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Review Article

Application of the Pyphagor's Theorem for Correction of 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 correction of the constants of inhibition (K_i) and activation (K_a) of enzymes. Examples of correction of constants are given.

Introduction

The study inhibition of enzymes helps to synthesize the drugs from poisoning of living organisms.

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 deduced. In articles [2-9] the equations of traditional form (*t.f.*) for calculation of the constants of activation (K_a) and absent in practice the equations of nontrivial types of biparametrical constants of inhibition (K_i) of enzymes (Table 1), was deduced [5].

This work is devoted to deduction of quadratic form (*q.f.*) of the equations for correction of biparametrical constants of inhibition K_i and activation K_a of enzymes (Table 1, *q.f.*), opening additional ability in the analysis of enzyme action what help of these equations.

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

Deduction of traditional form of equations

From Figures 1 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_i semiaxis will be determined by difference: (i- 0) parameters, The basic σ0 plane (Figure 2), actually is orthogonal projection of three-dimensional L vectors of (Figure 1), i.e. the scalar magnitudes (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

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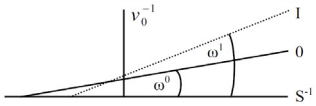
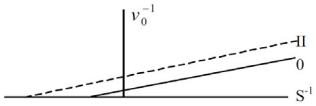
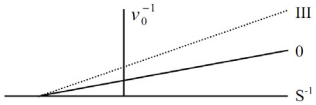
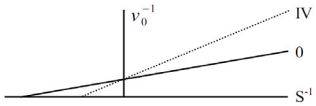
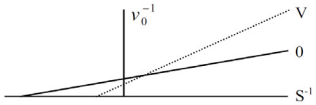
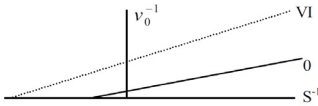
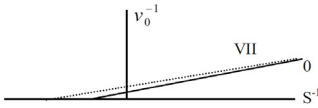
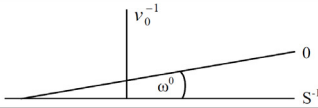
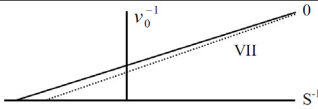
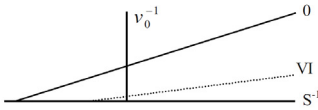
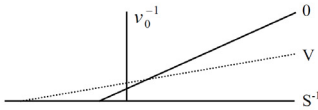
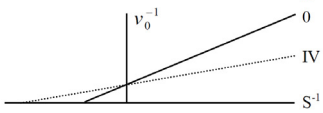
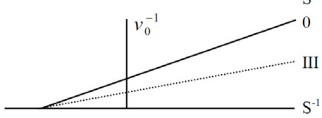
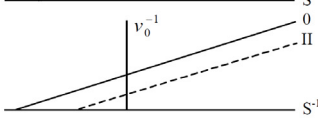
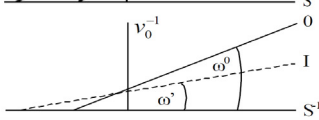
No	Effect	Type of effect	Correlation between $K_m^{'}$ and $V^{'}$ parameters	Graphs in (v_o^{-1}; S^{-1}) coordinates
1	Inhibition- ($i > 0$)	I_i	$K_m^{'} > K_m^0, V^{'} < V^0$	
2		II_i	$K_m^{'} < K_m^0, V^{'} < V^0$ $(tg\omega^{'} = tg\omega^0)$	
3		III_i	$K_m^{'} = K_m^0, V^{'} < V^0$	
4		IV_i	$K_m^{'} > K_m^0, V^{'} = V^0$	
5		V_i	$K_m^{'} > K_m^0, V^{'} > V^0$	
6		VI_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		VI_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		II_a	$K_m^{'} > K_m^0, V^{'} > V^0$ $(tg\omega^{'} = tg\omega^0)$	
*15		I_a	$K_m^{'} < K_m^0, V^{'} > V^0$	

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

*The symbol of a graph in Figure. 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 I_a type of activated enzymatic reaction etc.

