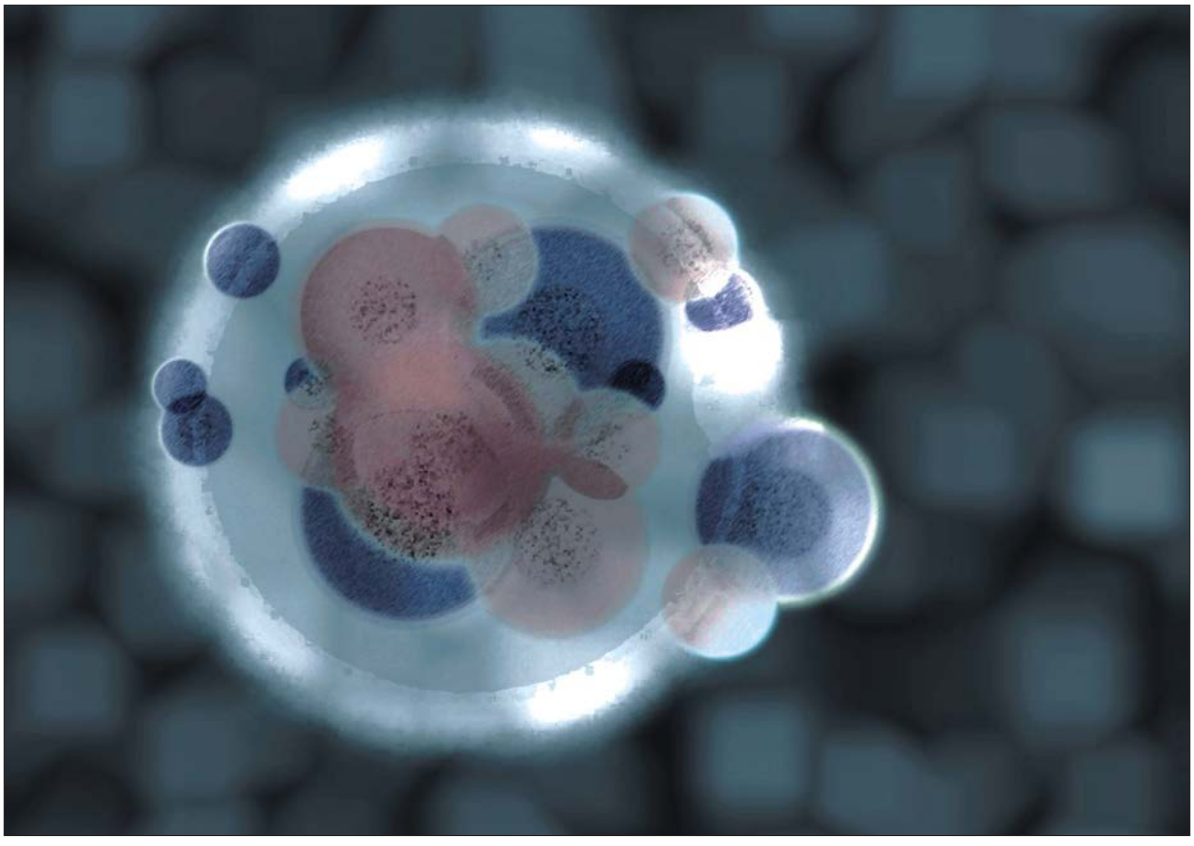

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Additional Possibility of Data Analysis of Enzyme Inhibition and Activation. 1: Equations for Calculation of the K_a and K_i Constants of Enzyme Activation and Nontrivial Types of Enzyme Inhibition

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Abstract: By taking into consideration symmetry of the position of L_i vectors of enzymatic inhibited reactions to L_a vectors of activated enzymatic reactions in the three-dimensional $K'_mV'I$ coordinate system and symmetric antidirectivity of tendencies in the course of change of K'_m and V' parameters of inhibited and activated enzymatic reactions similar by the type, a parametric classification of the types of enzymatic reactions was proposed, the equations for calculation of K_a constants of enzyme activation and K_i constants of nontrivial types of enzyme inhibition were obtained and some corrections introduced into practice of using the coordinates of intercepts and slopes for calculation of these constants. Examples of calculation of K_a and K_i constants are given.

Key words: Three-dimensional $K_m'V'I$ coordinate system, equation of K_a and K_i constants

INTRODUCTION

Enzymes are widely used in biotechnology and laboratory practice[1-3]. The search for new possibilities and improvement of the existing ones that have already become conventional for studying the properties and specificity of these biocatalysts will secure their more successful application.

In enzyme kinetics the procedures and methods of data processing were developed by taking into account antidirectivity of enzyme activation to enzyme inhibition, which is so simple in the course of change of initial reaction rates:

$$v_a > v_0,\ v_i < v_0 \quad (1)$$

Numerous endeavors to take into account this antidirectivity by using the coordinates of slopes[4-12] for data processing in enzyme activation:

$$\left(\frac{K'_m}{V'};\frac{1}{a}\right) \text{ and the coordinates of intercepts } \left(\frac{1}{V'};\frac{1}{a}\right) \quad (2)$$

both obtained by reversion of the concentrations of activator (1/a) in the known coordinates of slopes[6-11]:

$$\left(\frac{K'_m}{V'}:i\right) \text{ and intercepts: } \left(\frac{1}{V'}:i\right) \quad (3)$$

seem incorrect as it is unclear how this antidirectivity (Eq. 1) is realized in the course of change of the secondary K'_m and V' parameters. The incorrectness of this approach was proved by using a vector method of representation of enzymatic reactions[13-16].

