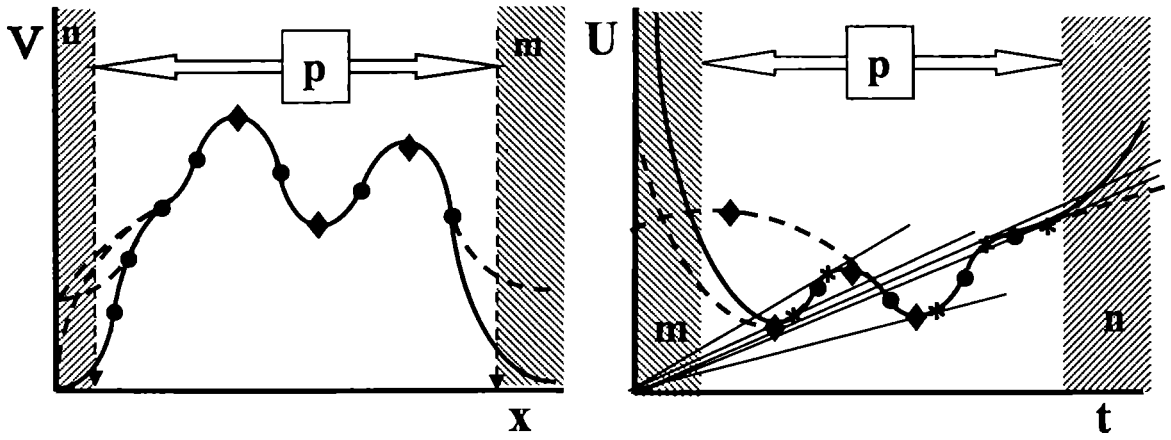


Fig. 5.1. Possible geometrical shapes of V=f(x) and U=f(t) functions

♦– the turning, • – inflection and * – the points at which the tangents go through the origin of coordinates. Delineated in the Fig. are the sites of n, m. p determination – estimation.

The method for determination of n and m parameters has been discussed in the previous chapter, as regards p parameter, determination of its numerical value is complicated. V=f(x) and U=f(t) functions may have curves with geometrical shapes of various complexity, depending on n, m, p, α_i and β_i quantities. With ligand's extremely small and large concentration ranges the curve shape is mainly determined by n and m parameters, while in the middle section it is p parameter that mainly accounts for the curve shape (Fig. 5.1). This conclusion has been made relying on the fact that p is the number of the sites assigned for partial effect modifiers and p does not participate in the formation of V=f(x) and U=f(t) curves in the constrained sites.

Let us examine equations U′= 0, U–tU′= 0, U″=0 and their respective roots ν, λ, μ. For this let us use the rule of Descartes' signs: the quantity of true roots of the equation is equal to the

number of sign change of the coefficients, or is less than this quantity by even number. The tables (5.1. 5.2, 5.3) below present the results of this examination. The following designations have been introduced in the tables.

$$DS \Rightarrow \left[\operatorname{sign}\left(\lim_{t\to+\infty} U'_t\right) = -\operatorname{sign}\left(\lim_{t\to 0} U'_t\right)\right]; \qquad IS \Rightarrow \left[\operatorname{sign}\left(\lim_{t\to+\infty} U'_t\right) = \operatorname{sign}\left(\lim_{t\to 0} U'_t\right)\right]$$

Table 5.1

$$\alpha_0 \neq 0;\ U'_t = \frac{t^{n-1}\left(\sum_{k=0}^{S+p}\Psi_k t^{-k}\right)}{\left(\sum_{i=0}^{p}\alpha_i t^{-i}\right)^2} = 0;\quad \sum_{k=0}^{s+p}\Psi_k t^{n-i} = 0 \quad (\nu\text{–root})$$

n>1 m≠0	Ψ_k	$\Psi_0 \ldots\ldots \Psi_n$	$\Psi_{n+1} \ldots\ldots \Psi_{n+2p-1}$	$\Psi_{n+2p} \ldots\ldots \Psi_{s+p}$
	sign	+ ..... +	± ..... ±	− ..... −
	ν	DS ⇒ $1 \le \nu \le (2p-1)$		
n>1 m=0	Ψ_k	$\Psi_0 \ldots\ldots \Psi_n$	$\Psi_{n+1} \ldots\ldots \Psi_{n+2p-1}$	Ψ_{n+2p}
	sign	+ ..... +	± ..... $\pm H_{01}$	0
	ν	DS ⇒ $1 \le \nu \le (2p-1)$ IS ⇒ $0 \le \nu \le (2p-2)$		
n=1 m≠0	Ψ_k	Ψ_0, Ψ_1	$\Psi_2 \ldots\ldots \Psi_{2p}$	$\Psi_{2p+1} \ldots\ldots \Psi_{2p+m+1}$
	sign	+ , +	± ..... ±	− ..... −
	ν	DS ⇒ $1 \le \nu \le (2p-1)$		
n=1 m=0	Ψ_k	Ψ_0, Ψ_1	$\Psi_2 \ldots\ldots \Psi_{2p}$	Ψ_{2p+1}
	sign	+ , +	± ..... $\pm H_{01}$	0
	ν	DS ⇒ $1 \le \nu \le (2p-1)$ IS ⇒ $0 \le \nu \le (2p-2)$		
n=0 m≠0	Ψ_k	Ψ_0	$\Psi_1 \ldots\ldots \Psi_{2p-1}$	$\Psi_{2p} \ldots\ldots \Psi_{2p+m}$
	sign	0	$\pm D_{10} \ldots\ldots \pm$	− ..... −
	ν	DS ⇒ $1 \le \nu \le (2p-1)$ IS ⇒ $0 \le \nu \le (2p-2)$		
n=0 m=0	Ψ_k	Ψ_0	$\Psi_1 \ldots\ldots \Psi_{2p-1}$	Ψ_{2p}
	sign	0	$\pm D_{10} \ldots\ldots \pm$	0
	ν	DS ⇒ $1 \le \nu \le (2p-3)$ IS ⇒ $0 \le \nu \le (2p-2)$		

Thus, if $n \neq 0$, the number of turning points is $1 \le \nu \le (2p-1)$ (DS) and $0 \le \nu \le (2p-2)$ (IS) and in the case when n=m=0 we have $1 \le \nu \le (2p-3)$ (DS) and $0 \le \nu \le (2p-2)$ (IS). Proceeding from this, it is possible to determine a minimal quantity (P_{min}) of p, which is required for the availability of ν

number of turning points. Similarly, one can define the number of points (μ) at which will go the tangents passing through the origin of coordinates (Table 5.2).

Table 5.2

$$\alpha_0 \neq 0;\ U - tU'_t = \frac{t^n\left(\sum_{k=0}^{S+p}\Phi_k t^{-k}\right)}{\left(\sum_{i=0}^{p}\alpha_i t^{-i}\right)^2} = 0;\qquad \sum_{k=0}^{s+p}\Phi_k t^{n-i} = 0 \quad (\mu\text{–root})$$

n>1 m≠0	Φ_k	Φ_0 ..... Φ_{n-1}	Φ_n..... Φ_{n+2p-2}	Φ_{n+2p-1} .....Φ_{s+p}
	sign	− ..... −	± ..... ±	+ ..... +
	μ	DS ⇒ $1 \le \mu \le (2p-1)$		
n>1 m=0	Φ_k	Φ_0 ..... Φ_{n-1}	Φ_{n+1}..... Φ_{n+2p-2}	Φ_{n+2p-1}, Φ_{n+2p}
	sign	− ..... −	± ..... ±	+, +
	μ	DS ⇒ $1 \le \mu \le 2p-1)$		
n=1 m≠0	Φ_k	Φ_0	Φ_1 ..... Φ_{2p-1}	Φ_{2p} ..... Φ_{2p+m+1}
	sign	0	$\pm D_{01}$ ..... ±	− ..... −
	μ	DS ⇒ $0 \le \mu \le (2p-2)$ IS ⇒ $1 \le \mu \le (2p-1)$		
n=1 m=0	Φ_k	Φ_0	Φ_1 ..... Φ_{2p-1}	Φ_{2p}, Φ_{2p+1}
	sign	0	$\pm D_{01}$ ..... ±	+, +
	μ	DS ⇒ $0 \le \mu \le (2p-2)$ IS ⇒ $0 \le \mu \le (2p-2)$		
n=0 m≠0	Φ_k	Φ_0	Φ_1 ..... Φ_{2p-2}	Φ_{2p-1}
	sign	+	± ..... ±	+
	μ	IS ⇒ $0 \le \mu \le (2p-2)$		
n=0 m=0	Φ_k	Φ_0	Φ_1 ..... Φ_{2p-2}	Φ_{2p-1}, Φ_{2p}
	sign	+	± ..... ±	+, +
	μ	IS ⇒ $0 \le \mu \le (2p-2)$		

As seen from the Table, the number of points at which goes the tangent passing through the coordinate origin also depends on p degree parameter, also according to n, m, DS and IS quantities respectively on $0 \le \mu \le (2p-2)$ or $1 \le \mu \le (2p-1)$. Proceeding from this, it is possible to define a minimal quantity of p (p_{min}) required for the availability of μ number of points. A far more complex picture is obtained while exploring the inclination points. They ought to be considered, for there might be such a curve which has no turning points and the tangents passing through the coordinate origin.

Table 5.3

$$\alpha_0 \neq 0;\quad U''_{tt} = \frac{t^{n-2}\left(\sum_{q=0}^{S+2p}\Lambda_q t^{-q}\right)}{\left(\sum_{i=0}^{p}\alpha_i t^{-i}\right)^3} = 0;\qquad \sum_{q=0}^{s+2p}\Lambda_q t^{-q} = 0 \quad (\lambda\mu\text{–root})$$

n>1 m≠0	Λ_q		$\Lambda_0 \ldots\ldots \Lambda_n$	$\Lambda_{n+1} \ldots\ldots \Lambda_{n+3p-2}$	$\Lambda_{n+3p-1} \ldots\ldots \Lambda_{s+2p}$
	sign		+ ..... +	± ..... ±	+ ..... +
	λ	Even p	IS ⇒ $0 \le \lambda \le (3p-1)$		
		Odd p	IS ⇒ $0 \le \lambda \le (3p-2)$		
n>1 m=0	Λ_q		$\Lambda_0 \ldots\ldots \Lambda_n$	$\Lambda_{n+1} \ldots\ldots \Lambda_{n+3p-2}$	Λ_{n+3p-1}, W_{n+3p}
	sign		+ ..... +	± ..... ±	0, 0
	λ	Even p	DS ⇒ $1 \le \lambda \le (3p-2)$, IS ⇒ $0 \le \lambda \le (3p-3)$		
		Odd p	DS ⇒ $1 \le \lambda \le (3p-3)$, IS ⇒ $0 \le \lambda \le (3p-2)$		
n=1 m≠0	Λ_q		Λ_0, Λ_1	$\Lambda_2 \ldots\ldots \Lambda_{3p-1}$	$\Lambda_{3p} \ldots\ldots \Lambda_{3p+m+1}$
	sign		0, 0	± ..... ±	+ ..... +
	λ	Even p	DS ⇒ $1 \le \lambda \le (3p-2)$, IS ⇒ $0 \le \lambda \le (3p-3)$		
		Odd p	DS ⇒ $1 \le \lambda \le (3p-3)$, IS ⇒ $0 \le \lambda \le (3p-2)$		
n=1 m=0	Λ_q		Λ_0, Λ_1	$\Lambda_2 \ldots\ldots \Lambda_{3p-1}$	Λ_{3p}, Λ_{3p-1}
	sign		0, 0	± ..... ±	0, 0
	λ	Even p	DS ⇒ $1 \le \lambda \le (3p-4)$, IS ⇒ $1 \le \lambda \le (3p-3)$		
		Odd p	DS ⇒ $0 \le \lambda \le (3p-3)$, IS ⇒ $0 \le \lambda \le (3p-4)$		
n=0 m≠0	Λ_q		Λ_0	$\Lambda_1 \ldots\ldots \Lambda_{3p-2}$	$\Lambda_{3p-1} \ldots\ldots \Lambda_{3p+m}$
	sign		0	± ..... ±	+ ..... +
	λ	Even p	DS ⇒ $1 \le \lambda \le (3p-2)$, IS ⇒ $0 \le \lambda \le (3p-3)$		
		Odd p	DS ⇒ $1 \le \lambda \le (3p-3)$, IS ⇒ $0 \le \lambda \le (3p-2)$		
n=0 m=0	Λ_q		Λ_0,	$\Lambda_1 \ldots\ldots \Lambda_{3p-2}$	Λ_{3p-1}, Λ_{3p}
	sign		0	± ..... ±	0, 0
	λ	Even p	DS ⇒ $1 \le \lambda \le (3p-4)$, IS ⇒ $0 \le \lambda \le (3p-3)$		
		Odd p	DS ⇒ $1 \le \lambda \le (3p-3)$, IS ⇒ $0 \le \lambda \le (3p-4)$		

Thus, the geometrical shape of U=f(t) curve is defined mainly: signs $\operatorname{sign}\left[\lim_{t\to\pm\infty}(U')\right]$, $\operatorname{sign}\left[\lim_{t\to\pm\infty}(U'')\right]$ and the number of turning (v) and inflection (λ) points and the number of the tangent crossing the origin of coordinates (μ).

5.2. Determination of p_{min} by the curve shape

Suppose n and m quantities are identical for two $U=f_1(t)$ and $U=f_2(t)$ functions and their p parameter is, respectively, p_1 and p_2, ($p_1<p_2$). $U=f_1(t=)$ function may have a definite number of curves of various shapes. According to the law of Descartes' signs, the number of curves of various shapes for $U=f_2(t)$ function may be greater, though among them availability of the curves of all shapes characteristic of $U=f_1(t)$ function is expected. So, with an increase of p parameter all the available curves are repeated and the curves of new geometrical shape are added.

From this it becomes evident that application of the method for the analysis of geometrical shape of curves it is impossible to accurately measure p parameter, but in the case of fixed n and m, when the number of v, μ and λ points as determinants of the curves geometrical shape is know n, it becomes possible to set up P_{min} quantity. By means of Table 7, presented below, P_{min} determination is possible.

Table 5.4

Interrelationship between the number of degree parameters (n, m, p) and special points (v, μ and λ), in the case of diverse (DS) and identical (IS) sign possessing limits $\lim_{t\to 0}U'$ & $\lim_{t\to\infty}U'$, $\lim_{t\to 0}(U-tU')$ & $\lim_{t\to\infty}(U-tU')$ ფა $\lim_{t\to 0}U''$ & $\lim_{t\to\infty}U''$

n & m		(U′=0)⇒ν	(U=tU′)⇒μ	(U″=0)⇒λ	
				Odd p	Even p
n > 1	DS	1 ≤ ν ≤ 2p-1	1 ≤ μ ≤ 2p-1	—	—
m ≠ 0	IS	—	—	0 ≤ λ ≤ 3p-1	0 ≤ λ ≤ 3p-2
n > 1	DS	1 ≤ ν ≤ 2p-1	1 ≤ μ ≤ 2p-1	1 ≤ λ ≤ 3p-2	1 ≤ λ ≤ 3p-3
m = 0	IS	0 ≤ ν ≤ 2p-2	—	0 ≤ λ ≤ 3p-3	0 ≤ λ ≤ 3p-2
n = 1	DS	1 ≤ ν ≤ 2p-1	1 ≤ μ ≤ 2p-1	1 ≤ λ ≤ 3p-2	1 ≤ λ ≤ 3p-3
m ≠ 0	IS	—	0 ≤ μ ≤ 2p-2	0 ≤ λ ≤ 3p-3	0 ≤ λ ≤ 3p-2
n = 1	DS	1 ≤ ν ≤ 2p-1	1 ≤ μ ≤ 2p-1	1 ≤ λ ≤ 3p-4	1 ≤ λ ≤ 3p-3
m = 0	IS	0 ≤ ν ≤ 2p-2	0 ≤ μ ≤ 2p-2	0 ≤ λ ≤ 3p-3	0 ≤ λ ≤ 3p-4
n = 0	DS	1 ≤ ν ≤ 2p-1	—	1 ≤ λ ≤ 3p-2	1 ≤ λ ≤ 3p-3
m ≠ 0	IS	0 ≤ ν ≤ 2p-2	0 ≤ μ ≤ 2p-2	0 ≤ λ ≤ 3p-3	0 ≤ λ ≤ 3p-2
n = 0	DS	1 ≤ ν ≤ 2p-3	—	1 ≤ λ ≤ 3p-4	1 ≤ λ ≤ 3p-3
m = 0	IS	0 ≤ ν ≤ 2p-2	0 ≤ μ ≤ 2p-2	0 ≤ λ ≤ 3p-3	0 ≤ λ ≤ 3p-4

If n and m parameters have been defined and represent constant values, then $P=P_{min}$ is such a quantity of the parameter, which provides U=f(t) with a definite number of special points v, μ and λ. P_{min} satisfies the principle of a minimal model, which implies a minimal number of enzyme

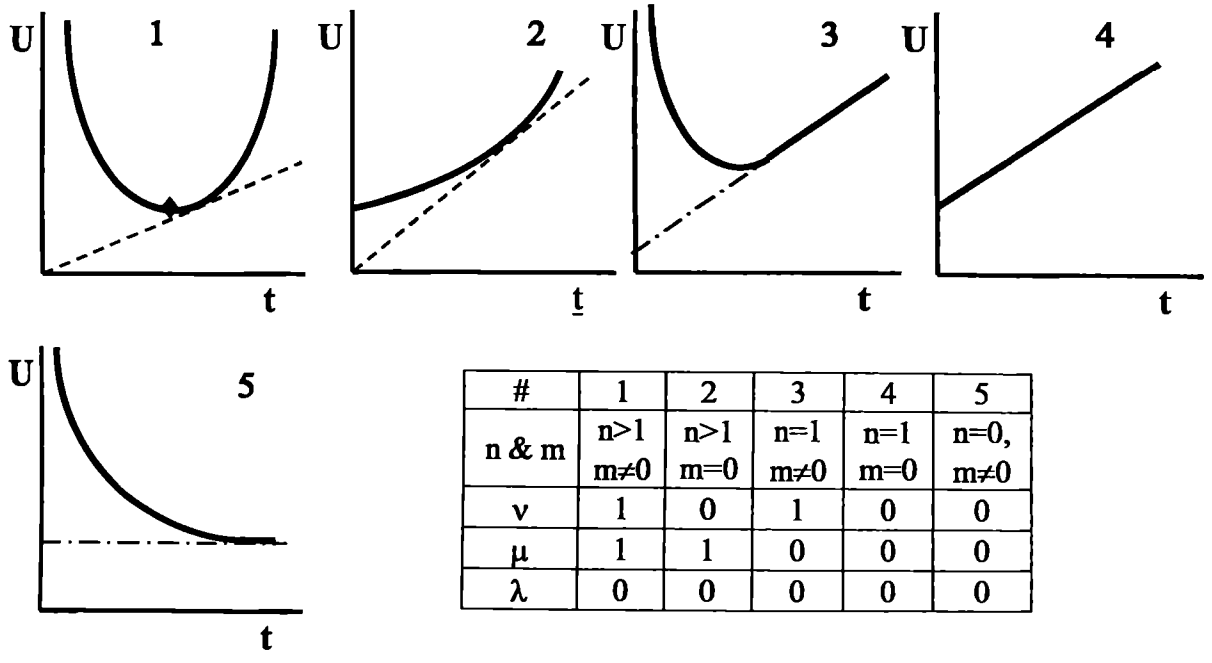
forms and reaction steps between them, their linkage under a certain rule. At any concentration of ligand P_{min} provides the coincidence of geometrical shape of theoretical and experimental curves. Genuine scheme and a minimal model have identical n, m, v, λ and μ, but may have different quantity of p and s. In the genuine availability of all sites of a minimal model, of enzyme forms and transitions between them is imperative, although it is not ruled out that there were additional forms and transitions, which have no effect on geometrical shape of experimental curve.

Thus, from the examined material, it is evident that we may establish P_{min} Vs curve turning point (v), vs. inclination point (λ), vs. the number of the points at which the tangent (μ) crosses the coordinates' origin, that would eventually lead to p_{min} determination.

It must be noted that the present analysis of p_{min} measurement is of a theoretical character and its implementation is rather difficult. Therefore, it is reasonable to present the readily applicable tables as a reference book which for definite quantities of n and m would exactly show the dependence between p_{min} and the number of special points v, μ, λ.

Let us examine U=f(t) curve whose p=0 (Fig. 5.2) $U = \left(t^n/\alpha_0\right)\sum_{i=0}^{s}\beta_i t^{-i}$

In this case, there may exist only 5 curves of various shapes. Such curves at any quantity of n and m have no inclination point. They may have one turning point and one tangent passing through the origin of coordinates (ØØ).

