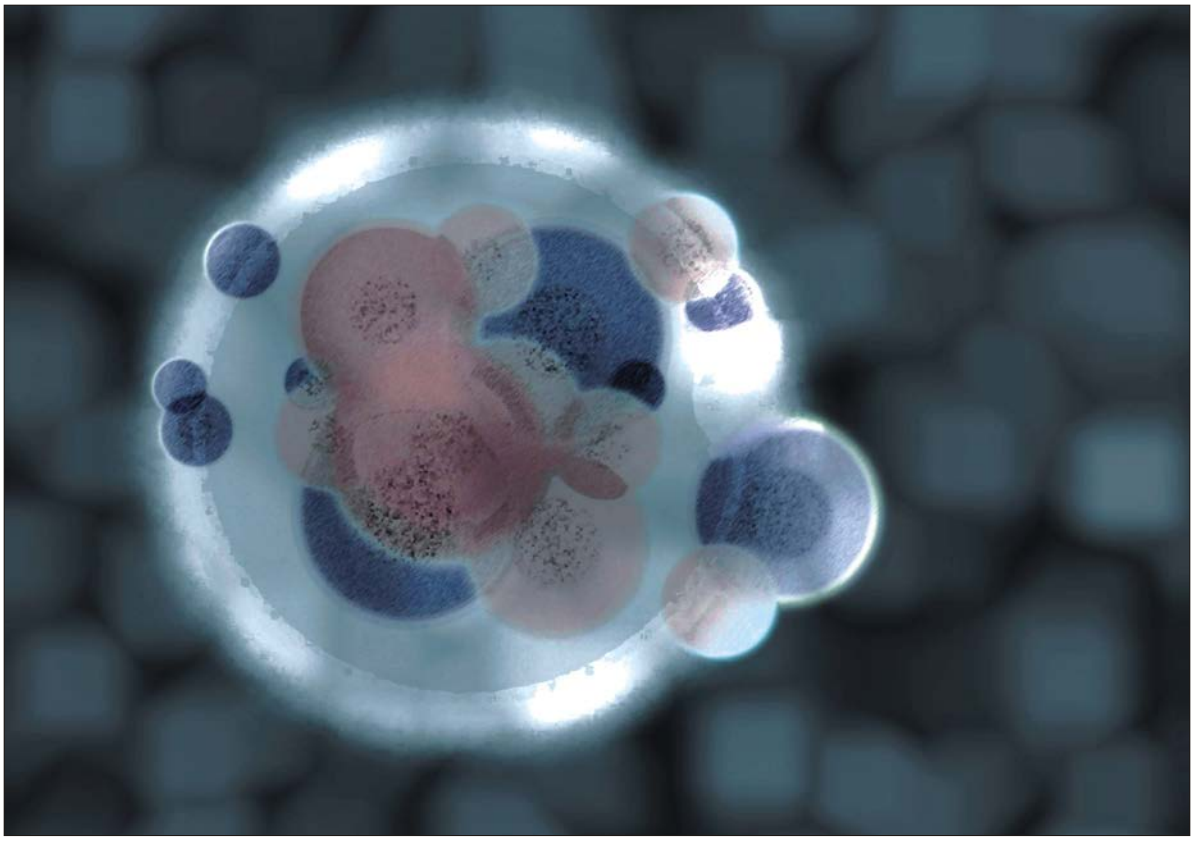

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Additional Possibility of Data Analysis of Enzyme Inhibition and Activation. 6. A Rule of Choice of the Equations and Coordinates of Slopes for Calculation of the K_i and K_a Constants of Enzyme Inhibition and Activation

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Abstract: The developed vector method of representation of enzymatic reactions in the three-dimensional coordinate $K'_mV'I$ system revealed the possibility of existence of 7 types of inhibited (I_i, II_i, III_i, IV_i, V_i, VI_i and VII_i), 7 types of activated (I_a, II_a, III_a, IV_a, V_a, VI_a and VII_a) and one type (I_0) of initial (uninhibited i=0 and nonactivated a=0) enzymatic reaction in a simple one-substrate scheme of Michaelis-Menten. Based on symmetry of vectors for inhibited (L_i) and activated (L_a) enzymatic reactions, the equations for calculation of K_{Ia}, K_{IIa}, K_{IIIa}, K_{IVa}, K_{Va}, K_{VIa} and K_{VIIa} constants of enzyme activation and K_{IIi}, K_{Vi}, K_{VIi} and K_{VIIi} constants of nontrivial types of enzyme inhibition were obtained and also the corrected coordinates of slopes for calculation of these constants. Taking into account the three known equations for calculation of K_{IVi}, K_{IIIi} and K_{Ii} constants of enzyme inhibition, it made up 14 equations for calculation of the K_i and K_a constants. A rule of choice of the equations and coordinates of slopes for calculation of these constants is given as well as the examples.

Key words: A rule of choice of the equations and coordinates of slopes for calculation of K_i and K_a constants, examples of calculation

INTRODUCTION

The developed vector method of representation of enzymatic reactions in the three-dimensional $K'_mV'I$ coordinate system (Fig. 1 and 2) allowed completion of a parametric classification of the types of enzymatic reactions[1,2], which takes into account parametricity of reactions, i.e., the number of changing, K'_m, V' (i or a) parameters and the direction of a course of their change.

