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Corrected Equations for Calculation of Constants in Enzyme Inhibition and Activation

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Abstract—Equations for calculation of the constants of biparametrical types of enzyme inhibition and activation were obtained that take into account a ratio of the lengths of L vector projections representing such reactions in the three-dimensional $K'_mV'I$ coordinate system. This allows higher accuracy of calculation and is more correct for comparison of these constants. Examples of data analysis of enzyme inhibition and activation by using the traditional equations (they do not take into account the lengths of vector projections) and corrected ones (they take into account the lengths of vector projections) are given. The corrected and traditional equations are used for calculation of the constants of biparametrical types of enzyme inhibition and activation.

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Equations for calculation of K_{IVi} constants of the associative IV_i type of enzyme inhibition (or competitive, according to the traditional terminology [1-3]):

$$K_{IVi} = \frac{i}{\frac{K'_m}{K^0_m} - 1}, \quad (1)$$

and the catalytic III_i (or noncompetitive) type of enzyme inhibition:

$$K_{IIIi} = \frac{i}{\frac{V^0}{V'} - 1} \quad (2)$$

are widely used for data analysis in studying such types of enzyme inhibition [4-7], where K'_m is the effective Michaelis constant, V' is the maximum reaction rate determined in the presence of inhibitor (i) relative to K^0_m and V^0 of the same parameters of initial (uninhibited i = 0 and nonactivated a = 0) enzymatic reaction. Most frequently, authors [4-6, 8-10] tend to plot dependences of a change in these parameters in the secondary coordinates of slopes (tan ω′; i), (K'_m; i) and intercepts ($1/V'$; i).

A parametric classification of the types of enzymatic reactions is given in Table 1 and in [11-14].

The equation for calculation of the K_{Ii} constant of the biparametrically coordinated I_i (or mixed) type of enzyme inhibition has long been known [3]:

$$K_{Ii} = \frac{i}{\frac{K'_mV^0}{K^0_mV'} - 1} = \frac{i}{\frac{\tan\omega'}{\tan\omega^0} - 1}, \quad (3)$$

where tan ω′ = K'_m/V' is a slope angle of the line representing any inhibited reaction to the abscissa axis, tanω^0 = K^0_m/V^0 is a slope angle of the line of initial (uninhibited) reaction to the same axis in the double reciprocal coordinates of Lineweaver–Burk (v^{-1}; S^{-1}) (Table 1). This equation is widely used for calculation of the K_{Ii} constants by plotting dependences in the secondary coordinates of slopes (K'_m/V'; i), (tanω′; i) and intercepts ($1/V'$; i) [6, 8, 10, 15-17].

Incorrectness of using the ($1/V'$; i) coordinates of intercepts in data analysis of the I_i type and other biparametrical (II_i, V_i, VI_i, and VII_i) types of enzyme inhibition (Table 1) was shown in [11-13, 18], because in this case a change in the K'_m parameter of such reactions is not taken into account.

A vector method representation of enzymatic reactions in the three-dimensional $K'_mV'I$ system (Fig. 1) revealed that a ratio of the length of a vector L projection of reaction under study on the *Pa*,*i* semiaxis of molar concentrations of *i* (or *a*) to a positive dimensionless difference of the orthogonal projection of this vector on the basic σ_0 plane is an equation for calculation of the $\overline{K_i}$ (or $\overline{K_a}$) constant of enzyme inhibition (or activation) [11-13, 18-20]. For example, by analyzing the position of L_{IIIi} vector of the catalytic III_i type of enzyme inhibition in the $K'_mV'I$ coordinate system (Fig. 1) one can easily see that Eq. (2) may be given in the following vector form:

$$K_{IIIi} = \frac{i}{\frac{V^0}{V'}-1} = \frac{i-0}{V^0-V'}\cdot V' = \frac{i-0}{\frac{V^0-V'}{V'}} = \frac{\mathrm{Pr}_{Pi}L_{IIIi}}{\mathrm{Pr}_{\sigma_0}\overline{L}_{IIIi}}, \quad (4)$$

where $\mathrm{Pr}_{Pi}L_{IIIi}$ is a projection of the L_{IIIi} vector on the coincident *Pa*,*i* (more exactly on *Pi*) semiaxis of molar concentrations of inhibitor (*i*, M), $\mathrm{Pr}_{\sigma_0}\overline{L}_{IIIi}$ is a vector projection on the coordinate $P0_{V'}$ semiaxis in the basic σ_0 plane expressed by the ratio $(V^0 - V')/V'$ of a positive difference of the coordinates of the vector L_{IIIi} projection to the V' coordinate of vector origin within positivity of this difference.

Deduction of an equation for calculation of the K_{IIIa} constant of the catalytic III_a type of enzyme activation. Having analyzed a ratio of the L_{IIIa} vector projection of the catalytic III_a type of enzyme activation on the *Pa* semiaxis of molar concentrations of activator $(a - 0)$ to a dimensionless positive difference of the coordinates $(V' - V^0)/V^0$ of this vector projection on the PV' semiaxis in the basic σ_0 plane (Fig. 1), we shall obtain the following equation for calculation of K_{IIIa} constant of catalytic enzyme activation:

$$\frac{\mathrm{Pr}_{Pa}L_{IIIa}}{\mathrm{Pr}_{\sigma_0}\overline{L}_{IIIa}} = \frac{a-0}{\frac{V'-V^0}{V^0}} = \frac{a}{\frac{V'}{V^0}-1} = K_{IIIa}. \quad (5)$$

Deduction of an equation for calculation of the K_{IVa} constant of the associative IV_a type of enzymes activation. Analysis of the position of L_{IVi} vector of the associative IV_i type of enzyme inhibition in the $K'_mV'I$ coordinate system (Fig. 1) shows that by detailing of a ratio of the $\mathrm{Pr}_{Pi}L_{IVi}$ projection of L_{IVi} vector on the *Pi* semiaxis $(i - 0)$ to a dimensionless positive difference of the coordinates $(K'_m - K^0_m)/K^0_m$ of this vector projection in the basic σ_0 plane or, which is the same, on the PK'_m semiaxis $(\mathrm{Pr}_{\sigma_0}\overline{L}_{IVi})$, we shall obtain the same form of the known equation [1-3] used for calculation of the K_{IVi} constant of associative enzyme inhibition:

$$\frac{\mathrm{Pr}_{Pi}L_{IVi}}{\mathrm{Pr}_{\sigma_0}\overline{L}_{IVi}} = \frac{i-0}{\frac{K'_m-K^0_m}{K^0_m}} = \frac{i}{\frac{K'_m}{K^0_m}-1} = K_{IVi}. \quad (6)$$