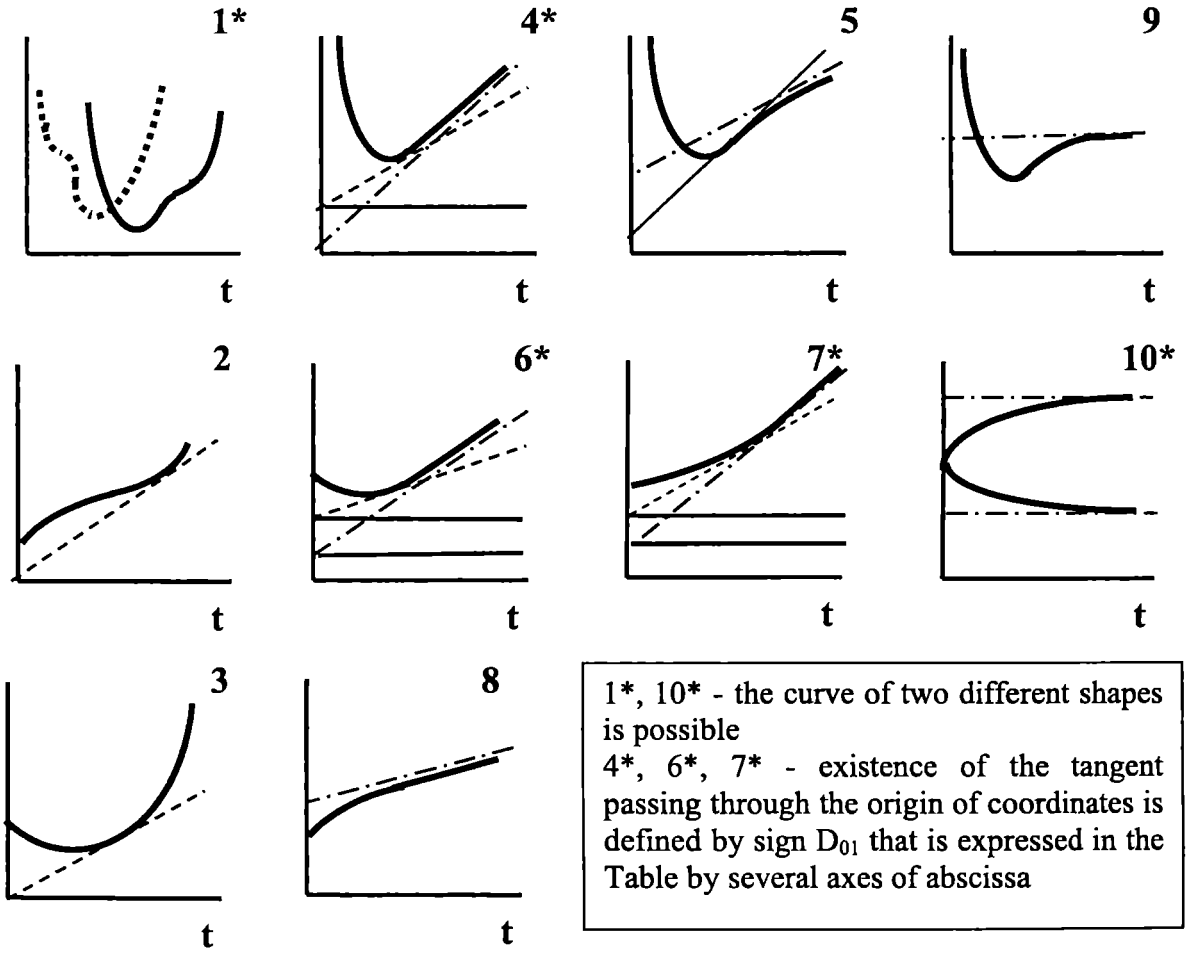


#	1	2	3	4	5
n & m	n>1 m≠0	n>1 m=0	n=1 m≠0	n=1 m=0	n=0, m≠0
v	1	0	1	0	0
μ	1	1	0	0	0
λ	0	0	0	0	0

Fig. 5.2. U=f(t) curves' shapes; p=0. —·— asymptote; ---- the tangent passing at ØØ.

U=f(t) function may have the curves of more complex shapes, when p=1, $U = \frac{t^n \sum_{i=0}^{s} \beta_i t^{-i}}{\alpha_0 + \alpha_1 t^{-1}}$.

In this case a curve of 22 diverse shapes may exist. 5 of them are repeated from p=0 case (Fig. 5.3). From the curves and Tables in Fig. 5.2 and 5.3 it is seen that there is a strict and unambiguous interrelation between P_{min}, ν, μ, λ numbers and n, m parameters.



1*, 10* - the curve of two different shapes is possible
4*, 6*, 7* - existence of the tangent passing through the origin of coordinates is defined by sign D_{01} that is expressed in the Table by several axes of abscissa

Fig. 5.3	n>0 m≠0	n>1 m=0		n=1 m≠0		n=1, m=0					n=1 m=0	n=0 m=0	
№	1	2	3	4	5	6		7		8	9	10	
ν	1	0	1	1	1	1	1	0	0	0	1	0	0
μ	1	1	1	1	1	1	0	1	0	0	0	0	0
λ	2	0	0	0	1	0	0	0	0	0	1	0	0
D_{01}				<0	>0	<0	>0	<0	>0	>0	<0	>0	<0
sign(U″)	>0	DS	>0	>0	DS	>0	>0	>0	>0	<0	DS	<0	>0
sign(U′)	DS	IS	DS	IS	IS	DS	DS	DS	IS	IS	DS	IS	IS

Fig. 5.3. Shapes of U=f(t) curves; p=1 (additional shapes, which U=f(t) is not in possession of, p=0). DS – different signs, IS – identical signs.

It should be specially pointed out that when n=0 and m≠0 for the geometrical shapes of a curve to be unequivocally displayed the sign of D_{01} expression is of importance. It must be noted that while defining n parameter D_{01} sign determines the sign of asymptote intersection of the ordinate axis and at the same time coincides with the sign of the regression A coefficient. The material presented in Fig. 5.2, 5.3 shows explicitly that with an increase in p_{min}, the number of curves increases substantially and complicates their geometrical shape. When p_{min}=0 (Table 5.2) the second derivative of U=f(t) function is positive and concave curves are obtained. Exception makes n=1 m=0 case, when we have to deal with single-sited enzyme systems and a straight line is obtained $\left(U''_{tt}=0\right)$. When n>1 and m≠0 the curve is concave and at small quantity of argument approximates the ordinate axis. When n>1 m=0 the concave curve at infinitesimal quantity of argument cuts the ordinate axis. When n=1 and m≠0 the concave curve at an infinite quantity of argument has an inclined asymptote. If n=0 at an infinite quantity of argument the function assumes limited value, therefore, the concave curve asymptotically approximates the given value. When p_{min}=1 (Table 5.2) the sign of the second derivative in the limit U=f(t) function may be as positive, so negative. Accordingly, the curves have both concave and convex shapes. When n>1, m≠0 geometrical shape of curves resembles the same situation in p_{min}=0 case, but in contrast to it, has two inclination points, which may be arranged consecutively on one or the other side of turning points. As seen from Table 8, in p_{min}=1 case compared to p_{min}=0, max value increases from 0 to 2, while max and maxμ value is constant and equals to 1. It is remarkable that when n=1 and m=0=λ=μ=0. Similar picture is observable in p_{min}=0 case, but the difference lies in the second derivative. When p_{min}=0 and when p_{min}=1, in the first case we will obtain a straight line, in the second – a convex curve, which has an asymptote at infinite quantity of argument. The geometrical shape of a curve gets rather complicated when p_{min}=2 (Table 5.5-5.10). Compared to p_{min}=1 increases max v number from 1 to 3, max λ number from 2 to 4, while maximal number from 1 to 3. Hence, on the basis of the tables (5.1; 5.10) it may be said that they actually represent a reference book with the help of which relying on the geometrical shape of the experimental curve it is possible to set up p_{min} quantity. And determination of n, m and p_{min} will enable to decipher the molecular mechanism for enzyme system (a minimal model).

Conclusion: If in the site of extremely small and large quantity of argument U=f(t) curve shape is known, then according to the number of turning and inclination points, as well as to that of tangents passing through the origin of coordinates p_{min} can be determined.

Appendix 5.1. By means of the tables given below, based on geometrical shape of U=f(t) curve, it is quite possible to find p_{min}.

1. On experimental U=f(t) curve v, μ, λ are verified.

2. Sign [U',U-tU',U''] (t→∞) and in (t→0) sites is verified.

3. The data corresponding p_{min} is sought.

Table 5.5

U=f(t) curve shape at extremely small and large quantity of argument

t→+∞	$\operatorname{sign}\left(\lim\limits_{t\to\infty} U'_t\right)$	$\operatorname{sign}\left(\lim\limits_{t\to\infty}(U-tU'_t\right)$	$\operatorname{sign}\left(\lim\limits_{t\to\infty} U''_{tt}\right)$
n > 1	+∞	−∞	+∞
n = 1	$\frac{\beta_0}{\alpha_0}>0$	$\pm\frac{D_{10}}{\alpha_0^2}$	$\frac{2(\alpha_0 D_{02}-\alpha_1 D_{01})}{\alpha_0^3}\left[\lim\limits_{t\to\infty} t^{-3}\right]=\pm 0$
n = 0	$\frac{D_{10}}{\alpha_0^2}\left(\lim\limits_{t\leftarrow\infty} t^{-1}\right)=\pm 0$	$\frac{\beta_0}{\alpha_0}>0$	$\frac{2D_{01}}{\alpha_0^2}\left(\lim\limits_{t\leftarrow\infty} t^{-3}\right)=\pm 0$
t→0	$\operatorname{sign}\left(\lim\limits_{t\to 0} U'_t\right)$	$\operatorname{sign}\left(\lim\limits_{t\to 0}(U-tU'_t)\right)$	$\operatorname{segn}\left(\lim\limits_{t\to 0} U''_{tt}\right)$
m ≠ 0	−∞	+∞	+∞
m = 0	$\pm\frac{H_{01}}{\alpha_p^2}$	$\frac{\beta s}{\alpha p}>0$	$\pm\left[\frac{2}{\alpha_p^3}\left(\alpha_p H_{02}-\alpha_{p-1}H_{01}\right)\right]$

Table 5.6

p_{min} determination (n>1)

$\left(\lim\limits_{t\to\infty} U''_{tt}\right)>0$	n>1, m≠0				n > 1, m=0									
	$\left(\lim\limits_{t\to 0} U''_{tt}\right)>0$				$\left(\lim\limits_{t\to 0} U''_{tt}\right)>0$						$\left(\lim\limits_{t\to 0} U''_{tt}\right)<0$			
ν	1	1	1	3	0	1	0	1	2	3	0	0	1	2
λ	0	2	4	2, 4	0		2, 4		2		1	3	1,3	
μ	1		1, 3		1		1,3				1	1, 3		
p_{min}	0	1	2		0	1	2				1	2		

Table 5.7

p_{min} determination (n=1, m≠0)

n =1, m≠0		$\left(\lim_{t\to\infty} U''_{tt}\right)>0$				$\left(\lim_{t\to\infty} U''_{tt}\right)<0$		
ν		1	1	1	3	1	1	3
λ		0	0	2, 4	2	1	3	3
μ	$D_{01}>0$	0	0	0, 2		0	2	0, 2
	$D_{01}=0$	–	0	0, 2		–	1	
	$D_{01}<0$	–	1	1, 3		–	1	
p_{min}		**0**	**1**	**2**		**1**	**2**	

Table 5.8

p_{min} determination (n =1, m=0)

n=1, m=0		ν=λ=μ=0 **p_{min}=0**	$\left(\lim_{t\to\infty} U''_{tt}\right)<0$				$\left(\lim_{t\to\infty} U''_{tt}\right)>0$				
$\left(\lim_{t\to 0} U''_{tt}\right)>0$	ν		0	0	1	2	–	0	1	2	–
	λ		0	0, 2		2	–	1, 3		1	–
	μ	$D_{01}>0$	0	0, 2			–	0, 2		0	–
		$D_{01}=0$	–	1			–	0, 2		1	–
		$D_{01}<0$	–	1			–	1, 3		0	–
$\left(\lim_{t\to 0} U''_{tt}\right)<0$	ν		–	0	1	–	1	0	1	2	3
	λ		–	1, 3		–	0	0, 2		2	2
	μ	$D_{01}>0$	–	0, 2		–	0	0, 2			
		$D_{01}=0$	–	1		–		0, 2			
		$D_{01}<0$	–	1		–		0, 2			
p_{min}			**1**	**2**			**1**	**2**			

Table 5.9

p_{min} determination (n =0, m≠0)

n=0 m≠0	$\left(\lim_{t\to\infty} U''_{tt}\right)<0$			$\left(\lim_{t\to\infty} U''_{tt}\right)>0$		
ν	0	0	2	1	1	3
λ	0	2	2	1	1	3
μ	0	0	0, 2	0	0, 2	0
p_{min}	**0**	**2**		**1**	**2**	

Table 5.10

p_{min} determination (n=0; m=0)

n=0; m=0		$\left(\lim_{t\to\infty} U''_{tt}\right)>0$				$\left(\lim_{t\to\infty} U''_{tt}\right)<0$			
$\left(\lim_{t\to 0} U''_{tt}\right)>0$	ν	0	–	1	2	–	0	1	–
	λ	0	–	2	2	–	1	1	–
	μ	0	–	0, 2	0, 2	–	0, 2	0, 2	–
$\left(\lim_{t\to 0} U''_{tt}\right)<0$	ν	–	0	1	–	0	–	1	2
	λ	–	1	1	–	0	–	2	2
	μ	–	0	0	–	0	–	0	0
p_{min}		**1**	**2**			**1**	**2**		

Table 5.11

U= f(t) function, p=3; possible quantities of ν, μ and λ.

n & m		(U′=0)⇒ν	(U=tU′)⇒μ	(U″=0)⇒λ
n > 1	DS	$1 \le \nu \le 5$	$1 \le \mu \le 5$	—
m ≠ 0	IS	—	—	$0 \le \lambda \le 8$
n > 1	DS	$1 \le \nu \le 5$	$1 \le \mu \le 5$	$1 \le \lambda \le 7$
m = 0	IS	$0 \le \nu \le 4$	—	$0 \le \lambda \le 6$
n = 1	DS	$1 \le \nu \le 5$	$1 \le \mu \le 5$	$1 \le \lambda \le 7$
m ≠ 0	IS	—	$0 \le \mu \le 4$	$0 \le \lambda \le 6$
n = 1	DS	$1 \le \nu \le 5$	$1 \le \mu \le 5$	$1 \le \lambda \le 5$
m = 0	IS	$0 \le \nu \le 4$	$0 \le \mu \le 4$	$0 \le \lambda \le 6$
n = 0	DS	$1 \le \nu \le 5$	—	$1 \le \lambda \le 7$
m ≠ 0	IS	$0 \le \nu \le 4$	$0 \le \mu \le 4$	$0 \le \lambda \le 6$
n = 0	DS	$1 \le \nu \le 3$	—	$1 \le \lambda \le 5$
m = 0	IS	$0 \le \nu \le 4$	$0 \le \mu \le 4$	$0 \le \lambda \le 6$

Chapter 6. FUNDAMENTAL KINETIC PARAMETERS FOR THE MULTI-SITED ENZYME SYSTEMS

For an exhaustive specification of the single-sited enzyme systems, as has been already stated (see chapter 2), it is necessary that only two parameters be determined (K_m and V_{max} – if ligand is an activator and K_I and V_{max} – if ligand is an inhibitor), as number of sites is a priori known and equals to 1. From this it naturally emerges that in order to fully and precisely describe the multi-sited enzyme systems at least two kinetic parameters and the overall number of sites should be defined for each site. Thus, for example, for the tri-sited enzyme systems 7 (6+1) parameter is to be determined. This task cannot be practically solved, therefore, for the analysis of multi-sited enzyme systems we ought to enter such a number of kinetic parameters which in a definite way, would fully describe the enzyme system and could be readily defined experimentally. To this end, it is primarily necessary to determine essential activators (n), full inhibitors (m) and, proceeding from the principle of a minimal model, the minimal number (p_{min}) of the sites to which partial effect modifiers (activators or inhibitors) are bound. At the next stage, one should introduce such parameters which represent an "averaged" value of kinetic parameters for all sites and characterize the summed effect of activation or inhibition.

While discussing the method for defining the number of essential activators (see chapter 4) it has been noted that in the extremely small concentration site of V=f(x) function, when r=n, $\sqrt[r]{U}=f(t)$ function has an asymptote $U_n = a_n + b_n t$. Its coefficients have the form:

$$a_n = \frac{D_{01}}{n\alpha_0\beta_0}\sqrt[n]{\frac{\beta_0}{\alpha_0}} = \frac{\alpha_0\beta_1 - \alpha_1\beta_0}{n\alpha_0\beta_0}\sqrt[n]{\frac{\beta_0}{\alpha_0}}; \qquad b_n = \sqrt[n]{\frac{\beta_0}{\alpha_0}}..$$

:t us introduce the designations:

$$V_A = \left(\frac{1}{a_n}\right)^n = \frac{\alpha_0}{\beta_0}\left(\frac{n\alpha_0\beta_0}{D_{01}}\right)^n \quad \text{and} \quad \frac{1}{K_A} = \frac{a_n}{b_n} = \frac{1}{n}\left(\frac{\beta_1}{\beta_0} - \frac{\alpha_1}{\alpha_0}\right); \qquad (6.1)$$

Plotting of n, K_A and V_A parameters represents an approximation of an experimental curve to the small concentration (correct) site

$$V_n = \frac{V_A}{\left(1 + \frac{K_A}{x}\right)^n} \qquad (6.2)$$

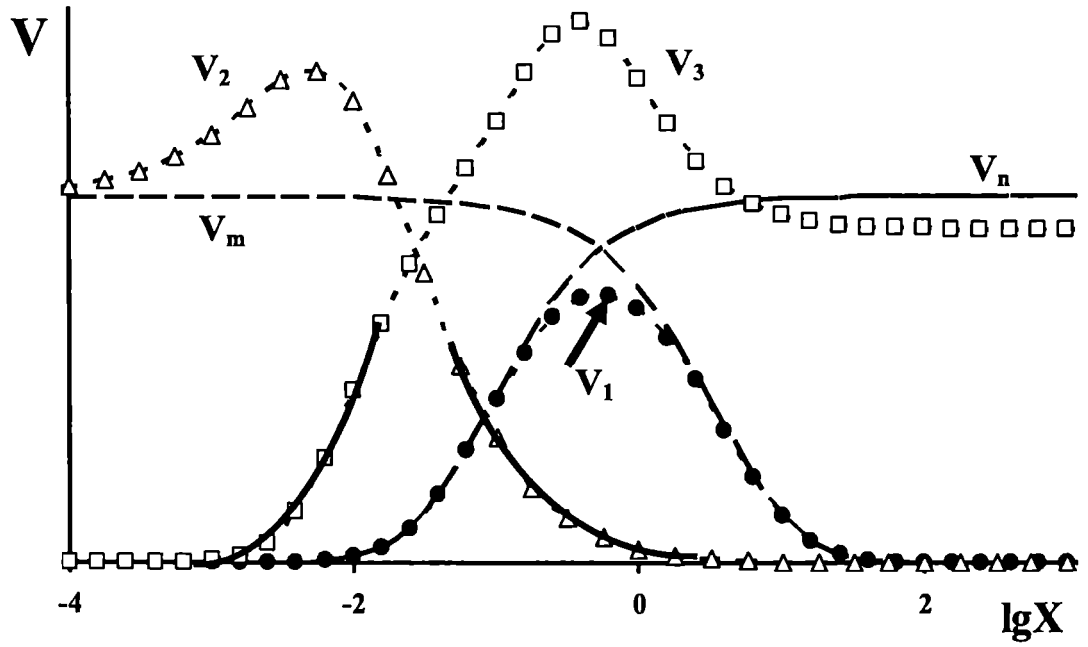
In the extremely high concentrations site of V=f(x) function m parameter can be determined (see chapter 4). Transformation of variables leads to function $\sqrt[r]{U}=f(x)$, which, given r=m, has an asymptote - $U_m = a_m + b_m x$, whose coefficients are::

$$a_m = \frac{H_{01}}{m\alpha_p\beta_s}\sqrt[m]{\frac{\beta_s}{\alpha_p}} = \frac{\alpha_p\beta_{s-1}-\alpha_{p-1}\beta_s}{m\alpha_p\beta_s}\sqrt[m]{\frac{\beta_s}{\alpha_p}}, \quad b_m = \sqrt[m]{\frac{\beta_s}{\alpha_p}}$$

Let us also introduce the designations and on the basis of them plot a curve:

$$V_m = \frac{V_I}{\left(1+\frac{x}{K_I}\right)^m} \quad (6.3),$$

where $V_I = \left(\frac{1}{a_m}\right)^m = \frac{\alpha_p}{\beta_s}\left(\frac{m\alpha_p\beta_s}{H_{01}}\right)^m$, $K_I = \frac{a_m}{b_m} = \frac{1}{m}\left(\frac{\beta_{s-1}}{\beta_s} - \frac{\alpha_{p-1}}{\alpha_p}\right)$, (6.4)



	n	K_n	m	K_m	η_1	K_{P1}	η_2	K_{P2}	η_{12}
V_1	3	0.03	3	10	--	--	--	--	--
V_2	0	--	3	10	2	0.01	2	0.01	0.01
V_3	3	0.03	0	--	2	10	2	10	0.9

$$V = k_n \frac{x^n}{K_n^n}\left[1+\left(\frac{\eta_1}{K_{p1}}+\frac{\eta_2}{K_{p2}}\right)x+\frac{\eta_{12}x^2}{K_{p1}K_{p2}}\right]\left(1+\frac{x}{K_n}\right)^{-n}\left(1+\frac{x}{K_{p1}}\right)^{-1}\left(1+\frac{x}{K_{p2}}\right)^{-1}$$

Fig. 6.1. $V_1=f(x)$, $V_2=f(x)$ and $V_3=f(x)$ experimental curves and their y(r,t) and y(r,x) transformation resultant $V_n=(a_n+b_n/x)^{-n}$ and $V_m=(a_m+bx)^{-m}$ curves (V_2 and V_3 darkened sites represent respective V_n and V_m functions). In graphs x is substituted by lgx

Fig. 6.1 represents illustrative examples of random binding of ligands under rapid equilibrium conditions. In the case of V_1 curve, the enzyme system possesses the sites assigned for essential activators and full inhibitors, while it lacks the site for the partial effect modifiers. As seen from Fig. 6.1, in the extremely small concentration site for ligand V_1 curve maximally coincides with the approximated V_n function. Similarly, in the extremely high concentration site for ligand there occurs a maximal coincidence of geometric shape of V_1 and approximated V_m functions. In the case of V_2 function, the enzyme system has the sites assigned for full inhibitors and partial effect modifiers, the number of sites for essential activators being n=0; for V_3 curve, the enzyme system has the sites for essential activators and partial effect modifier, while the number of sites for full inhibitors is m=0. In this case too there occur similar coincidences.