Type of effect	New name of enzy-matic 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	bipara-metrically coordina-ted inhibition	mixed inhibi-tion	$K_{Ii} = i / \left(\left(\frac{K_m' - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5}$	$K_{Ii} = 1/\left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
II_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-petitive inhibiton	$K_{IIIi} = \frac{i}{V^0/V' - 1} = \frac{i}{\frac{V^0 - V'}{V'}}$	$K_{IIIi} = 1/\left(\frac{1}{K_{IIIi}^2} + 0 \right)^{0,5}$
IV_i	associa-tive inhibition	com-petetive inhibi-tion	$K_{IVi} = \frac{i}{K_m'/K_m^0 - 1} = \frac{i}{\frac{K_m' - K_m^0}{K_m^0}}$	$K_{IVi} = 1/\left(\frac{1}{K_{IVi}^2} + 0 \right)^{0,5}$
V_i	pseudoin-hibition		$K_{Vi} = i / \left(\left(\frac{K_m' - K_m^0}{K_m^0} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{Vi} = 1/\left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
VI_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		$K_{VIIa} = a / \left(\left(\frac{K_m' - K_m^0}{K_m^0} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{VIIa} = 1/\left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
VI_a	discoor-dinated activation		$K_{VIa} = a / \left(\left(\frac{K_m' - K_m^0}{K_m^0} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{VIa} = 1/\left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2} \right)^{0,5}$
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	$K_{IVa} = \frac{a}{K_m^0/K_m' - 1} = \frac{a}{\frac{K_m^0 - K_m'}{K_m'}}$	$K_{IVa} = 1/\left(\frac{1}{K_{IVa}^2} + 0 \right)^{0,5}$
III_a	catalytic activation	noncom-petitve activa-tion	$K_{IIIa} = \frac{a}{V'/V^0 - 1} = \frac{a}{\frac{V' - V^0}{V^0}}$	$K_{IIIa} = 1/\left(\frac{1}{K_{IIIa}^2} + 0 \right)^{0,5}$
II_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	bipara-metrically coordina-ted activation	mixed activa-tion	$K_{Ia} = a / \left(\left(\frac{K_m^0 - K_m'}{K_m'} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5}$	$K_{Ia} = 1/\left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVa}^2} \right)^{0,5}$

Table 1 continued

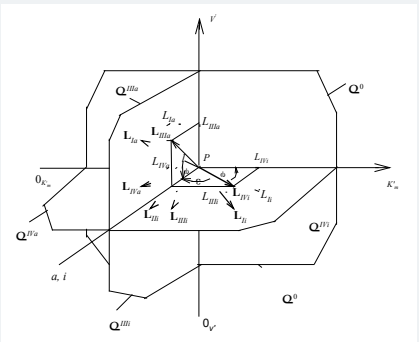


Figure 1: Three-dimensional (packed) $K_m' V' I$ system of rectangular coordinates with coincident P_i and P_a semiaxes of molar concentrations of inhibitor *i* and activator *a*. The symbols of kinetic parameters: K_m', V', three-dimensional vectors: $\mathbf{L}_{Ii}$, $\mathbf{L}_{IVi}$... $\mathbf{L}_{Ia}$, $\mathbf{L}_{IVa}$, $\mathbf{L}_{Ii}$, $\mathbf{L}_{IVi}$..., and their projections L_{Ii}, L_{IVi}... L_{Ia}, L_{IVa} on the basic σ_0 plane as well as the symbols of 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 the same as in the text.

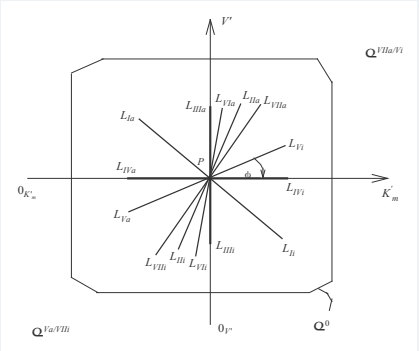


Figure 2: Two-dimensional (scalar) $K'_m V'$ coordinate system. The symbols of kinetic parameters: K'_m, V', K^0_m..., the projections *LIi, LIVi... LIa, LIVa* of three-dimensional vectors: **L***Ii*, **L***IVi*... **L***Ia*, **L***IVa* on the basic σ0 plane and symbols of PK'_m, $P0_{V'}$, $P0_{K'_m}$ and PV' coordinate semiaxes the same as in Figure 1 and in the text.

