NON-EXISTENCE OF SECONDARY COORDINATES OF INTERSECTS

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Keywords: noncompetitive enzyme inhibition; corrected coordinates for calculation of enzyme inhibition and enzyme activation constants; secondary coordinates of intersects

The analysis of algebraic forms of corrected equations for the calculation of constants of enzyme inhibition (K_i) and activation (K_a) have shown that the secondary intersects coordinates (1/ V'; i) may be use for calculation only K_{IIIi} constants of noncompetitive enzyme inhibition. A few examples of application of corrected coordinates are given in the present research article.

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Introduction

In the already available reports[1-4] the possibilities of plotting the ($\mathbf{L}_i$) vectors of inhibited enzymatic reactions and ($\mathbf{L}_a$) vectors of activated enzymatic reactions in the three-dimensional $K'_mV'I$ coordinate system[1] and also of using the properties of such vectors for plotting the parametric classification of types of enzymatic reactions[2] and the deduction of equations for calculation of initial rates of activated (v_a) and inhibited (v_i) enzymatic reactions and also of equations for calculation of (K_a) constants of enzyme activation and (K_i) constants of non-trivial types of enzyme inhibition unavailable in usual practice are considered.

The present paper is dedicated to the possibility of using the secondary coordinates of slopes that take into account the orthogonal projections of $\mathbf{L}_i$ and $\mathbf{L}_a$ vectors of inhibited and activated enzymatic reactions on the basic (σ_0) plane of the $K'_mV'I$ coordinate system (Figure 1) for calculation of K_i and K_a constants.

Correction of the coordinates of slopes and intersects for calculation of constants of enzyme inhibition and activation

Deduction of equations for calculation of K_i constants of enzyme inhibition and K_a constants of enzyme activation[4] that take into account the position of orthogonal projections of $\mathbf{L}_i$ vectors of enzyme inhibition and $\mathbf{L}_a$ vectors of activated enzymatic reactions on the basic (σ_0) plane (Figures 1 and 2) opens up the possibility of plotting the secondary (corrected) coordinates of slopes for more accurate estimation of such constants.

Example 1

Calculation of the K_{Ii} constant of enzyme inhibition. Let us consider the inhibitory effect of increasing concentrations of sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) on initial rates of pNPP cleavage catalyzed by calf alkaline phosphatase (EC 3.1.3.1).

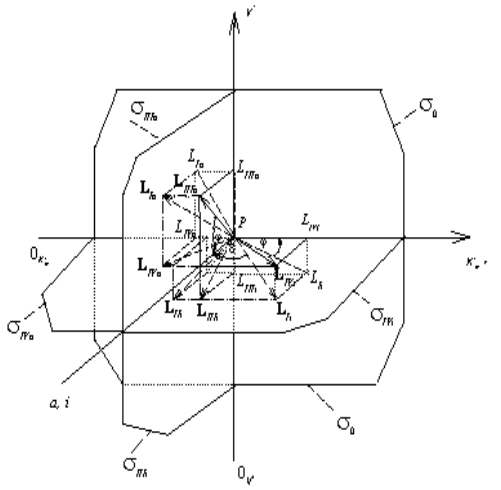


Figure 1. Three-dimensional (folded) $K'_mV'I$ coordinate system with coincident Pa,i semi-axis. The description of kinetic parameters: K'_m, K^0_m …; vectors $\mathbf{L}_{IVi}$ … $\mathbf{L}_{Ii}$ and their scalar projections L_{IVi} … L_{Ii} on the basic σ_0 plane and also of planes σ_{IVi}, σ_{IIIi}, $\sigma_{IV\alpha}$ is given in more detail in.[1,6]

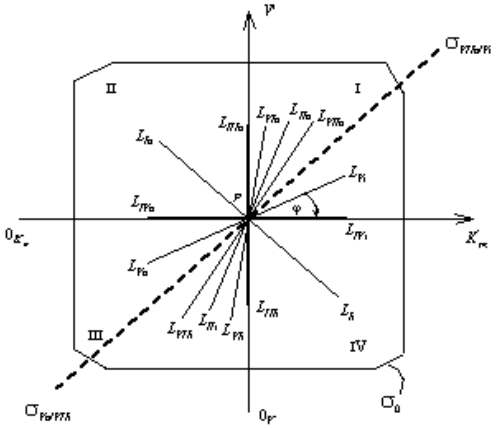


Figure 2. Two-dimensional (scalar) K'_mV' coordinate system. The description of kinetic parameters: K'_m, K^0_m, V' … and vector projections L_{IVi}, L_{Ii} … on the basic σ_0 plane. The projections of planes $\sigma_{VIIa/Vi}$ and $\sigma_{V\alpha/VIIi}$ of transient state between the $VII_a \Leftrightarrow V_i$ and $V_a \Leftrightarrow VII_i$ types of activated and inhibited enzymatic reactions on the σ_0 plane are marked with a dotted line.