On the strength of these numerical examples a conclusion can be drawn that V_A, K_A, n, V_1, K_1 and m parameters describe accurately enough $V=f(x)$ function in the domain of low and high concentrations.

The physical essence of V_A and V_1 parameters is easily comprehensible. They represent maximal velocities for the imaginary $V_n=f(x)$ and $V_m=f(x)$ functions and give a definite idea of the degree of activation and inhibition specific for $V=f(x)$ function. To fancy the physical significance of K_A and K_1 let us explore an illustrative example.

Suppose, the enzyme system is under rapid equilibrium, then the coefficients of the general velocity equation will have the form:

$$\beta_0=1, \quad \beta_1=\left[\sum_{i=1}^{n}\frac{1}{K_{ni}}+\sum_{i=1}^{m}\frac{1}{K_{mi}}+\sum_{i=1}^{p}\frac{1}{K_{pi}}\right], \ldots\ldots, \quad \beta_{s-1}=\frac{\sum_{i=1}^{s}\gamma_i K_i}{\prod_{i=1}^{s}K_i}, \quad \beta_s=\frac{\gamma_s}{\prod_{i=1}^{s}K_i}.$$

$$\alpha_0=\frac{k_n\gamma_n}{\prod_{i=1}^{n}K_{ni}}, \quad \alpha_1=\frac{\sum_{i=1}^{p}\frac{k_i\gamma_i}{K_{pi}}}{\prod_{i=1}^{n}K_{ni}}, \ldots\ldots, \quad \alpha_{p-1}=\frac{\sum_{i=1}^{p}k_{(p-1)i}K_{pi}\gamma_i}{\left(\prod_{i=1}^{n}K_{ni}\right)\left(\prod_{i=1}^{p}K_{pi}\right)}, \quad \alpha_p=\frac{k_p\gamma_p}{\left(\prod_{i=1}^{n}K_{ni}\right)\left(\prod_{i=1}^{p}K_{pi}\right)}.$$

If the given coefficients are integrated in 6.1 and 6.4 equalities, we obtain:

$$\frac{1}{K_A}=\frac{1}{n}\left[\sum_{i=1}^{s}\frac{1}{K_i}-\frac{1}{k_n}\sum_{i=1}^{p}\frac{\gamma_i k_i}{K_{pi}}\right]=\frac{1}{n}\left[\sum_{i=1}^{n}\frac{1}{K_{ni}}+\sum_{i=1}^{m}\frac{1}{K_{mi}}+\sum_{i=1}^{p}\frac{1-\lambda_i'}{K_{pi}}\right]; \qquad \lambda_i'=\frac{\gamma_i k_i}{\gamma_n k_n} \qquad (6.5).$$

$$K_I = \frac{1}{m}\left[\sum_{j=1}^{s}\frac{\gamma_j}{\gamma_s}K_j - \frac{1}{\gamma_p k_p}\sum_{i=1}^{p}\gamma_i k_{(p-1)i}K_{pi}\right] = \frac{1}{m}\left[\sum_{i=1}^{m+n}\frac{\gamma_j}{\gamma_s}K_j + \sum_{i=1}^{p}\frac{\gamma_i}{\gamma_s}(1-\lambda_i'')K_{pi}\right];\ \lambda_i'' = \frac{\gamma_i k_{(p-1)i}}{\gamma_p k_p} \tag{6.6}$$

Let us name K_A as activation constant (I/K_A would be an apparent averaged affinity). If we inspect equation 6.5, we'll see that the specific and catalytic constants are involved in it and they to a certain extent display affinity and catalytic degree of all sites.

Thus, the presented kinetic parameters depend on the kinetic parameters of sites not only of essential activators, but also on those of all sites (n, m and p types) and characterize the overall activation process.

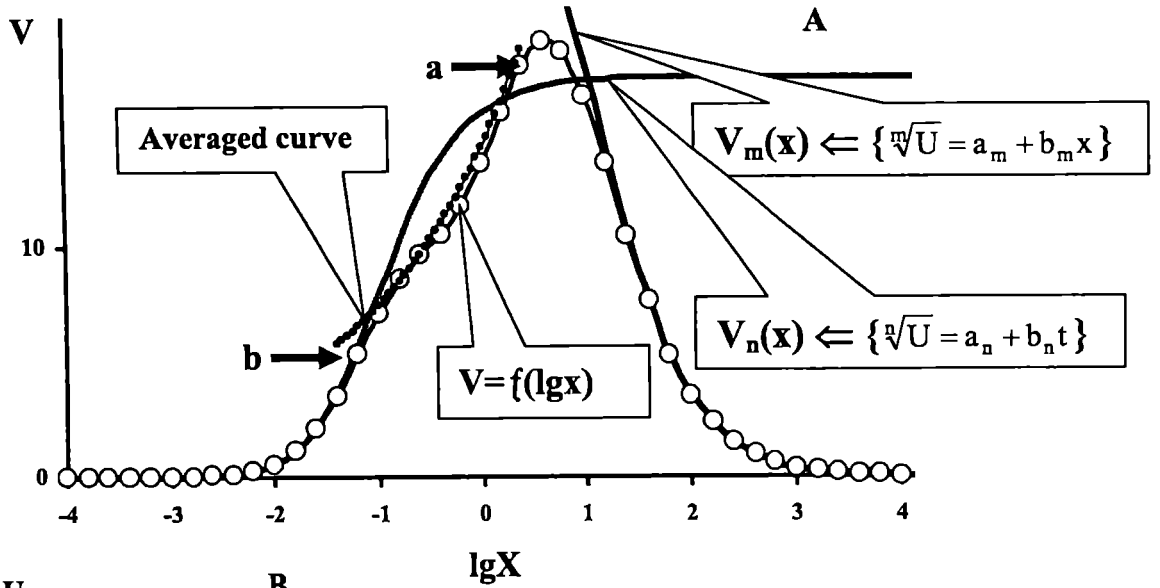
Equality 6.6 shows that the catalytic and specific constants also participate in K_I formation. It was named the inhibition constant (or imaginary averaged dissociation constant). As seen from equality 6.6, K_I depends not only upon the kinetic parameters of the sites for full inhibition, but also the kinetic parameter of all sites and specify the overall inhibition process.

The physical essence of kinetic parameters, as has been already pointed out, was determined under rapid equilibrium conditions. In the steady-state equilibrium determination of the physical essence of kinetic parameters is a difficult task to cope with. However, perhaps with a definite assumption, we can say that the physical essence of kinetic parameters in the steady-state too will be the same, but α_i and β_i coefficients will have other quantities.

Thus, in the site of extremely small concentrations the enzyme system is specified by the following parameters: n, I/K_A and V_A, while in the extremely large concentrations site by m, K_I and V_I.

Naturally, the enzyme systems differ from each other in this kinetic parameter that enables their relative specification.

As been stated earlier, in the middle concentration site for $V=f(x)$ function ($x_1 \le x \le x_2$), the curve's geometrical shape is mainly determined by p parameter (x_1 is determined by the first tangent of $V=f(x)$ function passing in the origin of coordinates). In this site we may have a stepwise activation or inhibition, when in the relative site several inflexion points are consecutively arranged. In the case if there is no need for a detailed characterization of this section we may restrict ourselves by imaginary reaction order (ω function). Fig. 6.2 presents geometrical shape of a theoretical kinetic curve for the multi-sited enzyme system in $V=f(\lg x)$ coordinate system. The stepwise activation section is distinctly seen in the plot. To characterize this section its transformation in InU/Int coordinate system is required.



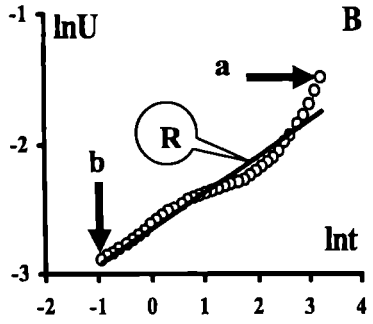


Fig. 6.2. V=f(x), an example of stepwise activation and its "averaged" curve. x is substituted by Igx in graphs.
R – a regression line drawn on the points of U=f(t) function transformed on the stepwise activation [ab] section.
$\{V=f(x), [a,b]\} \Rightarrow \ln U=f(\ln t) \Rightarrow \{\ln U = -\ln V_{st} + \omega_{st} \ln t\} \Rightarrow$
$\Rightarrow \{V = V_{st} x^{\omega_{st}}\}$

On the obtained points a regression line is drawn (Fig. 6.2 B) $\ln U=-\ln V_{st}+\omega_{st}$ Int. In V/x coordinate system the regression line assumes $V(x) = V_{st} x^{\omega_{st}}$ analytical shape. Fig. 6.2 shows that geometrical shape of $V(x)_{st} x_{\omega st}$ function maximally coincides that of V=f(Igx) function in the stepwise activation site. While in the extremely small and extremely large concentration site for ligand it maximally coincides with geometrical shape of V=f(Igx) function and of approximated V_n and V_m functions. Thus, in the case of stepwise activation or inhibition by the number of inflexion points P_{min} can be defined and accordingly, the given section will be characterized by the following parameters: P_{min} , V_{st} and ω_{st}. If, however, the stepwise activation or inhibition site is to be characterized in details, then in U=f(t) coordinate system the function is approximated by a rectilinear regression. The intersection points of the regression line on the axis of abscissa and ordinate ($1/K_{st}$; $1/V_{st}$) represent the parameters which may be specific for the present site. While the case of stepwise inhibition instead of U=f(t) transformation U=f(x) should be used.

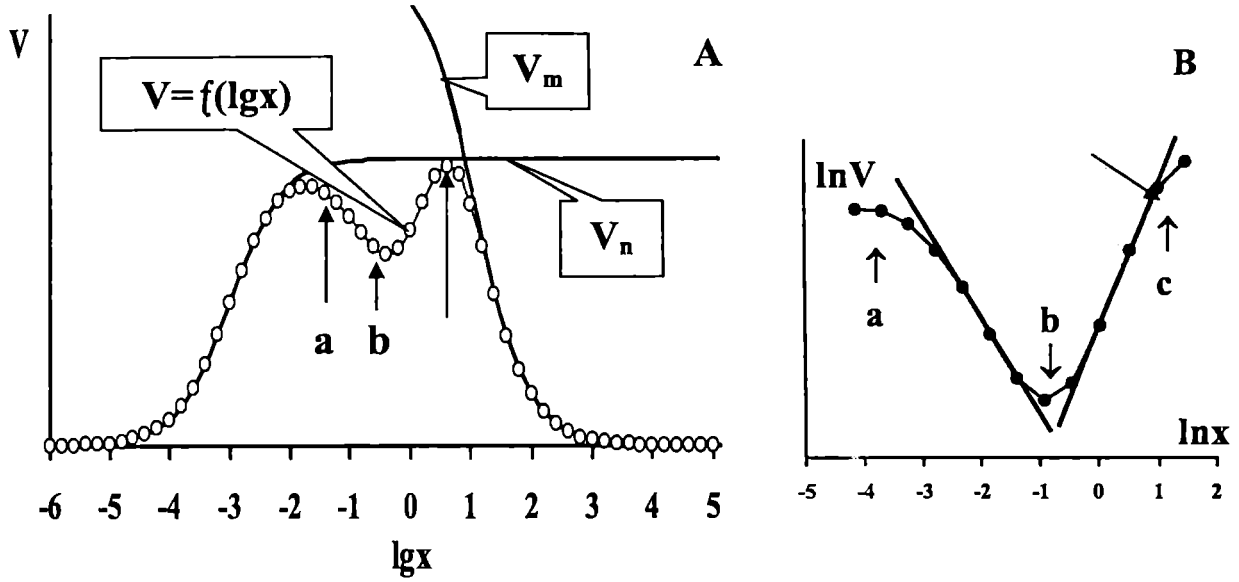


Fig. 6.3. **A** An example of V=f(x) function having turning points
$V_n(x) \Leftarrow \{\sqrt[n]{U} = a_n + b_n t\} V_m(x) \Leftarrow \{\sqrt[m]{U} = a_m + b_m x\}$

B Regression lines of InV=f(Inx) function drawn on [ab] and [bc] sections [ab]⇒ {V_{tp}=18.01, ω_{tp}=−0.126} and [bc]⇒ {V_{tp}=19.45, ω_{tp}=+0.225};
$\ln V = V_{tp} + \omega_{tp}(\ln x)$.
(for details see the text),

In the case, if in V=f(x) coordinate system several turning points are arranged in succession (Fig. 6.3), then analysis is made of the section existing between the turning points. Fig. 6.3 shows geometrical shape of theoretical kinetic curve for the multi-sited enzyme system in V/lgx coordinate system. Ligands bind with the enzyme system in a random way, without interaction. The curve shape allows for setting up the number of turning points P_{min}. For each section available between the turning points in InV_i=f(lnx) coordinate system a regression line (Fig. 6.3 B) $InV=\ln V_{tp}+\omega_{tp}\ln x$ is drawn. Then the entire section containing turning points would be characterized by P_{min} and for each site by the combination of V_{tp} and ω_{tp} parameters. Thus, it can be said that ω function permits to know as to with what acceleration the enzyme reaction proceeds. However, in order to characterize in more details the site with turning points it is primarily necessary to explore how many ligands are binding with the enzyme system in the site between extremum points – one or more than one. To this end, on the points

of the site in question in lnV/lnx coordinate system a regression line is drawn: $lnV=a+blnx$. If ($b=\omega_{tp}$) regression coefficient $b\leq1$, this means that one ligand is bound with the enzyme system, if $b>1$ then several ligands are bound with the enzyme system. In the former case, when $b\leq1$, the entire site, containing turning points, is considered. In this case, the enzyme system is linked by the ligands in succession, which alternatively would result in a positive or negative acceleration. Characterization of this site is possible in $U=f(t)$ coordinate system by means of rectilinear regression approximation of the parameter ($1/K_0=a/b$, $V_0=1/a$) obtained by the intersection of regression line of the axes of abscissa and ordinate. If $b>1$, then it is necessary that the origin of coordinates be transposed in the extremum point and the section between the extremum points be studied as an independent function. In the given site we may employ the methods of n and m determination.

The molecular mechanism for the multi-sited enzyme system may be structural in such a way that in $V=f(x)$ coordinate system may simultaneously be encountered the turning and inflexion points. In the given case to describe the kinetic curve of the relevant site it is better to turn to $InV=f(Inx)$ coordinate system and to draw a regression line $InV=V_o+\omega_o(Inx)$ in the site of turning and inflexion points. This function as a result of transformation in V/x coordinates, assumes the following form: $V = V_0x^{\omega_0}$ (Fig. 6.4). In the given case V_o and ω_o represent those kinetic parameters by which in the corresponding site the enzyme system may be described. Thus, for example, the regression line $InV=V_o+\omega_o(Inx)$ b coefficient (ω_o) furnishes information about the overall activation ($\omega_o>0$) or inhibition ($\omega_o<0$) of the enzyme system (Fig. 6.4).

Thus, we can finally fancy the kinetic parameters which enable to characterize the enzyme system in the site of extremely small, extremely large and medium concentrations:

I. For extremely small concentration site: n, K_A, V_A

II. For extremely large concentration site: m, K_1, ω_o

III. For medium concentration site: P_{min}, V_o, ω_o

IV (for a detailed characterization of medium concentration site):

P_{min}, V_{st}, ω_{st} ($\lambda\geq0$, stepwise activation or inhibition site)

P_{min}, V_{tp}, ω_{tp} ($v>1$, the site of turning points)

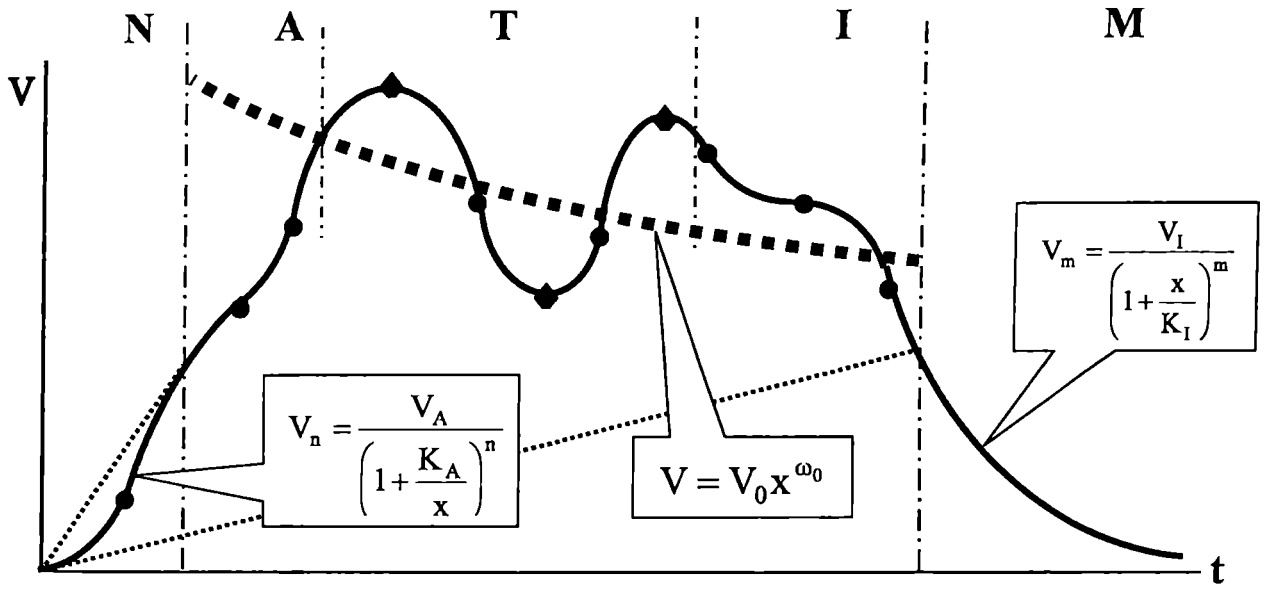


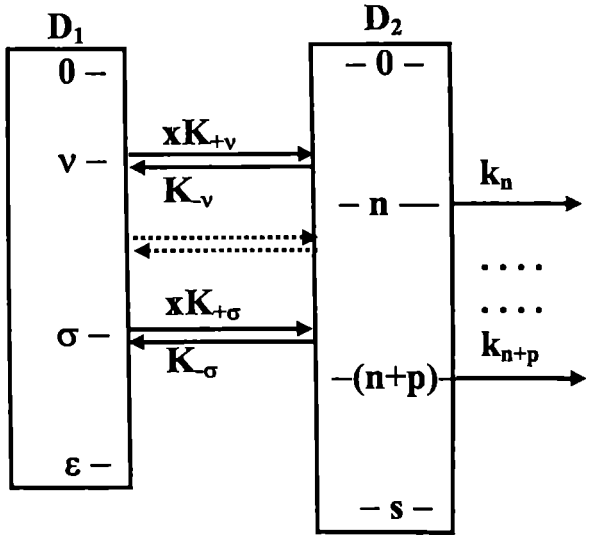
Fig. 6.4. V=f(x) function with sites of stepwise activation (A), inhibition (I) and turning points (T). A+T+1=P is the site of medium concentration, N of small and M of large concentrations