Thus, the equations for calculation of K_a constants of enzyme activation and the K_{IIi}, K_{Vi}, K_{VIi} and K_{VIIi}, constants of nontrivial types of enzyme inhibition were deduced[1-3]. Now their number is 14:7 equations for calculation of K_i constants (Eq. 1-7, Table 1) and 7 equations for calculation of K_a constants (Eq. 9-15). This required to formulate a rule of choice of the equations and coordinates of slopes needed for calculation of the constants in each particular case.

A rule of choice of the equations and coordinates of slopes for calculation of the K_i and K_a constants of enzyme inhibition and activation: The rule of choice is the same for calculation of both K_i and K_a constants of enzyme inhibition and activation:

a) let us take as an example the reaction of para-nitrophenyl phosphate (pNPP) cleavage by porcine alkaline phosphatase (EC 3.1.3.1) inhibited by sodium tungstate ($Na_2WO_4 \cdot 2H_2O$);
b) line 1 of the reaction under study is plotted by experimental points (Fig. 3A) and considered relative to line (0) of initial (uninhibited i=0) reaction in the double reciprocal (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk;
c) then, having found the analogous plot in Table 1, the respective type of reaction and the equation for K_i calculation (Eq. A1) are established as well as the secondary coordinates of slopes for calculation of this constant at increasing concentrations of inhibitor (i) (Table 2 and line 1).

ENZYME INHIBITION

Type I_i of enzyme inhibition: By comparing plots (Fig. 3A in the text and A1 in Table 1), it is easy to see that they are similar by their position in the (v^{-1}; S^{-1}) coordinates, which corresponds to the I_i type of porcine alkaline phosphatase inhibition. Hence, to calculate the K_{Ii} constant, one must use Eq. (A1). Substitution in this

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

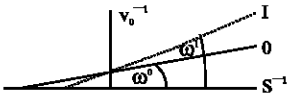
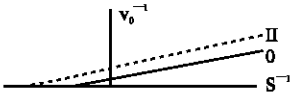
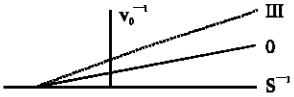
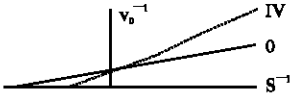
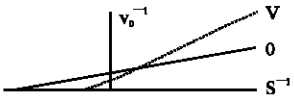
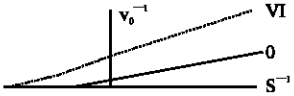
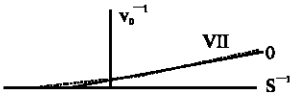
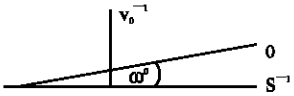
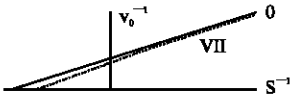
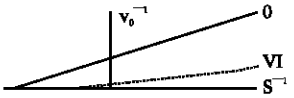
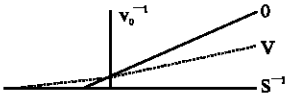
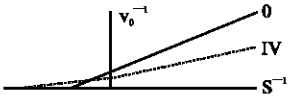
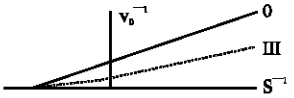
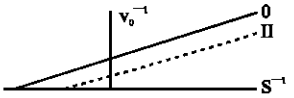
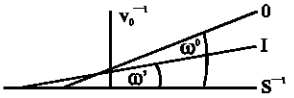
No.	Effect	Type of effect	Graphic in the (v_0^{-1}; S^{-1}) coordinates	Coerrelation between the K'_m and V' parameters
1	Inhibition (i>0)	I		$K'_m>K^0_m$, $V'<V^0$
2		II_i		$K'_m<K^0_m$, $V'<V^0$
3		III_i		$K'_m=K^0_m$, $V'<V^0$
4		IV_i		$K'_m>K^0_m$, $V'=V^0$
5		V_i		$K'_m>K^0_m$, $V'>V^0$
6		VI_i		$K'_m<K^0_m$, $V'<V^0$
7		VII_i		$K'_m<K^0_m$, $V'<V^0$
8	None	I_0		$K'_m=K^0_m$, $V'=V^0$
9	Activation (a>0)	VII_a		$K'_m>K^0_m$, $V'>V^0$
10		VI_a		$K'_m>K^0_m$, $V'>V^0$
11		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		$K'_m<K^0_m$, $V'>V^0$