The three-dimensional $K'_mV'I$ system of coordinates: If to construct the $K'_mV'I$ coordinate system in such a way that it would be possible to mark on the horizontal $0K'_m$ axis the numerical values of K'_m parameters of inhibited and activated enzymatic reactions and on the vertical $0V'$ axis - the numerical values of V' parameters of the same reactions; to intersect the axes belonging to the base σ_0 plane in the point $P(K^o_m;V^o;0)$ to restore from the obtained point the combined Pa,i semiaxis representing the molar concentrations of inhibitor (i) and activator (a); draw the σ_{IVi}, σ_{IIIi}, σ_{IVa}, σ_{IIIa} direction planes - which are reciprocally perpendicular between themselves and perpendicular to the base σ_0 plane - through this semiaxis and each of the direction PK'_m, $P0_v$ $P0_{Km}$, PV' semiaxes, one can obtain a coordinate system convenient for representation of data on enzyme inhibition and activation (Fig. 1), where, K'_m

and V′ are numerical values of the effective Michaelis constants and the maximum reaction rates determined in the presence of i (a); K°_m and V° are the parameters of initial (uninhibited, i=0 and nonactivated, a=0) enzymatic reaction (I_0). According to numerical values of K'_m and V′ parameters, every inhibited or activated enzymatic reaction in such coordinate system will have its own vector representation - a concrete (individual) three-dimensional L vector of this reaction (Fig. 1). The initial reaction (I_o) will be represented by a zero vector (L_0), the point $P(K^0_m;V^0;0)$ of intersection of the coordinate axes[13-16].

Thus,

a) the position of L_i vector in this system of coordinates will characterize the type of reaction
b) the length of L_i vector:

$$l_i = \sqrt{(K'_m - K^0_m)^2 + (V' - V^0)^2 + (i - 0)^2} \quad (4)$$

will characterize intensity of enzyme inhibition or activation
c) the area overlapped by the mobile end of L_i (or L_a) vector at change in the concentration of i(a) parameter:

$$S_i = 0.5.\left(\begin{vmatrix} K'_{m1} & V'_1 \\ K'_{m2} & V'_2 \end{vmatrix}^2 + \begin{vmatrix} V'_1 & i_1 \\ V'_2 & i_2 \end{vmatrix}^2 + \begin{vmatrix} i_1 & K'_{m1} \\ i_2 & K'_{m2} \end{vmatrix}^2 \right)^{0.5} \quad (5)$$

will be a measure of the overall effect of i(a) on the enzyme at change in the concentration
d) a trajectory made by the mobile end of L vector will represent an individual (characteristic) curve, i. e. a summarized geometrical portrait of the process under study (enzyme activation or inhibition)
e) constancy of the slope angles of the mobile end of L vectors at change in the concentration of i(a):

$$\text{tg } \varphi = \text{const and tg } \epsilon = \text{const} \quad (6)$$

will serve a criterion of stability of the mechanism of proceeding of the studied reaction.

Analysis of the position of L vectors in the $K'_mV'I$ coordinate system (Fig. 1) and the position of their *L* projections on the base σ_o plane in the scalar two-dimensional K'_mV' system of coordinates (Fig. 2) reveals that the number of types of activated enzymatic reactions is equal to the number of types of inhibited enzymatic reactions and that symmetric antidirectivity of the effect of enzyme activation to the effect of enzyme nhibition (Eq. 1) is realized by strictly symmetric antidirectivity in the course of change of K'_m and V′ parameters of inhibited enzymatic reactions versus activated enzymatic reactions similar by the type (Table 1, lines: A3 and A13; A4 and A12, etc.). This allows the construction of a parametric classification comprising fifteen types of reactions: seven types of inhibited enzymatic reactions (I_i, II_i, III_i, IV_i, V_i, VI_i, VII_i), seven types of activated enzymatic reactions (I_a, II_a, III_a, IV_a, V_a, VI_a, VII_a) and one type of initial (I_o) enzymatic reaction.

Symmetry of the position of reactions similar by the type (Table 1), symmetry of the position of L vectors of the same reactions by the respective elements in the three-dimensional $K'_mV'I$ coordinate system and also symmetry of vector projections in the two-dimensional K'_mV' coordinate system (Fig. 1 and 2) permit to take into account symmetric antidirectivity in the course of change of the K'_m and V′ parameters and slope angles of respective plots of inhibited and activated enzymatic reactions similar by the type (Table 1: lines A3 and A13, A4 and A12, etc.), which makes it possible to introduce the following corrections in data processing of enzyme activation: (1) If a course of change in the slope angles ($\text{tg } \omega' = K'_m/V'$) of respective plots for inhibited and activated enzymatic reactions similar by the type and the position of L vectors of these reactions in the $K'_mV'I$ coordinate system demonstrates symmetric antidirectivity of tendencies in the course of change of K'_m and V′ parameters of these reactions (Table 1), then, the conventional coordinates of slopes used for data processing of enzyme activation (Eq. 2) must be corrected by taking into account this antidirectivity, which may be expressed as:

$$\left(\frac{V'}{K'_m} : a \right) \text{ and the coordinates of intercepts-as: } (V';a) \quad (7)$$

instead of using the coordinates (Eq. 2)[6-11].
(2). As the known[17-21] equation for calculation of K_{IVi} constants of the associative (or competitive, according to the traditional classification) type of enzyme inhibition:

$$K_{IVi} = \frac{i}{\frac{K'_m}{K^0_m} - 1} = \frac{i}{\frac{K'_m V^0}{K^0_m V'} - 1} = \frac{i}{\frac{\text{tg}\omega'}{\text{tg}\omega^0} - 1} \quad (8)$$

includes multiplicity of increase in the slope angles ($\text{tg } \omega'$) of plots for reactions inhibited by IV_i type relative to the slope angle ($\text{tg } \omega^0$) of the plot of initial (uninhibited) reaction (line. A4). Hence, the equation for calculation of K_{IVa} constants of the associative IV_a type of enzyme

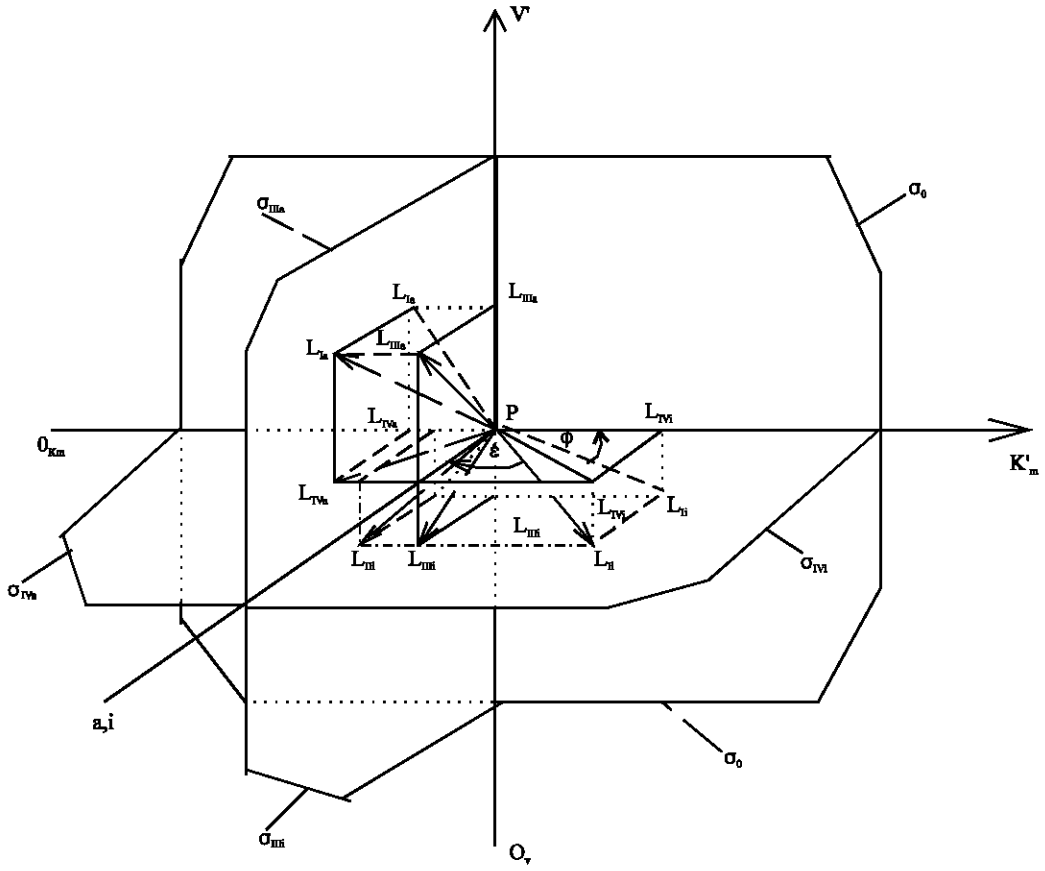


Fig. 1: Three-dimensional $K'_mV'I$ coordinate system with a coincident Pa, i semi-axis, the kinetic parameters: K'_m, K°_m, V' vectors: L_{IVi}, ... L_{Ii} and their scalar projections: L_{IVi}, L_{Ii}... and also of planes σ_{IVi}, σ_{IIIi}, σ_{IVa}... are given in the text

activation must include a symmetrically opposite multiplicity of decrease in the slope angles (tg ω°/ tg ω') of plots of the IV_a type of reactions, which is realized experimentally (Table 1, lines. A4 and A12):

$$K_{IVa} = \frac{a}{\frac{tg\omega^0}{tg\omega'} - 1} = \frac{a}{\frac{K'^0_m V'}{K'_m V^0} - 1} = \frac{a}{\frac{K^0_m}{K'_m} - 1} \quad (9)$$

Symmetric antidirectivity of the slope angles tg ω' and tg ω° in Eqs. (9) and (8) is in accord with the position of appropriate L_{IVa} and L_{IVi} vectors in the $K'_mV'I$ three-dimensional coordinate system, their L_{IVa} and L_{IVi} projections in the two-dimensional K'_mV' coordinate system (Fig. 1, 2) and the experimentally obtained position of plots of appropriate reactions in the double-reciprocal (v^{-1};S^{-1}) coordinates (lines. A12 and A4; A13 and A3, etc.). This allows derivation of the equations for calculation of respective K_a constants of enzyme activation (Eqs. A9-A15) and K_{Vi}, K_{IIi}, K_{VIi}, K_{VIIi} constants absent in practice of calculation of nontrivial V_i and II_i, VI_i, VII_i types of enzyme inhibition (Eqs. A5, A2, A6, A7).