◆ – is the inflexion point; ● – is the turning point

Chapter7. CLASSIFICATION OF MODIFIERS FOR MULTI-SITED ENZYME SYSTEM

7.1. The multi-sited modifying enzyme system with one substrate site

For the multi-sited systems a relatively simple example is a single-substrate system with multiple sites of modifiers. Naturally, in this case the enzyme velocity equation V=f(x, y) (x being substrate and y modifier's concentrations) is not in need of power transformation and even in inverse values in respect to substrate gives a rectilinear dependence (U=f(t), U=1.V, t=1/x=const.). The enzyme system of this kind can be schematically expressed in the following way::



$\varepsilon = \nu + \lambda + \mu, \quad \sigma = \nu + \lambda,$
$s = n+p+m, \quad h = n + p$

$D_1 = \sum_{i=0}^{\varepsilon} \beta_i y^i \qquad D_2 = \sum_{i=0}^{s} \alpha_i y^i$

$N_1 = \sum_{i=\nu}^{\sigma} K_{+i} \beta_i' y^i$

$N_2' = \sum_{i=\nu}^{\sigma} K_{-i} \alpha_i' y^i \qquad N_2'' = \sum_{i=n}^{n+p} k_i \alpha_i'' y^i$

x – substrate concentration
y – modifier's concentration

where K_n, ..., K_{n+p} are catalytic, while K_{+i} and K_{-i} are respectively substrate dissociation constants, while ν, σ, ε, n, p, s are the numbers of ligand binding sites. Suppose, there is no interaction between the substrate and modifier sites and the substrate imaginary dissociation constant depends upon the number of only bound modifier. At the same time the interaction between the modifiers' sites is permissible (γ – interaction coefficient). Then under the rapid equilibrium we'll have:

$$D_1 = \sum_{i=0}^{\varepsilon} \beta_i y^i = 1 + \sum_{i=1}^{\varepsilon} \frac{y}{K_{yi}} + \frac{y^2}{\prod_i^{\varepsilon} K_{yi}} \sum_{i=1}^{\varepsilon-1} \sum_{j=i+1}^{\varepsilon} \frac{K_{yi} K_{yj}}{\gamma_{ij}} + \cdots + \frac{y^{\varepsilon-1}}{\prod_i^{\varepsilon} K_{yi}} \sum_{i=1}^{\varepsilon} \frac{K_{yi}}{\gamma_i} + \frac{y^{\varepsilon}}{\left(\prod_i^{\varepsilon} K_{yi}\right)\gamma_{\varepsilon}} \qquad (7.1)$$

$$D_2=\sum_{i=0}^{s}\alpha_i y^i=1+\sum_{i=1}^{s}\frac{y}{K_{yi}^x}+\cdots+\frac{y^{s-1}}{\prod_i^s K_{yi}^x}\sum_{i=1}^{s}\frac{K_{yi}^x}{\gamma_i^x}+\frac{y^s}{\left(\prod_i^s K_{yi}^x\right)\gamma_s^x} \qquad (7.2)$$

Whereas without the interaction between the substrate and modifiers' sites we'll have: $\alpha=\alpha'=\alpha''$ and $\beta=\beta'$ as a result of which we'll have:

$$N_1=\sum_{i=v}^{\sigma}K_{+i}\beta_i y^i;\qquad N_2'=\sum_{i=v}^{\sigma}K_{-i}\alpha_i y^i;\qquad N_2''=\sum_{i=n}^{n+p}k_i\alpha_i y^i \qquad (7.3)$$

Under rapid equilibrium, velocity of the enzyme system of this lay-out will be expressed:

$$V=\frac{N_2''x}{D_1K_x+D_2x}\Rightarrow U=\frac{D_2}{N_2''}+\frac{D_1}{N_2''}\frac{K_x}{x};\quad \text{where}\quad K_x=\frac{K_{-i}}{K_{+i}}\quad \text{and}\quad U=1/V. \qquad (7.4)$$

Suppose, modifiers (y) compared to substrate (x), represent themselves molecules of smaller size and their binding with the enzyme system is realized more rapidly than of substrate, then in the steady-state the velocity equation can be solved using Cha method, in terms of which binding of modifiers is viewed under rapid equilibrium and of substrate, in the steady-state. In the given case, the rate equation in the reversed coordinate system will have the form:

$$U=\frac{D_2}{N_2''}+\frac{D_1(N_2'+N_2'')}{N_1N_2''}\left(\frac{1}{x}\right) \qquad (7.5)$$

In the end, the rate equation in the steady-state equilibrium in a complete form will look like:

$$U=\frac{\left(\sum_{i=0}^{\varepsilon}\beta_i y^i\right)\left[y^v\sum_{i=0}^{\lambda}K_{-(i+v)}\alpha'_{(i+v)}y^i+y^n\sum_{i=0}^{p}k_{(i+n)}\alpha''_{(i+n)}y^i\right]+xy^v\left(\sum_{i=0}^{s}\alpha_i y^i\right)\left[\sum_{i=0}^{\lambda}K_{(i+v)}\beta'_{(i+v)}y^i\right]}{xy^{v+n}\left(\sum_{i=0}^{\lambda}K_{+(i+v)}\beta'_{(i+v)}y^i\right)\left[\sum_{i=0}^{p}k_{(i+n)}\alpha''_{(i+n)}y^i\right]} \qquad (7.6)$$

On the strength of interrelationship of v and n parameters, by means of formula 7.6, the least power of denominator can be defined. If $v\le n$, the least power of denominator would be n. In the case when $n<v$, both the nominator and denominator of the fraction are cancelled by the least power having term (y^n), as a result, in the double reversed coordinate system, in the rate equation (7.6) the least power term of denominator would be y^v. The method for determination of the number of essential activators will allow to set up just this degree parameter, that is not the case in real situation. In reality, a substrate is linked with an enzyme after being bound with v modifiers and for further catalysis the necessary condition is release from the enzyme system of (v-n) modifiers. Therefore, v must be viewed as the number of a pseudo essential activator. When $v<n$, fraction cancels by the least power y^v term and y^n remains in denominator, the method defining the number of essential activators would enable to determine the real number of essential activators. In the present case, after occupation of v site, the enzyme system is linked with a substrate and thence for

a catalytically active form to be obtained the necessary condition would be binding again of (n-v) single modifier (in the case when n>v).

Similar situation holds for full inhibitors. As a result of division nominator and denominator in 7.6 equation by $y^{s+\varepsilon}$ term power term $1/ y^{\mu+m}$ is obtained in the denominator. Thus, if $m \geq \mu$ – the method of defining the number of full inhibitors (see Chapter 4) enables to determine the real number of full inhibitors (m). In the opposite case ($m<\mu$) the pseudonumber (μ) is determined.

Under the steady-state equilibrium $U=f(1/x)$ function is rectilinear (7.5), while value of its slope (SI) and of the point of intersection of ordinate (Int) and abscissa (t_0) depends upon modifiers' concentration (y). Determination of the enzyme system molecular mechanism and the type of a modifier requires a detailed analysis of these dependencies and determination of their limited values. These dependencies will give the different values (for more details see appendix 7.1).

In terms of the method for the analysis of geometrical shape of curves it is necessary to define the sign of these values and the limited quantities of their derivatives, of turning points and the first derivative. In the tables given below limited values and their dependency upon v, n, σ, h, μ and m parameters are shown.

With infinitely small value of argument, slope of the function and its derivative, proceeding from the interdependence of n and v parameters, assume various values. While at infinitely large values of argument, slope and the limit of its derivative depend upon μ, σ and h parameters. With infinitely small value of argument, the values of intersection on the abscissa (t_0) and its derivative, as is the case with slope of the function, depend upon n and v parameters. While with infinitely large values of argument, ordinate intersection and its derivative, depending upon μ, σ and h parameters, take on different value.

It should be noted that based on curve shape analysis of $Int=f(y)$, $Sl=f(y)$ and $t_0=f(y)$, with fixed y=const, it is possible to determine the position of intersection points of $U=f(t)=(Int)+(Sl)t$ straight lines.

Let us consider the examples of the enzyme system arranged in this way. This will enable to establish the interrelationship on the one hand between the molecular mechanism and on the other hand, between geometrical shape of $Int=f(y)$, $Sl=f(y)$ and $t_0=f(y)$ functions and the position of intersection points of $U=f(t)$ straight line.

Further on, finite positive number ($0<w<\infty$) will be designated by w.

Table 7.1

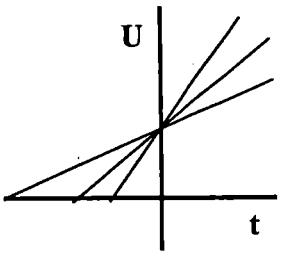
The shape of Int= f(y), SI= f (y) and t_o= f (y) function (w – positive finite number)

Shape of Int f(y) function			
$\lim_{y\to 0}(\mathrm{Int}) =$	$[n\neq 0] \Rightarrow +\infty$ $[n=0] \Rightarrow +w$	$\lim_{y\to\infty}(\mathrm{Int}) =$	$[m\neq 0] \Rightarrow +\infty$ $[m=0] \Rightarrow +w$
$\lim_{y\to 0}(\mathrm{Int})'_y =$	$[n\neq 0] \Rightarrow -\infty$ $[n=0] \Rightarrow \pm w$	$\lim_{y\to\infty}(\mathrm{Int})'_y =$	$[m\neq 0] \Rightarrow +\infty$ $[m=0] \Rightarrow \pm w$
Shape of SI=f(y) function			
$\lim_{y\to 0}(\mathrm{Sl}) =$	$[\nu<n] \Rightarrow +\infty$ $[\nu>n] \Rightarrow +\infty$ $[\nu=n\neq 0] \Rightarrow +\infty$ $[\nu=n=0] \Rightarrow +w$	$\lim_{y\to\infty}(\mathrm{Sl}) =$	$[\mu\neq 0]$ or $[\mu=0, \sigma>h] \Rightarrow +\infty$ $[\mu=0, \sigma\leq h] \Rightarrow +w$
$\lim_{y\to 0}(\mathrm{Sl})'_y =$	$[\nu<n] \Rightarrow -\infty$ $[\nu>n] \Rightarrow -\infty$ $[\nu=n\neq 0] \Rightarrow -\infty$ $[\nu=n=0] \Rightarrow \pm w$	$\lim_{y\to\infty}(\mathrm{Sl})'_y =$	$[\mu\neq 0]$ or $[\mu=0, \sigma>h] \Rightarrow +\infty$ $[\mu=0, \sigma<h] \Rightarrow -0$ $[\mu=0, \sigma=h] \Rightarrow \pm 0$
Shape of t_o=f(y) function			
$\lim_{y\to 0}(t_0) =$	$[\nu<n] \Rightarrow -w$ $[\nu=n] \Rightarrow -w$ $[\nu>n] \Rightarrow -0$	$\lim_{y\to\infty}(t_0) =$	$[\nu<n] \Rightarrow -w \lim_{y\to\infty} y^{m-\mu}$ $[\nu=n] \Rightarrow -w \lim_{y\to\infty} y^{s-\varepsilon}$ $[\nu>n] \Rightarrow -w \lim_{y\to\infty} y^{s-\varepsilon}$
$\lim_{y\to 0}(t_0)'_y =$	$[\nu<n] \Rightarrow \pm w$ $[\nu=n] \Rightarrow \pm w$ $[\nu>n] \Rightarrow -w$, $(\nu-n=1)$ $[\nu>n] \Rightarrow -0$, $(\nu-n>1)$	$\lim_{y\to\infty}(t_0)'_y =$	$[\nu<n] \Rightarrow w[\mu-m]\lim_{y\to\infty} y^{m-\mu-1}$; if $(m=\mu) \Rightarrow \pm 0$ $[\nu=n] \Rightarrow \pm w \cdot \lim_{y\to\infty} y^{m-\mu-1}$ $[\nu>n] \Rightarrow w[\varepsilon-s]\lim_{y\to\infty} y^{s-\varepsilon-1}$; if $(s=\varepsilon) \Rightarrow \pm 0$

A

Pure specific modifier

$(Sl)'_y < 0$ – **activator** ; $(Sl)'_y > 0$ – **inhibitor.**

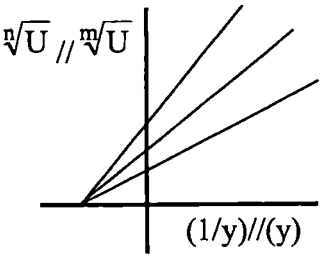


Necessary condition

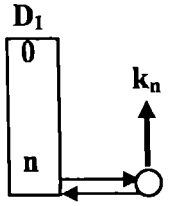
$$Int = \frac{1}{k};\ (Int)'_y = 0;$$

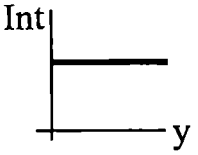
$$k_i = k,\ 0 \le n \le h$$

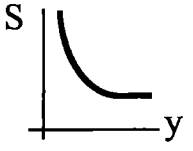
$$t_0 = -\frac{1}{k \cdot (Sl)};\ (t_0)'_y = \frac{(Sl)'_y}{k(Sl)^2}$$

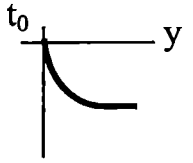


A1







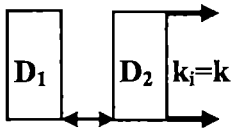


$$Sl = \frac{(K_{-n} + k_n)}{k_n K_{+n} \beta_n}\left[\frac{\beta_0}{y^n} + \frac{\beta_1}{y^{n-1}} + \cdots + \beta_n\right];\ (Sl)'_y \le 0;\ (Sl)''_y \ge 0$$

$$t_0 = -\frac{K_{+n}\beta_n}{(K_{-n} + k_n)}\frac{y^n}{(\beta_0 + \cdots + \beta_n y^n)};\quad (t_0)'_y \le 0;\ (t_0)''_y \ge 0$$

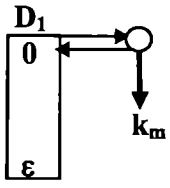
$$U(y) = \frac{K_{xn}(\beta_0 + \beta_1 y + \cdots + \beta_n y^n) + \beta_n x y^n}{k_n \beta_n x y^n};\ K_{xn} = \frac{K_{-n} + k_n}{K_{+n}}$$

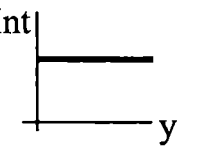
A1'

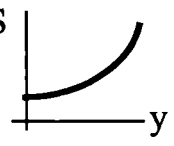


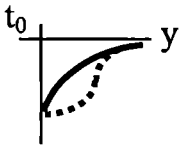
A1 and A1′ enzyme systems have the curves Int= f(y), Sl= f(y) and t_0= f(y) of identical shape.

A2









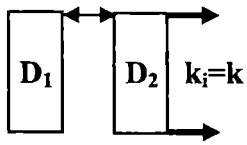
$$Sl = \frac{(K_{-0} + k_0)}{k_0 K_{+0}}\left[\beta_0 + \cdots + \beta_\varepsilon y^\varepsilon\right];\ (Sl)'_y \ge 0;\ (Sl)'_y \ge 0$$

$$\lim_{y\to 0}(Sl)'_y = +w;\ \lim_{y\to\infty}(Sl)'_y = +\infty;$$

$$t_0 = -\frac{K_{+0}}{(K_{-0} + k_0)}\frac{1}{(\beta_0 + \cdots + \beta_n y^\varepsilon)};\ (t_0)'_y \ge 0;$$

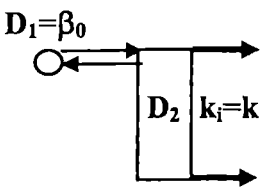
$$U(t) = \frac{(K_{-0} + k_0) + (\beta_m + \beta_{m-1} t + \cdots + \beta_0 t^m) + K_{-0}\beta_0 x t^m}{k_0 K_{-0} \beta_0 x t^m}$$

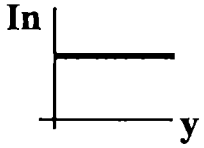
A2'

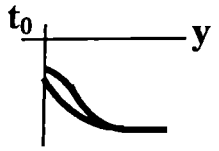


A2 and A2′ enzyme systems have the curves Int= f(y), Sl= f(y) and t_0= f(y) of identical shape.

A3

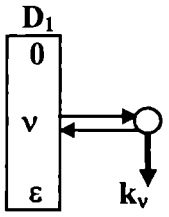


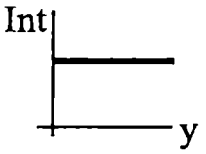


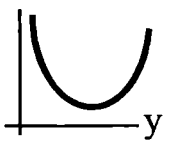


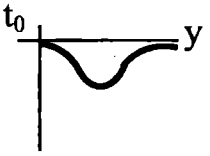
$$Sl=\frac{1}{K_{+0}}+\frac{K_{-0}\alpha_0}{kK_{+0}\left(\alpha_0+\alpha_1 y+\cdots+\alpha_h y^h\right)};\quad (Sl)'_y<0;\quad t_0=-\frac{1}{k(Sl)};\quad (t_0)'_y=\frac{(Sl)'_y}{k(Sl)^2}<0$$

A4







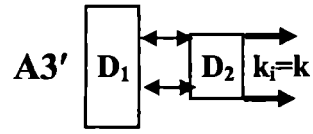


$$Sl=\frac{(K_{-\nu}+k_\nu)}{k_\nu K_{+\nu}\beta_\nu}\left[\frac{\beta_0}{y^\nu}+\cdots+\beta_\varepsilon y^{\varepsilon-\nu}\right];\quad (Sl)''_y\geq 0$$

$$t_0=-\frac{K_{+\nu}\beta_\nu}{(K_{-\nu}+k_\nu)}\frac{y^\nu}{\left(\beta_0+\cdots+\beta_\varepsilon y^\varepsilon\right)};$$

$$\lim_{y\to 0}(t_0)'_y=-0;\quad \lim_{y\to\infty}(t_0)'_y=+0;$$

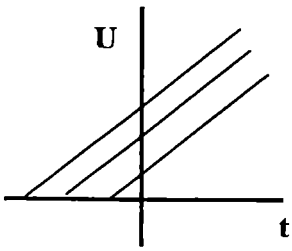
$$U(y)=\frac{(K_{-\nu}+k_\nu)\alpha_\nu\beta_0+\beta_1+\cdots+\alpha_\nu\beta_\nu^2K_{+\nu}xy^\nu+\cdots+\beta_\varepsilon y^\varepsilon}{k_\nu K_{+\nu}\alpha_\nu\beta_\nu xy^\nu}$$



A3 and A3′ enzyme systems have the curves Int= f(y), Sl= f(y) snd t_0= f(y) of identical shape.

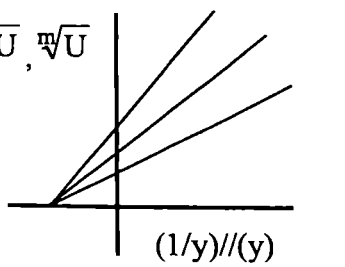
B

Pure catalytic modifier
$(Int)'_y<0$ – **activator;** $(Int)'_y>0$ – **inhibitor**

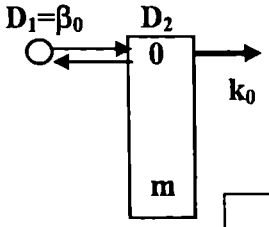


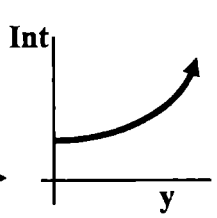
Sufficient condition

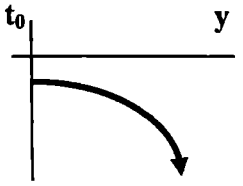
$$(Sl)'_y=0;\quad Sl=\frac{K_{-h}+k_p}{K_{+h}k_p}$$



B1



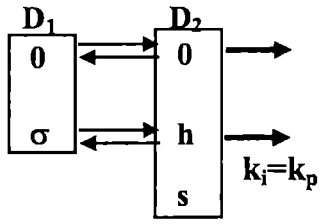




$$Int = \frac{1}{k_0\alpha_0}\left(\alpha_0 + \cdots + \alpha_m y^m\right);$$

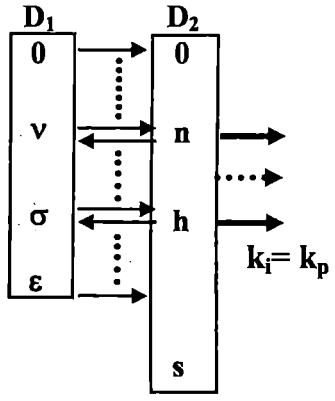
$$t_0 = -\frac{K_{+0\beta_0}}{(K_{-0} + k_0)\alpha_0}\left(\alpha_0 + \cdots + \alpha_m y^m\right)$$

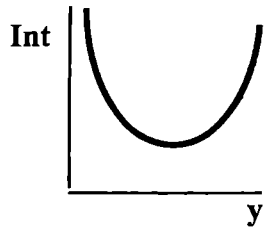
B1′

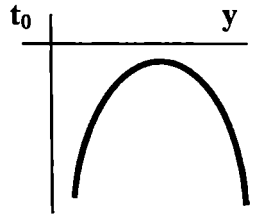


B1 and B1′ enzyme systems have the curves Int= f(y), Sl= f(y) and t_0= f(y) of identical shape

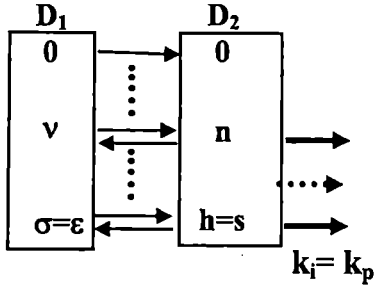
B2

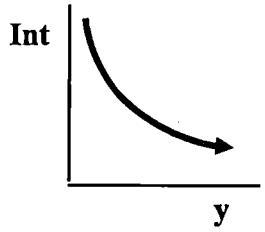


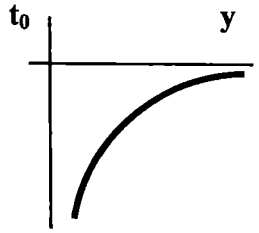




B3



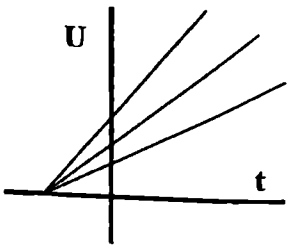


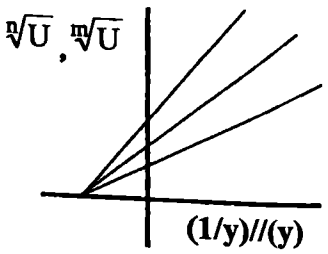


C

Mixed modifiers with constant affinity
$(Sl)'_y < 0$ – activator; $(Sl)'_y > 0$ – inhibitor

Necessary condition: $(t_0)'_y = 0$; $t_0 = -\dfrac{K_{+x}}{k + K_{-x}}$





Sufficient condition:

$D_1=D_2=D_0$, $\alpha_i=\beta_1$.