Having analyzed by analogy the position of L_{IVa} vector of the associative IV_a type of enzyme activation (Fig. 1) and bearing in mind that a positive difference of the coordinates of this vector projection on *Pa* semiaxis shall be $(a - 0)$ and a dimensionless positive difference of the coordinates of this vector projection on the $0K'_m$ semiaxis shall be $(K^0_m - K'_m)/K'_m$, we shall obtain an equation for calculation of the K_{IVa} constant of associative enzyme activation:

$$\frac{\mathrm{Pr}_{Pa}L_{IVa}}{\mathrm{Pr}_{\sigma_0}\overline{L}_{IVa}} = \frac{a-0}{\frac{K^0_m-K'_m}{K'_m}} = \frac{a-0}{K^0_m-K'_m}\cdot K'_m = \frac{a}{\frac{K^0_m}{K'_m}-1} = K_{IVa}. \quad (7)$$

The absence of Eqs. (5) and (7) in laboratory practice that were only deduced in 1986 [21] made it difficult to analyze experimental data of these types of enzyme activation. For such data analysis, the dependences were plotted in the secondary coordinates of slopes $(K'_m/V'; 1/a)$, $(\tan\omega'; 1/a)$ and intercepts $(1/V'; 1/a)$ [9, 22-24].

It was shown that use of the coordinates reversed by concentration of $1/a$ fails to take into account the symmetric antidirectivity of a tendency in a course of change in the K'_m and V' parameters of activated reactions relative to the same parameters of inhibited reactions of the same type. This can be taken into account by reversion of the K'_m and V' parameters relative to unreversed concentration of *a* in the coordinates of slopes $(V'/K'_m; a)$, $(1/\tan\omega'; a)$, and $(V'; a)$ [12, 13, 18].

Based on the established rule about a ratio of dimensionless positive difference of the coordinates of L vector

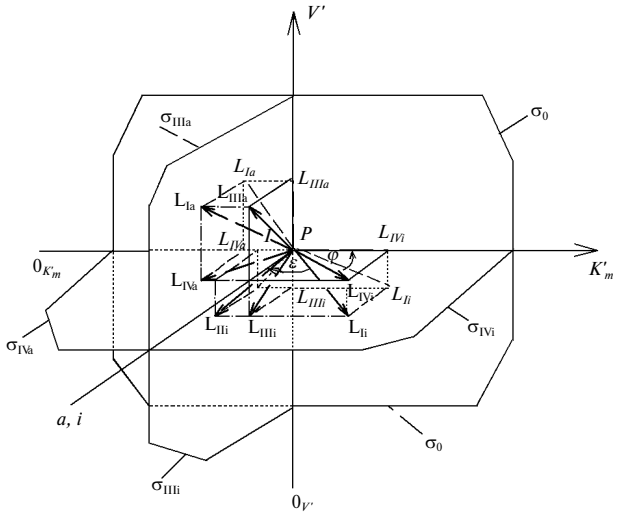


Fig. 1. Three-dimensional $K'_mV'I$ system of rectangular coordinates with the coincident *Pa* and *Pi* semiaxes of molar concentration of *i* and *a*. The symbols of kinetic parameters K'_m, V', K^0_m, three-dimensional vectors L_{Ii}, L_{IVi} ... L_{Ia}, L_{IVa}, and their orthogonal projections L_{Ii}, L_{IVi} ... L_{Ia}, L_{IVa} on the basic σ_0 plane and the orthogonal projections of directing planes σ_{IVi}, σ_{IIIi}, σ_{IVa}, σ_{IIIa} on the PK'_m, $P0_{V'}$, $P0_{K'_m}$, and PV' coordinate semiaxes are described in the text.

Table 1. Parametric classification of the types of enzymatic reactions and equations for calculation of the $\overline{K}_i$ and $\overline{K}_a$ constants