Table 1: Continue

New names of the types of enzymatic reactions	Traditional	Equations for calculation of the K_i and K_a constants	Number in Table 1
Biparametrically coordinated inhibition (coordinated)	Mixed inhibition	$K_{Ii}=\frac{i}{K'_m V^0/K^0_m V'-1}$	(A1)
Unassociative inhibition (unassociative)	Uncompetitive inhibition	$K_{IIi}=\frac{i}{K^0_m V^0/K'_m V'-1}$	(A2)
Catalytic inhibition (catalytic)	Noncompetitive inhibition	$K_{IIIi}=\frac{i}{V^0/V'-1}$	(A3)
Associative inhibition (associative)	Competitive inhibition	$K_{IVi}=\frac{i}{K'_m/K^0_m-1}$	(A4)
Pseudoinhibition (pseudoinhibition)		$K_{Vi}=\frac{i}{K'_m V^0/K^0_m V'-1}$	(A5)
Discoordinated inhibition (discoordinated)		$K_{VIi}=\frac{i}{K'_m V^0/K^0_m V'-1}$	(A6)
Transient inhibition (transient)		$K_{VIIi}=\frac{i}{K^0_m V'/K'_m V^0-1}$	(A7)
Initial (uninhibited i = 0 and nonactivated a = 0) enzymatic reaction			(A8)
Transient activation (transient)		$K_{VIIa}=\frac{a}{K'_m V^0/K^0_m V'-1}$	(A9)
Discoordinated activation (discoordinated)		$K_{VIa}=\frac{a}{K^0_m V'/K'_m V^0-1}$	(A10)
Pseudoactivation (pseudoactivation)		$K_{Va}=\frac{a}{K^0_m V'/K'_m V^0-1}$	(A11)
Associative activation (associative)	Competitive activation	$K_{IVa}=\frac{a}{K^0_m/K'_m-1}$	(A12)
Catalytic activation (catalytic)	Noncompetitive activation	$K_{IIIa}=\frac{a}{V'/V^0-1}$	(A13)
Unassocaitive activation (unassociative)	Uncompetitive activation	$K_{IIa}=\frac{a}{K'_m V'/K^0_m V^0-1}$	(A14)
Biparametrically coordinated activation (coordinated)	Mixed activation	$K_{Ia}=\frac{a}{K^0_m V'/K'_m V^0-1}$	(A15)

equation of all the required experimental parameters: $K'_m = 12.15\cdot10^{-5}$ M and V' = 4.95 μmol/min.μg protein obtained as a result of pNPP cleavage by the above enzyme in the presence of $(1.5\cdot10^{-5}$ M) WO_4^{2-}, $K^0_m=5.45\cdot10^{-5}$ M and V^0 = 9.36 μmol/min. μg protein of initial (uninhibited) reaction yields the expression:

$$K_{Ii}=\frac{i}{\frac{K'_m V^0}{K^0_m V'}-1}=\frac{1.5\cdot10^{-5}M}{\frac{12.15\cdot9.36}{5.45\cdot4.95}-1}=\frac{1.5\cdot10^{-5}}{3.216}=0.466\cdot10^{-5}M \quad (1)$$

Yet, in laboratory practice researchers tend to use the following technique: having established the i dependence of a course of change in the slope angles of lines 1, 2, 3 and 4 for inhibited reaction (Fig. 3B) within the concentrations of i: $0.25\cdot10^{-5}$- $2\cdot10^{-5}$ M, they calculate the K_{is} slope constants of enzyme inhibition from intersection of the abscissa axis in the $(K'_mV^0/K^0_mV'$; i) coordinates by the secondary plot:

$$\frac{K'_m V^0}{K^0_m V'}=\frac{1}{K_{is}}\cdot i+1, \quad (2)$$

where, $-i = K_{is} = K_{Ii}$ (Fig. 3C) or by the plot:

$$tg\omega' = tg\omega^0\cdot\frac{i}{K_{is}}+tg\omega^0, \quad (3)$$

intersecting the abscissa axis at the same point: $-i = K_{is} = K_{Ii}$ in the coordinates $(K'_m/V'$; i), if a series of increasing i concentrations are employed.

The explanation is simple, if to write Eq. (A1) in its angular form:

$$K_{Ii}=\frac{i}{\frac{K'_m V^0}{K^0_m V'}-1}=\frac{i}{\frac{tg\omega'}{tg\omega^0}-1}, \quad (4)$$

Table 2: The corrected coordinate slopes for calculation of the K_i and K_a constant of enzyme inhibition and activation

