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The Quadratic forms of Equations for Calculation of the K_i and K_a Constants of Enzyme Inhibition and Activation

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ABSTRACT

The analysis of dependence of the length projection of L_i vectors of biparametrical inhibited and activated (L_a) enzymatic reactions from the length projection of vectors of monoparametrical inhibited and activated enzymatic reactions on the basic σ_0 plane in three-dimensional $K_m^{'}V^{'}I$ coordinate system, allows to deduct the quadratic forms of equations for the calculation of the constants of inhibition (K_i) and activation (K_a) of enzymes. Examples of calculation of constants are given.

Introduction

In previous articles [1-9], devoted to construction of a vector method representation of enzymatic reactions in the three-dimensional $K_m^{'}V^{'}I$ coordinate system the properties of L vectors of enzymatic reactions was analyzed, from which the *parametriacal* classification of the types of enzymatic reactions and the equations for calculation of initial activated (V_a) and inhibited (V_i) reaction rates was suggested. In these article the equations of traditional form (t.f.) for calculation of the constants of activation (K_a) and absent in practice nontrivial types of biparametrical constants of inhibition (K_i) of enzymes (Table 1), was deduced.

This work is devoted to deduction of quadratic form (q.f.) of the equations for calculation of biparametrical constants of inhibition and activation of enzymes (Table 1t & 1f), opening additional ability in the analysis of enzyme action what help of quadratic forms of equation (Table 1q& 1f).

The examples of comparative using traditional and quadratic form of equations for calculation of K_i and K_a constants of inhibition and activation are given.

Deduction of Traditional form of Equations

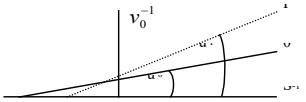
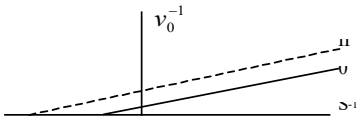
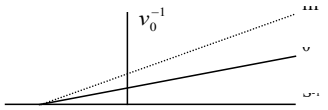
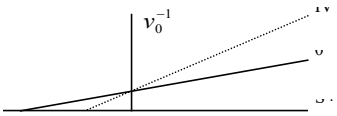
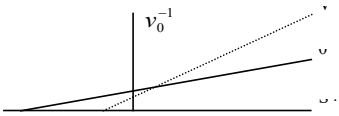
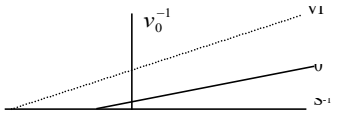
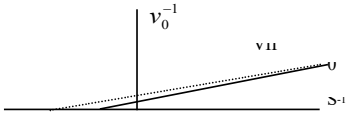
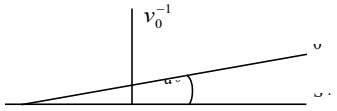
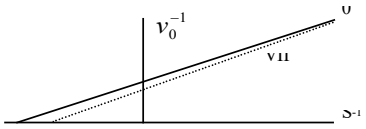
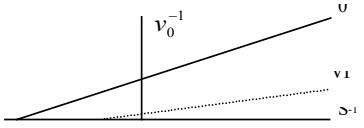
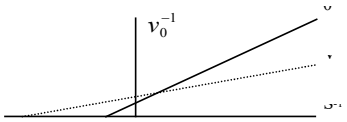
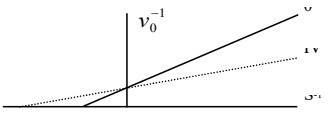
From (Figures 1, 1a and 2) it easy to see, that (l_I) length of (L_{Ii}) projection of L_{Ii} vector of biparametrically coordinated, I_i type (or mixed type [10 -12]) of enzyme inhibition on P semiaxis will be determined by divide (*i-0*) parameters on (l_I) length of (L_{Ii}) projection of L_{Ii} vector of – by summation of the quadratic (l^2) lengths (orthogonal between them self) L_{IIIi} and L_{IVi} projections of monoparametrical L_{IIIi} and L_{IVi} vectors of III_i and IV_i type of enzyme inhibition, (which also are the coordinate of these vectors) but in the same time they taking adjacent place relative to orthogonal L_{Ii} projection of L_{Ii} vector (Figure 2), determined by equation:

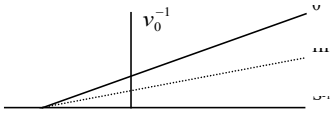
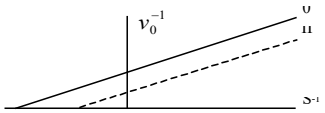
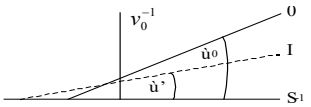
The l_I length of L_{Ii} projection on σ_0 plane of Figures.; (1 - 2) may be determined as

$$l_{Ii} = \sqrt{(l_{IIIi})^2 + (l_{IVi})^2} \qquad (1)$$

Having expressed from Eq. (2) the l_{IIIi} length of L_{IIIi} projection of $\mathbf{L}_{IIIi}$ vector on $P0_V$ semiaxis of $K_m^{'}V^{'}I$ coordinate (Figures 1, 1a):

Table 1: Equations for calculation of K_i and K_a constants (in traditional form).

No	Effect	Type of effect	Correlation between $K_m^{'}$ and $V^{'}$ parameters	Graphs in $(v_o^{-1}; s^{-1})$ coordinates
1	Inhibition- ($i > 0$)	I_i	$K_m^{'} > K_m^0, V^{'} < V^0$	
2		I_i	$K_m^{'} < K_m^0, V^{'} < V^0$ $(tg\omega^{'} = tg\omega^0)$	
3		III_i	$K_m^{'} = K_m^0, V^{'} < V^0$	
4		IV_i	$K_m^{'} > K_m^0, V^{'} = V^0$	
5		V_i	$K_m^{'} > K_m^0, V^{'} > V^0$	
6		V_i	$K_m^{'} < K_m^0, V^{'} < V^0$ $(tg\omega^{'} > tg\omega^0)$	
7		VII_i	$K_m^{'} < K_m^0, V^{'} < V^0$ $(tg\omega^{'} < tg\omega^0)$	
8	None	I_0	$K_m^{'} = K_m^0, V^{'} = V^0$	
9	Activation ($a > 0$)	VII_a	$K_m^{'} > K_m^0, V^{'} > V^0$ $(tg\omega^{'} > tg\omega^0)$	
10		V_a	$K_m^{'} > K_m^0, V^{'} > V^0$ $(tg\omega^{'} < tg\omega^0)$	
11		V_a	$K_m^{'} < K_m^0, V^{'} < V^0$	
12		IV_a	$K_m^{'} < K_m^0, V^{'} = V^0$	

13		III_a	$K_m^{'} = K_m^0, V^{'} > V^0$	
14		I_a	$K_m^{'} > K_m^0, V^{'} > V^0$ $(tg\omega^{'} = tg\omega^0)$	
15		I_a	$K_m^{'} < K_m^0, V^{'} > V^0$	

*The symbol of a graph in Figs. 1-15 corresponds to the type of reaction under study. For example: the line (0) characterizes the position of initial (nonactivated) enzymatic reaction, line I – the position of a graph representing the type of activated enzymatic reaction etc.

Table 1 (continuation).

Type of effect	New name of nzymatic reactions	Traditio-nal name	Traditional form (*t.f.*) of equation for calculation of K_i and K_a constants	Quadratic form (*q.f.*) of equations
I_i	Biparame-Trically coordi-na-ted inhibition	Mixed Inhi-bi-Tion	$K_{Ii} = i / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$	$K_{Ii} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
I_i	Unassoci-ative Inhibition	Uncom-petitive Inhibi-Tion	$K_{IIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$	$K_{IIi} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$
III_i	Catalytic Inhibition	Noncom-pe-titive Inhibiton	$K_{IIIi} = \frac{i}{V^0 / V^{'} - 1} = \frac{i}{\frac{V^0 - V^{'}}{V^{'}}}$	$K_{IIIi} = 1 / \left(\frac{1}{K_{IIIi}^2} \right)^{0,5} = K_{IIIi}$
IV_i	Associa-tive Inhibition	Com-petetive inhibi-Tion	$K_{IVi} = \frac{i}{K_m^{'} / K_m^0 - 1} = \frac{i}{\frac{K_m^{'} - K_m^0}{K_m^0}}$	$K_{IVi} = K_{IVi}$
V_i	Pseudoin-Hibition		$K_{Vi} = i / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{Vi} = 1 / \left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
V_i	Discoordi-nated Inhibition		$K_{VIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$	$K_{VIi} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$
VII_i	Transient Inhibition		$K_{VIIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$	$K_{VIIi} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$
I_0	Initial (i = 0 and a = 0) Enzymatic Reaction			
VII_a	Transient Activation		$K_{VIIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{VIIa} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$