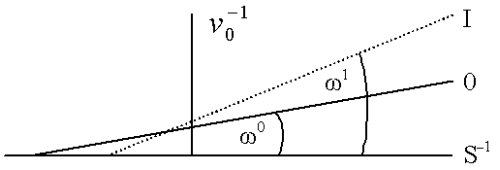
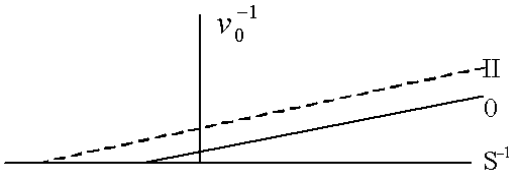
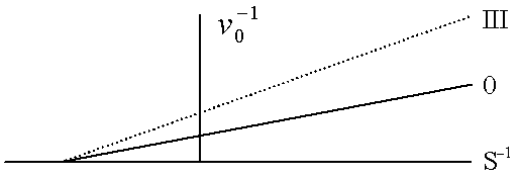
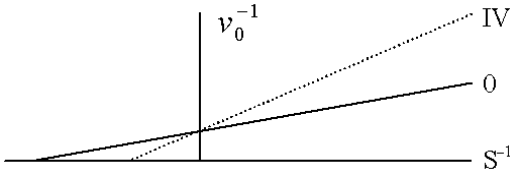
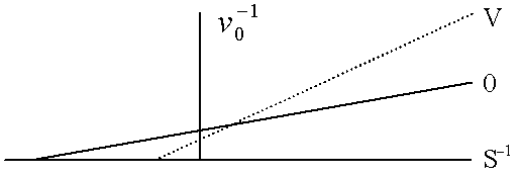
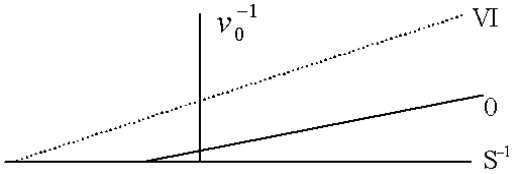
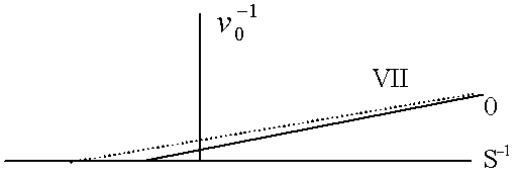
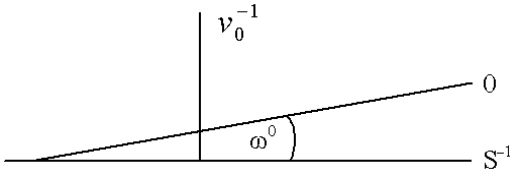
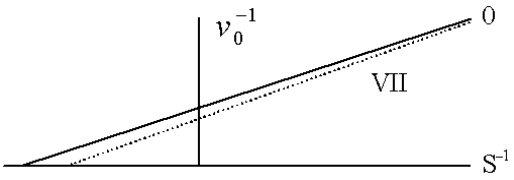
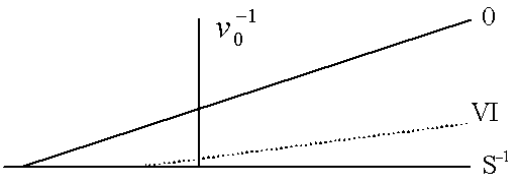
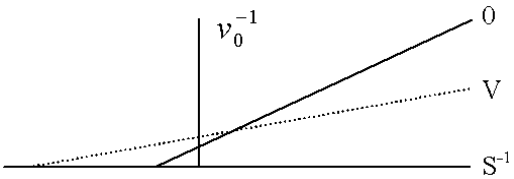
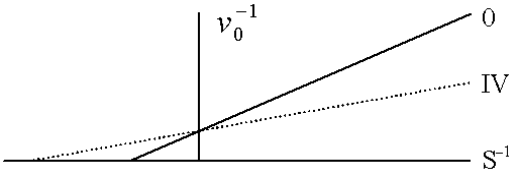
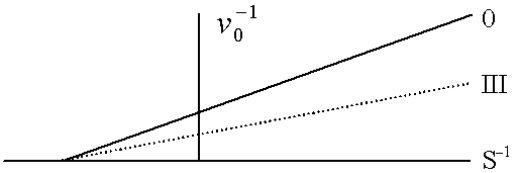
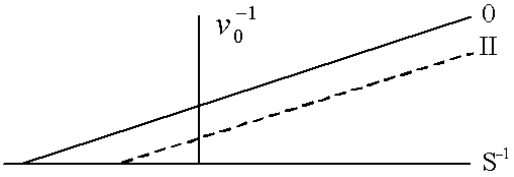
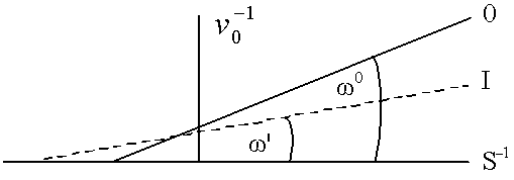
No.	Effect	Type of effect	Correlation between the K'_m and V' parameters	Graphs in the (v^{-1}; S^{-1}) coordinates
1	2	3	4	5
1	Inhibition ($i > 0$)	I_i	$K'_m > K^0_m$, $V' < V^0$	
2		II_i	$K'_m < K^0_m$, $V' < V^0$ ($\tan\omega' = \tan\omega^0$)	
3		III_i	$K'_m = K^0_m$, $V' < V^0$	
4		IV_i	$K'_m > K^0_m$, $V' = V^0$	
5		V_i	$K'_m > K^0_m$, $V' > V^0$	
6		VI_i	$K'_m < K^0_m$, $V' < V^0$ ($\tan\omega' > \tan\omega^0$)	
7		VII_i	$K'_m < K^0_m$, $V' < V^0$ ($\tan\omega' < \tan\omega^0$)	
8	None	I_0	$K'_m = K^0_m$, $V' = V^0$	

Table 1. (Contd.)

1	2	3	4	5
9	Activation ($a > 0$)	VII_a	$K'_m > K^0_m$, $V' > V^0$ ($\tan\omega' > \tan\omega^0$)	
10		VI_a	$K'_m > K^0_m$, $V' > V^0$ ($\tan\omega' < \tan\omega^0$)	
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	$K'_m > K^0_m$, $V' > V^0$ ($\tan\omega' = \tan\omega^0$)	
15		I_a	$K'_m < K^0_m$, $V' > V^0$	

Note: The designations of graphs Nos. 1-15 correspond to the types of reactions under study. For example, designation “0” corresponds to initial (nonactivated) enzymatic reaction, line “I” – to the Ia type of activated enzymatic reaction (figure No. 15), etc.

Table 1. (Contd.)

Type of effect	New name of the type of enzymatic reaction	Traditional name	Corrected equation for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants
1	2	3	4
I_i	biparametrically coordinated inhibition	mixed inhibition	$\bar{K}_{Ii} = i/\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
II_i	unassociative inhibition	uncompetitive inhibition	$\bar{K}_{IIi} = i/\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
III_i	catalytic inhibition	noncompetitive inhibition	$\bar{K}_{IIIi} = K_{IIIi} = \frac{i}{V^0/V' - 1} = \frac{i}{\frac{V^0 - V'}{V'}}$
IV_i	associative inhibition	competitive inhibition	$\bar{K}_{IVi} = K_{IVi} = \frac{i}{K_m'/K_m^0 - 1} = \frac{i}{\frac{K_m' - K_m^0}{K_m^0}}$
V_i	pseudoinhibition		$\bar{K}_{Vi} = i/\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$
VI_i	discoordinated inhibition		$\bar{K}_{VIi} = i/\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
VII_i	transient inhibition		$\bar{K}_{VIIi} = i/\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
I_0	initial (uninhibited ($i = 0$) and nonactivated ($a = 0$)) enzymatic reaction		
VII_a	transient activation		$\bar{K}_{VIIa} = a/\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$
VI_a	discoordinated activation		$\bar{K}_{VIa} = a/\left(\left(\frac{K_m' - K_m^0}{K_m^0}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$
V_a	pseudoactivation		$\bar{K}_{Va} = a/\left(\left(\frac{K_m^0 - K_m'}{K_m'}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
IV_a	associative activation	competitive activation	$\bar{K}_{IVa} = K_{IVa} = \frac{a}{K_m^0/K_m' - 1} = \frac{a}{\frac{K_m^0 - K_m'}{K_m'}}$
III_a	catalytic activation	noncompetitive activation	$\bar{K}_{IIIa} = K_{IIIa} = \frac{a}{V'/V^0 - 1} = \frac{a}{\frac{V' - V^0}{V^0}}$

Table 1. (Contd.)

1	2	3	4
II_a	unassociative activation	uncompetitive activation	$\bar{K}_{IIa} = a/\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$
I_a	biparametrically coordinated activation	mixed activation	$\bar{K}_{Ia} = a/\left(\left(\frac{K^0_m - K'_m}{K'_m}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$

projections of inhibited and activated enzymatic reactions in the $K'_mV'I$ coordinate system, one may obtain all the other equations for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants of nontrivial (biparametrical) types of enzyme inhibition and activation.