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:

$$l_I = \sqrt{(l_{IIIi})^2 + (l_{IVi})^2} \tag{1}$$

It is analogous for length of adjacent projections of L_{IIIi}, L_{Vi} ... and L_{Ia}, L_{IIa}, L_{Va} ... for all other L_{IIIi}, L_{Vi} ... , L_{Ia}, L_{IIa}, L_{Va} ... three-dimensional vectors of biparametrical reactions (Figure 2).

Having expressed from Eqn. (2)

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

the l_{IIIi} length of dimensionless of L_{IIIi} projection of L_{IIIi} vector on $P0_{V'}$ semiaxis of K'_m V' I coordinate (Figure 1) and from Eqn. (3)

$$l_{IVi} = \frac{K'_m - K^0_m}{K^0_m} = \frac{i}{K_{IVi}} \tag{3}$$

the l_{IVi} length of the second adjacent dimensionless of L_{IVi} vector projection on PK'_m semiaxis and substituted them in Eqn. (4):

$$K_{Ii} = \mathrm{Pr}_{P_i} L_{Ii} / \mathrm{Pr}_{\sigma_0} L_{Ii}, \tag{4}$$

we shall obtain traditional form (*t.f.*) of equation for calculation of the K_{Ii} 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 1:

$$K_{Ii} = \frac{i}{\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}}. \tag{5}$$

Similarly for deduction of all biparametrical equations of table 1 [5,7,8].

Deduction of quadratic form of equations

From analysis of equations (1 – 4) one can easily see that substitution in Eqn. (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

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

then it is not difficult to become the quadratic forms of equations for correction

of K_i and K_a constants of biparametrical types of inhibition and activation of enzymes (Table 1).

For example, such as:

$$l_{Ii} = \frac{i_I}{K_I}, \quad (7)$$

this substitution will leads to equation:

$$K_{Ii} = i/l_{Ii} = i/(i/\left(\frac{1}{K^2_{IIIi}} + \frac{1}{K^2_{IVi}}\right)^{0,5}) = 1/\left(\frac{1}{K^2_{IIIi}} + \frac{1}{K^2_{IVi}}\right)^{0,5}, \quad (8)$$

or, in quadratic form:

$$\frac{1}{K^2_I} = \frac{1}{K^2_{IIIi}} + \frac{1}{K^2_{IVi}}, \quad (9)$$

convenient for correction of K_{Ii} constant inhibition of enzymes (Eqn. 1, *q.f.*, Table 1).

It is analogous for all the other equations of biparametrical types of inhibition (Eqns. 2, 5 – 7), and activation (Eqns. 9 – 11 and 14, 15) of enzymes, (Table 1, *q.f.*) taking into account, orthogonal projections of tree-dimensional L vectors on the basic σ_0 plane of (Figure 1) by data analysis of correspond position two-dimensional scalar L projections of L vectors on these vectors in $K'_m V'$ coordinate system (Figure 2). For example, the orthogonal projection length of L_{Ia} vector of, I_a type, activation will be determined by analogous common equation (1, text) of enzyme activation that is located in the σ_0 plane of scalar $K'_m V'$ coordinate system (Figure 2, in IInd quadrant) and edged by two L_{IIIa} and L_{IVa} lengths of edged projection of this vector on the PV' and $P0_{Km}$ semiaxes ($l_{Ia} = \sqrt{(l_{IIIa})^2 + (l_{IVa})^2}$),

a) in equation of l_{IIi} length projection – by two l_{IVa} and l_{IIIi} lengths of edged vector projections ($l_{IIi} = \sqrt{(l_{IIIi})^2 + (l_{IVa})^2}$);

b) in equation of l_{Vi} length projection – l_{IVi} and l_{IIIa} lengths of edged vector projections ($l_V = \sqrt{(l_{IIIa})^2 + (l_{IVi})^2}$) and so on.

Examples of constants correction

Example 1: Calculation of K_{Ii} constant inhibition.