 DOI: 10.17628/ECB.2014.3.815

Enzyme Activity Assay

Reactions were performed in 0.05 M Tris-HCl buffer (pH 9.0) at ionic strength 0.1 by NaCl of high purity under constant mixing[5] in a thermostat at 37 ^{0}C. The final concentrations of pNPP were varied within $2.94\cdot10^{-5}$ - $9.8\cdot10^{-5}$ M, the concentration of enzyme was constant 1.13 μg mL^{-1} and that of (10^{-4} M) $Na_2MoO_4\cdot2H_2O$ was varied within 0.0625 – 0.25·(Figure 3A). The course of pNPP cleavage by calf alkaline phosphatase was recorded by a CF-4 DR double-beam spectrophotometer (Optica Milano, Italy). Reactions were registered at the wave length ($+\Delta D_{400}$) of solution containing substrate, enzyme and inhibitor against the solution of the same composition, but without the enzyme.

Initial reaction rates ν_0 of pNPP cleavage were determined by the slope angles of tangents to initial segments of curves representing a course of reaction change in not less than five sets of parallel experiments.

The kinetic V and K_m parameters of calf alkaline phosphatase inhibition were calculated by plots in the (ν^{-1}, S^{-1}) coordinates of Lineweaver-Burk by using the program SigmaPlot, version 2000 (USA). Root-mean-square deviations at five-fold determination were as follows: ν=±2.5 %; K_m and V = ± 7.5 %, K_i (and K_a) = ± 10 %.

Results

As clearly observed from the results given in Figure 3A and Table 1 the increasing concentrations of MoO_4^{2-} exhibit all the features of the biparametrically coordinated I_i type ($K'_m>K^0_m$, $V'<V^0$, $i>0$) of enzyme inhibition and hence, we shall use (Eq. 1 of Table 2):

$$K_{Ii} = \frac{i}{\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}} \qquad (1)$$

for calculation of the K_{Ii} constant of enzyme inhibition.

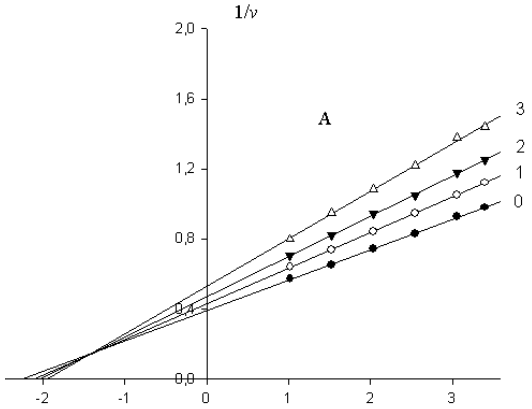


Figure 3A. Plots of inhibitory effect of anions MoO_4^{2-} on initial rates of pNPP cleavage by calf alkaline phosphatase. Designation, the concentration of MoO_4^{2-} (10^{-4} M) is: 0.0625 – line 1; 0.125 – line 2 and 0.25 – line 3. Line 0 – the inhibitor is absent; V μmol·min^{-1} μg protein^{-1}.

Table 1. Inhibitory effect of increasing concentrations of anions MoO_4^{2-} on calf alkaline phosphatase

Inhibitor, 10^{-4} M	**K'_m, 10^{-5} M**	**V' μmol·min^{-1} μg protein^{-1}**	**K_{Ii}, 10^{-4} M**	***A****
0	4.45	2.56		
0.0625	4.75	2.33	0.523	0.1195
0.125	4.91	2.13	0.551	0.2268
0.250	5.14	1.89	0.646	0.3869

**A* – according to Eq. (1), it is a dimensionless value.

Substitution of appropriate parameters in this equation yields the following values of the constant of enzyme inhibition: K_{Ii} (10^{-4} M): 0.523, 0.551 and 0.646 – by the first, second and third concentration of MoO_4^{2-}.