Analysis of line. A2 (Table 1) reveals that in the case of calculation of K_{IIi} constants of the unassociative type enzyme inhibition the projections of vector L_{IIi} on the base σ_0 plane will have the following correlation in the interval of their positivity:

$$K^0_m - K'_m, V^0 - V', \quad (10)$$

and the equation for calculation of K_{IIi} constants of this type of enzyme inhibition:

$$K_{IIi} = \frac{i}{\frac{K^0_m V^0}{K'_m V'} - 1} \quad (11)$$

which does not operate the slope angles tg ω' and tg ω° angles of plots II and 0 (line. A2). From this equation it follows that in the $(\frac{K_m^0 V^0}{K`_m V`};i)$ or $(\frac{1}{K`_m V`};i)$ coordinates the appropriate graphs will intersect the abscissa in the point: -i=K_{Ii} and this equation restricts application of $(\frac{1}{K`_m};i), (\frac{1}{V`};i)$ and other analogous coordinates, which are widely used for calculation of K_{Ii} constants of enzyme inhibition[22-24].

From the position of graphs VI and 0; graphs VII and 0 (Lines. A6 and A7, Table 1) characterizing the types VI_i and VII_i of discoordinated and transient enzyme inhibition, respectively, it is easy to see that all these cases are manifested experimentally by change in the slope angles: tg ω'>tg ω° (Line A6) and tg ω'<tg ω° (Line A7) and hence, the equation for calculation of constants of the type VI_i of enzyme inhibition will have the form:

$$K_{VIi} = \frac{i}{\frac{tg\omega'}{tg\omega^0} - 1}. \quad (12)$$

and the equation for calculation of K_{VIIi} constants of the VII_i type of enzyme inhibition will have the form:

$$K_{VIIi} = \frac{i}{\frac{tg\omega^0}{tg\omega'} - 1}, \quad (13)$$

A situation with the position of graph V of the V_i type of enzyme inhibition relative to graph 0 of initial (uninhibited) reaction is more simple; graph V of inhibited reaction is located above graph 0 and intersects it in the point to the right of the ordinate at tg ω'> tg ω° (Line A5). The equation for calculation of K_{Vi} constants of the V_i type of enzyme pseudoinhibition that takes into account the experimental position of graphs V and 0 will have the form:

$$K_{Vi} = \frac{i}{\frac{tg\omega'}{tg\omega^0} - 1} = \frac{i}{\frac{K'_m V^0}{K_m^0 V'} - 1}. \quad (14)$$

It follows from Eq. 14 that the coordinates $(K'_m/V';i)$ used by enzymologists for determination of K_{vi} (but actually K_{IVi}) constants of enzyme inhibition ignore the correlation between V′ and V° parameters[25,26].

The coordinates of intercepts: In enzyme kinetics the equation (À1, Table 1) for calculation of K_{Ii} constants of the coordinated I_i type of enzyme inhibition:

$$K_{Ii} = \frac{i}{\frac{K'_m V^0}{K_m^0 V'} - 1} = \frac{i}{\frac{tg\omega'}{tg\omega^0} - 1}. \quad (15)$$

has long been known. In the monograph[21] it is given in the form:

$$\frac{K'_m}{V'} = \frac{K_m^0}{V^0}.\left(1 + \frac{i}{K_{is}}\right) \quad (16)$$

for calculation of the position of graphs I and 0 (Fig. A1) in the $(K`_m/V`;i)$ coordinates of slopes, but it is more frequently used in its angular form:

$$\frac{tg\omega'}{tg\omega^0} = \frac{1}{K_{Ii}}.i + 1 \quad (17)$$

or in the form:

$$tg\omega' = tg\omega^0.\frac{1}{k_{Ii}}.i + tg\omega^0 \quad (18)$$

for calculation of the K_{is} slope constants of enzyme inhibition[6-10].

In data processing of such type of enzyme inhibition both the coordinates of slopes and the coordinates of intercepts (Eq. 3) for calculation of the so-called K_{is}- slope and - intercept constants of enzyme inhibition are widely used[5-12].