The inhibitory effect of Tungstic acid anions WO_4^{2-} ($0.5 \cdot 10^{-4}$ M) on the initial rate of pNPP cleavage by calf alkaline phosphatase figure 3 shows that the presence $0.5 \cdot 10^{-4}$ M of these anions in the enzyme-substrate system makes the binding of the enzyme to the substrate cleaved ($K^0_m = 4.45 \cdot 10^{-5}$ M, $K'_m = 6.56 \cdot 10^{-5}$ 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 ($K'_m > K^0_m$, $V' < V^0$, $i > 0$) of the biparametrically coordinated, I_i type, of enzyme inhibition (Table 1, line 1). Hence, to calculate the K_{Ij} constant of this enzyme inhibition it is necessary to use Eqn. (5, text), or (Eqn. 1, *t.f.*, Table 1).

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:

$$K_{Ii} = \frac{0.5 \cdot 10^{-4} M}{\left(\left(\frac{6.56 - 4.45}{4.45}\right)^2 + \left(\frac{2.56 - 1.74}{1.74}\right)^2\right)^{0.5}} = 7.48 \cdot 10^{-5} \text{ M}. \quad (10)$$

Substitution of these parameters rewritten to forms with ($K_{IIIi} = 1.062\ 10^{-4}$ M, $K_{IVi} = 1.055\ 10^{-4}$ M) in (Eqn. 1, *q.f.*, Table 1)

$$\frac{1}{K^2_I} = \frac{1}{K^2_{IIIi}} + \frac{1}{K^2_{IVi}} = \left(\frac{1}{1.062^2} + \frac{1}{1.055^2}\right), \quad (11)$$

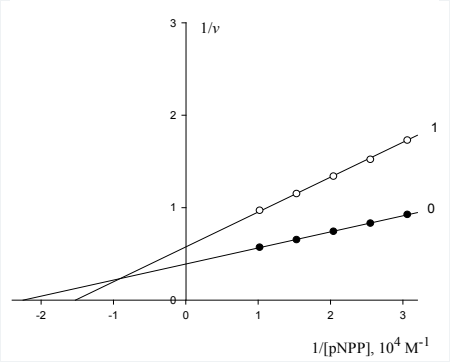


Figure 3: Inhibitory effect of anions WO_4^{2-} on the initial rate v_0, μmol/(min per μg protein) of pNPP cleavage by calf alkaline phosphatase.

Note: line 1 – the concentration of WO_4^{2-} is 0.5·10-4 M; line (0) – the inhibitor is absent.

result in to the same value of the constant of enzyme inhibition:

$$K_{Ii} = \frac{1}{\left(\frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2}\right)^{0.5}} = \left(\frac{(K_{IVi}^2 \cdot K_{IIIi}^2)\cdot(10^{-4})^2\cdot(10^{-4})^2}{(K_{IIIi}^2 + K_{IVi}^2)\cdot(10^{-4})^2}\cdot\frac{M^4}{M^2}\right)^{0.5} =$$

$$\sqrt{0.5602}\cdot\sqrt{(10^{-4})^2}\cdot\sqrt{M^2} = 0.7485\cdot10^{-4}\cdot\text{M}. \quad (12)$$

From Eqns. (10 – 12) it follows that dimension of K_{Ii} constants in all cases, are the molar concentration of inhibitor:

$$K_{Ii} = \sqrt{i^4/i^2} = i\ [\text{M}]. \quad (13)$$

Correction. Determine the value of the K_{IVi} constant of this experiment (Figue 3) by values of K_{Ii} and K_{IIIi} constants.

From equation (11), rewritten to the form,

$$\left(\frac{1}{K_i^2} = \frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2}\right) = \left(\frac{1}{0.7485^2} = \frac{1}{K_{IVi}^2} + \frac{1}{1.062^2}\right), \quad (14)$$

it follows that:

$$K_{IVi} = \left(\frac{K_i^2\cdot K_{IIIi}^2}{K_{IIIi}^2 - K_i^2}\right)^{0.5}\ 10^{-4}\ \text{M}. \quad (15)$$

Substitution the necessary parameters from (Eqn. 14) to (Eqn. 15), we find that:

$$K_{IVi} = \left(\frac{0.7485^2\cdot1.062^2}{1.062^2 - 0.7485^2}\right)^{0.5}\cdot10^{-4}\ \text{M} = \left(\frac{0.5595\cdot1.1278}{1.1276 - 0.5595}\right)^{0.5}\cdot10^{-4}\ \text{M} =$$

$$1.11295^{0,5}\cdot10^{-4}\text{M} = 1.0549\cdot10^{-4}\ \text{M}, \quad (16)$$

which is in good agreement with the experimental value of this constant (Eqn. 10).