However, Eq. (1) provides for another more preferable option for calculation of such constants: plotting of dependencies of change in the values of denominators of this equation in the coordinates (A;i), where K_{Ii} of enzyme inhibition can be calculated by the slope angle (tg a) of the experimental line (Figure 3B):

$$A = \frac{1}{K_{Ii}} i + 0 \qquad (2)$$

to the abscissa axis:

$$K_{Ii} = \frac{1}{tga} \qquad (3)$$

where

A – a course of change of the denominator of Eq. (1) on increasing concentrations of MoO_4^{2-}.

Data analysis of Figure 3A by using the program Sigma Plot, version 2000 (USA) shows:

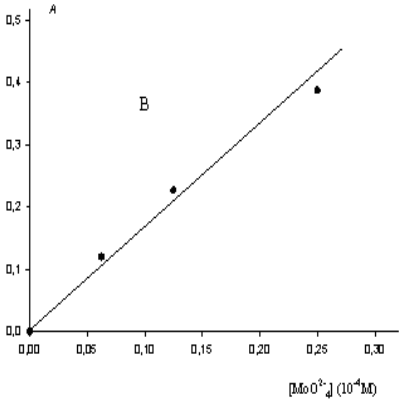


Figure 3B. Dependence of A parameters of Eq. (2) (Figure 3A) on increasing concentration of anions MoO_4^{2-} in the coordinates (A;i).

that the line of Eq. (2) goes via the origin of the coordinates at the slope angle to the abscissa axis (tg a) = [b(1) = $1.575\cdot10^4$ M^{-1}]: where: b(1) – a parameter of the program SigmaPlot 2000 (USA).

 DOI: 10.17628/ECB.2014.3.815

This gives the following (average) value of the K_{Ii} constant of calf alkaline phosphate: $K_{Ii}=1/(1.575\cdot10^4\ M^{-1})=0.635\cdot10^{-4}$ M.

At analogous data analysis (Figure 3A) a technique of plotting the dependencies of change in the ratio K'_m/V' of inhibited enzymatic reactions on molar concentrations of inhibitor i_{Ii} in the secondary coordinates of slopes (K'_m/V'; i) and the coordinates of intersects ($1/V'$; i) used for calculation of the K_{is} - slope constant and K_{ii} - intersects constant in enzyme inhibition is widely employed.[7-11]

Plotting the dependencies of change in the ratio K'_m/V' (Figure 3A) on the molar concentrations of i_{Ii} in the coordinates of slopes (K'_m/V'; i) (see Figure 3C, line 2) for calculation of K_{is} - slope-constant and the coordinates of intersects ($1/V'$; i) (Figure 3C, line 1) for calculation of K_{ii} - intersect-constant of enzyme inhibition.

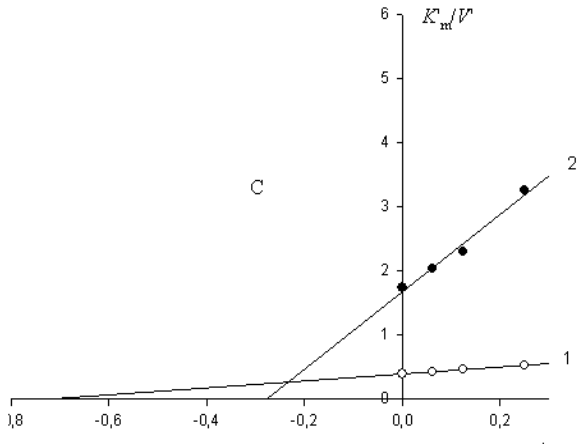


Figure 3C. Dependence of change in the ratio K'_m/V' (Figure 3A) on the concentration of MoO_4^{2-} in the coordinates of slopes (K'_m/K^0_m; i) – line 2 and the coordinates of intersects ($1/V'$; i) – line 1.

This gives the following values: $K_{is}= 0.275\cdot10^{-4}$ M in the first case and $K_{ii} = 0.715\cdot10^{-4}$ M – in the second case (Figure 3C), which neither in the second case nor in the first one would correspond the actual value: $K_{Ii} = 0.635\cdot10^{-4}$ M calculated by Eqn. 3.

Deviation between the values of constants K_{Ii} and K_{is}, K_{ii} can be explained as: at calculation of the latter, the lengths of orthogonal projections of $\mathbf{L}_{Ii}$ vectors on the basic σ_0 plane in the $K'_mV'I$ coordinate system (Figures 1 and 2) were not taken into account, and besides this, as shown below (Example 3), the coordinates of intersects ($1/V'$; i) are a simplified form (K'_m/V'; i) of the coordinates of slopes (Table 3), and plotting the dependencies in these coordinates does not take into account changes K'_m parameters of the biparametrical reactions, to which the data of Figure 3A are referred.