No.	Type of effect	Coordinates for calculation of the K_i and K_a constants
1	I_i	$\left(\frac{K_m' V^0}{K_m^0 V'};i\right), \left(\frac{K_m'}{V'};i\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};i\right)$ and $\left(tg\cdot\omega';i\right)$
2	II_i	$\left(\frac{K_m^0 V^0}{K_m' V'};i\right), \left(\frac{1}{K_m' V'};i\right), \left(\frac{1}{n_i^2};i\right), \left(\left(\frac{V^0}{V'}\right)^2;i\right)$ and $\left(\left(\frac{K_m^0}{K_m'}\right)^2;i\right)$
3	III_i	$\left(\frac{K_m' V^0}{K_m^0 V'};i\right), \left(\frac{V^0}{V'};i\right), \left(\frac{1}{V'};i\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};i\right)$ and $\left(tg\cdot\omega';i\right)$
4	IV_i	$\left(\frac{K_m' V^0}{K_m^0 V'};i\right), \left(\frac{K_m'}{K_m^0};i\right), \left(K_m';i\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};i\right)$ and $\left(tg\cdot\omega';i\right)$
5	V_i	$\left(\frac{K_m' V^0}{K_m^0 V'};i\right), \left(\frac{K_m'}{V'};i\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};i\right)$ and $\left(tg\cdot\omega';i\right)$
6	VI_i	$\left(\frac{K_m' V^0}{K_m^0 V'};i\right), \left(\frac{K_m'}{V'};i\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};i\right)$ and $\left(tg\cdot\omega';i\right)$
7	VII_i	$\left(\frac{K_m^0 V'}{K_m' V^\circ};i\right), \left(\frac{V'}{K_m'};i\right), \left(\frac{tg\omega^\circ}{tg\omega'};i\right)$ and $\left(\frac{1}{tg\omega'}\right)$
8	I_0	
9	VII_a	$\left(\frac{K_m' V^0}{K_m^0 V'};a\right), \left(\frac{K_m'}{V'};a\right), \left(\frac{tg\cdot\omega'}{tg\cdot\omega^0};a\right)$ and $\left(tg\cdot\omega';a\right)$
10	VI_a	$\left(\frac{K_m^0 V'}{K_m' V^0};a\right), \left(\frac{V'}{K_m'};a\right), \left(\frac{tg\cdot\omega^0}{tg\cdot\omega'};a\right)$ and $\left(\frac{1}{tg\cdot\omega'};a\right)$
11	V_a	$\left(\frac{K_m^0 V'}{K_m' V^0};a\right), \left(\frac{V'}{K_m'};a\right), \left(\frac{tg\cdot\omega^0}{tg\cdot\omega'};a\right)$ and $\left(\frac{1}{tg\cdot\omega'};a\right)$
12	IV_a	$\left(\frac{K_m^0 V'}{K_m' V^0};a\right), \left(\frac{K_m^0}{K_m'};a\right), \left(\frac{1}{K_m'};a\right), \left(\frac{tg\cdot\omega^0}{tg\cdot\omega'};a\right)$ and $\left(\frac{1}{tg\cdot\omega'};a\right)$
13	III_a	$\left(\frac{K_m^0 V'}{K_m' V^0};a\right), \left(\frac{V'}{V^0};a\right), \left(V';a\right), \left(\frac{tg\cdot\omega^0}{tg\cdot\omega'};a\right)$ and $\left(\frac{1}{tg\cdot\omega'};a\right)$
14	II_a	$\left(\frac{K_m' V'}{K_m^0 V^0};a\right), \left(K_m' V';a\right), \left(n_a^2;a\right), \left(\left(\frac{V'}{V^0}\right)^2;a\right)$ and $\left(\left(\frac{K_m'}{K_m^0}\right)^2;a\right)$
15	I_a	$\left(\frac{K_m^0 V'}{K_m' V^0};a\right), \left(\frac{V'}{K_m'};a\right), \left(\frac{tg\cdot\omega^0}{tg\cdot\omega};a\right)$ and $\left(\frac{1}{tg\cdot\omega'};a\right)$

where:

$$tg\omega' = \frac{K_m'}{K_m^0} \tag{5}$$

and

$$tg\omega^0 = \frac{K_m^0}{V^0} \tag{6}$$

are the slope angles of plots of inhibited reaction (Eq. 5) and line (0) (Fig. A1) of initial reaction to the abscissa axis (Fig. 3B). As seen from Fig. 3C, the experimental line (Eq. 2) intersects the abscissa axis at the point: $-i = K_{is} = K_{Ii} = 0.513 \cdot 10^{-5}$ M, the value, which was obtained by use of the program Sigma Plot 4.0, USA as:

$$-i = K_{is} = K_{Ii} = b(0)/b(1), \quad (7)$$

where, b(0) and b(1) - are symbols of the parameters taken from in Linear Regression of the program.

The deviation of K_{Ii} values in the 1st ($0.466 \cdot 10^{-5}$ M) and 2nd case ($0.513 \cdot 10^{-5}$ M) does not exceed the experimental error (±9.2%). However, the 2nd approach is more preferable as it takes into account the scattering of K_{Ii} values at separate concentrations of inhibitor.

Besides, for data processing (Fig. 3B, as an example) in the (K'_m/V'; i) coordinates[4-6], researchers often tend to plot the i dependencies of a course of change in the 1/V' parameters in the secondary (1/V'; i) coordinates of intercepts for calculation of K_{ii} intercept constants of inhibition of the same enzyme by intersection of such plots at: $-i = K_{ii}$[4-7].

It follows from line 3 (Table 1) that due to the equality $K'_m = K^0_m$ in data processing of the III_i type of enzyme inhibition the classical coordinates of slopes ($tg\omega'/tg\omega^0$; i) may be simplified to the form of the (1/V'; i) coordinates of slopes:

$$\left(\frac{tg\omega'}{tg\omega^0}; i\right) \rightarrow \left(\frac{K'_m V^0}{K^0_m V'}; i\right) \rightarrow \left(\frac{V^0}{V'}; i\right) \rightarrow \left(\frac{1}{V'}; i\right). \quad (8)$$

Hence, it is incorrect to use the monoparametrical (1/V'; i) coordinates of slopes for data processing of the I_i type and other biparametrical II_i[8] and VI_i[9] types of enzyme inhibition, because a course of change in the K'_m parameters of such reactions is not taken into consideration.

Equation (A1) has long been known in literature[10], yet in most cases investigators who obtain experimental data analogous to those given in Fig. 3B usually calculate the K'_{Ii} constants by Eq. A3 (Table 1) and characterize this type as noncompetitive inhibition or use Eq. (A4) and call such type as competitive inhibition etc. Obviously, a change in the K'_m and V' parameters of such reactions will not be taken into account in the 1st (Eq. A3) and 2nd case (Eq. A4) that will lead to greater errors of the calculation of K'_{Ii} in both cases.