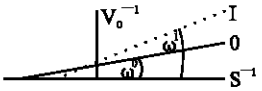
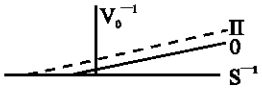
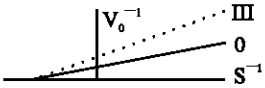
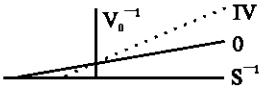
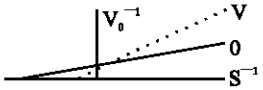
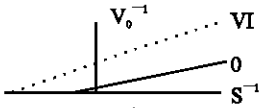
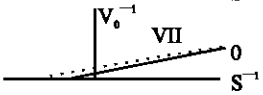
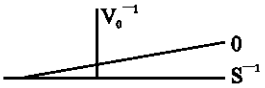
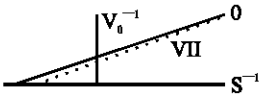
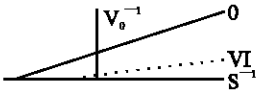
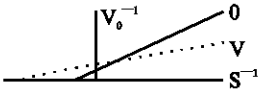
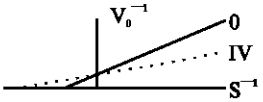
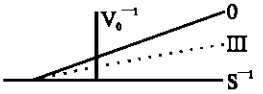
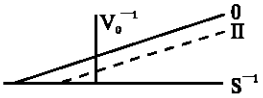
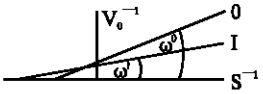
By comparing Eqs. (À3), (À1) and Eq. (16), one can easily see that if to take into account the ratio of parameters ($K'_m = K_m^{\circ}$, $V'<V^{\circ}$) characterizing the catalytic III_i type of enzyme inhibition, it will be right to simplify the ratio of the coordinates of slopes as follows:

$$(\frac{tg\omega'}{tg\omega^0};i) \rightarrow (\frac{K'_m V^0}{K_m^0 V'};i) \rightarrow \left(\frac{V^0}{V'};i\right) \rightarrow \left(\frac{1}{V'};i\right). \quad (19)$$

Hence, the $(1/V';i)$ coordinates of intercepts can only be used in data processing of the III_i type of enzyme inhibition and it will be incorrect to use these coordinates in case of the I_i type and other types (II_i, V_i, VI_i, VII_i) of biparametrical enzyme inhibition, because, as it follows from Eqs. 15 and 16:

$$\frac{1}{V'} = \frac{K_m^0}{K'_m V^0}.\frac{1}{K_{is}}.i + \frac{K_m^0}{K'_m V^0}, \quad (20)$$

Table 1: Equation for calculation of the constants of enzyme activation and inhibition

No	Effect	Type of effect	Graphs in the (v_o^{-1}; S^{-1}) coordinates	Correlation between the K'_m and V' parameters
1	inhibition (i>0)	I_i		$K'_m>K^0_m$, $V'<V^0$
2		II_i		$K'_m<K^0_m$, $V'<V^0$
3		III_i		$K'_m=K^0_m$, $V'<V^0$
4		IV_i		$K'_m>K^0_m$, $V'=V^0$
5		V_i		$K'_m>K^0_m$, $V'>V^0$
6		VI_i		$K'_m<K^0_m$, $V'<V^0$
7		VII_i		$K'_m<K^0_m$, $V'<V^0$
8	None	I_o		$K'_m=K^0_m$, $V'=V^0$
9		VII_a		$K'_m>K^0_m$, $V'>V^0$
10		VI_a		$K'_m>K^0_m$, $V'>V^0$
11	Activation (a>0)	V_a		$K'_m<K^0_m$, $V'<V^0$
12		IV_a		$K'_m<K^0_m$, $V'=V^0$
13		III_a		$K'_m=K^0_m$, $V'>V^0$
14		II_a		$K'_m>K^0_m$, $V'>V^0$
15		I_a		$K'_m<K^0_m$, $V'>V^0$

Continued

New name of the types of enzymatic reaction	Traditional	Equation for calculating the K_i and K_a constants	Number in Table 1
Biparametrically coordinated inhibition (coordinated)	Mixed inhibition	$K_{Ii} = \frac{i}{K`_m V^0/K^0_m V` - 1}$	(A 1)
Unassociative inhibition (unassociative)	Uncompetitive inhibition	$K_{IIi} = \frac{i}{K^0_m V^0/K`_m V` - 1}$	(A 2)
Catalytic inhibition (catalytic)	Noncompetitive inhibition	$K_{IIIi} = \frac{i}{V^0/V` - 1}$	(A 3)
Associative inhibition (associative)	Competitive inhibition	$K_{IVi} = \frac{i}{K`_m/K^0_m - 1}$	(A 4)
Pseudoinhibition (pseudoinhibition)		$K_{Vi} = \frac{i}{K`_m V^0/K^0_m V` - 1}$	(A 5)
Discoordinated inhibition (discoordinated)		$K_{VIi} = \frac{i}{K`_m V^0/K^0_m V` - 1}$	(A 6)
Transient inhibition (transient)		$K_{VIIi} = \frac{i}{K^0_m V`/K`_m V^0 - 1}$	(A 7)
Initial (uninhibited $i = 0$ and nonactivated $a = 0$) enzymatic reaction			(A 8)
Transient activation (transient)		$K_{VIIa} = \frac{a}{K`_m V^0/K^0_m V` - 1}$	(A 9)
Discoordinated activation (discoordinated)		$K_{VIa} = \frac{a}{K^0_m V`/K`_m V^0 - 1}$	(A 10)
Pseudoactivation (pseudoactivation)		$K_{Va} = \frac{a}{K^0_m V`/K`_m V^0 - 1}$	(A 11)
Associative activation (associative)	Competitive activation	$K_{IVa} = \frac{a}{K^0_m/K`_m - 1}$	(A 12)
Catalytic activation (catalytic)	Noncompetitive activation	$K_{IIIa} = \frac{a}{V`/V^0 - 1}$	(A 13)
Unassocaitive activation (unassociative)	Uncompetitive activation	$K_{IIa} = \frac{a}{K`_m V`/K^0_m V^0 - 1}$	(A 14)
Biparametrically coordinated activation (coordinated)	Mixed activation	$K_{Ia} = \frac{a}{K^0_m V`/K`_m V^0 - 1}$	(A 15)

the course of change in the K'_m parameters of such reactions will turn out unchanged.