Example 2: Calculation of K_{Vi} 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 110^{-3} M PDTA the parameters $K_m^0 = 4.69\cdot10^{-5}$ M and $V^0 = 2.921$ μmol/(min per μg protein) change as follows: $K_m' = 11.26\cdot10^{-5}$ M and $V' = 3.616$ μmol/(min per μg protein) (Figure 4). This corresponds to the, V_i type, of enzyme pseudoinhibition ($K_m' > K_m$, $V' > V$, $i > 0$) (Table 1, line 5) and Eqn. (5, *t.f.*) is applicable for calculation of the K_{Vi} constant of enzyme

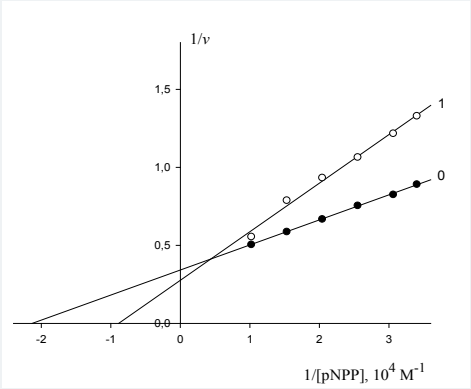


Figure 4: Inhibitory effect of PDTA on the initial rate v_0, μmol/(min per μg protein) of pNPP cleavage by canine alkaline phosphatase.
Note: line 1 – the concentration of PDTA is $1 \cdot 10^{-3}$ M; line (0) – the inhibitor is absent.

inhibition. Substitution all necessary parameters in this equation allow calculation of this constant of enzyme inhibition:

$$K_{Vi} = \frac{1 \cdot 10^{-3} M}{\left(\left(\frac{11.26 - 4.69}{4.69}\right)^2 + \left(\frac{3.616 - 2.92}{2.92}\right)^2\right)^{0.5}} = 7.04 \cdot 10^{-4} \text{ M.} \quad (17)$$

Substitution all necessary parameters from of recalculated parameters of (Figure 4) to (Eqn. 5, Table 1, *q.f.*) – result in to value of K_{Vi} constant inhibition:

$$\frac{1}{K_V^2} = \left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2}\right), \quad (18)$$

rewritten to the forms (K_{IVi} = 0.714 10^{-3} M and K_{IIIa} = 4.203 10^{-3} M)

from which it follows that

$$K_{Vi} = \frac{1}{\left(\frac{1}{K_{IIIai}^2} + \frac{1}{K_{IVi}^2}\right)^{0.5}} = \left(\frac{(K_{IVi}^2 \cdot K_{IIIa}^2) \cdot (10^{-4})^2 \cdot (10^{-4})^2}{(K_{IIIai}^2 + K_{IVI}^2) \cdot (10^{-4})^2} \cdot \frac{M^4}{M^2}\right)^{0.5} =$$

$$\left(\frac{0.51 \cdot 17.67}{0.51 + 17.67}\right)^{0.5} = \left(\frac{9.093}{18.175}\right)^{0.5} = \sqrt{0.496} = 0.7041 \cdot 10^{-3} \text{ M.} \quad (19)$$

Example 3: Calculation of K_{Va} constant activation.

The results of study presented in figure 5 show that: the parameters of initial non-activated reaction of pNPP cleavage by alkaline phosphatase K_m^0 = $5.45 \cdot 10^{-5}$ M, V^0 = 9.363 μmol/(min μg protein) in the presence of 0.001 M of activator change as follows: $K_m^{'}$ = $3.47 \cdot 10^{-5}$ M, V' = 8.803 μmol/(min μg protein), which satisfies all the features of type V_a of enzyme pseudoactivation (line 11, Table 1).