$N_1 = K_{+x} \cdot N_0$; $N'_2 = K_{-x} \cdot N_0$; $N''_2 = k \cdot N_0$

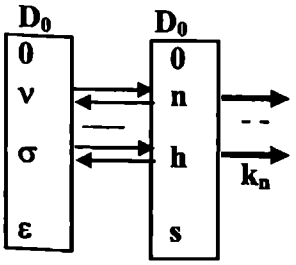
$\varepsilon=s$, $\nu=n$, $\sigma=h$

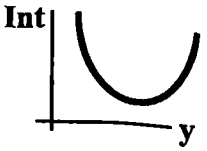
$$D_0 = \sum_{i=1}^{s} \alpha_i y^i; \quad N_0 = \sum_{i=n}^{h} \alpha_i y^i; \quad U = f(y) \cdot \varphi(x);$$

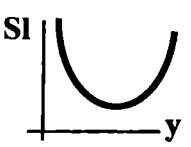
$$U(x,y) = \left[\frac{\alpha_0 + \alpha_1 y + \cdots + \alpha_s y^s}{k y^n \left(\alpha_0 + \alpha_1 y + \cdots + \alpha_p y^p\right)}\right]\left(1 + \frac{k + K_{-x}}{x K_{+x}}\right)$$

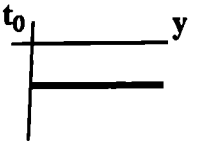
$$U(x,t) = \left[\frac{\alpha_0 t^s + \cdots + \alpha_{s-1} t + \alpha_s}{k t^m \left(\alpha_0 t^p + \cdots + \alpha_{s-1} t + \alpha_s\right)}\right]\left(1 + \frac{k + K_{-x}}{x K_{+x}}\right)$$

C1





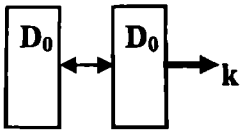




$$Int = \frac{\left(\alpha_0 + \alpha_1 y + \cdots + \alpha_\varepsilon y^\varepsilon\right)}{k_n \left(\alpha_n + \cdots + \alpha_h y^{h-n}\right) y^n}; \quad (Int)''_y > 0$$

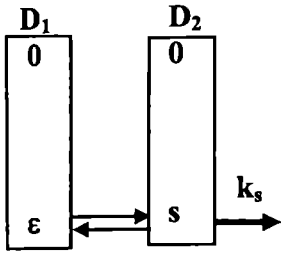
$$Sl = \frac{\left(\alpha_0 + \alpha_1 y + \cdots + \alpha_\varepsilon y^\varepsilon\right)\left(K_{-s} + k_n\right)}{k_n \left(\alpha_n + \alpha_{n+1} y + \cdots + \alpha_h y^{h-n}\right) y^n}; \quad (Sl)''_y > 0.$$

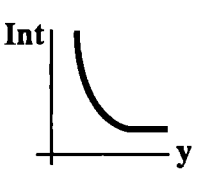
C1′

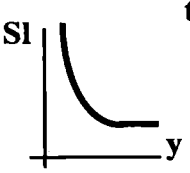


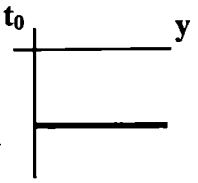
C1 and C1′ enzyme systems have the curves Int= f(y), Sl= f(y) and t_0= f(y) of identical shape

C2







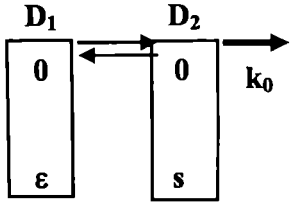


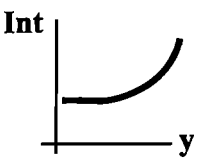
$$\mathrm{Int}=\frac{\left(\alpha_0+\alpha_1 y+\cdots+\alpha_\varepsilon y^{\varepsilon}\right)}{k_s\alpha_s y s};\quad \mathrm{Sl}=\frac{\left(\alpha_0+\alpha_1 y+\cdots+\alpha_\varepsilon y^{\varepsilon}\right)\left(K_{-s}+k_p\right)}{k_{+s}\alpha_s y^{s}};$$

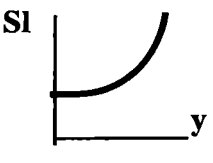
$$(\mathrm{Int})'_y\le 0\ ;\ (\mathrm{Int})'_y\ge 0;\qquad (\mathrm{Sl})'_y\le 0;\quad (\mathrm{Sl})'_y\ge 0;$$

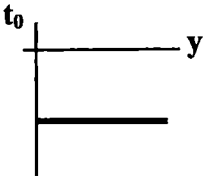
$$U(y)=\frac{\left(\left[k_s+K_{-s}\right]+K_{+s}x\right)\left(\alpha_0+\alpha_1 y+\cdots+\alpha_\varepsilon y^{\varepsilon}\right)}{k_s K_{+s}\left(\alpha_n+\cdots+\alpha_h y^{p}\right)xy^{n}}.$$

C3









$$\mathrm{Int}=\frac{\left(\alpha_0+\alpha_1 y+\cdots+\alpha_\varepsilon y^{\varepsilon}\right)}{k_0\alpha_0};\qquad \mathrm{Sl}=\frac{\left(\alpha_0+\alpha_1 y+\cdots+\alpha_\varepsilon y^{\varepsilon}\right)\left(K_{-s}+k_p\right)}{k_0\alpha_0 K_{+0}};$$

$$(\mathrm{Int})'_y\ge 0;\ (\mathrm{Int})'_y\le 0;\qquad (\mathrm{Sl})'_y\ge 0;\ (\mathrm{SL})'_y\le 0$$

$$U(y)=\frac{\left[\left(k_0+K_{-s}\right)+K_{+s}x\right]}{K_{+s}k_0 x}\cdot\frac{\alpha_\varepsilon+\cdots+\alpha_0 t^{\varepsilon}}{t^{m}\left(\alpha_h+\cdots+\alpha_n t^{h-n}\right)};$$

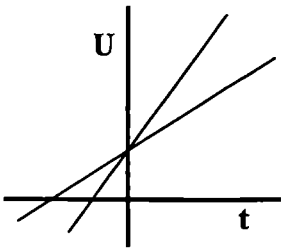
D

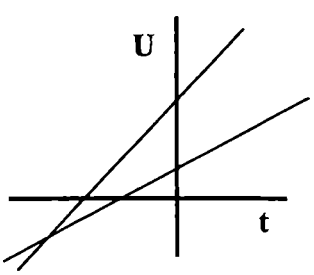
Mixed action modifiers

1. Activator with decreasing affinity, $(t_0)'_y\ge 0;\ (\mathrm{Sl})'_y<0.\Rightarrow$ DA
2. Inhibitor with decreasing affinity, $(t_0)'_y\ge 0;\ (\mathrm{Sl})'_y>0.\Rightarrow$ DA
3. Activator with increasing affinity, $(t_0)'_y\le 0;\ (\mathrm{Sl})'_y<0.\Rightarrow$ IA
4. Inhibitor with increasing affinity, $(t_0)'_y\le 0;\ (\mathrm{Sl})'_y>0.\Rightarrow$ IA

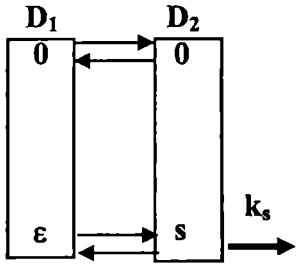
Necessary condition:

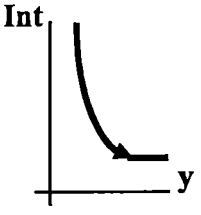
$\mathrm{sign}(\mathrm{Int})'_y=\mathrm{sign}(\mathrm{Sl})'_y;\ \varepsilon=s$

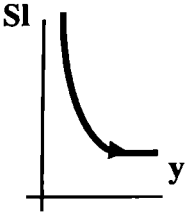


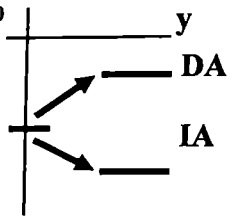


D1









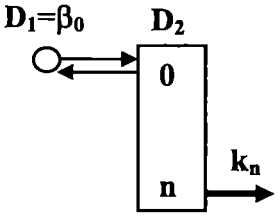
$$\mathrm{Int}=\frac{1}{k_s\alpha_s}\left(\frac{\alpha_0}{y^s}+\cdots+\alpha_s y^s\right);\ \lim_{y\to 0}(\mathrm{Int})=+\infty;\ \lim_{y\to\infty}(\mathrm{Int})=\frac{1}{k_s};\ (\mathrm{Int})'_y\le 0;$$

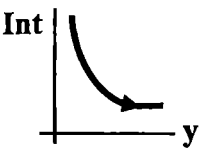
$$\mathrm{Sl}=\frac{\left(\beta_0+\cdots+\beta_\varepsilon y^\varepsilon\right)\left(K_{-0}\alpha_0+\cdots+\left[K_{-s}+k_s\right]\alpha_s y^s\right)}{k_s\alpha_s y^s\left(K_{+0}\beta_0+\cdots+K_{+\varepsilon}\beta_\varepsilon y^\varepsilon\right)};\ \lim_{y\to 0}(\mathrm{Sl})=+\infty;\ \lim_{y\to\infty}(\mathrm{Sl})=+w;\ (\mathrm{Sl})'_y\le 0;$$

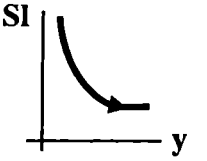
$$t_0=-\frac{\left(\alpha_0+\cdots+\alpha_s y^s\right)\left(K_{+0}\beta_0+\cdots+K_{+\varepsilon}\beta_\varepsilon y^\varepsilon\right)}{\left(\beta_0+\cdots+\beta_\varepsilon y^\varepsilon\right)\left(K_{-0}\alpha_0+\cdots+\left[K_{-s}+k_s\right]\alpha_s y^s\right)};\ \lim_{y\to 0}(t_0)=-\frac{K_{+0}}{K_{-0}};\ \lim_{y\to\infty}(t_0)=\frac{-K_{+\varepsilon}}{k_s+K_{-s}};$$

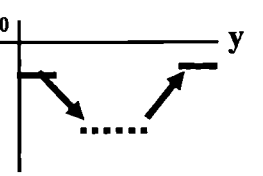
$$\mathrm{sign}(t_0)'_y\le 0\Rightarrow \mathrm{DA};\ \mathrm{sign}(t_0)'_y\ge 0\Rightarrow \mathrm{IA}.$$

D2





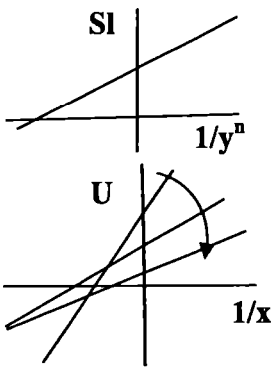


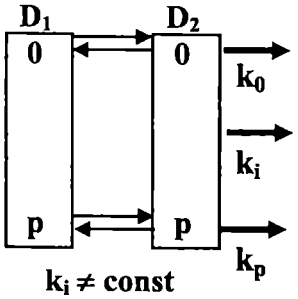


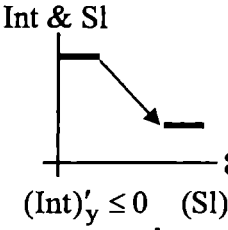
$$\mathrm{Int}=\frac{\alpha_0+\cdots+\alpha_n y^n}{k_n\alpha_n y^n};\quad (\mathrm{Int})'_y\le 0;\quad (\mathrm{Int})'_y\ge 0$$

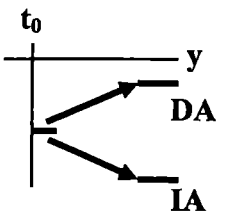
$$\mathrm{Sl}=\frac{1}{k_n K_{+0}\alpha_n}\left(\frac{K_{-0}\alpha_0}{y^n}+k_n\alpha_n\right);\qquad (\mathrm{Sl})'_y\le 0;\quad (\mathrm{Sl})'_y\ge 0$$

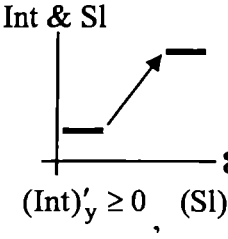
$$t_0=-\frac{\left(\alpha_0+\cdots+\alpha_n y^n\right)K_{+0}\beta_0}{\beta_0\left[K_{-0}\alpha_0+k_n\alpha_n y^n\right]};\ \lim_{y\to 0}(t_0)'_y<0;\ \lim_{y\to\infty}(t_0)'_y>0$$









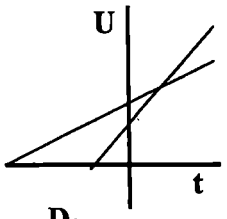


D3a

D3b

E

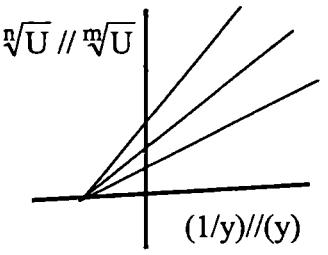
Modifier with a dual effect
(with concentration-dependent activation and inhibition sites)

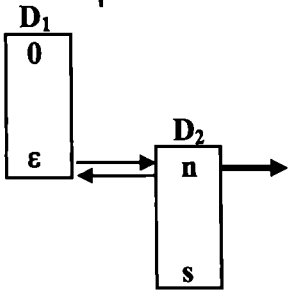


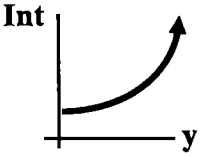
Necessary condition

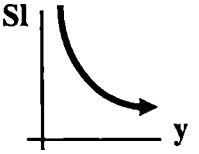
$\text{sign}(\text{Int})'_y = -\text{sign}(\text{Sl})'_y$;

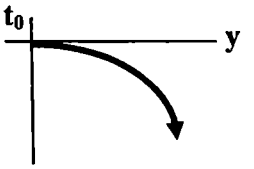
$\text{sign}(\text{Sl})'_y = \text{sign}(t_0)'_y$.







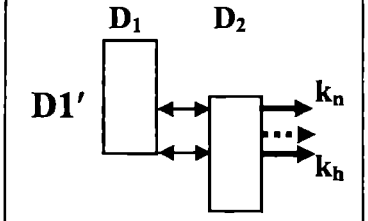




$$\text{Int} = \frac{1}{k_\varepsilon \alpha_\varepsilon}\left(\alpha_\varepsilon + \cdots + \alpha_s y^{s-\varepsilon}\right);\ (\text{Int})'_y \geq 0;\ (\text{Int})''_y \geq 0.$$

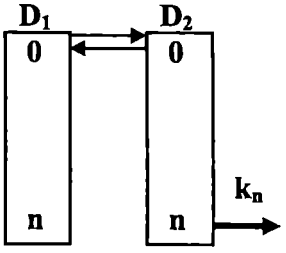
$$\text{Sl} = \frac{(K_{-\varepsilon} + k_\varepsilon)}{K_{+\varepsilon} k_\varepsilon \beta_\varepsilon}\left[\frac{\beta_0}{y^\varepsilon} + \cdots + \frac{\beta_{\varepsilon-1}}{y} + \beta_\varepsilon\right];\ (\text{Sl})'_y \leq 0;\ (\text{Sl})''_y \geq 0.$$

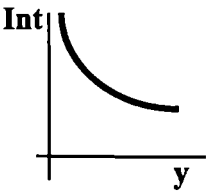
$$t_0 = \frac{K_{+\varepsilon}\beta_\varepsilon\left(\alpha_\varepsilon + \cdots + \alpha_s y^{s-\varepsilon}\right)}{\alpha_\varepsilon\left(K_{-\varepsilon} + k_\varepsilon\right)\left(\beta_0 + \cdots + \beta_\varepsilon y^\varepsilon\right)};\ (t_0)'_y \leq 0;\ (t_0)''_y \leq 0.$$

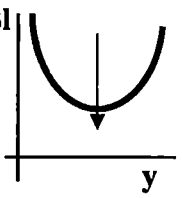


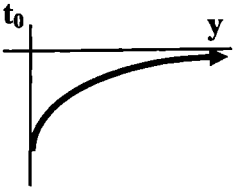
D1 and D1 enzyme systems have the curves Int= f(y), Sl= f(y) and t_0= f(y) of identical shape.