V_a	Discoor-dinated Activation		$K_{VIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{VIa} = 1 / \left(\frac{1}{K_{IIIa}} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
V_a	Pseudo-Activation		$K_{Va} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$	$K_{Va} = 1 / \left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$
IV_a	Associa-Tive Activation	Competi-Tive Activa-Tion	$K_{IVa} = \frac{a}{K_m^0 / K_m^{'} - 1} = \frac{a}{\frac{K_m^0 - K_m^{'}}{K_m^{'}}}$	$K_{IVa} = \left(\frac{1}{K_{IVa}^2} \right)^{-0,5} = K_{IVa}$
III_a	Catalytic Activation	Noncom-Petitve Activa-Tion	$K_{IIIa} = \frac{a}{V^{'} / V^0 - 1} = \frac{a}{\frac{V^{'} - V^0}{V^0}}$	$K_{IIIa} = K_{IIIa}$
I_a	Unassocia-tive Activation	Uncom-Petitive Activa-Tion	$K_{IIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{IIa} = 1 / \left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
I_a	Biparame-Trically Coordina-Ted Acti-vation	Mixed Activa-tion	$K_{Ia} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{Ia} = 1 / \left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$

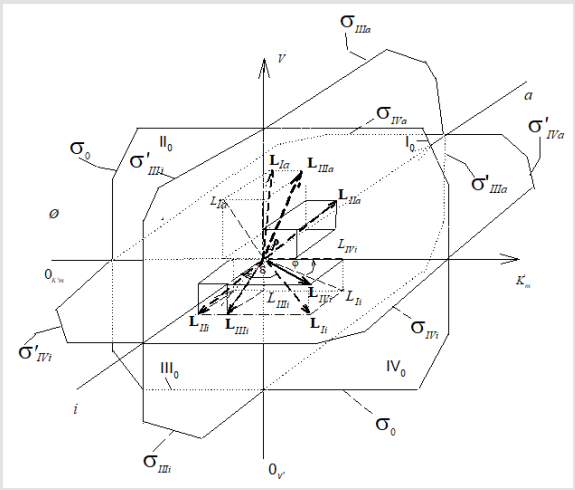


Figure 1: Three dimensional (incompletely) system of rectangular coordinate with separately Pi and Pa semiaxes of molar concentrations of [i] inhibitor and [a] activator. Only 8 L vectors of enzymatic reactions (the symbols: L_{Ii}, L_{IVi}, L_{IIIi}, L_{IIi}, L_{Ia}, L_{IIIa}, LIIa placed in appropriate parallelepipeds and four orthogonal projections of these L vectors (the symbols: L_{Ii}, L_{IVi}, L_{IIIi} and L_{Ia}) are placed on basic σ_0 plane (I_o, II_o … quadrants of this plane), the magnitude of φ angle about 3400.

$$l_{IIIi} = \frac{V^0 - V^{'}}{V^{'}} = \frac{i}{K_{IIIi}}, \qquad (2)$$

from Eq. (3) – the l_{IVi} length of the second adjacent of L_{IVi} vector projection on $PK_m^{'}$ semiaxis:

$$l_{IVi} = K_{Ii} = \mathrm{Pr}_{pi} L_{Ii} / \mathrm{Pr}_{\sigma 0} L_{Ii}, = \frac{i}{K_{IVi}} \qquad (3)$$

and substituted them in Eq. (4):

$$K_{Ii} = \mathrm{Pr}_{pi} L_{Ii} / \mathrm{Pr}_{\sigma 0} L_{Ii}, \qquad (4)$$

we shall obtain traditional form (t.f.) of equation for calculation of the K_I constant of biparametrically coordinated, I_i type, inhibition of enzymes, taking in to consideration the l_{Ii} length of orthogonal projection of L_{Ii} vector on basic σ_0 plane of Figure (1a):

$$K_{Ii} = (i - 0) l_{Ii} = K_{Ii} = (i - 0) l_{Ii} \frac{i}{\left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}} \qquad (5)$$

where $l_{Ii} = \sqrt{(l_{IIIi})^2 + (l_{IVi})^2}$, as it follows from (Figures 1&2).