Substitution of the experimental parameters of (Figure 5, in Eqn. 11, *t.f.*, Table 1) gives the following value of K_{Va} constant:

$$K_{Va} = \frac{1 \cdot 10^{-3} M}{\sqrt{\left(\frac{5.45 - 3.47}{3.47}\right)^2 + \left(\frac{9.363 - 8.803}{8.803}\right)^2}} = 1.74 \cdot 10^{-3} \text{ M,} \quad (20)$$

or according Eq. 11, Table 1)

$$K_{Va} = \frac{1}{\left(\frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2}\right)^{0.5}} = \left(\frac{(K_{IVi}^2 \cdot K_{IIIa}^2) \cdot (10^{-4})^2 \cdot (10^{-4})^2}{(K_{IIIa}^2 + K_{IV_i}^2) \cdot (10^{-4})^2} \cdot \frac{M^4}{M^2}\right)^{0.5} =$$

$$\left(\frac{1.753^2 \cdot 15.72^2}{1.753^2 + 15.72^2}\right)^{0.5} 10^{-3}\ \text{M} = \left(\frac{759.39}{258.19}\right)^{0.5} = 1.742 \cdot 10^{-3}\ \text{M}. \quad (21)$$

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 1, *t.f.*), rewritten to the form (22)

$$\left(\frac{1}{K_I^2} = \frac{1}{K_{IIIi}^2} + \frac{1}{K_{IVi}^2}\right) = \left(\frac{1}{0.7485^2} = \frac{1}{K_{IIIi}^2} + \frac{1}{1.055^2}\right), \quad (22)$$

it follows that:

$$K_{IVi} = \left(\frac{K_I^2 \cdot K_{IIIi}^2}{K_{IIIi}^2 - K_I^2} \cdot M^2\right)^{0,5}. \quad (23)$$

Having substituted all necessary parameters from (Eqn. 22) into (Eqn. 23), the next value of this constant is received:

$$K_{IIIi} = \left[\left(\frac{0.748^2 \cdot 1.055^2}{1.055^2 - 0.748^2} \cdot (10^{-4})^2 \cdot M^2\right)\right]^{0,5} = \left(\frac{0.5595 \cdot 1.113}{1.113 - 0.5595}\right)^{0,5} \cdot 10^{-4}\ \text{M} =$$

$$1.125^{0.5} \cdot 10^{-4}\ \text{M} = 1.061 \cdot 10^{-4}\ \text{M}. \quad (24)$$

Example 5: Calculate the value of K_{IIIa} constant of experiment (Figure 5), by value of K_{Va} and K_{IVi} constants.

From equation (11, Table 1, *t.f.*), rewritten to the form (22)

$$\left(\frac{1}{K_{Va}^2} = \frac{1}{K_{IIIa}^2} + \frac{1}{K_{IVi}^2}\right) = \left(\frac{1}{1.742^2} = \frac{1}{K_{IIIa}^2} + \frac{1}{1.753^2}\right), \quad (25)$$

it follows that:

$$K_{IIIa} = \left(\frac{K_{Va}^2 \cdot K_{IVi}^2}{K_{IVi}^2 - K_{Va}^2} \cdot M^2\right)^{0.5} = \left(\frac{1.753^2 \cdot 1.742^2}{1.753^2 - 1.742^2}\right)^2 10^{-3}\ \text{M} =$$

$$\left(\frac{9.435}{0.038}\right)^{0.5} 10^{-3}\ \text{M} = 245.4^{0.5}\ 10^{-3}\ \text{M} = 15.66\ 10^{-3}\ \text{M} \quad (26)$$

It is no desirable to put K_{Va} = $1.74 \cdot 10^{-3}$ M from (Eqn. 20) in (Eqn. 25), because calculation leads to K_{IIIa} = $14.43\ 10^{-3}$M (instead $15.66\ 10^{-3}$ M), such as the first constant is not in Pythagorean´s «bundle» (Egn. 25).

It is analogous for all biparametrical types of catalyzed reactions (Table 1).