This fact gives grounds not to use the name of (1/V′;i) coordinates as "the coordinates of intercepts" and to remain the name "the coordinates of slopes" as they really are (Eq. 19) and use them only in data processing of the catalytic III_i type of enzyme inhibition with the name: the (1/V′;i) coordinates of slopes.

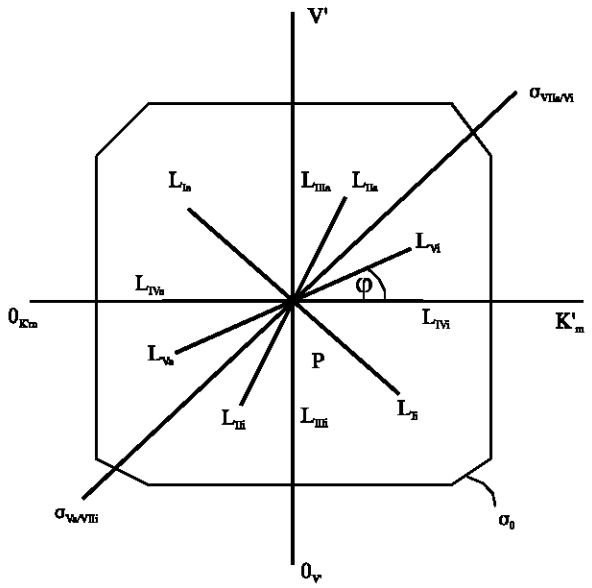


Fig. 2: Two-dimensional K'_mV' coordinate system. The kinetic K'_m, K^0_m, V' parameters and vector projections: L_{IVi}, L_{Ii} ... on the base σ_0 plane are given in Fig. 1. The projections of planes $\sigma_{VIIa/Vi}$ and $\sigma_{Va/VIIi}$ of a transient state between the $VII_a \Leftrightarrow V_i$ and $V_a \Leftrightarrow VII_i$ types of enzymatic reaction on the base plane are indicated with a dash line

The equations for calculation of the constants of enzyme activation (Table 1) were deduced and the coordinates of intercepts and slopes corrected (Eq. 7).

As seen in this case, the rejected name the (V′;a) coordinates of "intercepts" obtained as a result of ($K'_m = K_m^0$, $V'<V^0$) simplification of the more general form of the corrected coordinates of slopes:

$$\left(\frac{tg\omega^0}{tg\omega'};a\right)\rightarrow\left(\frac{V'K_m^0}{V'_mV^0};a\right)\rightarrow\left(\frac{V'}{V^0};a\right)\rightarrow(V';a) \qquad (21)$$

is only used in data processing of the III_a catalytic type of enzyme activation with retention of their name "the (V′;a) coordinates of slopes".

MATERIALS AND METHODS

Chemicals: Bovine pyrimidine specific RNase B (EC 3.1.27.5), a product of Sigma (USA).

Substrate: Cytidine-2′;3′-monophosphate sodium salt (C>p), a product of Serva (Germany).

Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), a crystalline salt of domestic make (high purity grade).

The process of substrate cleavage (C>p) was recorded on a CF-4 double-beam spectrophotometer (Optica Milano, Italy) by registering increase in the absorption density ($+\Delta A_{286}$) of a solution containing the substrate, enzyme and activator (MoO^{2-}_4) against a solution of the same composition without the enzyme. Reactions were carried out in 0.01M MES buffer, pH 6.0, with ionic strengh 0.1 by NaCl at constant stirring in a thermostat (21°C).

The concentration (C>p) was changed within $1.49 \cdot 10^{-4}$-$4.46 \cdot 10^{-4}$ M, the concentration of enzyme was kept constant -1.29 μg μL^{-1}. The concentration of MoO^{2-}_4 is given in the legend to Fig. 3.

Calf intestinal alkaline phosphatase (EC 3.1.3.1) - a product of Fluka (Switzerland).

Substrate: p-nitrophenylphosphate 2CH-salt (pNPP) - a product of Serva (Germany).

Inhibitor: pyrrolidine dithiocarboxylic acid (PDTA) - a crystalline salt of high purity grade.

The concentration of pNPP was changed within $2.94 \cdot 10^{-5}$-$9.8 \cdot 10^{-5}$ M and that of the enzyme was kept constant-1.96 μg mL^{-1}. Selection of substrates in experiments was stipulated by an interval of minimum error in the determination of K_m and V parameters[27].

The process of pNPP cleavage was recorded by the same spectrophotometer. Reactions were carried out in 0.05 M Tris-HCl buffer, pH 9.0, with ionic strength 0.1 (by NaCl of high purity) at constant stirring by registering the absorption increment ($+\Delta D_{400}$) of a solution containing the substrate and enzyme versus a solution of the same concentration, but without the enzyme.

Determination of enzyme activity: The initial rate (v) of p-nitrophenylphosphate cleavage by phosphatase and (C>p) by RNase B was calculated by a slope angle of tangents to the initial segments of curves representing the course of reactions determined in not less than 5 parallel experiments.