Equations for calculation of the $\bar{K}_{Ii}$ and $\bar{K}_{Ia}$ constants of biparametrical types of enzyme inhibition and activation. As seen from Figs. 1 and 2, the l_{Ii} length of the L_{Ii} vector projection on the *Pi* semiaxis shall be determined by a difference $(i - 0)$; but the length of the orthogonal projection on the basic σ_0 plane shall be determined by a rule about the summation of lengths (orthogonal between themselves) of L_{IIIi} and L_{IVi} projections of L_{IIIi} and L_{IVi} vectors of the III_i and IV_i types of enzyme inhibition (these projections are at the same time the coordinates of L_{Ii} vector):

$$l_{Ii} = \sqrt{(l_{IIIi})^2 + (l_{IVi})^2} \quad . \qquad (8)$$

Having expressed from Eq. (2) the l_{IIIi} length of the dimensionless L_{Ii} projection on the $P0_{V'}$ semiaxis in the $K'_mV'I$ coordinate system as:

$$l_{IIIi} = \frac{V^0 - V'}{V'} = \frac{i}{K_{IIIi}} \quad , \qquad (9)$$

and by Eq. (1) – the l_{IVi} length of the above projection on the PK'_m semiaxis:

$$l_{IVi} = \frac{K'_m - K^0_m}{K^0_m} = \frac{i}{K_{IVi}} \qquad (10)$$

and having substituted them in Eq. (11):

$$\bar{K}_{Ii} = \frac{\mathrm{Pr}_{Pi} L_{Ii}}{\mathrm{Pr}_{\sigma_0} \bar{L}_{Ii}} \quad , \qquad (11)$$

we shall obtain an equation for calculation of the $\bar{K}_{Ii}$ constant of the I_i type of enzyme inhibition:

$$\bar{K}_{Ii} = \frac{i}{\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}} \quad , \qquad (12)$$

that takes into account the length of the dimensionless orthogonal L_{Ii} vector projection on the basic σ_0 plane (Fig. 1).

Such form of the equation is convenient, since there is no need to express the K_m and V parameters of Eq. (12) in conventional units (c.u.), which otherwise would have require their unification for the purpose of comparing the constants.

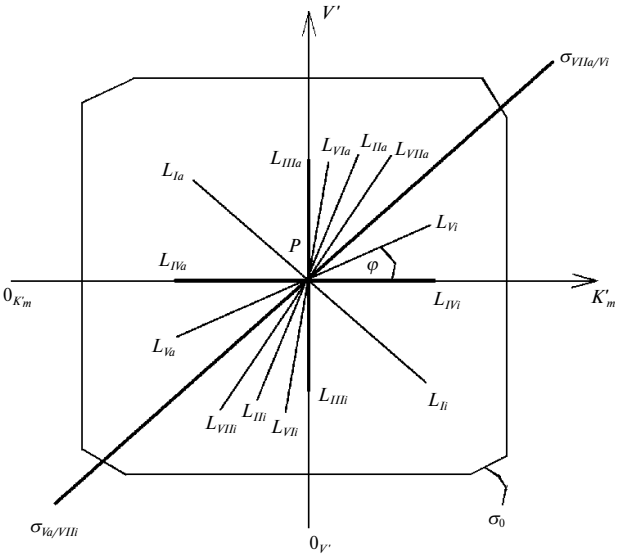


Fig. 2. Two-dimensional (scalar) K'_mV' coordinate system. The symbols of kinetic parameters K'_m, V', K^0_m, orthogonal projections L_{Ii}, L_{IVi} ... L_{Ia}, L_{IVa} of the respective three-dimensional vectors, and the designations of coordinate semiaxes PK'_m, $P0_{V'}$, $P0_{K'_m}$, and PV' on the basic σ_0 plane are as in Fig. 1.

Table 2. Equations for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants of biparametrical types of enzyme inhibition and activation with *i* and *a* parameters given in non-obvious view

Type of effect	New name of the type of enzymatic reaction	Traditional name	Corrected equation for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants
1	2	3	4
I_i	biparametrically coordinated inhibition	mixed inhibition	$\bar{K}_{Ii} = \left(\frac{K_{IIIi}^{\ 2} \cdot K_{IVi}^{\ 2}}{K_{IIIi}^{\ 2} + K_{IVi}^{\ 2}} \right)^{0.5}$
II_i	unassociative inhibition	uncompetitive inhibition	$\bar{K}_{IIi} = \left(\frac{K_{IIIi}^{\ 2} \cdot K_{IVa}^{\ 2}}{K_{IIIi}^{\ 2} + K_{IVa}^{\ 2}} \right)^{0.5}$
III_i	catalytic inhibition	noncompetitive inhibition	$\bar{K}_{IIIi} = K_{IIIi} = \frac{i}{V^0/V' - 1}$
IV_i	associative inhibition	competitive inhibition	$\bar{K}_{IVi} = K_{IVi} = \frac{i}{K_m'/K_m^0 - 1}$
V_i	pseudoinhibition		$\bar{K}_{Vi} = \left(\frac{K_{IIIa}^{\ 2} \cdot K_{IVi}^{\ 2}}{K_{IIIa}^{\ 2} + K_{IVi}^{\ 2}} \right)^{0.5}$
VI_i	discoordinated inhibition		$\bar{K}_{VIi} = \left(\frac{K_{IIIi}^{\ 2} \cdot K_{IVa}^{\ 2}}{K_{IIIi}^{\ 2} + K_{IVa}^{\ 2}} \right)^{0.5}$
VII_i	transient inhibition		$\bar{K}_{VIIi} = \left(\frac{K_{IIIi}^{\ 2} \cdot K_{IVa}^{\ 2}}{K_{IIIi}^{\ 2} + K_{IVa}^{\ 2}} \right)^{0.5}$
I_0	initial (uninhibited ($i = 0$) and nonactivated ($a = 0$)) enzymatic reaction		
VII_a	transient activation		$\bar{K}_{VIIi} = \left(\frac{K_{IIIa}^{\ 2} \cdot K_{IVi}^{\ 2}}{K_{IIIa}^{\ 2} + K_{IVi}^{\ 2}} \right)^{0.5}$
VI_a	discoordinated activation		$\bar{K}_{VIa} = \left(\frac{K_{IIIa}^{\ 2} \cdot K_{IVi}^{\ 2}}{K_{IIIa}^{\ 2} + K_{IVi}^{\ 2}} \right)^{0.5}$
V_a	pseudoactivation		$\bar{K}_{Va} = \left(\frac{K_{IIIi}^{\ 2} \cdot K_{IVa}^{\ 2}}{K_{IIIi}^{\ 2} + K_{IVa}^{\ 2}} \right)^{0.5}$
IV_a	associative activation	competitive activation	$\bar{K}_{IVa} = K_{IVa} = \frac{a}{K_m^0/K_m' - 1}$
III_a	catalytic activation	noncompetitive activation	$\bar{K}_{IIIa} = K_{IIIa} = \frac{a}{V'/V^0 - 1}$

Table 2. (Contd.)