It is analogous for length of adjacent projections:

$$l_{VIIi}=l_{VIIi}=\sqrt{(l_{IIIi})^2+(l_{IVa})^2},l_{Vi}=\sqrt{(l_{IIIa})^2+(l_{IVi})^2},l_{IIa}=\sqrt{(l_{IIIa})^2+(l_{IVi})^2},l_{Va}=\sqrt{(l_{IIIi})^2+(l_{IVa})^2},l_{Vi}=\sqrt{(l_{IIIa})^2+(l_{IVi})^2},l_{IIa}=\sqrt{(l_{IIIa})^2+(l_{IVi})^2},l_{Va}=\sqrt{(l_{IIIi})^2+(l_{IVa})^2}$$

for all other l_{IIa}, l_{Va} ... of vectors projections of biparametrical reactions (Figures 1,1a & 2).

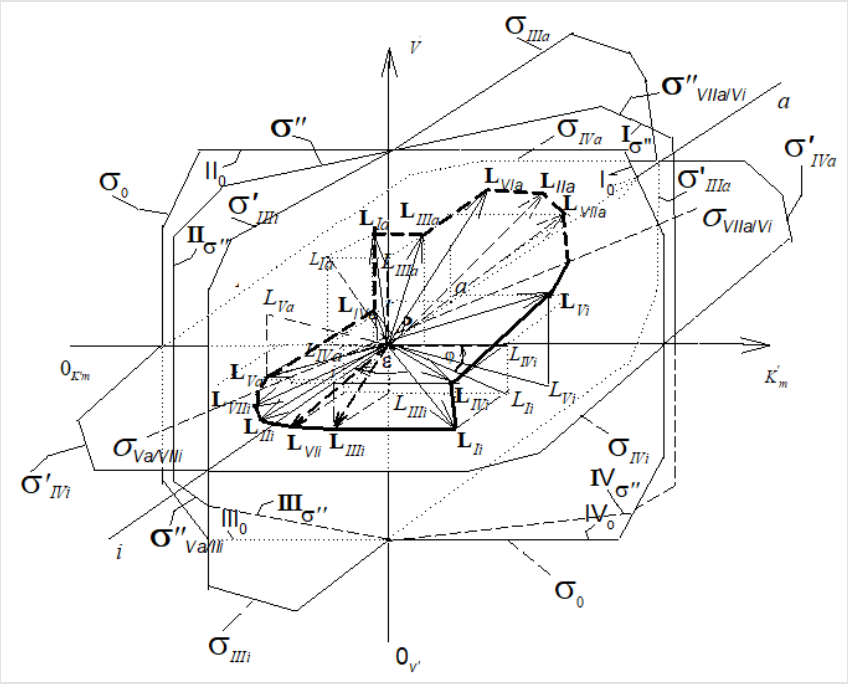


Figure 1a: Three dimensional (completely) $K'_mV'I$ coordinate system, (the same as Figure 1), bat with all 14 branched L vectors (7 type of additional L vectors placed without of appropriate arallelepipeds. The ends of all mobile L vectors are joined by dash line (broken part – activated, unbroken part– L vectors of inhibited reactions). The 15th L_0 vector of initial reaction (and it L_0 projection take place in P point of coordinate intersection. The all 14 orthogonal L_{Ii}, L_{IVi} … L_{Ia} , L_{Ia} projections of L vectors on basic σ_0 plane, are placed completely in (Figure 2). $\sigma^{"}_{\frac{IIIa}{V}}$ – first $(I_{\sigma^{"}})$ and $\sigma^{"}_{Va/IIIi}$ – third $(III_{\sigma^{"}})$ quadrants of transient $\sigma^{"}$ plane, $\sigma_{VIIa/Vi}$ and $\sigma_{Va/VIIi}$ – beginning and finishing ends of the line of orthogonal $\sigma^{"}$ transient plane projection on basic σ_0 plane (in Figures.1a and 2, market by broken lines), the magnitude of φ angle about 3400.