Examples of using the coordinates *(K'_m/V'; i) and ($1/V'$;i)* to data analysis of such type (Figure 3A) of enzyme inhibition are numerous in literature.[7-11]

The K^0_m and V^0 parameters of initial reaction (i= 0, or a =0) are present in all the equations of Table 2 used for calculation of K_i and K_a constants of enzyme inhibition and activation. This may lead to difficulties, for example, in search of a response to a query as to how shall we use these equations, if the values K^0_m and V^0 parameters of initial reaction have not been determined ? Such data are often available in experimental part of the reports in literature.[7,12,14,16]

The best possible answer here follows from analysis of plots of Figures 3A and 3B. Thus, as clearly observed from Figure 3B, the slope angles (tg a) of rectilinear segments of dependencies A = f(i) well coincide by tested intervals (Δi) of the concentrations of inhibitor: for $\Delta(i_1 - 0)$ = (0.0625 – 0) 10^{-4} M, where: $K_{Ii(0)} = 0.523\cdot10^{-4}$ M, for $\Delta(i_2 - i_1)$ = (0.125 – 0.0625) 10^{-4} M, whereе $K_{Ii(2)} = 0.551\cdot10^{-4}$ M, for $\Delta(i_3 - i_2)$ = (0.25 – 0.125) 10^{-4} M where $K_{Ii(3)} = 0.646\cdot10^{-4}$ M (see Table 1). Such coincidence of the results is evident.

Example 2

Calculation of the K_{VIa} constant of enzyme activation. Earlier, the activating effect of arginine-containing activator (ArgA), on initial rates of P_9 polyphosphate cleavage by vacuolar Mg^{2+} - independent polyphosphate hydrolase from the fungus, *Neurospora crassa* (E.C. 3.6.11) which exhibits the maximum activity at pH 6.4. The conditions of enzyme isolation and study of its activity are given in the reference.[12]

Results

The results of this experiment presented in Figure 4A show that in the presence of 1.1 μM activator the activity of P_9 polyphosphate cleavage by vacuolar polyphosphate hydrolase under study had the following parameters: $K'_m=3.810\cdot10^{-4}$ M, $V'=2.110\cdot\mu E\cdot min^{-1}$; in the presence of 2.20 μM – by $K'_m= 4.359\cdot10^{-4}$ M, $V'=2.773\cdot\mu E\cdot min^{-1}$; in the presence of 3.3 μM – parameters: $K'_m = 4.836\cdot10^{-4}$ M, $V'= 3.494\cdot\mu E\cdot min^{-1}$.

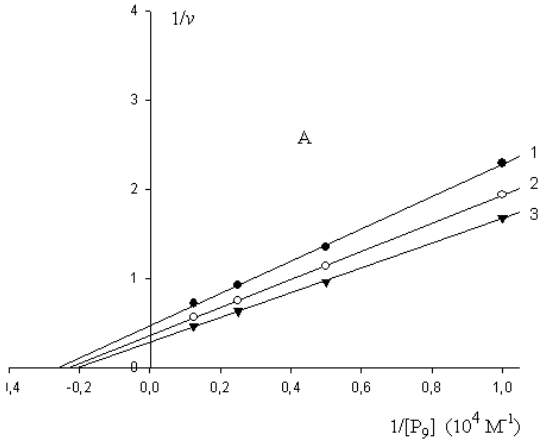


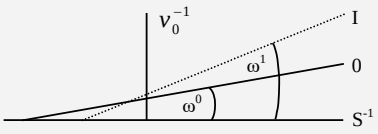
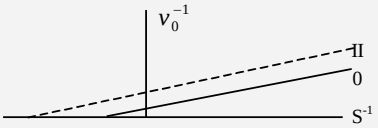
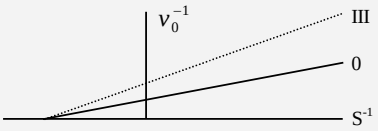
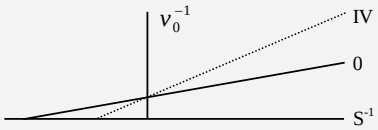
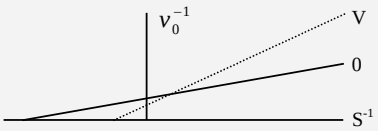
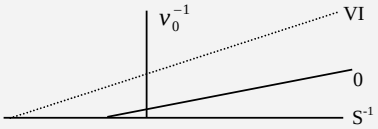
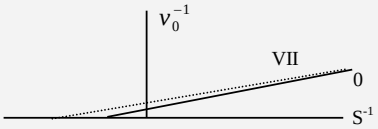
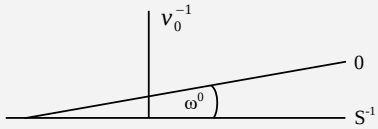
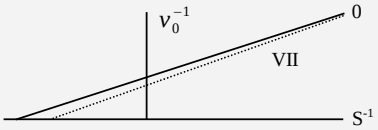
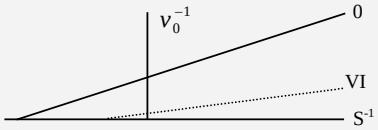
Figure 4A. Activating effect of ArgA on initial rates v_{VIa} of P_9 polyphosphate cleavage catalyzed by vacuolar polyphosphatase from *N. crassa*: the concentration of ArgA; line 1 – 1.1 μM, line 2 – 2.2 μM, and line 3 – 3.3 μM. Designation: v $\mu E\cdot min^{-1}$.