1	2	3	4
II_a	unassociative activation	uncompetitive activation	$\bar{K}_{IIa} = \left(\frac{K_{IIIa}^{\ 2} \cdot K_{IVi}^{\ 2}}{K_{IIIa}^{\ 2} + K_{IVi}^{\ 2}} \right)^{0.5}$
I_a	biparametrically coordinated activation	mixed activation	$\bar{K}_{Ia} = \left(\frac{K_{IIIa}^{\ 2} \cdot K_{IVa}^{\ 2}}{K_{IIIa}^{\ 2} + K_{IVa}^{\ 2}} \right)^{0.5}$

The alternative forms of calculation of the $\bar{K}_{\mathrm{Ii}}$ constants and the constants of other biparametrical types of enzyme inhibition and activation are given in Table 2.

Having analyzed the position of the L_{Ia} orthogonal projection of L_{Ia} vector of the biparametrically coordinated I_a type of enzyme activation that is located in the σ_0 plane and edged by this vector L_{IIIa} and L_{IVa} projections on the PV' and $P0_{K'_m}$ coordinate semiaxes, which are also the orthogonal projections of L_{IIIa} and L_{IVa} vectors of the monoparametrical III_a and IV_a types of enzyme activation (Figs. 1 and 2), it is easy to see that its length shall be calculated by the same rule of summation of the l_{IIIa} and l_{IVa} lengths of L_{IIIa} and L_{IVa} vector projections on the PV' and $P0_{K'_m}$ semiaxes:

$$l_{\mathrm{Ia}} = \sqrt{(l_{IIIa})^2 + (l_{IVa})^2} \quad . \qquad (13)$$

Having expressed the lengths of l_{IIIa} and l_{IVa} projections for calculation of the K_{IIIa} and K_{IVa} constants from Eqs. (5) and (7) as follows:

$$l_{\mathrm{IIIa}} = \frac{V' - V^0}{V^0} = \frac{a}{K_{IIIa}} \quad , \qquad (14)$$

and

$$l_{\mathrm{IV}a} = \frac{K_m^0 - K'_m}{K'_m} = \frac{a}{K_{IVa}} \qquad (15)$$

and substituted them in Eq. (16):

$$\bar{K}_{Ia} = \frac{\mathrm{Pr}_{Pa} L_{Ia}}{\mathrm{Pr}_{\sigma_0} \bar{L}_{Ia}} \quad , \qquad (16)$$

we shall obtain an equation for calculation of the $\bar{K}_{\mathrm{Ia}}$ constant of biparametrically coordinated enzyme activation in the form:

$$\bar{K}_{Ia} = \frac{a}{\left(\left(\frac{K_m^0 - K'_m}{K'_m} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5}} \quad . \qquad (17)$$

The taking into account the projections of L vectors of monoparametrical and all the other biparametrical types of enzyme inhibition and activation in the basic σ_0 plane (Fig. 2) permits to establish by summation of the length of which L vector projections of monoparametrical reactions the length of the L orthogonal projection of L vector of each biparametrical reaction of enzyme inhibition and activation shall be determined.

For example, as easily seen from Fig. 2, the length of L_{IIi} orthogonal projection of L_{IIi} vector of the unassociative II_i type of enzyme inhibition shall be surrounded by the L_{IVa} and L_{IIIi} projections (orthogonal between themselves), which are the coordinates of L_{IIi} vector of this reaction on the $P0_{K'_m}$ and $P0_{V'}$ semiaxes in the basic σ_0 plane. Having expressed the l_{IIIi} length of the dimensionless L_{IIIi} projection by Eq. (9) and that of the l_{IVa} projection by Eq. (15) and substituted them in Eq. (18):

$$\bar{K}_{IIi} = \frac{\mathrm{Pr}_{Pi} L_{IIi}}{\mathrm{Pr}_{\sigma_0} \bar{L}_{IIi}} \quad , \qquad (18)$$

we shall obtain an equation for calculation of the $\bar{K}_{IIi}$ constant of the II_i type of enzyme inhibition:

$$\bar{K}_{IIi} = \frac{i}{\left(\left(\frac{K_m^0 - K'_m}{K'_m} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5}} \qquad (19)$$

(Table 1, line 2).

Having analyzed the position of L_{VIa}, L_{IIa}, and L_{VIIa} orthogonal projections of the three-dimensional L_{VIa}, L_{IIa}, and L_{VIIa} vectors on the first quadrant of the σ_0 plane

(Fig. 2), one can see that each of these projections is surrounded by the L_{IIIa} and L_{IVi} projections of L_{IIIa} and L_{IVi} vectors of the monoparametrical types of enzyme activation and inhibition. At the same time these projections are the coordinates of each of the L_{VIa}, L_{IIa}, and L_{VIIa} vectors representing the VI_a, II_a, and VII_a types of enzyme activation. This is why the equations for calculation of the $\overline{K}_{VIa}$, $\overline{K}_{IIa}$, and $\overline{K}_{VIIa}$ constants of the biparametrically discoordinated types of enzyme activation have the same subradical values (Table 1, lines 10, 14, and 9). Each of these types of enzyme activation is characterized by its own individual ratio of $\tan\omega'/\tan\omega^0$ – the angles of slope of experimental plots (Table 1).

It is by analogy for the L_{VIi}, L_{IIi}, and L_{VIIi} orthogonal projections and equations for calculation of the $\overline{K}_{VIi}$, $\overline{K}_{IIi}$, and $\overline{K}_{VIIi}$ constants of the biparametrically discoordinated types of enzyme inhibition (Figs. 1 and 2; Table 1, lines 6, 2, and 7).