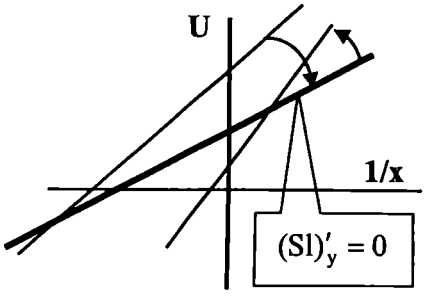
E1











In II quadrant (t*<0, U*>0) there are no intersection points

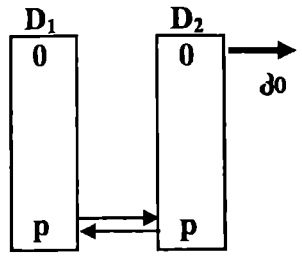
$$\text{Int} = \frac{1}{k_n \alpha_n}\left[\frac{\alpha_0}{y^n} + \cdots + \alpha_n\right];$$

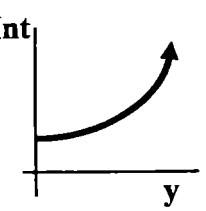
$$\text{Sl} = \frac{\left(\beta_0 + \cdots + \beta_n y^n\right)\left(K_{-0}\alpha_0 + k_n \alpha_n\right)}{K_{+0} k_n \alpha_n \beta_0};$$

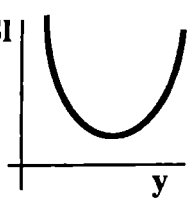
$$t_0 = -\frac{\left(\alpha_0 + \cdots + \alpha_n y^n\right)K_{+0}\beta_0}{\left(K_{-0}\alpha_0 + k_n \alpha_n y^n\right)\left(\beta_0 + \cdots + \beta_n y^n\right)};$$

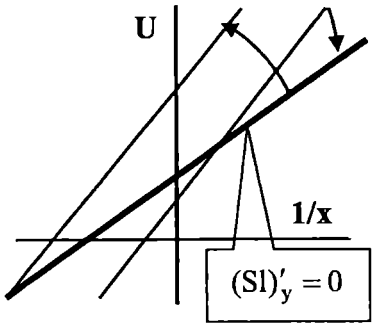
$$\text{sign}(t^*) = -\frac{\text{sign}(\text{Int})'_y}{\text{sign}(\text{Sl})'_y};\ \text{sign}(U^*) = \frac{\text{sign}(t_0)'_y}{\text{sign}(\text{Sl})'_y}$$

E2









In II quadrant (t*<0, U*>0) there are no intersection points

$$Int = \frac{1}{k_0\alpha_0}\left[\alpha_0 + \cdots + \alpha_n y^p\right];$$

$$Sl = \frac{\left(\beta_0 + \cdots + \beta_p y^p\right)\left(K_{-p}\alpha_p y^p + k_0\alpha_0\right)}{K_{+p}k_0\alpha_0\beta_p y^p};$$

$$t_0 = -\frac{\left(\alpha_0 + \cdots + \alpha_p y^p\right)K_{+p}\beta_p y^p}{\left(K_{-p}\alpha_p y^p + k_0\alpha_0\right)\left(\beta_0 + \cdots + \beta_p y^p\right)}$$

$$sign(t^*) = -\frac{sign(Int)'_y}{sign(Sl)'_y};\quad sign(U^*) = \frac{sign(t_0)'_y}{sign(Sl)'_y}.$$

Thus, from the examples examined above it is clear that differing from each other are five diverse classes of modifiers: 1) Pure specific modifier; 2) Pure catalytic one; 3) Mixed type with constant affinity; 4) Mixed type with variable affinity; 5) Modifier with dual effect. Each type is characterized by distinct shapes for Int= f(y), SI= f (y) and t_o= f (y) functions that enables plotting of graphs in U= f(t) coordinate system characteristic of each type of modifier and delineate them from each other. Thus, for instance, for pure specific modifiers it is necessary that $(Int)'_y = 0$ condition be fulfilled, on the basis of which in U= f(t) coordinate system at different concentrations of a modifier the curves intersect each other on the ordinate axis. In the case of a pure catalytic modifier condition $(Sl)'_y = 0$ is fulfilled, giving thus in U= f(t) coordinate system the parallel to each other lines. With a constant affinity mixed modifier, $(t_0)'_y = 0$ (t_o), as a result, the graphs plotted in U= f(t) coordinate system, in the presence of varying concentration of the modifier, intersect on the abscissa. In the case of mixed modifier with variable affinity the condition $sign(Int)'_y = sign(Sl)'_y$ and s=ε is necessarily fulfilled, resulting in the intersection in U=f(t) coordinate system of circles in the second and third quadrants. As to the modifiers with a dual effect, it is necessary that $sign(Int)'_y = sign(Sl)'_y$ and $sign(Sl)'_y = sign(t_0)'_y$ condition be fulfilled, as a result, in U= f(t) coordinate system the circles intersect in the first or third quadrant. So, it is

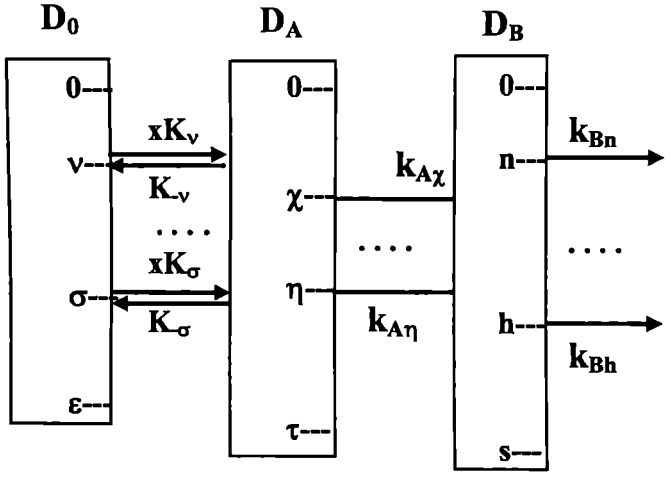
evident that during the influence exerted by different types of modifier on the enzyme system a graphical drawing specific for each type is obtained in U=f(t) coordinate system, which, relying on experimental evidence, enables to formulate the molecular mechanism of the action of a modifier.

7.2. Stepwise catalytic single-sited substrate and multi-sited modifier enzyme system

The transport ATPases have a crucial role in the cell functioning. From this viewpoint P type ATPases, having the phosphorylated intermediate condition (P-intermediate), are remarkable. The catalysis realized by them takes two steps: with phosphorylation and dephosphorylation.

$$E+ATP\leftrightarrow EATP\rightarrow EP+ADP\rightarrow E+P_{in}$$

Each step of P types ATPase reaction is, as a rule, activated and inhibited by modifiers (ions). In a general way, the reaction of this kind has been schematically presented in the below diagram:



$\varepsilon=\nu+\lambda+\mu$, $\sigma=\nu+\lambda$, $s=n+p+m$, $h=n+p$,
$0\le\nu\le\sigma\le\varepsilon$, $0\le\chi\le\eta\le\tau$, $0\le n\le h\le s$.

$$D_0=\sum_{i=0}^{\varepsilon}\beta_i y^i;\quad N_0=\sum_{i=\nu}^{\sigma}K_{+i}\beta'_i y^i;$$

$$D_A=\sum_{i=0}^{\tau}\delta_i y^i;\quad N'_A=\sum_{i=\nu}^{\sigma}K_{-i}\delta'_i y^i;$$

$$N''_A=\sum_{i=\chi}^{\tau}k_{Ai}\delta''_i y^i;$$

$$D_B=\sum_{i=0}^{s}\alpha_i y^i;\quad N''_B=\sum_{i=n}^{h}k_{Bi}\alpha''_i y^i$$

Where $K_A,\ldots,K_3$ are catalytic, while K_{+i} and K_{-i} are respectively substrate (x) dissociation constants, as to ν, σ, ε, χ, η, τ, n, p and s, they are numbers of ligand (modifier) binding sites. Suppose that there is no interaction between the substrate and modifier (y) sites and the substrate imaginary dissociation constant depends only upon the number of bound modifiers, while the interaction between modifying sites is permissible ($y\neq 1$). Then, under rapid equilibrium we'll have:

$$D_0=\sum_{i=0}^{\varepsilon}\beta_i y^i=1+\sum_{i=1}^{\varepsilon}\frac{y}{K_{yi}}+\sum_{i=1}^{\varepsilon-1}\left[\frac{y^2}{K_{yi}}\sum_{j=i+1}^{\varepsilon}\frac{\gamma_{ij}}{K_{yi}}\right]+\cdots+\frac{y^{\varepsilon-1}}{\prod_{i}^{\varepsilon}K_{yi}}\sum_{i=1}^{\varepsilon}\gamma_i K_{yi}+\frac{y^{\varepsilon}}{\prod_{i}^{\varepsilon}\gamma_i K_{yi}}$$

$$D_A=\sum_{i=0}^{\tau}\delta_i y^i=1+\sum_{i=1}^{\tau}\frac{y}{K_{yi}}+\cdots+\frac{y^{\tau-1}}{\prod_{i}^{\tau}K_{yi}}\sum_{i=1}^{\tau}\gamma_i K_{yi}+\frac{y^{\tau}}{\prod_{i}^{\tau}\gamma_i K_{yi}}\qquad(7.8)$$

$$D_B = \sum_{i=0}^{s} \alpha_i y^i = 1 + \sum_{i=1}^{s} \frac{y}{K_{yi}} + \cdots + \frac{y^{s-1}}{\prod_{i}^{s} K_{yi}} \sum_{i=1}^{s} \gamma_i K_{yi} + \frac{y^s}{\prod_{i}^{s} \gamma_i K_{yi}}$$

In D_0, D_A and D_B equations K_{yi} constants have the same physical and diverse numerical quantities, and with no interaction between the substrate and modifier sites we'll have: $\alpha = \alpha' = \alpha''$ $\delta = \delta' = \delta''$ and $\beta = \beta'$, resulting in

$$N_0 = \sum_{i=v}^{\sigma} K_{+i}\beta_i y^i; \quad N'_A = \sum_{i=v}^{\sigma} K_{-i}\delta_i y^i; \quad N''_A = \sum_{i=\chi}^{\tau} k_{Ai}\delta_i y^i; \quad N''_B = \sum_{i=n}^{h} k_{Bi}\alpha_i y^i \qquad (7.9)$$

If modifiers, compared to substrate, represent the molecules smaller in size and their binding with the enzyme system occurs more rapidly, then under the steady-state equilibrium the velocity equation may be worked with Cha method, according to which binding of modifiers is viewed as occurring under the rapid equilibrium and of substrate, in the steady-state. In the given case, the velocity equation in the reversed coordinate system will assume the following form:

$$U = a + bt = (Int) + (Sl)t = \left(\frac{D_A}{N''_A} + \frac{D_B}{N''_B}\right) + \frac{[N'_A + N''_A]\cdot D_0 D_B}{N_0 N''_A N''_B}\frac{1}{x},$$

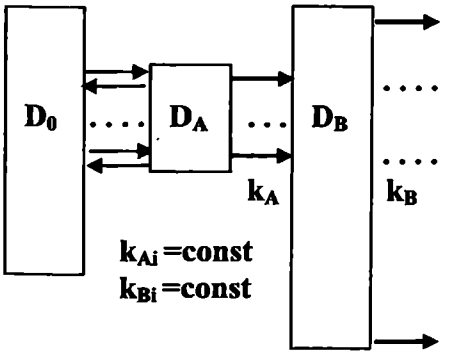
where $U=1/V$, $t=1/x$; $(Int) = a = \left(\frac{D_A}{N''_A} + \frac{D_B}{N''_B}\right)$; $(Sl) = b = \frac{[N'_A + N''_A]\cdot D_0 D_B}{N_0 N''_A N''_B}$. (7.10)

where $t_0 = -\frac{a}{b} = -\frac{(Int)}{(Sl)} = \frac{[D_A N''_B + D_B N''_A]N_0}{[N'_A + N''_A]D_0 D_B} = -\frac{N_0}{D_0}\left(\frac{D_A}{N'_A + N''_A}\right)\left(\frac{N''_A}{D_A} + \frac{N''_B}{D_B}\right)$.

Let us examine a few examples of the enzyme system organized in this way.

A **Pure specific modifier**

Necessary condition: $(Int)'_y = 0$



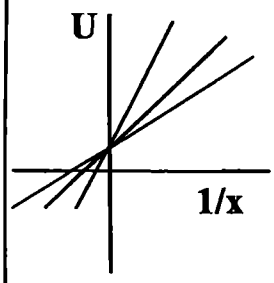
$N''_A = k_A D_A;$

$N''_B = k_B D_B;$

$Sl = \frac{1}{k_B}\left(1 + \frac{N'_A}{N''_A}\right)\frac{D_0}{N_0};$

$t_0 = \left(\frac{1}{k_A} + \frac{1}{k_B}\right)\frac{N_0}{D_0}\left(\frac{1}{k_A} + \frac{D_A}{N'_A}\right).$

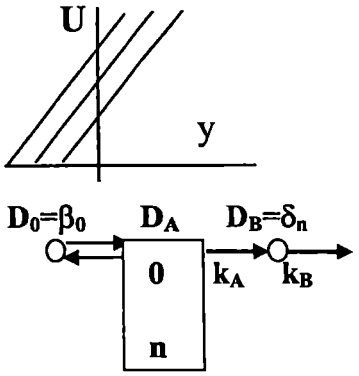
It is possible $D_A = D_B$, $D_A \neq D_B$.



B

Pure catalytic modifier

Necessary condition: $(Sl)'_y = 0$



$$Sl = \frac{(K_{-0} + k_{A0})}{K_{+0} k_{A0} k_{B0}} = \text{const};$$

$$Int = \frac{1}{k_{A0}} + \frac{(\delta_0 + \cdots + \delta_m y^m)}{k_{B0}}; \quad (Int)'_y \geq 0; \quad (Int)''_y > 0;$$

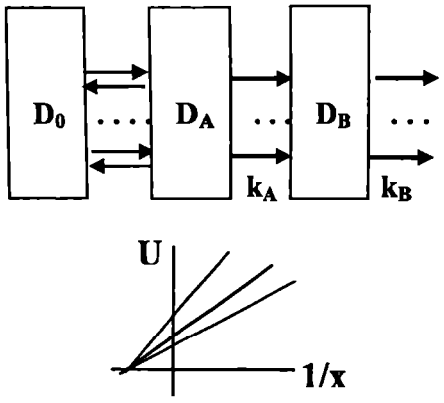
$$t_0 = -\frac{(\delta_0 + \cdots + \delta_m y^m) K_{-0}}{(K_{-0} + k_{A0})} \left(k_{B0} + \frac{k_{A0}\delta_0}{(\delta_0 + \cdots + \delta_m y^m)} \right);$$

$$(t_0)'_y \leq 0; \quad (t_0)''_y < 0.$$

C

Mixed action modifier constant affinity

Necessary condition: $(t_0)'_y = 0$



$\nu=\chi=n$, $\sigma=\eta=h$, $\varepsilon=\tau=s$

$k_{Ai}=k_A$; $k_{Bi}=k_B$, $K_{-i}=K_-$, $K_{+i}=K_+$.

$$D_0 = D_A = D_B = D; \; D = \sum_{i=0}^{s} \beta_i y^i; \; N = \sum_{i=n}^{h} \alpha_i y^i$$

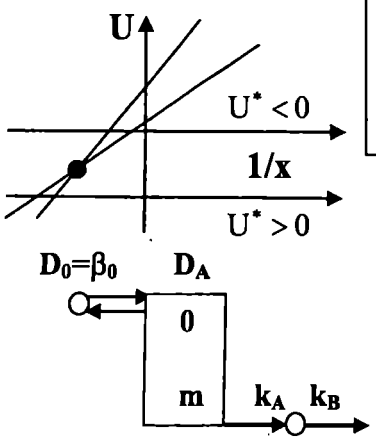
$$N_0 = K_+ N; \; N'_A = K_- N; \; N''_A = k_A N; \; N''_B = k_B N;$$

$$U = \left[\left(\frac{1}{k_A} + \frac{1}{k_B} \right) + \frac{K_- + k_A}{k_A k_B K_+} \right] \frac{D}{N}$$

D

Mixed type modifier

1. Activator with decreasing affinity: $(t_0)'_y \geq 0$, $(Sl)' < 0$.
2. Inhibitor with decreasing affinity: $(t_0)'_y \geq 0$, $(Sl)' < 0$.
3. Activator with increasing affinity: $(t_0)'_y \leq 0$, $(Sl)' < 0$.
4. Inhibitor with increasing affinity: $(t_0)'_y \leq 0$, $(Sl)' > 0$.



Necessary condition: $\text{sign}(Int)'_y = \text{sign}(Sl)'_y$

$$Int = \frac{1}{k_{B0}} + \frac{(\delta_0 + \cdots + \delta_n y^n)}{k_{B0}\delta_n y^n}; \quad (Int)'_y \leq 0; \; (Int)''_y > 0;$$

$$Sl = \frac{1}{k_{Bn}} \left(1 + \frac{K_{+0}\delta_0}{k_{A0}\delta_n y^n} \right); \; (Sl)'_y \leq 0; \; (Sl)''_y > 0;$$

$$t_0 = -\frac{(\delta_0 + \cdots + \delta_n y^n) \cdot k_{Bn} K_{+0}}{(K_{-0}\delta_0 + k_{A0}\delta_n y^n)}$$

$(y \to 0) \Rightarrow -K_{+0}/K_{-0}$.

$(y \to \infty) \Rightarrow -K_{+0} k_{Bn}/k_{An}$

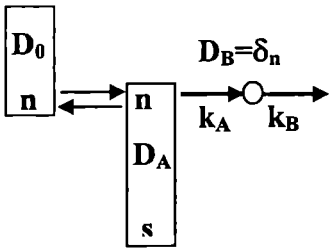
$(t_0)'_y \geq 0 \Rightarrow$ DA, $(U^* < 0)$; $(t_0)'_y \leq 0 \Rightarrow$ IA, $(U^* > 0)$.

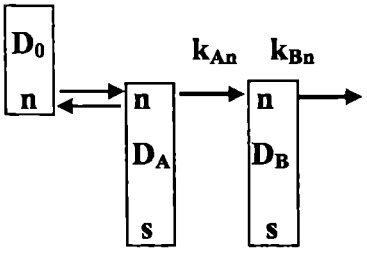
E

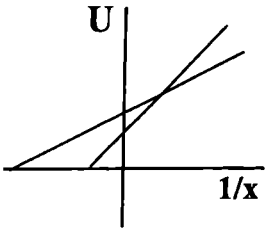
Modifier with a dual effect

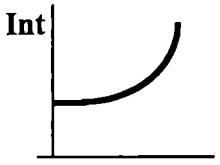
Necessary condition:

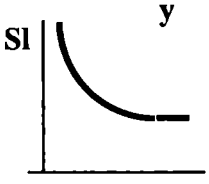
$$\mathrm{sign}(\mathrm{Int})'_y = -\mathrm{sign}(\mathrm{Sl})'_y; \quad \mathrm{sign}(\mathrm{Sl})'_y = \mathrm{sign}(t_0)'.$$

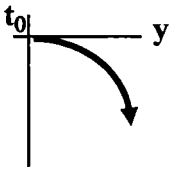












$$\mathrm{Int} = \frac{1}{k_{Bn}} + \frac{\left(\delta_0 y^n + \cdots + \delta_s y^s\right)}{k_{An}\delta_n y^n}; \qquad (\mathrm{Int})'_y \geq 0 \;;\; (\mathrm{Int})''_y > 0$$

$$\mathrm{Sl} = \frac{\left(\beta_0 + \cdots + \beta_n y^n\right)}{K_{+n}\beta_n y^n}\frac{\left(K_{-n} + k_{An}\right)}{k_{An}k_{Bn}}; \qquad (\mathrm{Sl})'_y \leq 0 \;;\; (\mathrm{Sl})''_y > 0$$

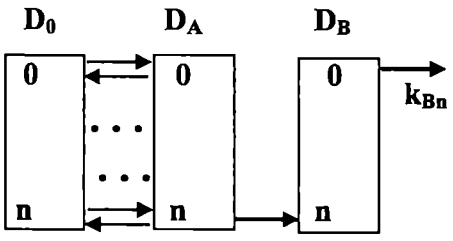
$$t_0 = -\left(\frac{1}{k_{Bn}} + \frac{\left(\delta_0 y^n + \cdots + \delta_s y^s\right)}{k_{An}\delta_n y^n}\right)\frac{K_{+n}\beta_n y^n}{\left(\beta_0 + \cdots + \beta_n y^n\right)}\frac{k_{An}k_{Bn}}{\left(K_{-n} + k_{An}\right)};$$

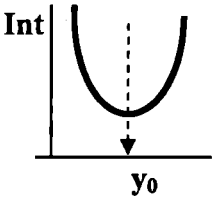
$$(t_0)'_y \leq 0; \; (t_0)''_y < 0.$$

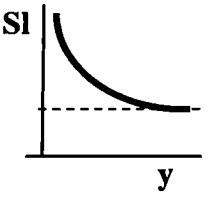
E1

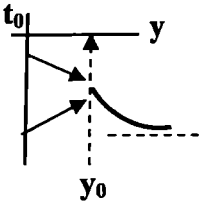
Modifier with a dual effect

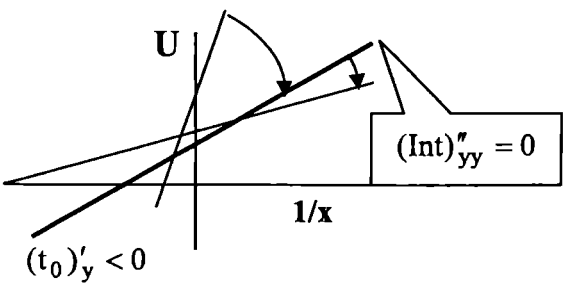
P-type transport ATPase model (phosphorylated intermediate)

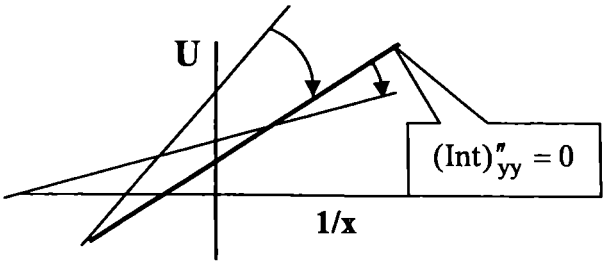












$$Int = \frac{\delta_0 + \cdots + \delta_n y^n}{k_{An}\delta_n y^n} + \frac{\alpha_0 + \cdots + \alpha_n y^n}{k_{Bn}\alpha_0};\ (Int)''_y > 0.$$

$$Sl = \frac{(\beta_0 + \cdots + \beta_n y^n)}{K_{+n}(\beta_0 + \cdots + \beta_n y^n)} \cdot \frac{[K_{-0}\delta_0 + \cdots + (K_{-n} + k_{An})\delta_n y^n]}{k_{An}\delta_n y^n} \cdot \frac{(\alpha_0 + \cdots + \alpha_n y^n)}{k_{B0}\alpha_0};\ (Sl)'_y < 0;$$

$$t_0 = \frac{K_{+n}[\delta_0 + \cdots + \delta_n y^n]}{[K_{-0}\delta_0 + \cdots + (K_{-n} + k_{An})\delta_n y^n]} \left[\frac{k_{An}\delta_n y^n}{(\delta_0 + \cdots + \delta_n y^n)} + \frac{k_{B0}\alpha_0}{(\alpha_0 + \cdots + \alpha_n y^n)}\right];$$

$$(y > y_0) \Rightarrow (t_0)'_y < 0, \quad (y = y_0) \Rightarrow (t_0)'_y > 0, \quad (y < y_0) \Rightarrow (t_0)'_y = \pm w.$$

Thus, the examples examined above makes it imminent that in the case of stepwise catalysis too the same pattern is seen as has been dealt with in Chapter 7.1. Five diverse classes are distinguished of modifiers, which in U= f(t) coordinate system yield the graphical drawing similar to that considered in the previous chapter. The difference is seen in the case of dual-effect modifiers, which additionally gives intercepts in the second quadrant of U= f(t) coordinate system.