 DOI: 10.17628/ECB.2014.3.815

Table 2. Equations for calculation of the K_i and K_a constants

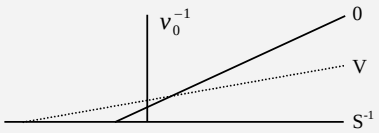
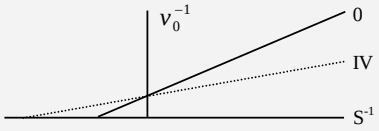
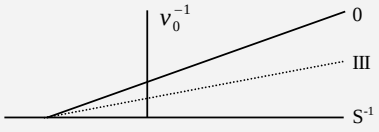
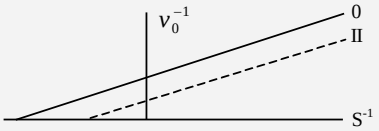
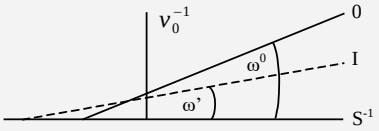
Type of effect	New name of the types of enzymic reactions	Traditional name	Equation for calculation of the K_i and K_a constants
I_i	biparametrically coordinated inhibition	mixed inhibition	$K_{Ii} = \frac{i}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
II_i	unassociative inhibition	uncompetitive inhibition	$K_{IIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
III_i	catalytic inhibition	noncompetitive inhibition	$K_{IIIi} = \frac{i}{V^0/V^{'} - 1}$
IV_i	associative inhibition	competitive inhibition	$K_{IVi} = \frac{i}{K_m^{'}/K_m^0 - 1}$
V_i	pseudoinhibition		$K_{Vi} = \frac{i}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
VI_i	discoordinated inhibition		$K_{VIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
VII_i	transient inhibition		$K_{VIIi} = \frac{i}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
I_0	initial (uninhibited $i = 0$ and nonactivated) enzymatic reaction		
VII_a	transient activation		$K_{VIIa} = \frac{a}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
VI_a	discoordinated activation		$K_{VIa} = \frac{a}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
V_a	pseudoactivation		$K_{Va} = \frac{a}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}}\right)^2\right)^{0.5}}$
IV_a	associative activation	competitive activation	$K_{IVa} = \frac{a}{K_m^0/K_m^{'} - 1}$
III_a	catalytic activation	noncompetitive activation	$K_{IIIa} = \frac{a}{V^{'}/V^0 - 1}$
II_a	unassociative activation	uncompetitive activation	$K_{IIa} = \frac{a}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$
I_a	biparametrically coordinated activation *	mixed activation	$K_{Ia} = \frac{a}{\left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}}\right)^2 + \left(\frac{V^{'} - V^0}{V^0}\right)^2\right)^{0.5}}$

Table 3. The coordinates of slopes for calculation of K_i and K_a constants of enzyme inhibition and activation

No	Effect	Type	Plots in (v^{-1}; S^{-1}) coordinates	Coordinates of slopes for calculation of K_i and K_a constants
1	Inhibition ($i > 0$)	I_i		(A; i) (see Figure 3B in the text)
2		II_i		(A; i)
3		III_i		$\left(\frac{K_m'}{V'};i\right);\left(\frac{1}{V'};i\right)$ or $\left(\frac{tg\omega'}{tg\omega^0};i\right)$ and $\left(tg\omega';i\right)$ (see Figure 5B in the text)
4		IV_i		$\left(\frac{K_m'}{V'};i\right)$ and $\left(K_m';i\right)$
5		V_i		(A; i)
6		VI_i		(A; i)
7		VII_i		(A; i)
8	None	I_0		None
9	Activation ($a > 0$)	VII_a		(A; a)
10		VI_a		(A; a) (see Figure 4B in the text)

 DOI: 10.17628/ECB.2014.3.815

Cont. Table 3.