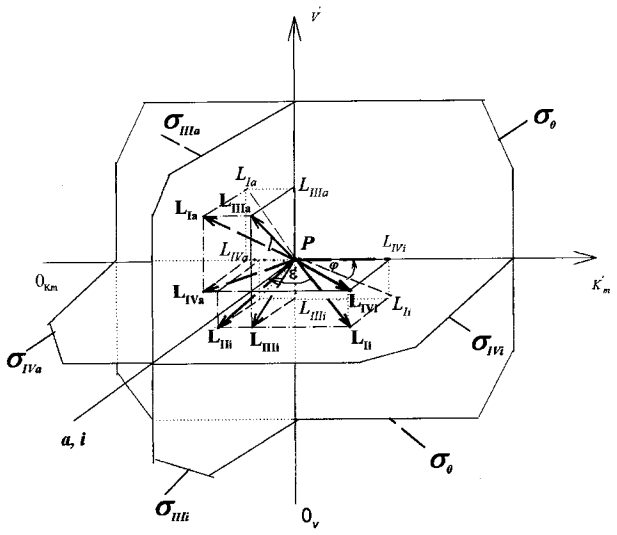


Fig. 1: Three-dimensional $K'_m V'I$ coordinate system with a coincident Pa, i semi-axis, the description of kinetic parameters: K'_m, K^0_m; V', vectors: L_{IVi}, ... L_{Ii} and their scalar projections: L_{IVi}... L_{Ii} and also of planes σ_{IVi}, σ_{IIIi}, σ_{IVa}... are given in the text

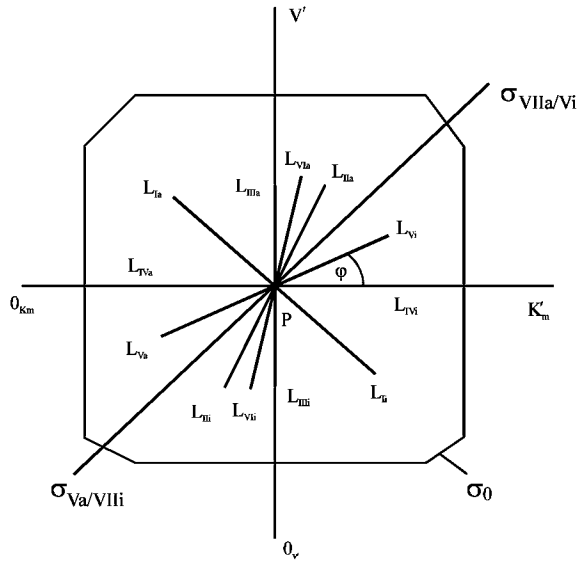


Fig. 2: Two-dimensional $K'_m V'$ coordinate system. The K'_m, K^0_m; V', parameters and vector projections: L_{IVi}...L_{Ii} on the base σ_0 plane are given in Fig. 1. The projections of planes $\sigma_{VIIa/Vi}$ and $\sigma_{Va/VIIi}$ of a transient state between the $VII_a \Leftrightarrow V_i$ and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

Type II_i of enzyme inhibition: This type is characterized by $tg\omega' = tg\omega^0$ - the equality of slope angles of line II of inhibited reaction located parallel to and over line (0) of initial (uninhibited i = 0) reaction (Table 1 and line 2).

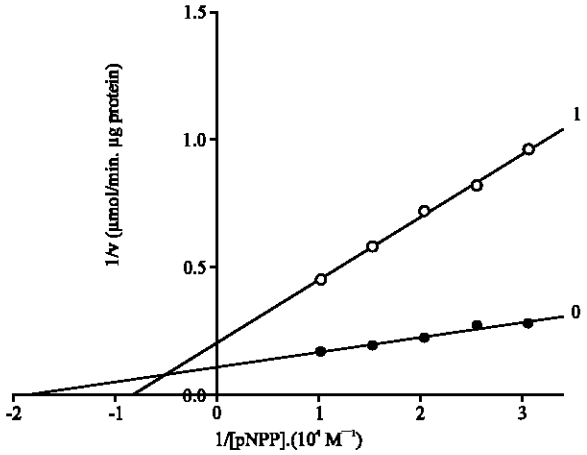


Fig. 3A: Inhibitory effect of WO_4^{2-} on initial rates of pNPP cleavage catalysed by porcine alkaline phosphatase. Line 1 - the concentration of WO_4^{2-} is $1.5 \cdot 10^{-5}$ M. Line 2 - the inhibitor is absent