As seen in Fig. 2, the line in the first (I) and third (III) quadrants that runs from the right downwards through the center of the coordinates indicates the presence of projections orthogonal to the σ_0 plane: the $\sigma_{VIIa/Vi}$ plane of existence of $L_{VIIa/Vi}$ vectors of a transient state between the $VII_a \Leftrightarrow V_i$ types of enzyme activation and inhibition (a projection on the first quadrant of the σ_0 plane) and the $\sigma_{VIIi/Va}$ plane of existence of $L_{VIIi/Va}$ vectors of a transient state between the $VII_i \Leftrightarrow V_a$ types of enzyme inhibition and activation (a projection on the third quadrant of the σ_0 plane). The position of these planes in the $K'_mV'I$ coordinate system is considered in detail in the monograph [11].

Let us consider a few examples that illustrate the applicability of corrected and traditional equations for calculation of constants of enzyme inhibition and activation.

MATERIALS AND METHODS

Calf intestinal alkaline phosphatase (EC 3.1.3.1) (Fluka, Switzerland) and canine alkaline phosphatase (EC 3.1.3.1) (Sigma, USA) were used as enzymes.

p-Nitrophenylphosphate (pNPP) di(cyclohexylammonium) salt (Serva, Germany) was used as a substrate.

The process of pNPP cleavage by alkaline phosphatases was recorded on a CF-4 double-beam spectrophotometer (Optica Milano, Italy). Reactions were carried out in 0.05 M Tris-HCl buffer, pH 9.0, at ionic strength 0.1 (by NaCl of high purity) under constant stirring in a thermostat (37°C) [11] at the wavelength 400 nm of a solution containing the substrate, inhibitor, and enzyme against a solution of the same composition without the enzyme.

The initial reaction rate (v) was calculated by the angle of slope of tangents to initial segments of curves representing the process of substrate cleavage determined in not less than five parallel experiments.

Isolation and characterization of the activity of chitinsynthase (EC 2.4.1.17) from *Saccharomyces carlsbergensis* are given in [20, 25].

The kinetic V and K_m parameters of enzyme inhibition and activation were determined by plots in the (v^{-1}; S^{-1}) coordinates of Lineweaver–Burk using the computer program Sigma Plot, version 2000 (USA).

The root-mean-square deviation at five measurements was estimated using the same program: $v = \pm 2.5\%$; K_m and $V = \pm 7.5\%$; K_i and $K_a = \pm 10\%$.

RESULTS AND DISCUSSION

The results and examples of the calculation of corrected ($\overline{K}_i$ and $\overline{K}_a$) and traditional (K_i and K_a) constants are given in Figs. 3-6.

Example 1. The inhibitory effect of tungstic acid anions WO_4^{2-} on the initial rate of pNPP cleavage by calf alkaline phosphatase (Fig. 3) shows that the presence $0.5\cdot10^{-4}$ M of these anions in the enzyme–substrate system makes the binding of the enzyme to the substrate cleaved ($K_m^0 = 4.45\cdot10^{-5}$ M, $K'_m = 6.56\cdot10^{-5}$ M) difficult and leads to a decrease in the maximum reaction rate ($V^0 = 2.56$, $V' = 1.74$ μmol/min per μg protein). This meets all the features ($K'_m > K_m^0$, $V' < V^0$, $i > 0$) of the biparametrically coordinated I_i type of enzyme inhibition (Table 1, line 1). Hence, to calculate the $\overline{K}_{Ii}$ constant of this phosphatase inhibition it is necessary to use Eq. (12) from the text or Eq. (1) in Table 1.

Substitution in this equation of the parameters K'_m, K_m^0, V', V^0, and i obtained by data analysis (Fig. 3) allows the calculation of this constant of enzyme inhibition:

$$\bar{K}_{Ii} = \frac{\mathrm{Pr}_{Pi}\,L_{Ii}}{\mathrm{Pr}_{\sigma_0}\,L_{Ii}} = \frac{0.5\cdot10^{-4}\ \mathrm{M}}{\left(\left(\frac{6.56-4.45}{4.45}\right)^2+\left(\frac{2.56-1.74}{1.74}\right)^2\right)^{0.5}} =$$

$$= 7.48 \pm 0.52\cdot10^{-5}\ \mathrm{M}. \qquad (20)$$

Substitution of the same parameters in the known Eq. (3) gives a decreased value of the K_{Ii} constant of enzyme inhibition:

$$K_{Ii} = \frac{i}{K'_mV^0 - K_m^0V'}\cdot K_m^0V' = \frac{0.5\cdot10^{-4}\ \mathrm{M}}{\frac{6.56\cdot2.56}{4.45\cdot1.76}-1} =$$

$$= 4.28 \pm 0.33\cdot10^{-5}\ \mathrm{M}, \qquad (21)$$

which is by $[(7.48 - 4.28)/4.28]\cdot100 = 75\%$ lesser than the actual value of $\overline{K}_{Ii}$. This is caused by inadequacy that

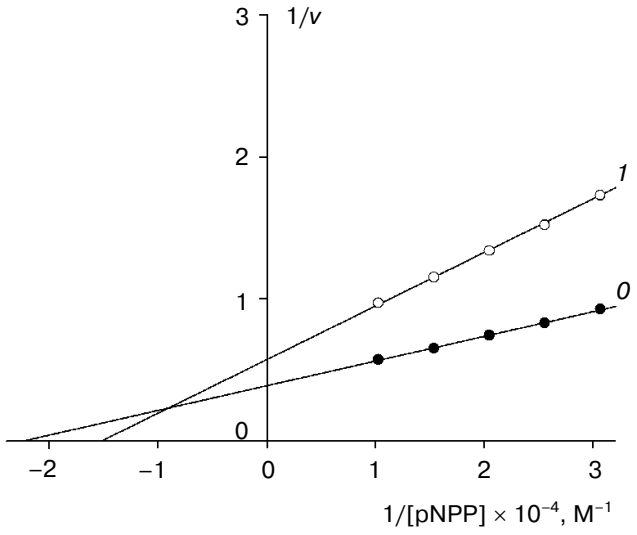


Fig. 3. Inhibitory effect of anions WO_4^{2-} on the initial rate (v, μmol/min per μg protein) of pNPP cleavage by calf alkaline phosphatase: *1*) the concentration of WO_4^{2-} is $0.5 \cdot 10^{-4}$ M; *0*) the inhibitor is absent.

occurs when the l_{Ii} length of L_{Ii} vector projection of reaction under study is not taken into consideration. Data analysis revealed that this deviation augments when the concentration of i and a difference between K'_m and K^0_m, V' and V^0 parameters increase.