7.3. General classification of the multi-sited enzyme system modifiers

Full kinetic estimation of single-sited enzyme systems, as has been shown in Chapter 2, is determined only by two kinetic parameters (K_m and V_{max} in the case of an activator and K_I and V_{max} in the case of an inhibitor, that furnishes information about the catalytic and specific constants). The third parameter, the number of sites is a priori known and equals to 1. Proceeding from this, at one glance, it is logical that for the full kinetic estimation of the multi-sited enzyme systems it is required that the number of modifier binding sites be defined and each site be characterized by two kinetic parameters. As has been demonstrated earlier, the multi-sited enzyme systems are affected by different types of modifiers: essential activators, full inhibitors and partial-effect modifiers. That is, for full characteristic of the multi-sited enzyme systems rather a great number of kinetic parameters are required and to characterize an enzyme by these parameters is a practically unsolvable task and is devoid of any sense. Therefore, one needs to formulate such principles of modifiers' classification that would facilitate sufficiently full kinetic analysis of the multi-sited enzyme systems. As a result of analysis of the examples dealt with in the previous sections, it is possible to find the way out and to relate the modifiers' classification principles with the alteration of the position of intercepts at different concentrations of a m modifier (coordinates: U*, t*) and inflexion (SI) and intersection points with the coordinate axes (intersection point Int with the ordinate axis or t_o with the axis of abscissa). In these examples, the enzyme reaction rate vs. substrate concentration, in inverse values, is circular and classification according to the location of intercepts at different concentrations of a modifier is easy, this being rather difficult for the multi-

sited enzyme systems, the more so when we deal with the experimental points. In this case, the dependency is frequently curvilinear and it is rather complex to determine, on the strength of experimental data, the intersection points of curves. As regards the classification, it requires a simple procedure.

In Chapter 6 the fundamental kinetic parameters were considered and on V=f(x) graph three of them were singled out: small, large and medium concentration sites. The first two sites are described, respectively, in terms of $\sqrt[n]{U}=f(1/x)$ and $\sqrt[m]{U}=f(x)$ functions, representing circles. In order to describe the medium concentration site, one may use InV=f(Inx) function, averaging of which occurs by means of a regression line and is expressed in the following way: $\ln V = a + b \ln x$, where a may be fancied as $\ln V_{max}$, while b represents averaged ω function. An alternate method can be also applied to characterize $\ln V = a + b \ln x$ function for the medium concentrations' site, that is, averaging of U=f(t) function with rectilinear regression, as a result of which we may obtain an ascending (b>0), descending (b<0) or a horizontal circle. (In the case of increasing curve, the function is approximated by means of a rectilinear regression in U=f(t) coordinate system, while in decreasing case we can employ U=f(x) transformation). If in the middle of V=f(t) function several turning points are arranged in succession, then analysis may be made of the sections between the turning points and averaging of these sections be carried out.

Thus, as a result of power transformation of V=f(y) function for each site circles are obtained. This would, perhaps, facilitate relatively the classification of modifiers for the multi-sited enzyme systems, according to the intercepts obtained in the conditions of various concentrations of modifiers, this, in turn, being associated with the alteration of catalytic and specific constants in the velocity equation.

Let us examine the multi-sited enzyme system, whose velocity is dependent on two ligands, V=f(x, y). Assume that y is considered as a modifier in respect with x, then in [y]=const case, U=f(x) function may be grouped into three classes according to small, large and medium [x] concentrations and make their kinetic description by means of the relevant functions, (U=1/V):

1. Small concentrations' site, N type;

$U_n=f(t) \Rightarrow U_n=f(t) \Rightarrow \sqrt[n]{U} = a_n + b_n(1/x) = (Int) + (Sl)(1/x)$;

2. Large concentrations' site, M type;

$U_m=f(x) \Rightarrow U_m=f(x) \Rightarrow \sqrt[m]{U} = a_m + b_m(x) = (Int)_m + (Sl)_m(x)$;

3. Medium concentrations' site, P type; $V = f(x) \Rightarrow \overline{V} = a_0 + b_0(x)$

3.1 ($b_P \geq 0$) $U_P=f(t) \Rightarrow \overline{U} = a_P + b_P(1/x) = (Int)_p + (Sl)_p(1/x)$

3.2 ($b_P<0$) $U_P=f(x) \Rightarrow \overline{U} = a_P + b_P(x) = (Int)_p + (Sl)_p(x)$

Therefore, based on geometrical shape of Int=f(y), SI=f (y) vs. t_o=f (y), it is possible to classify y modifier and single out respective subgroups.

Thus, at various concentrations of modifiers (y), according to the above listed intercepts' coordinates (U*, t*), three major groups (N, M and P types) of modifiers can be distinguished and the respective to each one subgroups. The characteristic and to be explored circles of these groups are: U_n=f(t), U_m=f(x) and U_p=f(x).

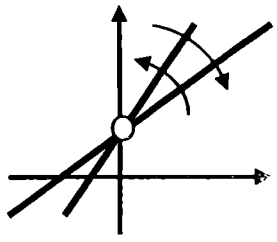
A — N, M and P type pure specific modifiers

Necessary condition: $(Int)'_y = 0$

$(Sl)'_y < 0 \Rightarrow$ aqtivatori

$(Sl)'_y > 0 \Rightarrow$ inhibitori

$(Sl)'_y = 0 \Rightarrow \neq f(y)$



U_n=f(t)

U_m=f(x)

U_P=f(t) or U_P=f(x)

$U^*>0$, $t^*=0$.

A subgroup comprises the modifiers whose action results in intersection of relevant circular functions on the ordinate axis (($U^*>0$, $t^*=0$)), i.e. by the effect of a modifier changes slope (if a modifier is an activator $(Sl)'_y<0$, while if an inhibitor – $(Sl)'_y>0$). and don't changes ordinate intersection point – $(Int)'_y=0$. And this, as been already stated, means that the modifier acts on the specific constant and has no effect on the catalytic constant. Therefore, such modifiers have been attributed by us to the subgroup of pure specific modifiers.

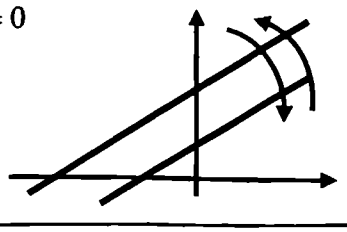
B — N, M and P type pure catalytic modifiers

Necessary condition: $(Sl)'_y = 0$

$(Int)'_y<0 \Rightarrow$ activator

$(Int)'_y>0 \Rightarrow$ inhibitor i

$(Int)'_y=0 \Rightarrow \neq f(y)$



U_n=f(t)

U_m=f(x)

U_P=f(t) or U_P=f(x)

$U^*\approx\infty$, $t^*\approx\infty$

B subgroup comprises the modifiers whose different concentrations corresponding power circular functions are parallel to each other, i.e. do not change slope, $(Sl)'_y = 0$ and alter the intersection point with ordinate axis (for activators $(Int)'_y < 0$ and for inhibitors $(Int)'_y > 0$), that means that the modifier changes the catalytic constant and has no effect on the specific constant. From this it follows that the modifiers of this subgroup have been attributed by us to the pure catalytic ones.

C and **D** subgroups unite the modifiers under the influence of which alters the inclination of the respective circular functions (if a modifier is an activator $(Sl)'_y < 0$ and if an inhibitor – $(Sl)'_y > 0$), as well as changes the ordinate axis intersection point (for activators $(Int)'_y < 0$, and for inhibitors – $(Int)'_y > 0$, i.e. the modifiers of this subgroup affect both the specific and catalytic constants, that's why we have attributed them to the mixed modifiers. In this subgroup, in turn, three cases are distinguished:

C, D

N, M and P type mixed-effect modifiers

Necessary condition: $\text{sign}(Int)'_y = \text{sign}(Sl)'_y$

D1. Activator with decreasing affinity
$(t_o)'_y \geq 0$, $(Sl)'_y < 0$

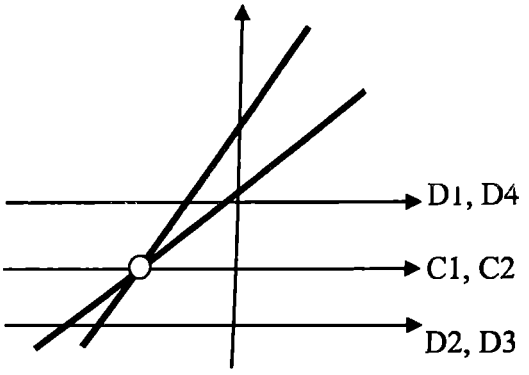
D2. Inhibitor with decreasing affinity
$(t_o)'_y \geq 0$, $(Sl)'_y > 0$

C1. Activator with constant affinity
$(t_0)'_y = 0$; $(Sl)'_y < 0$.

C2. Inhibitor with constant affinity
$(t_0)'_y = 0$; $(Sl)'_y > 0$.

D3. Activator with increasing affinity
$(t_0)'_y \leq 0$; $(Sl)'_y < 0$.

D4 Inhibitor with increasing affinity
$(t_0)'_y \leq 0$; $(Sl)'_y > 0$.



$U_n = f(t)$, $U_m = f(x)$, $U_P = f(t)$ an $U_P = f(x)$

$t^* < 0$; $U^* > 0$, $U^* = 0$, $U^* < 0$

D2, D3 – when intersection of circles occurs in the second quadrant of the coordinate system ($U^* > 0$), $t^* < 0$), the mixed type modifiers are predominantly specific. In this case, if the modifier is an activator, then by its action the intersection point with the axis of abscissa diminishes ($(t_0)'_y < 0$) and the mixed type modifier will be preferentially specific with increasing affinity, and if a modifier is an inhibitor, then the intersection point with abscissa increases ($(t_0)'_y > 0$ and the mixed type modifier will be preferentially specific, with increasing affinity.

E subgroup unites the modifiers, under the action of which the intersection of the respective circular functions occurs in the first quadrant of the coordinate system ($U^* > 0$, $t^* > 0$), i.e. at this time, if the inclination decreases ($(Sl)'_y < 0$), the intersection point with the ordinate axis increases ($(Int)'_y > 0$) and vice versa, if inclination increases ($(Sl)'_y > 0$), the intersection point with the ordinate decreases ($(Int)'_y < 0$). We have attributed such modifiers to the dual-effect ones.

E

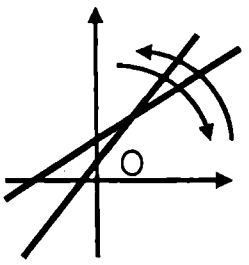
N, M and P type dual-effect modifiers

(with concentration-dependent activation and inhibition sites)

Necessary condition: $-\text{sign}(\text{Int})'_y = \text{sign}(\text{Sl})'_y$;

1. With an increase in modifier's concentration activation passes into inhibition
2. With an increase in modifier's concentration inhibition passes into activation

$U_n = f(t)$, $U_m = f(x)$, $U_P = f(t)$ an $U_P = f(x)$



$U^*>0, t^* > 0.$

In the multi-sited enzyme system, when p≥1, there may create a situation when the sites assigned for the partial effect modifiers would have distinctly greater effect on the enzyme velocity than those assigned for essential activators or full inhibition (let us designate this enzyme feature by a symbol PP). The situation of this kind may exist without modifiers and may also be created, or, on the contrary, be eliminated, as a result of binding of a modifier. the necessary condition for this situation to exist is:

$$D_{01} = \left(\frac{\beta_1}{\beta_0} - \frac{\alpha_1}{\alpha_0}\right) < 0 \quad \text{or} \quad H_{01} = \left(\frac{\beta_{s-1}}{\beta_s} - \frac{\alpha_{p-1}}{\alpha_p}\right) < 0 \qquad (7.\ 11\ )$$

As a result of which we'll have: $\lim_{\tau \to +\infty} \frac{d\omega}{d\tau} \le 0$ and $(\text{Int})_n < 0$ or $\lim_{\tau \to -\infty} \frac{d\omega}{d\tau} \le 0$ and $(\text{Int})_m < 0$.

This results in substantial changes in the geometrical shape of some important functions (Fig. 7.1.).

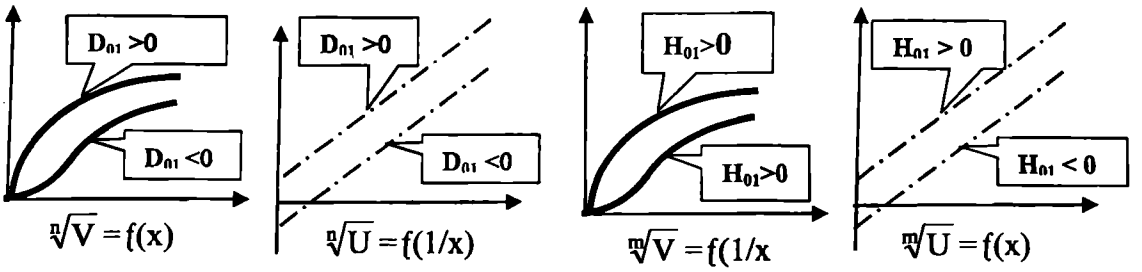
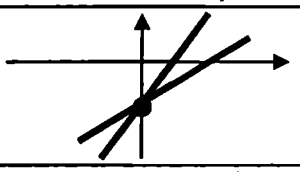
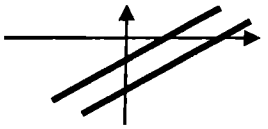
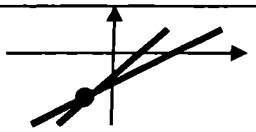
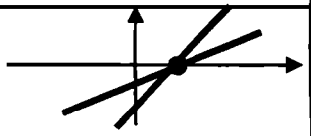
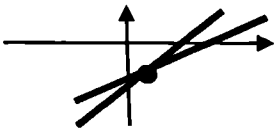


Fig. 7.1. Importance of sign_{01} and $\text{sign}H_{01}$ for the formation of geometrical shape of $U_n = f(t)$ and $U_m = f(x)$ functions

Bearing in mind the above-said, two new subgroups can be formed:

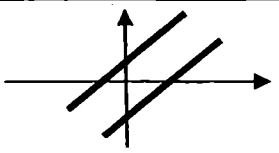
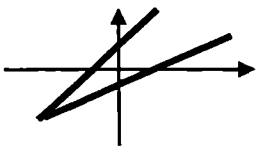
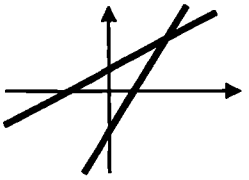
1) The enzyme mechanism operates by a priority of P-type sites and modifiers cannot alter this situation (PP).

2) Priority of P-type sites in the enzyme mechanism is regulated by modifiers (RP).

PP	**P-type sites' priority nonregulating modifiers in N, M and P type sited enzyme system**			
Type	Necessary condition	Location of intersection point U^*, t^*		Modifiers
PPA	$(Int)'_y = 0$		$U^* < 0$ $t^* = 0$	$(Sl)'_y < 0$ – Activator $(Sl)'_y > 0$ – Inhibitor
PPB	$(Sl)'_y = 0$		$U^* \to \infty$ $t^* \to \infty$	$(Int)'_y < 0$ – Activator $(Int)'_y > 0$ – Inhibitor
PPD	$\text{sign}(Int)'_y = \text{sign}(Sl)'_y$		$U^* < 0$ $t^* < 0$	$(Sl)'_y < 0$ – Activator $(Sl)'_y > 0$ – Inhibitor
PPE	$\text{sign}(Int)'_y = -\text{sign}(Sl)'_y$ $(t_0)'_y = 0$		$U^* = 0$ $t^* > 0$	$(Sl)'_y < 0 - \delta$ Activator $(Sl)'_y > 0$ – Inhibitor
	$\text{sign}(Int)'_y = -\text{sign}(Sl)'_y$ $\text{sign}(Sl)'_y = \text{sign}(t_0)'_y$		$U^* < 0$ $t^* > 0$	$(Sl)'_y < 0$ – Activator $(Sl)'_y > 0$ – Inhibitor

In these subgroups united are the modifiers under the influence of which intersection of circles occurs in the fourth quadrant of the coordinate system ($U^*<0$, $t^*>0$), or on the abscissa – by the coordinates ($U^*=0$, $t^*>0$), or on the ordinate axis – by coordinates ($U^*<0$, $t^*=0$). Such a position of intercepts is possible only in the case of multi-sited enzyme systems and depends on the parameters stipulated by multisitedness. Therefore, the modifiers of these subgroups can be referred to as the modifiers with multisitedness effect. It must be noted that in a similar situation the condition $D_{01}=0$ or $H_{01}=0$ is fulfilled.

The subgroup with adjustable P-type site priority varies from the first subgroup in that one of the circles does not cross the fourth quadrant and respectively, the intersection points therein are not located in the fourth quadrant, while another crossing circle will by all means pass the fourth quadrant.

RP	**P-type sites' priority regulating modifiers in the enzyme system with N, M and P-type sites**			
Type	Necessary condition	Location of intersection point U^*, t^*		Modifiers' characteristics
BRP	$(Sl)'_y = 0$		$U^* \to \infty$ $t^* \to \infty$	$(Int)'_y<0$ – activator, stimulates primal activity of partial-effect sites; (0→P) $Int)'y>0$ – inhibitor, inhibits primal activity of partial-effect sites; (P→0)
DRP	$\text{sign}(Int)'_y = \text{sign}(Sl)'_y$		$U^* < 0$ $t^* < 0$	
ERP	$\text{sign}(Int)'_y = -\text{sign}(Sl)'_y$		$U^* > 0$ $t^* > 0$	

Appendix 7.1

$$Int = \frac{D_2}{N_2''} = \frac{\alpha_0 + \alpha_1 y + \cdots + \alpha_{S-1} y^{S-1} + \alpha_s y^S}{y^n \left(k_n \alpha_0 + k_{n+1} \alpha_{n+1} y + \cdots + k_{n+p} \alpha_{n+p} y^{n+p}\right)} \tag{7.7}$$

$$Sl = \frac{(N_2' + N_2'')D_1}{N_2' N''} = \frac{y^{\nu}\left[K_{-\nu}\alpha_{\nu}\beta_0 y^{\nu} + \cdots + K_{-\sigma}\alpha_{\sigma}\beta_{\varepsilon} y^{\varepsilon+\varpi}\right] + y^n\left[k_n\alpha_n\beta_0 + \cdots + k_h\alpha_h\beta_{\varepsilon} y^{n+p}\right]}{y^{n+\nu}\left[k_n K_{\nu}\alpha_n\beta_{\nu} + \cdots\cdots + k_h K_{\nu+\lambda}\alpha_n\beta_{\nu+\lambda} y^{\lambda+p}\right]}$$

$$t_0 = -\frac{Int}{Sl} = -\frac{D_2 N_1}{(N_2' + N_2'')D_1} = \frac{\left[K_{\nu}\alpha_0\beta_{\nu} y^{\nu} + \cdots + K_{\sigma}\alpha_S\beta_{\sigma} y^{S+\sigma}\right]}{\left[K_{-\nu}\alpha_{\nu}\beta_0 y^{\nu} + \cdots + K_{-\sigma}\alpha_{\sigma}\beta_{\varepsilon} y^{\varepsilon+\varpi}\right] + \left[k_n\alpha_n\beta_0 y^n + \cdots + k_h\alpha_h\beta_{\varepsilon} y^{n+p+n}\right]}$$

Let us enter the following designations:

$A_{\nu} = K_{-\nu}\alpha_{\nu}\beta_0$;
$A_{\nu+1} = K_{-(\nu+1)}\alpha_{\nu+1}\beta_0 + K_{-\nu}\alpha_{-\nu}\beta_1$;
$A_{\varepsilon+\sigma-1} = K_{-(\sigma-1)}\alpha_{\varepsilon-1}\beta_{\varepsilon} + K_{-\sigma}\alpha_{\varepsilon}\beta_{\varepsilon-1}$,
$A_{\varepsilon+\sigma} = K_{-\sigma}\alpha_{\varepsilon}\beta_{\varepsilon}$
$B_n = k_n\alpha_n\beta_0$;
$B_{n+1} = k_{n+1}\alpha_{n+1}\beta_0 + k_n\alpha_n\beta_1$;
$B_{\varepsilon+h-1} = k_{h-1}\alpha_{h-1}\beta_{\varepsilon} + k_h\alpha_h\beta_{\varepsilon-1}$
$B_{\varepsilon+n+p} = k_h\alpha_h\beta_{\varepsilon}$
$C_{n+\nu} = k_n K_{\nu}\alpha_n\beta_{\nu}$;
$C_{n+\nu+1} = k_n K_{\nu+1}\alpha_n\beta_{\nu+1} + k_{n+1}K_{\nu}\alpha_{n+1}\beta_{\nu}$.
$C_{\sigma+h} = k_h K_h\alpha_h\beta_{\varpi}$
$C_{\sigma+h-1} = k_h K_{\sigma-1}\alpha_h\beta_{\sigma-1} + k_{h-1}K_{\sigma}\alpha_{h-1}\beta_{\sigma}$
$F_{\nu} = K_{\nu}\alpha_0\beta_{\nu}$;
$F_{s+\sigma} = K_{\sigma}\alpha_s\beta_{\sigma}$
$F_{\nu+1} = K_{\nu}\alpha_1\beta_{\nu} + K_{\nu+1}\alpha_0\beta_{\nu+1}$
$F_{\nu+1} = K_{-(\sigma-1)}\alpha_{\sigma-1}\beta_{\varepsilon} + K_{-\sigma}\alpha_{\sigma}\beta_{\varepsilon-1}$