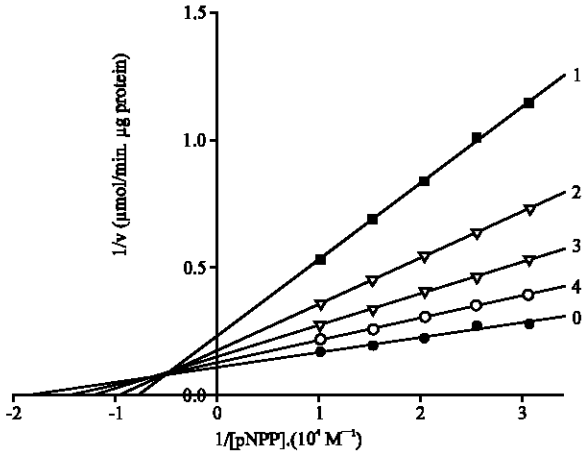


Fig. 3B: Inhibitory effect of increasing concentrations of WO_4^{2-} on pNPP cleavage catalysed by porcine alkaline phosphatase. Line 1 - the concentration is $2 \cdot 10^{-5}$ M, line 2-$1 \cdot 10^{-5}$ M, line 3-$0.5 \cdot 10^{-5}$ M, line 4-$0.25 \cdot 10^{-5}$ M, line 0 - the inhibitor is absent

For calculation of K_{IIi} constants of enzyme inhibition, Eq. (A2) is applicable, if the single concentration of i is used, or either the secondary ($K'_mV'/K^0_mV^0$; i) coordinates, where the plot is built by the expression:

$$\frac{K'_mV'}{K^0_mV^0} = \frac{1}{K_{IIi}} \cdot i + 1, \qquad (9)$$

or the (K'_mV'; i) coordinates; where, the 2nd plot:

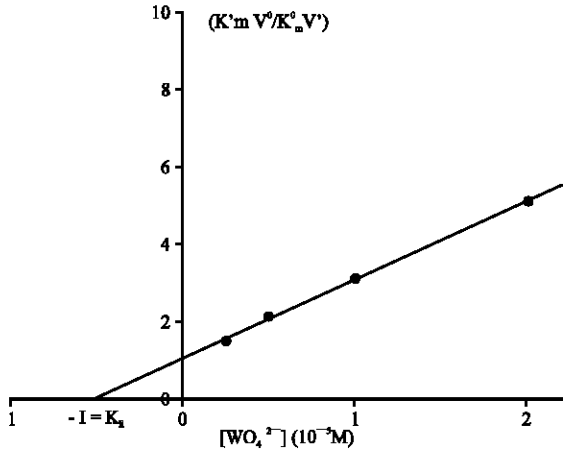


Fig. 3C: Dependence of a course of change in the ratio of parameters K'_mV^0/K^0_mV' of (Fig. 3B) upon the concentration of WO_4^{2-} in the $\left(\frac{K'_mV^0}{K^0_mV'}; i\right)$ coordinates

$$K'_mV' = K^0_mV^0 \cdot \frac{1}{K_{IIi}} \cdot i + K^0_mV^0 \qquad (10)$$

will intersect the abscissa axis at: $-i = K_{IIi}$.

As seen from Fig. A2, at parallel transfer of line II of inhibited reaction upwards of line (0) of initial reaction, i.e., when K'_m and V' parameters of inhibited reaction decrease by n_i times: $K'_m = (n_i) \cdot K^0_m$ and $V' = (n_i) \cdot V^0$ at $n_i < 1$, then, the denominator of Eq. (A2) may be transformed to:

$$K_{IIi} = \frac{i}{\frac{K^0_mV^0}{K'_mV'} - 1} = \frac{i}{\frac{1}{n_i^2} - 1}, \qquad (11)$$

or:

$$K_{IIi} = \frac{i}{\frac{K^0_mV^0}{K'_mV'} - 1} = \frac{i}{\left(\frac{V^0}{V'}\right)^2 - 1} = \frac{i}{\left(\frac{K^0_m}{K'_m}\right)^2 - 1}. \qquad (12)$$

Hence, to calculate the K_{IIi} constants of this type of enzyme inhibition one can also choose to plot the dependencies in either of these coordinates (Table 2 and line 2) as alternative instead of using the ($1/K'_m$; i) or ($1/V'$; i) coordinates recommended in very many studies[11,12].

Type III_i of enzyme inhibition: Its characteristic feature is that line III of inhibited reaction and line (0) of initial reaction intersect the abscissa axis in the double reciprocal (v^{-1}; S^{-1}) coordinates at: $K'_m = K^0_m$ (Table 1 and line 3). Data processing in this case has no difficulty: either the known Eq. (A3) for calculation of the K_{IIIi}

constants is used at the single i concentration and any of a variety of the secondary coordinates of slopes (Eq. 8), if a series of increasing concentrations are employed[13-15].

There are examples of using the data of this type of enzyme inhibition simultaneously in the coordinates of slopes and those of intercepts - actually, the same coordinates of slopes (Eq. 8), which should be manifested by coincidence of the points of intersection of the abscissa axis by both lines[5].

A great interest of enzymologists to use of Eq. (A3) is due to high informative value of the result obtained, i.e. the absence of interaction between the inhibitor and the active enzyme centre. This made them to apply that equation for data processing of some other biparametrically types of enzyme inhibition: the I_i[12,13] and VI_i types[12,13], which is incorrect as in this case a course of change in the K'_m parameters of these reactions is not taken into account.

Type IV_i of enzyme inhibition: The characteristic feature: line IV in Table 1 of inhibited reaction is located above line (0) of initial reaction and intersects it at the point ($V'=V^0$) on the ordinate axis in the double reverse (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk (Fig. A4). For calculation of i constants, Eq. (A4) is applicable, if the single concentration of i is used and any of the secondary coordinates of slopes (Table 2 and line 4), if increasing concentrations of i are used, where the experimental line will intersect the abscissa axis: $-i = K_{IVi}$. The latter technique is widely applied for calculation of the K_{IVi} constants[13,15,16].