Examples of using Eqs. (4) and (3) from Table 1 for data analysis of such type of enzyme inhibition (Fig. 3) are often found in practice. The values obtained in our experiment $K'_{IVi} = 1.054 \cdot 10^{-4}$ M and $K'_{IIIi} = 1.061 \cdot 10^{-4}$ M would exceed the $\bar{K}_{Ii}$ constant of enzyme inhibition by 10.6/7.48 = 1.4 times, because in the first case (Eq. (4)) the ratio V^0/V' would not be taken into account, while in the second one (Eq. (A3)) – the ratio K'_m/K^0_m.

Example 2. The inhibitory effect of pyrrolidine dithiocarbonic acid (PDTA) on the initial rate of pNPP cleavage by canine alkaline phosphatase shows that in the presence of 10^{-3} M PDTA the parameters $K^0_m = 4.69 \cdot 10^{-5}$ M and $V^0 = 2.921$ μmol/min per μg protein change as follows: $K'_m = 11.26 \cdot 10^{-5}$ M and $V' = 3.616$ μmol/min per μg protein (Fig. 4). This corresponds to the V_i type of enzyme pseudoinhibition ($K'_m > K^0_m$, $V' > V^0$, $i > 0$) (Table 1, line 5) and Eq. (5) from the Table 1 is applicable for calculation of the $\bar{K}_{Vi}$ constant of enzyme inhibition. Substitution of all the parameters in this equation allows calculation of this constant of enzyme inhibition:

$$\bar{K}_{Vi} = \frac{\mathrm{Pr}_{Pi} L_{Vi}}{\mathrm{Pr}_{\sigma_0} L_{Vi}} = \frac{1 \cdot 10^{-3}\,\mathrm{M}}{\left(\left(\frac{11.26-4.69}{4.69}\right)^2 + \left(\frac{3.616-2.92}{2.92}\right)^2\right)^{0.5}} =$$

$$= 7.04 \pm 0.51 \cdot 10^{-4}\,\mathrm{M}. \qquad (22)$$

Comparison of the $\bar{K}_{Vi}$ constant of enzyme inhibition with the K^0_m parameter of initial reaction permits to establish that affinity of the inhibitor under study for canine alkaline phosphatase by $(\bar{K}_{Ii}/K^0_m) = 15$ times weaker than the binding of enzyme to substrate. It is quite explicable because PDTA has no features of structural similarity to pNPP.

Example 3. The activating effect of guanosine (Guo) on canine alkaline phosphatase (Fig. 5) shows that in the presence of 10^{-3} M Guo the parameters of initial reaction of pNPP cleavage, i.e. $K^0_m = 4.69 \cdot 10^{-5}$ M, $V^0 = 2.921$ μmol/min per μg protein, change as follows: $K'_m = 5.67 \cdot 10^{-5}$ M, $V' = 3.527$ μmol/min per μg protein. This corresponds to the II_a type of unassociative enzyme activation. Hence, to calculate the $\bar{K}_{IIa}$ constant of enzyme activation, one should use Eq. (14) from Table 1. Substitution of the obtained values of parameters in this equation allows calculation of this constant of enzyme activation:

$$\bar{K}_{IIa} = \frac{\mathrm{Pr}_{Pa} L_{IIa}}{\mathrm{Pr}_{\sigma_0} \bar{L}_{IIa}} = \frac{1 \cdot 10^{-3}\,\mathrm{M}}{0.2945} = 3.4 \pm 0.26 \cdot 10^{-3}\,\mathrm{M}. \qquad (23)$$

In the literature, there are data on study of the effect of nucleosides, Guo included, on bovine acid phosphatase (EC 3.1.3.2) [26]. The results of our experiment reveal that as to this enzyme, Guo exhibits the analogous mechanism of the unassociative II_a type of activation ($K'_m > K^0_m$, $V' > V^0$, $a > 0$). As seen from Fig. 5, the line *1* of activated enzymatic reaction goes below and parallel to line (*0*) of initial reaction.

The authors [26] established that a ratio of the maximum reaction rates at 10^{-3} M Guo was $V'/V^0 = 1.5$. As seen from the above figure, this ratio is equal to 1.21 for

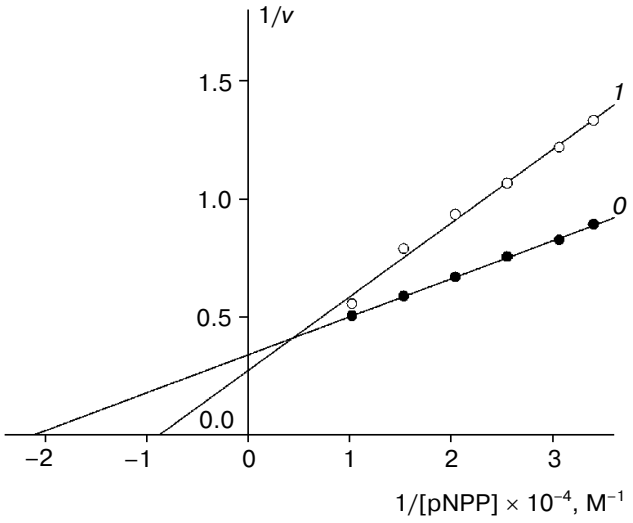


Fig. 4. Inhibitory effect of PDTA on the initial rate (v, μmol/min per μg protein) of pNPP cleavage by canine alkaline phosphatase: *1*) the concentration of PDTA is 10^{-3} M; *0*) the inhibitor is absent.

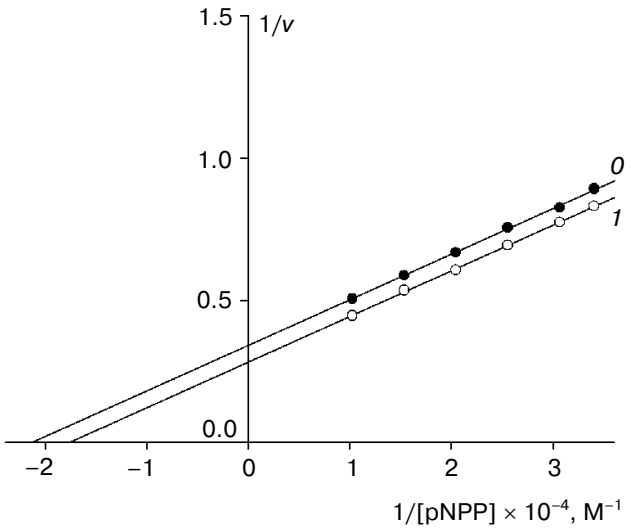


Fig. 5. Activating effect of Guo on the initial rate (v, μmol/min per μg protein) of pNPP cleavage by canine alkaline phosphatase: *1*) the concentration of Guo is 10^{-3} M; *0*) the activator is absent.