The kinetic V and K_m parameters of enzyme activation were calculated by plots in the (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk. The root-mean-square deviation at five measurements of v was ±2.5% and that of K_m, V, K_i and K_a parameters was ±7.5% (estimated by using the program Sigma Plot, Version 4.0.)

RESULTS AND DISCUSSION

Calculation of the K_{IIIa} constant of RNase B activation: Conventional analysis of the results (Fig. 3) reveals that enzyme activation enhanced in the presence of MoO^{2-}_4.

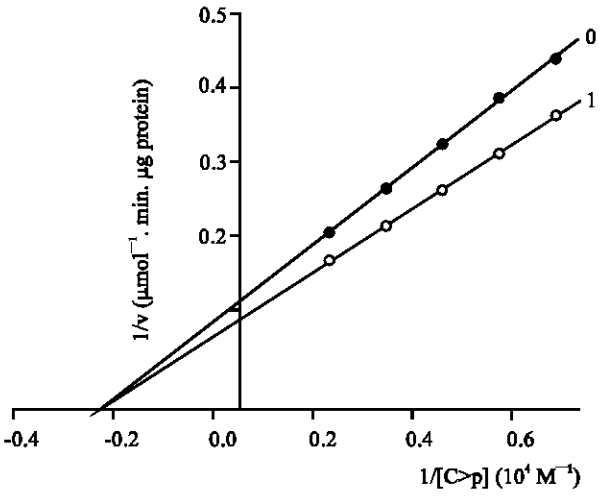


Fig. 3: Plots representing the activating effect of anions MoO^{2-}_4 on RNase B in the two-dimensional ($v^{-1};S^{-1}$) coordinates of Lineweaver-Burk. Designations: line 1 - the concentration of MoO^{2-}_4 is $1\cdot10^{-3}$ M. Line 0 - the activator is absent.

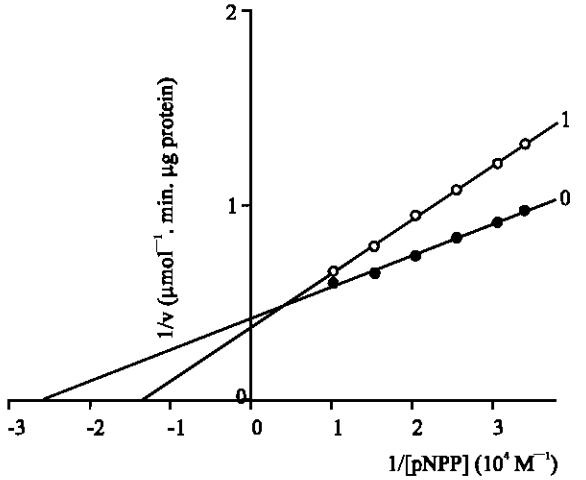


Fig. 4: Plots representing the inhibitory effect of PDTA on alkaline phosphatase in the two-dimensional ($v^{-1};S^{-1}$) coordinates of Lineweaver-Burk. Designations: line 1 - the concentration of PDTA is $1\cdot10^{-3}$ M. Line 0 - the inhibitor is absent

The experimental line 1 intersected line 0 of initial reaction in one point on the abscissa at a lesser slope angle to the abscissa. This is an example of the catalytic III_a type of enzyme activation (Table 1, line 13) and here, Eq. A13 must be used for calculation of the K_{IIIa} constant of enzyme activation. Substitution of $V'=10.62\cdot$µmol/min·µg protein determined in the presence of $1.0\cdot10^{-3}$ M of MoO^{2-}_4 and $V^0=8.83\cdot$ µmol/min·µg protein determined in the absence of activator ($K'_m = K^0_m = 4.35\cdot10^{-4}$ M) permits to estimate the constant:

$$K_{IIIa} = \frac{a}{\frac{V'}{V^0} - 1} = \frac{1.10^{-3}M}{\frac{10.62}{8.83} - 1} = 4.93\cdot10^{-3}\cdot M \quad (22)$$

However, in experimental practice and literature Eq. A13 is unknown and enzymologysts undertake attempts to calculate the K_{IIIa} constant of enzyme activation by construction of graphs in the $(tg\omega';\frac{1}{a})$ slope or $(\frac{1}{V'};\frac{1}{a})$ intercept coordinates[10,22]. It is easy to see that Eq. (A13) may be transformed as follows:

$$\frac{V^0}{V' - V^0} = K_{IIIa}\cdot\frac{1}{a} \quad (23)$$

and the experimental line in the $(\frac{V^0}{V' - V^0};\frac{1}{a})$ coordinates allows calculation of the K_{IIIa} constant as a slope angle of the line (Eq. 23) to the abscissa. But it is simpler to use the $(1/tg\omega';a)$ slope coordinates where the straight line:

$$\frac{1}{tg\omega'} = \frac{1}{tg\omega^0}\cdot\frac{1}{K_{IIIa}}.a + \frac{1}{tg\omega^0} \quad (24)$$

intersects the abscissa in the point: $-a = K_{IIIa}$.