Equations (A4) and (A3) are principal for calculation of the K_i constants of enzyme inhibition. Enzymologists have made numerous endeavors to apply these equations even in such cases, even when the experimental points of v^{-1} dependencies upon S^{-1} cannot be located on the plots, which corresponds to the IV_i[17] or III_i[17,18] types of enzyme inhibition. This explains the great interest of researchers to these equations and their disappointment by unavailability of information about the other equations of Table 1 and the coordinates for their calculation (Table 2) in any of the journals in the field of enzymology.

All the equations of Table 1 were published in Russian in 1986 and 1990[1,2].

Type V_i of enzyme inhibition: The characteristic feature: line V in Table 1 of inhibited reaction intersects line (0) of initial reaction at a point located to the right of the ordinate axis (Fig. A5). For calculation of the K_{Vi} constants of enzyme inhibition, Eq. (A5) can be applied and any of the secondary coordinates (Table 2 and line 5).

In most cases the investigators tend to refer the data on the V_i type of enzyme inhibition to the competitive IV_i type[19-21] and to use Eq. (A4) for calculation of K'_{Vi} constants, which is incorrect as then, a change of V' parameters is not taken into consideration.

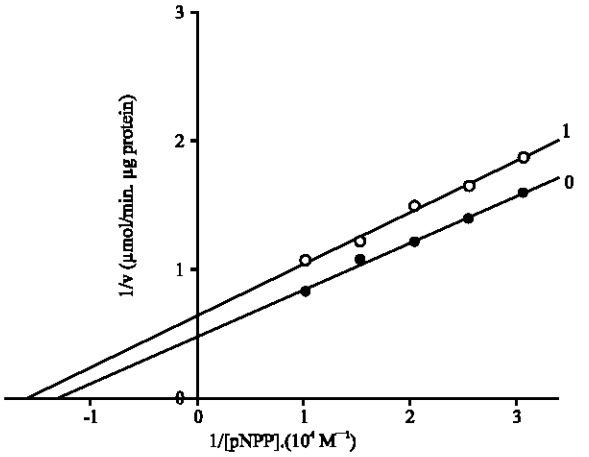


Fig. 4: Inhibitory effect of pNP on initial rates of pNPP cleavage catalysed by rabbit alkaline phosphatase. Line 1 - the concentration of pNP is $5 \cdot 10^{-5}$ M, line 0 - the inhibitor is absent

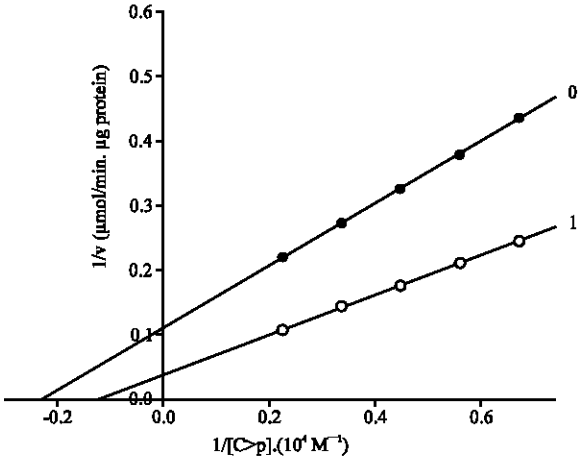


Fig. 5: Activating effect of $(NH_4)_6Mo_7O_{24}$ on the initial rates of C>p cleavage catalysed by bovine pyrimidine-specific RNase. Line 1 - the concentration of $(NH_4)_6Mo_7O_{24}$ is $5 \cdot 10^{-4}$ M, line 0 - the activator is absent

Type VI_i of enzyme inhibition: Interestingly, line VI in Table 1 of inhibited reaction intersects line (0) of initial reaction at the point located to the left below the abscissa axis (Fig. A6). For calculation of K_{VIi} constants, Eq. (A6) can be applied and any of the secondary coordinates (Table 2 and line 6).

In most cases enzymologists tend to refer the data on the VI_i type of enzyme inhibition to the noncompetitive III_i

type[9,14,15,22,23], but this is a biparametrical type of enzyme inhibition (Table 1) and use of the monoparametrical coordinates for data processing of the VI_i type is incorrect, because a change of K'_m into account.

Example of calculation of the K_{VIi} constant: As follows from experimental data (Fig. 4), the parameters $K^0_m = 7.58 \cdot 10^{-5}$ M and $V^0 = 2.09$ μmol/min. μg protein of pNPP cleavage by rabbit alkaline phosphatase (EC 3.1.3.1) in the presence of $5 \cdot 10^{-5}$ M para-nitrophenol (pNP) change their values to: $K'_m = 6.27 \cdot 10^{-5}$ M, $V' = 1.57$ μmol/min. μg protein. The position of lines is typical (Fig. A6 and Table 1): they ntersect in the 3rd quadrant of the double reciprocal (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk.