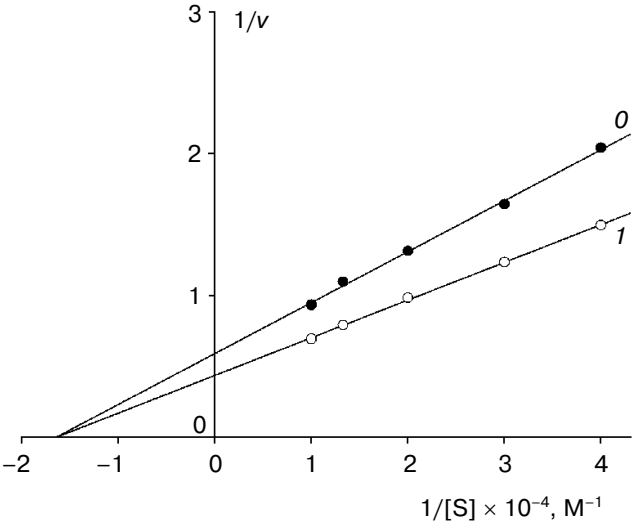


Fig. 6. Activating effect of ATP on the initial rate (v, mU/mg protein) of chitin biosynthesis (substrate UDP-N-acetylglucosamine) by chitinsynthase of *Sacch. carlsbergensis*: *1*) the concentration of ATP is $0.5\cdot10^{-3}$ M; *0*) the activator is absent.

tested alkaline phosphatase, which in both cases indicates a weak activating effect of Guo on both enzymes.

Comparison of the K_m^0 and $\bar{K}_{IIa}$ values shows that the affinity of Guo for canine alkaline phosphatase is by $\bar{K}_{IIa}/K_m^0 = 73$ times weaker than of this enzyme to the substrate cleaved. The reason is in the fact that there is no similarity between the molecule of Guo and a negatively charged phosphate residue of pNPP. Evidently, it would have been more difficult to make such a conclusion without calculation of the $\bar{K}_{IIa}$ constant of enzyme activation.

Example 4. The study of activating effect of ATP on chitinsynthase of *Sacch. carlsbergensis* (Fig. 6) [20, 25] reveals that the parameters of initial reaction of chitin biosynthesis: $K_m^0 = 6.1\cdot10^{-4}$ M, $V^0 = 1.69$ mU/mg protein in the presence of $5\cdot10^{-4}$ M ATP, change as follows: $K'_m = 6.102\cdot10^{-4}$ M, $V' = 2.36$ mU/mg protein. This corresponds to the III_a type of catalytic enzyme activation ($K'_m = K_m^0$, $V' > V^0$, $a > 0$). For calculation of the $\bar{K}_{IIIa}$ constant of enzyme activation that takes into account the length of L_{IIIa} vector projection of this reaction on the basic σ_0 plane (Figs. 1 and 2), Eq. (5) from the text or Eq. (13) from Table 1 should be used.

Analysis of the L_{IIIa} vector position representing this reaction in the three-dimensional $K'_mV'I$ coordinate system (Fig. 1) shows that as in this case the L_{IIIa} vector projection is located on the PV' coordinate semiaxis in the basic σ_0 plane, there is no need to determine it by Eq. (13), because the length l_{IVa} of the second vector projection is absent. As seen from a vector form of Eq. (5), such form allows taking into account the length of L_{IIIa} vector orthogonal projection on the basic σ_0 plane (Fig. 1). Substitution of V', V^0, and a parameters of chitinsynthase activation in Eq. (5) allows calculation of the respective constant of enzyme activation:

$$\bar{K}_{IIIa} = \frac{0.5\cdot10^{-3}\,\text{M}}{\dfrac{2.36-1.69}{1.69}} = \frac{0.5\cdot10^{-3}\,\text{M}}{0.3965} = 1.26\pm0.09\cdot10^{-3}\,\text{M}. \quad (24)$$

The result indicates weak interaction of ATP with catalytically active amino acid residues of the enzyme, which does not prevent its binding to substrate.

Use of the corrected equations for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants of biparametrical types of enzyme inhibition and activation (Table 1) increases the accuracy of calculation of these constants and makes more correct comparative analysis of these constants and the K_i and K_a constants of monoparametrical types of enzyme inhibition and activation that permits taking into account the length of vector orthogonal projections of appropriate enzymatic reactions just from the start.

APPENDIX

By comparing Eqs. (8)-(11), one can easily see that substitution in Eq. (8) of the dimensionless coordinates of the lengths of L_{IIIi} and L_{IVi} vector projections is equal to substitution in this equation of the i/K_{IIIi} and i/K_{IVi} parameters. Having substituted them in Eq. (8):

$$\bar{l}_{Ii} = \sqrt{\left(\frac{i}{K_{IIIi}}\right)^2 + \left(\frac{i}{K_{IVi}}\right)^2} \quad (A1)$$

we shall obtain that:

$$\bar{K}_{Ii} = \left(\frac{K_{IVi}^{2} \cdot K_{IIIi}^{2}}{K_{IVi}^{2} + K_{IIIi}^{2}} \right)^{0.5} . \qquad \text{(A2)}$$

One can deduce by analogy all the other equations of a similar form for calculation of the $\bar{K}_i$ and $\bar{K}_a$ constants of the biparametrical types of enzyme inhibition and activation (Table 2). For example, as seen from Figs. 1 and 2, the length of orthogonal projection $\bar{L}_{IIi}$ of L_{IIi} vector on the basic σ_0 plane shall be determined by a vector principle of summation of projection lengths of this vector on the $P0_{V'}$ and $P0_{K'_m}$ coordinate semiaxes. Hence, in the equation for calculation of the $\bar{K}_{IIi}$ constant of the II_i type of enzyme inhibition the constituent parameters shall be the constants K_{IIIi} (Eq. (3) in Table 1) and K_{IVa} (Eq. (12) in Table 1) of the monoparametrical types of enzyme inhibition and activation:

$$\bar{K}_{IIi} = \left(\frac{K_{IVa}^{2} \cdot K_{IIIi}^{2}}{K_{IVa}^{2} + K_{IIIi}^{2}} \right)^{0.5} . \qquad \text{(A3)}$$

Substitution in Eq. (A2) the values of K_{IIIi} and K_{IVi} constants (Fig. 3) gives the same value ($\bar{K}_{Ii}$ = $0.748 \cdot 10^{-4}$ M) of this constant of enzyme inhibition.

It makes no difficulty to obtain by analogy all the other equations of Table 2.

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