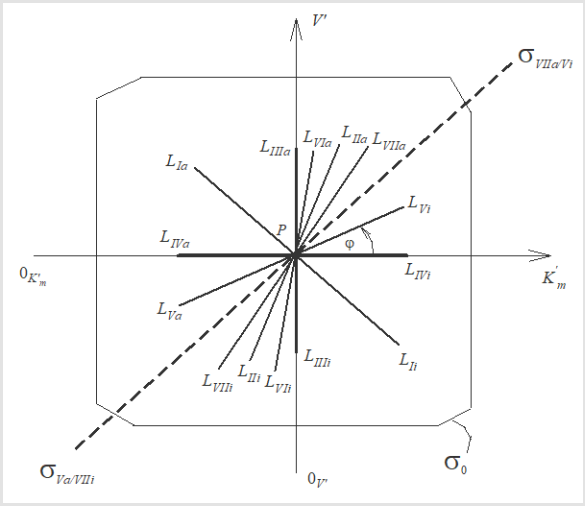


Figure 2: Two-dimensional (scalar) K'_mV' coordinate system. The symbols of kinetic parameters: K'_m, V', K^0_m the projections L_{Ii}, L_{IVi}… L_{Ia}, L_{IVa} of three-dimensional vectors: L_{Ii}, L_{IVi}… L_{Ia}, L_{IVa} on the basic σ_0 plane and symbols of $PK'_m, P0_{V'}, P0_{K'_m}$ and PV' coordinate semiaxes the same as in Figure. 1 and in the text, the magnitude of φ angle about 150.

Deduction of Quadratic form of Equations

From analysis of Equations (1-4) one can easily see that substitution in Eq. (4) 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 then it is not difficult to become the alternative equations for calculation of K_i and K_a constants of biparametrical types of inhibition and activation of enzymes. Having substituted in Eq. (4) 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.

$$l_{Ii}=\sqrt{\left(\frac{i}{K_{IIIi}}\right)^2+\left(\frac{i}{K_{IVi}}\right)^2} \tag{6}$$

we find that such as:

$$l_{Ii}=l_{Ii}=\frac{i_{Ii}}{K_{Ii}} \tag{7}$$

this substitution will lead to equation:

$$K_{Ii}=i/l_{Ii}=i/(i/K_{Ii}=i/l_{Ii}=i/(i/\left(\frac{1}{K^2_{IIIi}}+\frac{1}{K^2_{IVi}}\right)^{0.5})=1/\left(\frac{1}{K^2_{IIIi}}+\frac{1}{K^2_{IVi}}\right)^{0.5}=1/\left(\frac{1}{K^2_{IIIi}}+\frac{1}{K^2_{IVi}}\right)^{0.5} \tag{8}$$

or, in quadratic form:

$$\frac{1}{K^2_{Ii}}=\frac{1}{K^2_{IIIi}}=\frac{1}{K^2_{IVi}}=\frac{1}{K^2_{IIIi}}+\frac{1}{K^2_{IVi}} \tag{9}$$

convenient for calculation of constant inhibition of enzymes (Eq. 1, q.f., in Table 1).

It is analogous for all the other equations of biparametrical types of inhibition (Eqs: 2, 5 – 7), and activation (Eqs: 9 – 11 and 14-15 of enzymes, Table 1q & 1f) such as orthogonal projections of correspond **L** vectors on the basic σ_0 plane, easy to determine by data of two-dimensional (scalar) K'_mV' coordinate system (Figure 2), taking into account orthogonal L projections of tree-dimensional **L** vectors on basic σ_0 plane of (Figures 1a).

Examples of constants calculation.

Example 1: Calculation of Constant Inhibition

The inhibitory effect of Tungstic acid anions $WO_4^{2-}(0.5\times10^{-4}M)$ on the initial rate of pNPP cleavage by calf alkaline phosphatase (Figure 3).

shows that the presence $0.5\times10^{-4}M$ of these anions in the enzyme-substrate system makes the binding of the enzyme to the substrate cleaved $(K^0_m=4.45\times10^{-5}M, K'_m=6.56\times10^{-5}M)$ difficult and leads to a decrease in the maximum reaction rate (V^0= 2.56, V'= 1.74μmol/ (min per μg protein). This meets all the features $(K'_m>K^0_m, V'<V^0, i>0)$ of the biparametrically coordinated, I_i type, of enzyme inhibition (Table 1, line 1). Hence, to calculate the K_i constant of this phosphatase inhibition it is necessary to use Eq. (5, text), or (Eq. 1, Table 1t & 1f).