11	V_a		$(A; a)$
12	IV_a		$\left(\frac{V'}{K'_m}; a\right); \left(\frac{1}{K'_m}; a\right); \left(\frac{tg\omega^0}{tg\omega'}; a\right)$ and $\left(\frac{1}{tg\omega'}; a\right)$
13	III_a		$\left(\frac{V'}{K'_m}; a\right); \left(V'; a\right); \left(\frac{tg\omega^0}{tg\omega'}; a\right)$ and $\left(\frac{1}{tg\omega'}; a\right)$
14	II_a		$(A; a)$
15	I_a		$(A; a)$

It satisfies type VI_a of enzyme activation (line 10, Table 3) and hence. Eqn. 10 of Table 2, is applicable here for calculation of K_{VIa} constant of enzyme activation), as the parameters of initial reaction (K^0_m and V^0) in this case were not determined, because of weak enzyme activity in the absence of activator.

Use of this equation gives the following values of constants of polyphosphatase hydrolase activation: K_{VIa}=3.182 μM – in the interval ArgA (2.2-1.1=1.1) μM and K_{VIa} = 3.103 μM – in the interval ArgA (3.3–1.1 = 2.2) μM, where $K_{m(2.2)}=K'_m$, $K_{m(1.1)}=K^0_m$ and so on.

By having marked according to the equation:

$$K_{Ia} = \frac{1}{tga} = \frac{1}{A/a} = \frac{a}{A} \qquad (4)$$

the numerical values of A denominators by intervals of tested concentrations of ArgA. One shall obtain that the values of A parameters are in the following functional dependence on the intervals of tested concentrations of activator (Figure 4B):

Hence, the average value of this constant (by intervals of ArgA concentrations as activator) shall be the value:

$$K_{VIa} = 1/b(1) = 1/3.2227\ (\mu M)^{-1} = 0.3103\ \mu M, \qquad (5)$$

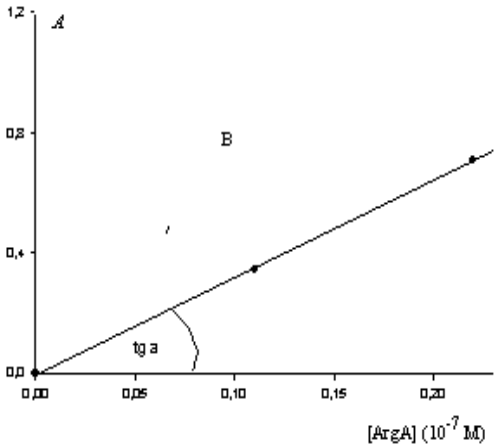


Figure 4B. Dependence of A parameters of Eqn. 5 (Figure 4A) on increasing concentrations of ArgA in the coordinates (A; [ArgA]). Designation: the intervals of tested concentrations ArgA were 1.1 and 2.2 μM.

where, $b(1)$ = 3.2227 – a parameter of the subprogram statistics of the computer program SigmaPlot, version 2000.

Probably, this technique may also be used in the case of single intervals of Δa and Δi parameters of both the biparametrical and monoparametrical K_a and K_i constants of enzyme inhibition and activation (Table 2).

At data analysis of the VI_a type of enzyme activation (the positions of plots are typical of Figure 4A).

The many authors[7,13-16] used to plot such dependences of a course of change of the slope angles (tg ω′) and the intersection points (1/*V'*) of the ordinate axis as a function of reverse concentrations of activator 1/*a* in the coordinates of : (tg ω′;1/*a*) slopes and (1/*V*';1/*a*) intersects. It is incorrect. Plotting the dependencies of change of K'_m/V' on 1/*a* in the coordinates of slopes (K'_m/V'; 1/*a*) and coordinates of intersects (*1/V'*; 1/*a*) does not take into account symmetric counter-directivity of effects of enzyme activation to → enzyme inhibition, which is so evident at the comparison of the positions of one-type $\mathbf{L}_a$ and $\mathbf{L}_i$ and their L_a and L_i projections in the three-dimensional (Figure 1) and two-dimensional (Figure 2) systems of coordinates. It is taken into account by reversion of V'/K'_m parameters relative to non-reversed concentrations of activator (*a*) in the corrected coordinates of slopes (Table 3, lines 12 and 13). So, one must use either (V'/K'_m; *a*) or (*V'*; *a*) coordinates of slopes instead of (K'_m/V'; *a*) coordinates for calculation of only monoparametrical K_{IIIa} constants of enzyme activation. The coordinates of slopes for calculation of biparametrical K_a constants of enzyme activation are given in Table 3 and their application is established in Examples 1 and 2.