For calculation of the K_{VIi} constant, Eq. (A6) is applicable. Substitution of the obtained parameters allows calculation of the constant as:

$$K_{VIi} = \frac{i}{\frac{K'_m V^0}{K^0_m V'} - 1} = \frac{5 \cdot 10^{-5} M}{\frac{6.27 \cdot 2.092}{7.58 \cdot 1.57} - 1} = 4.902 \cdot 10^{-4} \text{ M}, \quad (13)$$

from where, it follows that the strength of enzyme binding to the neutral moiety of substrate cleaved is by (48.92/7.58 = 6.5) times weaker.

Using Eq. (A3) for calculation of the K'_{VIi} constant would have given the value $K'_{VIi} = 1.5 \cdot 10^{-4}$ M.

Type VII_i of enzyme inhibition Line VII in Table 1 of inhibited reaction intersects line (0) of initial reaction at a point located to the right of the interval of a scale of reverse concentrations of cleaved substrate in the double reciprocal (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk (Fig. A7)[23,24]. For calculation of K_{VIIi} constants, Eq. (A7) is applied and any of the secondary coordinates (Table 2 and line 7), where the experimental line will intersect the abscissa axis at $-i = K_{VIIi}$.

This type of enzyme inhibition is called a transient type in the parametric classification[2] because any transition of the point of intersection of line VII and line (0) (Table 1 and line 7) to the left along the abscissa axis will lead to the V_a type of enzyme activation after the middle point in the scale of reverse concentrations of cleaved substrate and vice verse (Fig. 7, 9 and Table 1). The interval of the scale of reverse concentrations of cleaved substrate should not be arbitrary. It must correspond to the conditions of minimal error in the calculation of K^0_m[25], i.e., the point of intersection of the abscissa axis by this constant must be located in the middle of the scale of reverse concentrations of cleaved substrate. If to turn to the right by 180^0 an extrapolated segment of this line coming out from the ordinate axis, it should intersect the scale of reverse concentrations of substrate on the positive branch of the abscissa axis by 180^0, at a transient point, where: $1/K^0_m = 1/S^{-1}_{mid}$ or in immediate proximity to this point. The position of vector trajectories marked in Fig. 2 with an extended line of a transient state between the types of reactions: $VII_i \Leftrightarrow V_a$ and $V_i \Leftrightarrow VII_a$ is determined by concrete (coincident) two: V_i and VII_a (or VII_i and V_a) parameters of the transient types of reactions.

ENZYME ACTIVATION

Analysis of the position of L_i vectors of inhibited reactions and L_a vectors of activated enzymatic reactions in the three-dimensional $K'_m V'I$ coordinate system (Fig. 1) as well as their projections on the base σ_0 plane (Fig. 2) permitted to establish that symmetric antidirectivity of the effects of enzyme activation and inhibition:

$$v_a > v_0 \qquad v_i < v_0 \quad (14)$$

is realized by symmetric antidirectivity of tendencies to a change in the K'_m and V' parameters and the slope angles ($tg\omega'$ and $tg\omega^0$) of experimental lines in reactions of enzyme activation and inhibition similar by the type (Table 1, Fig. A15 and A1; Fig. A13 and A3 etc). It was found that instead of the traditional coordinates of slopes:

$$\left(\frac{K'_m}{V'}; \frac{1}{a}\right) \text{ and intercepts } \left(\frac{1}{V'}; \frac{1}{a}\right), \quad (15)$$

it is necessary to use the corrected coordinates of slopes[3]:

$$\left(\frac{V'}{K'_m}; a\right) \text{ and } (V'; a), \quad (16)$$

taking into account symmetric antidirectivity of the effects (Eq. 14). Besides, some corrections in the practice of using the secondary coordinates of slopes for calculation of the K_a and K_i constants were made (Table 2).

Earlier, it was noted (see the I_i type of enzyme inhibition) that a rule of choice of the equations for calculation of the K_a constants of enzyme activation (Table 1) is the same as for calculation of the K_i constants of enzyme inhibition.

Example of calculation of the K_{VIa} constant: The activating effect of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ on the initial rate of cytidine-2′;3′-monophosphate cleavage catalysed by pyrimidine-specific RNase A (E.C 3.1.27.4) revealed that the parameters of initial reaction $K^0_m = 4.4 \cdot 10^{-4}$ M and $V^0 = 8.96$ μmol/min. μg protein in the presence of $5 \cdot 10^{-4}$ M ammonium molybdate changed as follows: $K'_m = 8.036 \cdot 10^{-4}$ M, $V' = 25.91$ μmol/min. μg protein (Fig. 5).

The position of lines is typical ($tg\omega' < tg\omega^0$) and is the same as in Table 1, Fig. A10. The lines intersect in the 3rd quadrant below the abscissa axis. This is the VI_a type of enzyme activation and Eq. A10 is quite applicable here for calculation of the K_{VIa} constant of RNase A activation.

Substitution of all the appropriate parameters of enzyme activation in this equation shows that:

$$K_{VIa} = \frac{a}{\frac{K_m^0 V'}{K_m' V^0} - 1} = \frac{5 \cdot 10^{-5} M}{\frac{4.4 \cdot 25.91}{8.03 \cdot 8.96} - 1} = \frac{5 \cdot 10^{-5} M}{0.563} = 8.85 \cdot 10^{-4} M \quad (17)$$

the strength of binding of the complex salt (ammonium molybdate) to the enzyme is twice as weaker than that of the enzyme to the substrate cleaved.

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