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

$$K_{Ii}=K_{Ii}=\frac{0.5\cdot10^{-4}M}{\left(\left(\frac{6.56-4.45}{4.45}\right)^2+\left(\frac{2.56-1.74}{1.74}\right)^2\right)^{0.5}}=7.48.10^{-5}M=7.48\times10^{-5}M \tag{10}$$

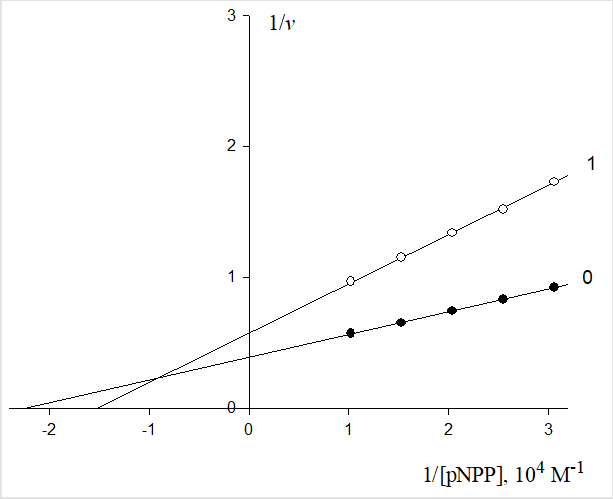


Figure 3: Inhibitory effect of anions WO_4^{2-} on the initial rate V_0, μ mol/ (min per μ g protein) of pNPP cleavage by calf alkaline phosphatase. Note: line 1 – the concentration of WO_4^{2-} is $0.5\times10^{-4}M$; line (0) – the inhibitor is absent.

Substitution the same parameters (recalculated to values of $K_{IIIi}=1.602\times10^{-4}M$ and $K_{IVi}=1.055\times10^{-4}M$ constants) in equation (1 of Table 1t & 1f), result into next value of this K_I constant:

$$K_{Ii}=K_{Ii}=\frac{0.5\cdot10^{-4}M}{\sqrt{0.447}}=\frac{0.5\cdot10^{-4}M}{0.669}=0.747.10^{-4}M=\frac{0.5\times10^{-4}M}{0.669}=0.747\times10^{-4}M \quad (11)$$

Substitution of these parameters in (Eq. 1, q.f., Table 1)

$$\frac{1}{K_{Ii}^2}=\frac{1}{K_{IIIi}^2}+\frac{1}{K_{IVi}^2}=\frac{1}{K_{Ii}^2}=\frac{1}{K_{IIIi}^2}=\frac{1}{K_{IVi}^2}=\left(\frac{1}{1.062^2}+\frac{1}{1.055^2}\right) \quad (12)$$

result into the same value of the constant of enzyme inhibition:

$$K_{Ii}=K_{Ii}=\sqrt{\frac{1}{1.7851}}=\sqrt{0.5602}=0.7485.10^{-4}M=\sqrt{0.5602}=0.7485\times10^{-4}M, \quad (13)$$

From Eqs. (10 -13) it follows that dimension of constants in all cases, are the molar concentration of inhibitor:

$$K_{Ii}=\sqrt{i^4/i^2}=i[M] \quad (14)$$

Control. Determine the value of the K_{IIIi} constant of this experiment (Figure 3) by values of K_I and K_{IVi} constants.

From equations (11) and (12), rewritten to the form,

$$\left(\frac{1}{K_{Ii}^2}=\frac{1}{K_{IIIi}^2}+\frac{1}{K_{IVi}^2}\right)=\left(\frac{1}{0.7485^2}=\frac{1}{K_{IIIi}^2}+\frac{1}{1.055^2}\right) \quad (15)$$

it follows that:

$$K_{IIIi}=\left(\frac{K_{Ii}^2\cdot K_{IVi}^2}{K_{IVi}^2-K_{Ii}^2}\right)^{0.5}10^{-4}M \quad (16)$$

Substitution the necessary parameters from (Eq. 15) to (Eq. 16), we find that:

$$K_{IIIi}=\left(\frac{0.748^2\cdot1.055^2}{1.055^2-0.748^2}\right)^{0.5}.10^{-5}M=\left(\frac{0.5595\times1.113}{1.113-0.5595}\right)^{0.5}.10^{-5}M=1{,}125^{0.5}.10^{-5}M=1.061\times10^{-5}M, \quad (17)$$

which is in good agreement with the experimental value of this constant (Eq. 12).