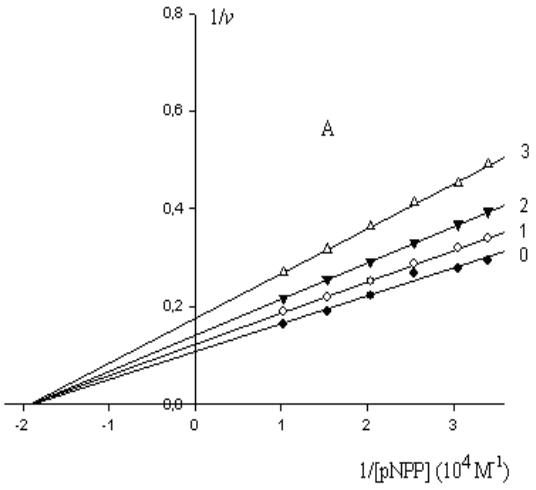


Figure 5A. Inhibitory effect of $K_3Fe(CN)_6$· on initial rates of pNPP cleavage catalyzed by porcine alkaline phosphatase. Designation: line 1 –$0.25\cdot10^{-3}$ M, line 2 – $0.5\cdot10^{-3}$ M, line 3 - $1.0\cdot10^{-3}$ M. Line 0 – the inhibitor is absent, ν μmol·min^{-1} μg protein^{-1}.

Example 3

Calculation of K_{IIIi} constant of enzyme inhibition. Let us use the data obtained at study of the inhibitory effect of potassium ferrocyanide $K_3Fe(CN)_6$ on initial rates of pNPP cleavage catalyzed by porcine alkaline phosphatase (EC 3.1.3.1) for calculation of K_{IIIi} constants of enzyme inhibition. The enzyme is produce of Sigma (USA).

The concentration of pNPP in the experiment was varied within $0.294\cdot10^{-4}$ – $0.98\cdot10^{-4}$ M, the concentration of enzyme was kept constant 1.13 μg mL^{-1}. The other conditions are same as in Example 1.

Results

The results of study showed that the kinetic parameters of initial reaction (K^0_m= $5.31\cdot10^{-5}$ M, V^0= 9.321 μmol·min^{-1} μg protein^{-1}) in the presence of 0.25 10^{-3} M $K_3Fe(CN)_6$ change as follows: K'_m=$5.23\cdot10^{-5}$ M, *V'*= 8.158 μmol·min^{-1} μg protein^{-1}, in the presence of 0.5 ·10^{-3} M $K_3Fe(CN)_6$: K'_m=$5.25\cdot10^{-5}$ M, *V'*= 5.70 μmol·min^{-1} μg protein^{-1} and in the presence of (1 10^{-3} M) $K_3Fe(CN)_6$ –K'_m= $5.27\cdot10^{-5}$ M, *V'*= 7.085 μmol·min^{-1} μg protein^{-1} (Figure 5A).

It satisfies all the features ($K'_m=K^0_m$, $V'<V^0$, $i>0$) of the catalytic *III*$_i$ type of enzyme inhibition. As seen from Figure 5A, lines 1, 2 and 3 of inhibited reactions go above line 0 of initial reaction in the first quadrant of the (ν^{-1}, S^{-1}) coordinates in the point (-1/K'_m; 0) located on the continuation of the abscissa axis. The positions of lines 1, 2 and 3 (Figure 5A) are typical to such of lines III and 0 (Figure 3 in Table 3); hence, for calculation of K_{IIIi} constant of enzyme inhibition we shall use the equation:

$$K_{IIIi} = \frac{i}{V^0/V' - 1} \qquad (6)$$

of (Table 2, line 3).[4]