Then, in inverse values of U= f(1/x)=(Int)+(SI)(1/x) coefficients in the enzyme velocity vs. substrate equation, as y functions, their derivatives and limits will have the following form:

$$\mathrm{Int}=\frac{D_2}{N_2''}=\frac{\alpha_0+\alpha_1 y+\cdots+\alpha_{s-1}y^{s-1}+\alpha_s y^s}{y^n\left(k_n\alpha_n+k_{n+1}\alpha_{n+1}y+\cdots+k_{n+p}\alpha_{n+p}y^p\right)}$$

$$\lim_{y\to 0}\mathrm{Int}=\frac{\alpha_0}{k_n\alpha_n}\lim_{y\to 0}y^{-n};\qquad \lim_{y\to\infty}\mathrm{Int}=\frac{\alpha_s}{k_h\alpha_h}\lim_{y\to\infty}y^m$$

$$(\mathrm{Int})'_y=\frac{y^{n-1}\left[-nk_n\alpha_0\alpha_n-\begin{pmatrix}(n+1)k_{n+1}\alpha_0\alpha_{n+1}+\\+(n-1)k_n\alpha_1\alpha_n\end{pmatrix}y+\cdots+\begin{pmatrix}(m+1)k_{h-1}\alpha_{h-1}\alpha_s+\\+(m-1)k_h\alpha_1\alpha_{s-1}\end{pmatrix}y^{s+p-1}+mk_h\alpha_h y^{s+p}\right]}{y^{2n}\left(k_n\alpha_n+k_{n+1}\alpha_{n+1}y+\cdots+k_{n+p}\alpha_{n+p}y^p\right)^2}$$

$$\lim_{y\to 0}\left((\mathrm{Int})'_y\right)=\frac{-n\alpha_0}{k_n\alpha_n}\lim_{y\to 0}y^{-n-1}+\frac{\left[(n+1)k_{n+1}\alpha_0\alpha_{n+1}+(n-1)k_n\alpha_1\alpha_n\right]}{(k_n\alpha_n)^2}\lim_{y\to 0}y^{-n}$$

$$\lim_{y\to\infty}\left((\mathrm{Int})'_y\right)=\frac{m\alpha_s}{k_h\alpha_h}\lim_{y\to\infty}y^{m-1}+\frac{\left[(m+1)k_{h-1}\alpha_{h-1}\alpha_s+(m-1)k_h\alpha_h\alpha_{s-1}\right]}{(k_h\alpha_h)^2}\lim_{y\to\infty}y^{m-2}$$

$$\mathrm{Sl}=\frac{(N_2'+N_2'')D_1}{N_1N_2''}=\frac{y^{\nu}\left[K_{-\nu}\alpha_\nu\beta_0+\cdots+K_{-\sigma}\alpha_\sigma\beta_\varepsilon y^{\varepsilon+\sigma}\right]+y^n\left[k_n\alpha_n\beta_0+\cdots+k_h\alpha_h\beta_\varepsilon y^{\varepsilon+p}\right]}{y^{n+\nu}\left[k_nK_\nu\alpha_n\beta_\nu+\cdots\cdots+k_hK_{\nu+\lambda}\alpha_h\beta_{\nu+\lambda}y^{\lambda+p}\right]}$$

$$\lim_{y\to 0}\mathrm{Sl}=\lim_{y\to 0}\frac{y^{\nu}A_\nu+y^nB_n}{y^{n+\nu}C_{n+\nu}},\ (n=\nu\neq 0 \text{ an } n>\nu \text{ an } n<\nu);$$

$$\lim_{y\to 0}\mathrm{Sl}=\frac{A_\nu+B_n}{C_{n+\nu}}>0,\ (n=\nu=0)$$

$$\lim_{y\to\infty}\mathrm{Sl}=\lim_{y\to\infty}\frac{y^{\mu+\sigma-h}\left[A_{\varepsilon+\sigma}\right]+y^{\mu}\left[B_{\varepsilon+h}\right]}{C_{\sigma+h}}\ (\mu\neq 0);$$

$$\lim_{y\to\infty}\mathrm{Sl}=\lim_{y\to\infty}\frac{y^{\sigma-h}\left[A_{\varepsilon+\sigma}\right]+\left[B_{\varepsilon+h}\right]}{C_{\sigma+h}},\ (\mu=0,\ \sigma\leq h);$$

$$(\mathrm{Sl})'_y=\frac{\begin{matrix}-nA_\nu C_{n+\nu}y^{2\nu+n-1}-\left[(n+1)A_\nu C_{n+\nu+1}+(n-1)A_{\nu+1}C_{n+\nu}\right]y^{2\nu+n}+\cdots\\ -\nu B_n C_{n+\nu}y^{2n+\nu-1}-\left[(\nu+1)B_nC_{n+\nu+1}+(\nu-1)B_{n+1}C_{n+\nu}\right]y^{2n+\nu}+\cdots\end{matrix}}{y^{2(n+\nu)}\left(C_{\nu+n}+\cdots+C_{\sigma+h}y^{\lambda+p}\right)^2}\quad \text{an}$$

$$(\mathrm{Sl})'_y=\frac{\begin{matrix}(\varepsilon-h)A_{\varepsilon+\sigma}C_{\sigma+h}y^{\varepsilon+2\sigma+h-1}+\left[(\varepsilon-h+1)A_{\varepsilon+\sigma}C_{\sigma+h-1}+(\varepsilon-h-1)A_{\varepsilon+\sigma-1}C_{\sigma+h}\right]y^{\varepsilon+2\sigma+h-2}+\cdots\\ +(\varepsilon-\sigma)B_{\varepsilon+h}C_{\sigma+h}y^{\varepsilon+2h+\sigma-1}+\left[(\varepsilon-\sigma+1)B_{\varepsilon+h}C_{\sigma+h-1}+(\varepsilon-\sigma-1)B_{\varepsilon+h-1}C_{\sigma+h}\right]y^{\varepsilon+2h+\sigma-2}+\cdots\end{matrix}}{y^{2(n+\nu)}\left(C_{\nu+n}+\cdots+C_{\sigma+h}y^{\lambda+p}\right)^2}$$

$$\lim_{y\to 0}\left((\mathrm{Sl})'_y\right)=\lim_{y\to 0}\frac{-nA_\nu C_{n+\nu}y^{\nu-1}-\nu B_nC_{n+\nu}y^{n-1}}{y^{n+\nu}(C_{\nu+n})^2}\quad (n=\nu\neq 0 \text{ an } n>\nu \text{ an } n<\nu)$$

$$\lim_{y\to 0}\left((\mathrm{Sl})'_y\right)=\frac{-\left[(n+1)A_\nu C_{n+\nu+1}+(n-1)A_{\nu+1}C_{n+\nu}\right]-\left[(\nu+1)B_nC_{n+\nu+1}+(\nu-1)B_{n+1}C_{n+\nu}\right]}{(C_{\nu+n})^2}\quad (n=\nu=0)$$

$$\lim_{y\to\infty}\left((Sl)'_y\right)=\lim_{y\to\infty}\frac{A_{\sigma+\varepsilon}y^{\mu+(\sigma-h)}+B_{h+\varepsilon}y^{\mu}}{C_{\sigma+h}}=+\infty\quad(\mu\neq0;\ \text{an}\ \mu=0,\ \sigma>h)$$

$$\lim_{y\to\infty}\left((Sl)'_y\right)=\lim_{y\to\infty}\frac{\left[A_{\varepsilon+\sigma.}+B_{\varepsilon+\sigma}\right]C_{\sigma+h-1}-\left[A_{\varepsilon+\sigma-1}+B_{\varepsilon+h-1}\right]C_{\sigma+h}}{y^2\left(C_{\sigma+h}\right)^2}=\pm0,\ (\mu=0,\ \sigma=h)$$

$$\lim_{y\to\infty}\left((Sl)'_y\right)=\lim_{y\to\infty}\frac{-A_{\varepsilon+\sigma}}{y^{h-\sigma}\left(C_{\sigma+h}\right)}=-0,\ (\mu=0,\ \sigma<h);$$

$$t_0=-\frac{Int}{Sl}=-\frac{D_2N_1}{(N'_2+N''_2)D_1}=-\frac{\left[K_\nu\alpha_0\beta_\nu y^\nu+\cdots+K_\sigma\alpha_s\beta_\sigma y^{s+\sigma}\right]}{\left(K_{-\nu}\alpha_\nu\beta_0y^\nu+\cdots+K_{-\sigma}\alpha_\sigma\beta_\varepsilon y^{\varepsilon+\sigma}\right)+\left(k_n\alpha_n\beta_0y^n+\cdots+k_h\alpha_h\beta_\varepsilon y^{\varepsilon+h}\right)}$$

$$\lim_{y\to0}t_0=-\frac{F_\nu}{A_\nu},\ (\nu<n);\quad \lim_{y\to0}t_0=-\frac{F_\nu}{A_\nu+B_n},\ (\nu=n)\quad \lim_{y\to0}t_0=-\frac{F_\nu}{B_\nu}\lim_{y\to0}y^{\nu-n},\ (\nu>n)$$

$$\lim_{y\to\infty}t_0=-\frac{F_{s+\sigma}}{B_{\varepsilon+h}}\lim_{y\to\infty}y^{m-\mu},\ (\sigma<h);\quad \lim_{y\to\infty}t_0=-\frac{F_{s+\sigma}}{A_{\varepsilon+\sigma}}\lim_{y\to\infty}y^{s-\varepsilon},\ (\sigma>h);$$

$$\lim_{y\to\infty}t_0=-\frac{F_{s+\sigma}}{A_{\varepsilon+\sigma}+B_{\varepsilon+h}}\lim_{y\to\infty}y^{s-\varepsilon},\ (\sigma=h);$$

$$(t_0)'_y=\frac{\begin{array}{l}-y^{2\nu}\left[(F_{\nu+1}A_\nu-F_\nu A_{\nu+1})+\cdots+(s-\varepsilon)F_{s+\sigma}A_{s+\sigma}y^{s+\varepsilon+2\lambda-1}\right]-\\-y^{n+\nu+1}\left[(\nu-n)F_\nu B_n+\cdots+(m+\mu)F_{s+\sigma}B_{\varepsilon+h}y^{s+\varepsilon+h-1}\right]\end{array}}{y^{2\nu}\left[A_\nu+\cdots+A_{\varepsilon+\sigma}y^{\varepsilon+\lambda}\right]^2+y^{n+\nu}2\left[A_\nu B_n+\cdots+A_{\varepsilon+\sigma}B_{\varepsilon+p}y^{2(s+\varepsilon+2\lambda-1}\right]+y^{2n}\left[B_n+\cdots+B_{\varepsilon+p}y^{\varepsilon+p}\right]^2}$$

$$\lim_{y\to0}(t_0)'_y=\frac{F_\nu A_{\nu+1}-F_{\nu+1}A_\nu}{[A_\nu]^2},\ (\nu<n);\quad \lim_{y\to0}(t_0)'_y=\frac{(\nu-n)F_\nu}{[B_\nu]^2}\lim_{y\to0}y^{\nu-n-1},\ (\nu>n);$$

$$\lim_{y\to0}(t_0)'_y=-\frac{F_{\nu+1}A_\nu}{[A_\nu+B_n]^2},\ (\nu=n);\quad \lim_{y\to\infty}t_0=\frac{(\varepsilon-s)F_{\varepsilon+\sigma}}{A_\nu}\lim_{y\to\infty}y^{s-\varepsilon-1}(\sigma>h)$$

$$\lim_{y\to\infty}t_0=\frac{(\mu-m)F_{\varepsilon+\sigma}}{B_{\varepsilon+h}}\lim_{y\to\infty}y^{m-\mu-1}(\sigma<h,\ m\neq\mu);\quad (m=\mu)\Rightarrow\lim_{y\to\infty}t_0=\frac{\pm D}{B_{\varepsilon+h}}\lim_{y\to\infty}y^{-2};D=\text{const})$$

$$\lim_{y\to\infty}t_0=\frac{\left[(\varepsilon-s)B_{\varepsilon+\sigma}+(\mu-m)F_{s+\sigma}\right]F_{s+\sigma}}{A_\nu+B_{\varepsilon+h}}\lim_{y\to\infty}y^{s-\varepsilon-1}$$

Appendix 7.2

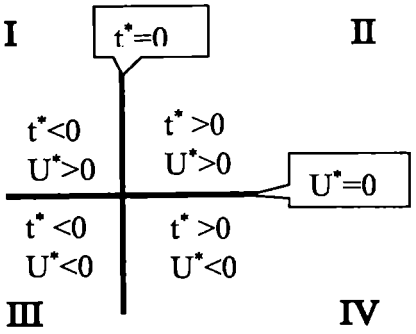
For an alternative classification of modifiers (see Chapter 2) it is required to determine the position of the intersection points. The coordinates of the intersection point of two lines ($U=a_1+b_1t$, $U=a_2+b_2t$) are:

$$t^*=-\frac{a_2-a_1}{b_2-b_1}\ \text{ and }\ U^*=b_1b_2\frac{t_{02}-t_{01}}{b_2-b_1},$$

while t* and U* sign and respectively, the location of intersection point in the coordinate plane is determined by the signs of derivatives $(Int)'_y$, $(Sl)'_y$, $(t_0)'_y$, (Fig. 7.2).

$$sign(t^*) = -\frac{sign(Int)'_y}{sign(Sl)'}; \quad sign(U^*) = sign(b_1 b_2) \cdot \frac{sign(t_0)'_y}{sign(Sl)'_y}$$

if $b_1>0$ and $b_2>0$, then $\Rightarrow sign(U^*) = \frac{sign(t_0)'_y}{sign(Sl)'_y}$; if $b_1=0$ or $b_2=0$, then $\Rightarrow U^*=0$



sign(t*)		sign(Sl)'y			sign(U*) b1>0, b2>0		sign(Sl)'y		
		>0	=0	<0			>0	=0	<0
sign(Int)'y	>0	–	±∞	+	sign(t0)'y	>0	+	±∞	–
	=0	0	≠f(y)	0		=0	0	≠f(y)	0
	<0	+	±∞	–		<0	–	±∞	+

Fig. 7.2. The location of intersection points vs. $(Int)'_y$, $(SI)'_y$ and $(t_o)'_y$ derivatives' signs.

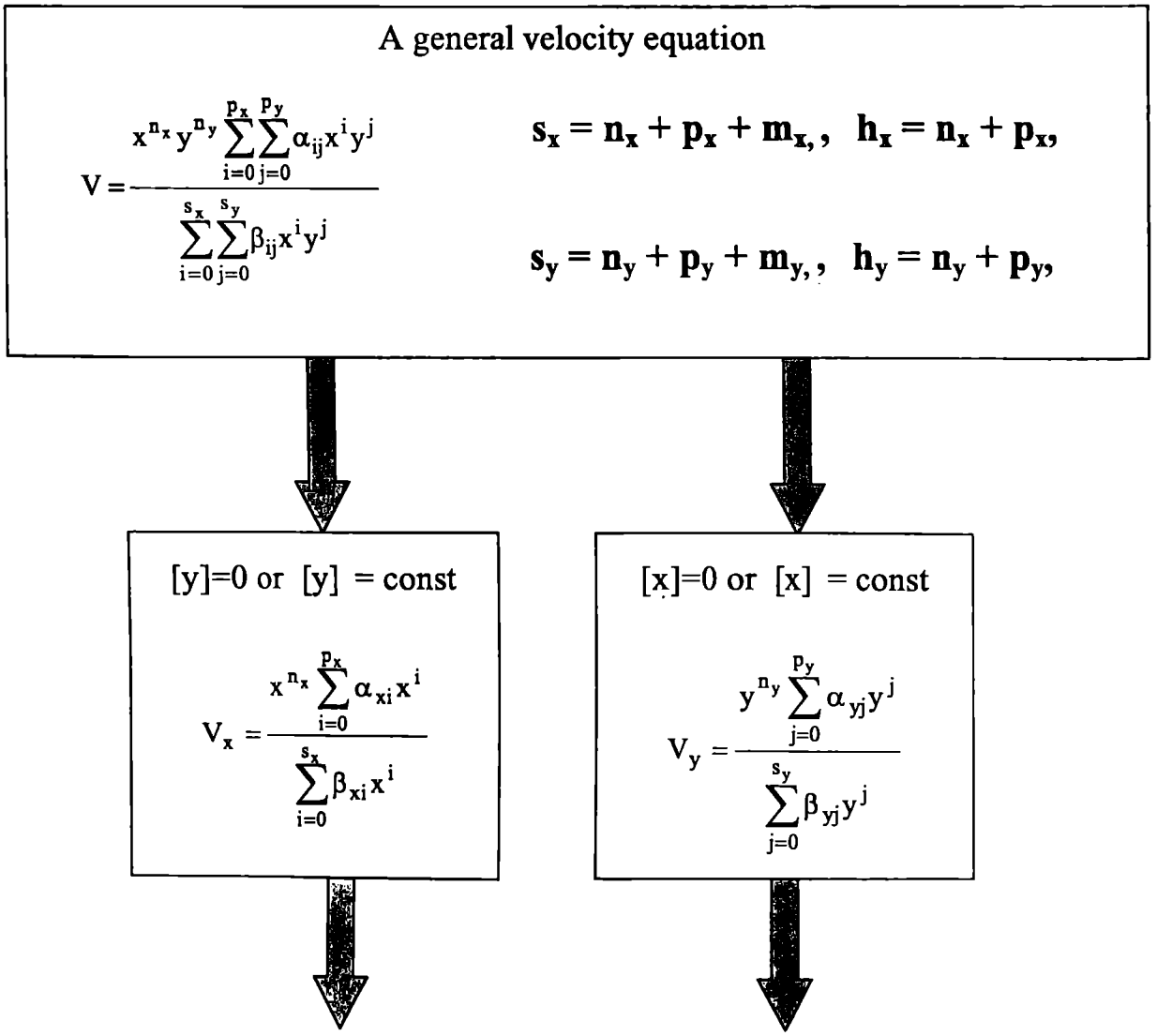
Z. Kometiani – "Kinetic analysis of the multi-sited enzyme systems"

At the start of the last century a kinetic theory of single-site enzyme systems was formulated and the technique for their full kinetic analysis was perfected. Its essence consisted in the transformation of a hyperbolic Function into linear dependence that is characteristic only for a single-site systems. It has been found later that many enzymes because of their multi-site character are characterized by a curve-linear kinetic dependence. This necessitated to formulate the principles and elaborate new kinetic parameters for the analysis of such curves.

Z. Kometiani in his monograph presents for the first time the theoretical bases of complete deciphering of molecular mechanism of the multi-site enzyme system and based on experimental date develops a statistically valid method for the measurement of main kinetic parameters.

Summary. Scheme of exploration of multi-sited enzyme systems.

Suppose the enzyme velocity depends on two arguments $V=f(x,y)$, of which one (x) represents a substrate or one of the modifiers, while the other (y) represents a modifier in relation to the substrate or to the first modifier. A complete kinetic study of such enzyme system proceeds in terms of following scheme:



For $V_x = f(x)$

1. Determination of the number of sites for essential activators and main kinetic parameters of activation.

$\sqrt[n]{(1/V)} = a_n + b_n(1/x) \Rightarrow n, K_A$ და V_A. (§ 4.4)

2. Determination of the number of sites for full inhibitors and main kinetic parameters of inhibition.

$\sqrt[m]{(1/V)} = a_m + b_m(x) \Rightarrow m, K_I$ და V_I. (§ 4.5)

3. Determination of the minimal number of sites for the ligands of partial effect of activation or inhibition and their main kinetic parameters.

$(U' = 0 \ , \ U'' = 0) \Rightarrow p_{min}$,

$\{\overline{\ln V} = \ln V_p + \bar{\omega}(\ln x)$ or $\overline{U} = a_p + b_p(1/x)\} \Rightarrow \overline{V_p}, \overline{K_p}$ (Chapter 6)

The same for $V_y = f(y) \Rightarrow$ [n, K_A and V_A];
[m, K_I and V_I];
[p_{min}, $\overline{V_p}$ and $\overline{K_p}$]

Analysis of geometrical shape of $Int = f(y)$, $Sl = f(y)$ and $t_0 = f(y)$ functions and establishment of classification group and subgroup of y modifier. (§ 7.1, 7.2 and 7.3)

Deciphering of the enzyme system molecular mechanism.

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