Example 2: Calculation of Constant Inhibition

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 $1\times10^{-3}M$ PDTA the parameters $K_m^0=4.69\times10^{-5}M$ and V^0= 2.921 μmol/(min per μg protein) change as follows $K_m^{'}=11.26\times10^{-5}M$ and $V^{'}=3.616$ μmol/(min per μg protein) (Figure 4). This corresponds to the, V_i type, of enzyme pseudoinhibition $(K_m^{'}>K_m^0, V^{'}>V^0, i>0)$ (Table 1, line 5) and Eq. (5, t.f.) is applicable for calculation of the K_V constant of enzyme inhibition. Substitution all necessary parameters in this equation allows calculation of this constant of enzyme inhibition:

$$K_{Vi}=\frac{1\cdot10^{-3}M}{\left(\left(\frac{11.26-4.69}{4.69}\right)^2+\left(\frac{3.616-2.92}{2.92}\right)^2\right)^{0.5}}=7.04\times10^{-4}M \quad (18)$$

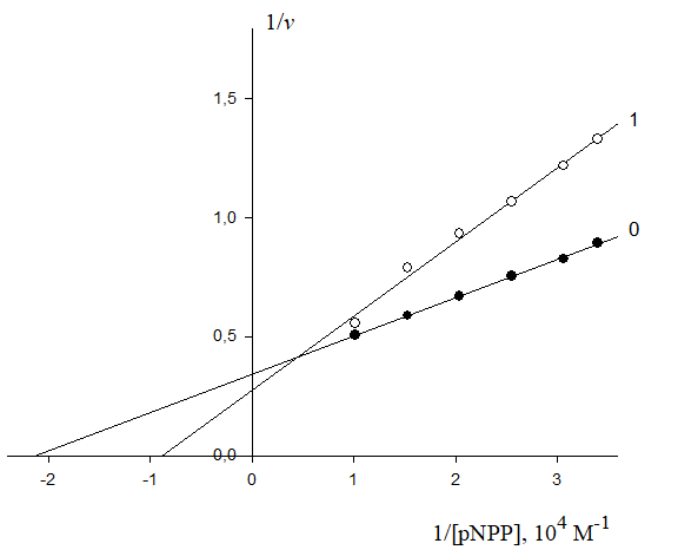


Figure 3: Inhibitory effect of PDTA on the initial rate V_0, μ mol/(min per μ g protein) of pNPP cleavage by canine alkaline phosphatase. Note: line 1 – the concentration of PDTA is $1\times10^{-3}M$; line (0) – the inhibitor is absent.

Substitution of recalculated parameters of (Figure 4) $K_{IVi}=0.7138\times10^{-3}M$ and $K_{IIIa}=4.2029\times10^{-3}M$ to (Eq. 5, Table 1, q.f.) – result into value of K_V constant inhibition:

$$\frac{1}{K_{Vi}^2}=\frac{1}{K_{IIIa}^2}+\frac{1}{K_{IVi}^2}=\left(\frac{1}{4.2029^2}+\frac{1}{0.7138^2}\right)=(2.0192)^{0.5}\times10^{-4}M= \quad (19)$$

$$K_{Vi}=\sqrt{1/2.0192}=\sqrt{0.4951}=7.036\times10^{-4}M$$

Example 3: Calculation of Constant Activation

The activating effect of Guanosine (Guo) on canine alkaline phosphatase (Figure 5) shows that in the presence of $1\times10^{-3}M$ Guo the parameters of initial reaction of pNPP cleavage, i. e $K_m^0=4.69\times10^{-5}M, V^0=2.92$ μmol/(min per μg protein), change as follows: $K_m^{'}=5.67\times10^{-5}M, V^{'}=3.527$ μmol/(min per μg protein). This corresponds to the, I_a type, of unassociative enzyme activation.