Calculation of K_{Vi} constant of the V_i type of enzyme pseudinhibition: The experimental line 1 of enzyme inhibition is located above line 0 of initial reaction and intersects it in the point to the right of the ordinate (Fig. 4). This is a feature of the V_i type of enzyme pseudoinhibition (Line A5, Table 1). Eq. A5 must be used for calculation of the K_{Vi} constant of calf alkaline phosphatase inhibition by PDTA. Substitution of $K'_m = 7.3\cdot10^{-5}$ M, $V' = 2.67\cdot$µmol/min·µg protein determined in the presence of $1.0\cdot10^{-3}$ M of PDTA and $K^0_m = 3.83\cdot10^{-5}$ M, $V^0 = 2.39$ µmol/min·µg protein determined in the absence of inhibitor allows estimation of the constant:

$$K_{Vi} = \frac{1.10^{-3}M}{\frac{K'_m V^0}{K^0_m V'} - 1} = \frac{1.10^{-3}M}{\frac{7.3\cdot2.39}{3.83\cdot2.67} - 1} = 1.42\cdot10^{-3}\,M \quad (25)$$

Eq. A4 is very often used for calculation of constants of such type of enzyme. Inhibition, which is referred to

competitive inhibition[25,26]. Use of Eq. A4 (instead of Eq. 25) for calculation of the K'_{IVi} constant of enzyme inhibition leads to decrease in their values, because the correlation between V° and V′ parameters is not taken into consideration.

Other examples of calculation of the constants of enzyme activation and nontrivial types of inhibition: If authors only use the K'_m, $K°_m$, V′ and V° parameters and do not constract the respective plots in the (v^{-1};S^{-1}) coordinates of Lineweaver-Burk, the choice of equations for calculation of the K_i and K_a constants may present difficulty, especially in data processing of nontrivial types of enzyme inhibition and activation.

Example 1: Marchesini *et al.*[28] studied the effect of dithiothreitol on the activity of neutral spingomyelinase2 (nSMase2) and revealed that the initial parameters of enzyme activity ($K°_m$=27 μM, V° = 15.8 nmol/h·μg) in the presence of 5 μM dithiothreitol changed as follows: K'_m = 2.4 μM, V′ = 0.43 nmol/h·μg. In this article the respective plot demonstrating the effect of DTT is given. By having found the position of experimental line 0 of initial reaction, it is easy to establish by the coordinates of points of its intersection with the abscissa: $1/K°_m = -1/S_0 = -0.037\ \mu M^{-1}$ and $1/V° = 0.063\ (nmol/h·\mu g)^{-1}$ that the studied effect of dithiothreitol on nSMase2 is an example of discoordinated VI_i type of enzyme inhibition ($K'<K_m°, V'<V°$), since here: $K'_m/V'>K°_m/V°$ (see the position of lines VI and 0, Line A6) and hence, to calculate the K_{VIi} constant of enzyme inhibition, the equation (A6) is applicable:

$$K_{VIi} = \frac{5\,\mu M}{\frac{2.4\cdot 15.8}{27\cdot 0.43} - 1} = 2.20\,\mu M \qquad (26)$$

from where it follows that the strength of binding of dithiotreitol to the enzyme ($K°_m/K_{VIi}$) exceeds by 12.27 times that of the enzyme to the substrate.

Example 2: Hirano *et al.*[29] studied the effect of ribosomal protein L5 isolated from rat liver ribosomes as the L5-5 S RNA complex on activity of protein phosphatase type 1 (PP1) and found that the activity of enzyme ($K°_m$= 4.4 μM, V° = 0.65 μmol/min·mg protein) changed in the presence of 0.5 μM of L5 as follows; K'_m = 3.7 μM, V′ = 1.14 μmol/min·mg·protein. This is an example of coordinated I_a type of enzyme activation ($K'_m<K°_m$, $V'>V°$) (Line A15). Substitution of all necessary parameters in the equation (A15):

$$K_{Ia} = \frac{0.5\,\mu M}{\frac{4.4\cdot 1.14}{3.7\cdot 0.65} - 1} = 0.46\,\mu M \qquad (27)$$

shows that strength of binding of L5 protein to the enzyme ($K°_m/K_{Ia}$) exceeds by 9.6 times that of the enzyme to the substrate.

Nomenclature

- K'_m and V′ - effective Michaelis constant and maximum reaction rate determined in the presence of the inhibitor (i) or activator (a)
- $K°_m$ and V° - the same parameters of initial (uninhibited, i=0 and nonactivated, a=0) enzymatic reaction
- (I_i, II_i, III_i, IV_i, V_i, VI_i, VII_i), - the types of inhibited enzymatic reactions
- (I_a, II_a, III_a, IV_a, V_a, VI_a, VII_a)- the types of activated enzymatic reactions
- v_i and v_a - initial (unspecified) rate of inhibited and activated enzymatic reactions
- v_{IVi} - the initial rate of enzymatic reaction inhibited by the associative IV_i type of enzyme inhibition
- K_{IVi} - the constant of associative enzyme inhibition
- L_i - the a three-dimensional vector of inhibited reactions
- L_a - a three-dimensional vector of activated reactions
- L_i and L_a - the scalar projections of these vectors on the base σ_0 plane
- L_{IVi} - the three-dimensional vector of reaction inhibited by the associative IV_i type of enzyme inhibition.
- II_i - earlier suggested II''_i,
- VI_i - earlier suggested II'''_i,
- VII_i - earlier suggested II'_i[13,30,31]

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