To calculate the value of the constant of enzyme inhibition independently of deviations due to sue of separate (individual) concentrations of $K_3Fe(CN)_6$, it is also desirable to employ already above-descript techniques (Examples 1 and 2) – it is study of effect of increasing concentrations of inhibitor on initial rates of substrate cleavage by plotting dependencies in one (or several) variants of the secondary coordinates of slopes, which proceed from transformation of Eq. (6). It is easy to see that in the (V^0/V; *i*) coordinates of slopes the experimental line:

$$\frac{V^0}{V'} = \frac{1}{K_{IIIi}} \cdot i + 1 \qquad (7)$$

,

explained by this equation (Figure 5B, line 2) or in the (1/*V*; *i*) variant of the same coordinates of slopes the line:

$$\frac{1}{V'} = \frac{1}{V^0} \cdot \frac{1}{K_{IIIi}} \cdot i + \frac{1}{V^0} \qquad (8)$$

(Figure 5B, line 1) shall intersect the continuation of the abscissa axis of molar concentrations of inhibitor in the point: $-i_{IIIi} = K_{IIIi}$.

Analysis of the positions of the intersection points of the abscissa of Figure 5A using the subprogram Statistics of the computer program SigmaPlot, version 2000 (USA) gives the following of K_{IIIi} = $1.550\cdot10^{-3}$ M in the first case (line 2) and K_{IIIi} = $1.552\cdot10^{-3}$ M in the second case (line 1).

Experimental data of catalytic type of enzyme inhibition in literature there are.[7, 8,19,20] Now it becomes necessary to discuss the question of using the secondary (1/*V'*; *i*) coordinates of intersects for experimental data analysis on enzyme inhibition, which follows from transformation of Eq. (6) to Eq. (8) due to the equality $K'_m=K^0_m$ of parameters characteristic of only this *III*$_i$ type of enzyme inhibition (Table 3, line 3).

 DOI: 10.17628/ECB.2014.3.815

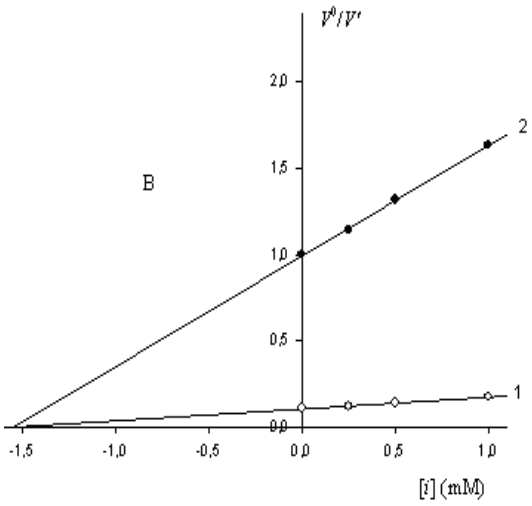


Figure 5B. Data representation of Figure 5A in the coordinates (V^0/V'; i) of slopes – line 2 and in the ($1/V'$; i) variant of the same coordinates – line 1,

It becomes evident from analysis of the Eqns. 6, 7 and 8 of the text that ($1/V'$; i) coordinates can be used for data analysis of only III_i type of enzyme inhibition characterized by change of only one $V'<V^0$ reaction parameter, and as the coordinates of slopes for calculation of K_{is} slope constants of this type of enzyme inhibition, but not K_{ii} intersects coordinates that actually do not exist (Table 3).

Apparently, using the ($1/V'$; i) coordinates of intersects for data analysis of types I_i and other biparametrically II_i, V_i, VI_i and VII_i types of enzyme inhibition that exhibit themselves by change of two (K'_m and V') reaction parameters seems incorrect, as the course of change of K'_m parameters of such reactions shall not be taken into account.

Examples of simultaneous use of the coordinates of slopes and intersects for calculation of K_{is} slope constants and K_{ii} intersect constants of biparametrical types of enzyme inhibition are numerous in literature.[6-11,17,18]

Besides, the authors do not discuss the question that, what does the value of K_{ii} constant of enzyme inhibition characterize, if K_{is} constant is attached the sense of constant of dissociation of the enzyme-inhibitor complex, especially in those cases, when their values do not coincide ? This question can be put in another way that, could one and the same inhibitor exhibit different binding to enzyme under the same experimental conditions?

Probably, not. The situation gets simplified at refusal of calculation and using symbols of K_{ii} intersect constants of enzyme inhibition, because they are the K_{is} constants of enzyme inhibition, But then, the necessity of using symbols of K_{is} slope constants of enzyme inhibition is also of no use, and only one symbol remains – it is the K_i constant of enzyme inhibition (for the convenience, it is preferable to indicate the type of inhibited reaction). It is analogous in enzyme activation (Tables 3 and 2).

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Received: 11.06.2014.
Accepted: 30.07.2014.