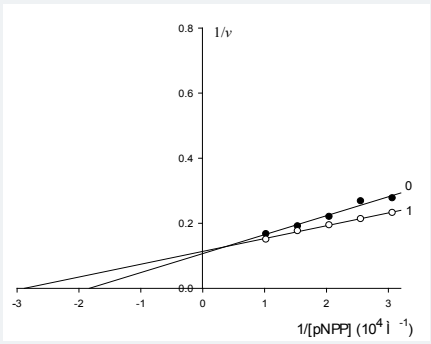


Figure 5: Activating effect of Guo on the initial rate V_0, μmol/(min per μg protein) of pNPP cleavage by canine alkaline phosphatase.
Note: line 1 – the concentration of Guo is $1 \cdot 10^{-3}$ M; line (0) – the activator is absent.

Discussion

The analysis of data obtained shows that:

1) The values of the constants of biparametrical types of inhibition (Eqns. 1, 2, 5 – 7) and activation (Eqns. 9 – 11, 14, 15), are not subjected to additive dependencies on the values of the constants of monoparametrical types of inhibition (Eqns. 3, 4) and activation (Eqns. 12, 13) of the enzymes (Table 1);

$$K_{Ii} \neq K_{IVi} + K_{IIIi}. \quad (27)$$

They subjected to geometrical relationships (Pyphagorean theorem):

$$(1/K_{\mathbf{I}})^2 = (1/K_{IVi})^2 + (1/K_{IIIi})^2, \quad (28)$$

2) this opens an array of possibilities for calculation and correction of the values of K_i and K_a constants (Examples 1 – 4).

References

1. Krupyanko VI. A vector method of representation of enzymic reactions. 3. Three-dimensional system of the K'_m V' I coordinates convenient for representation of enzyme inhibition and activation. Proc Biochem. 2004; 39: 825- 832. **Ref.:** https://goo.gl/dqMjBF
2. Krupyanko VI. Perspectives of Data Analysis of Enzyme Inhibition and Activation. Part 1: Use of the Three-Dimensional K'_m V' I Coordinate System for Data Analysis of Enzyme Inhibition and Activation. J Biochem Mol Toxicol. 2009; 23: 97-100. **Ref.:** https://goo.gl/BK2nqc
3. Krupyanko VI. Perspectives of Data Analysis of Enzyme Inhibition and Activation. Part 2: Parametrical Classification of Types of Enzymatic Reactions. J Biochem Mol Toxicol. 2009; 23: 101-107. **Ref.:** https://goo.gl/vRPeFp
4. Krupyanko VI. Perspectives of data analysis of enzyme inhibition and activation, Part 3: Equations for calculation of the initial rates of enzymatic reactions. J Biochem Mol Toxicol. 2009; 23: 108-118. **Ref.:** https://goo.gl/yyowYP
5. Krupyanko VI. Perspectives of Data Analysis of Enzyme Inhibition and Activation. Part 4: Equations for Calculation of Constants of Enzyme Activation and Inhibition. J Biochem Mol Toxicol. 2010; 24: 145-154. **Ref.:** https://goo.gl/F6m6Tf
6. Krupyanko VI. Determination of intensity of enzyme inhibition and activation. Eur Chem Bull. 2014; 3: 582-586. **Ref.:** https://goo.gl/RxX2M2
7. Krupyanko VI. Non-existence of secondary coordinates of intersects. Eur Chem Bull. 2014; 3: 815-822. **Ref.:** https://goo.gl/i8qkUU
8. Krupyanko VI. Correction of Dixon Plots. Eur Chem Bull. 2015; 4: 142-153. **Ref.:** https://goo.gl/4bfDVP
9. Krupyanko VI. Correction of Data Analysis Equations Relating to Two-Substrate Enzyme Catalyzed Reactions. Eur Chem Bull. 2016; 5: 354-363. **Ref.:** https://goo.gl/5P9gDz
10. Webb L. Enzyme and metabolic inhibitors. Moscow: Mir Publishers. 1963; 6-862. **Ref.:** https://goo.gl/Ae4JrU
11. Segel IH. Enzyme kinetics. J Wiley: New York. 1975; 3-957. **Ref.:** https://goo.gl/A54Kz6
12. Dixon M, Webb EC. Enzymes. Moscow: Mir Publishers. 1982; 2: 3-481.