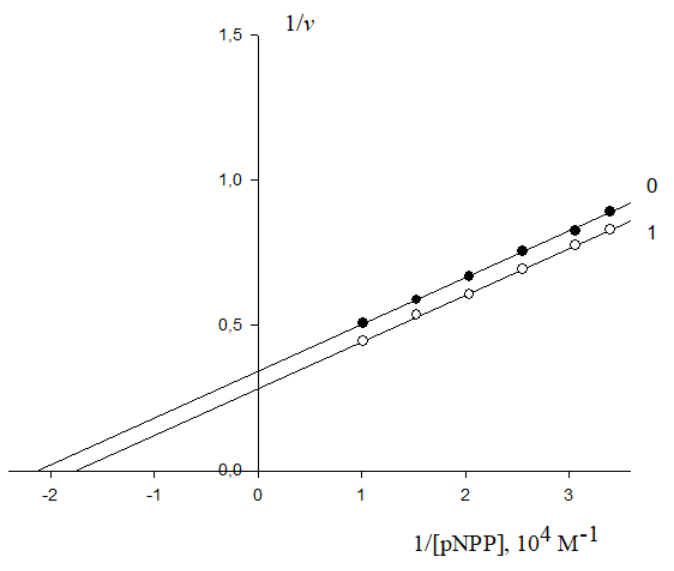


Figure 5: Activating effect of Guo on the initial rate v_0, μ mol/(min per μ g protein) of pNPP cleavage by canine alkaline phosphatase. Note: line 1 – the concentration of Guo is $1\times10^{-3}M$; line (0) - the activator is absent.

Hence, to calculate the K_{IIa} constant of enzyme activation, one should use Eq. (14, t.f., Table 1).

Substitution of the obtained values of parameters in this equation allows calculation of this constant of enzyme activation:

$$K_{IIa}=a/\left(\left(\frac{K_m^{'}-K_m^0}{K_m^0}\right)^2+\left(\frac{V^{'}-V^0}{V^0}\right)^2\right)^{0.5}=1/\sqrt{0.0867}=3.3965\times10^{-3}M \quad (20)$$

Substitution of the: $K_{IVi}=4.785\times10^{-3}M$ and $K_{IIIa}=4.819\times10^{-3}M$ parameters of this experiment (Figure 5) in (Eq. 14, q.f., Table 1), result in to:

$$\left(\frac{1}{K_{IIa}}\right)^2=\left(\frac{1}{K_{IIIa}}\right)^2+\left(\frac{1}{K_{IVi}}\right)^2=\frac{1}{23.223}+\frac{1}{23.766}=0.0852\times10^6M^{-2}, \quad (21)$$

as it was to be expected, result in to the same value of activation constant:

$$K_{IIa}=\frac{1}{\sqrt{0.0852\times10^6,M^2}}=\frac{1}{0.2919}10^{-3}M=3.426\times10^{-3}M \quad (22)$$

From the length parts of equations: (12), (15), (19) and (21) may to see that all they obeys to the signs of Pifagor's theorem and this may be used as for calculation any of the third constants by the two others known already and for correction the constants, determined by using any other equations.

Example 4: Calculate the value of K_{IIIi} constant of experiment (Figure 3), by value of K_{Ii} and K_{IVi} constants. From equation (1, Table 1t & 1f), rewritten to the quadratic form (23).

$$\frac{1}{K_{Ii}^2}=\frac{1}{K_{IIIi}^2}+\frac{1}{K_{IVi}^2}=\frac{1}{0,7485^2}=\frac{1}{K_{IIIi}^2}+\frac{1}{1,055^2} \quad (23)$$

it follows that:

$$K_{IIIi}=\left(\frac{K_{Ii}^2\cdot K_{IVi}^2}{K_{IVi}^2-K_{Ii}^2}\right)^{0.5}M. \quad (24)$$

Having substitution all necessary parameters from (Eq. 23) to (Eq. 24), we shall become, that:

$$K_{IIIi}=\left(\frac{0.748^2\cdot1.055^2}{1.055^2-0.748^2}\right)^{0.5}\times10^{-5}M=\left(\frac{0.5595\cdot1.113}{1.113-0.5595}\right)^{0.5}\times10^{-5}M=1.125^{0.5}\times10^{-5}M=1.061\times10^{-5}M. \quad (25)$$

Ii is analogous for all biparametrically types of catalyzed reactions (Table 1).

Introduction in practice of quadratic forms of equations for calculation of K_i and K_a constants, will facilitates for many authors

to interpret obtained data of nontrivial types of inhibition and activation by such definition as «essentially competitive inhibition», «similarly to competitive inhibition » and so on [13 -17].

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