ISSN: 2640-7477

Research Article

Journal of Gastroenterology & Digestive Systems

On Impermissibility of the Use of Any Equation Instead of the Corresponding One for Calculation of Rate Constants of Enzyme Inhibition and Activation

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Submitted: 20 Apr 2022; **Accepted**: 27 Apr 2022; **Published**: 06 May 2022

Citation: *V I Krupyanko. (2022). On Impermissibility of the Use of Any Equation Instead of the Corresponding One for Calculation of Rate Constants of Enzyme Inhibition and Activation. J Gastro & Digestive Systems, 6(1): 84-90*

Abstract

The use of any other equation instead of the corresponding one for calculation of constants of enzyme inhibition and activation , is not allowed. Example of such substitution printed.

Keywords: Impermissibility of The Using of Other Equations

Running Title: Impermissibility of the using of other equations

Introduction

The development of fundamentals of vector method representation of enzymatic reactions (1-10) has opened up new possibilities for calculation of a wide array of kinetic parameters applying new approaches such as:

1. The perception of the presence of a symmetry between inhibited and activated of enzymatic reactions, this can be seen when comparing the initial rates of reactions

$$V_i < V_0 \; V_a < V_0 \qquad (1)$$

but correlation of the secondary parameters of these reactions on the level of correlation of K_m, V, K^0_m and V^0 values still needs further follow-up.

2. In the works (3-7) was shown that such symmetry is proved based on the dependence of the ratio of the effective K_m and V parameters determined in the presence of an inhibitor (i) or activator (a) relative to K^0_m and V^0 parameters of the initial (neither inhibited i = 0, nor activated a = 0) enzymatic reaction rate (Table 1) and that L_i vectors of correspondent enzymatic inhibited reaction take oppositely directed pace to that (similar by type) of L_a vectors of activated reactions.

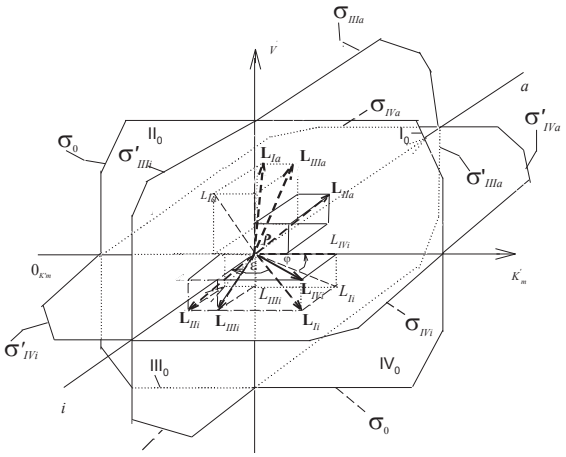


Figure 1: Three dimensional (non complete) $K_m VI$ system of rectangular coordinate with separately P_i and P_a semiaxes of molar concentrations of (i) inhibitor and (a) activator, where only LIi, L_{IVi}, L_{IIIi}, L_{IIi}, L_{Ia}, L_{IIIa}, L_{IIa} vectors of enzymatic reactions placed in appropriate parallelepipeds, L_{II}, L_{IVi}, L_{IIIi} and L_{Ia} are projections

of these ***L*** vectors on basic σ_0 plane. The magnitude of φ angle is about 3400.

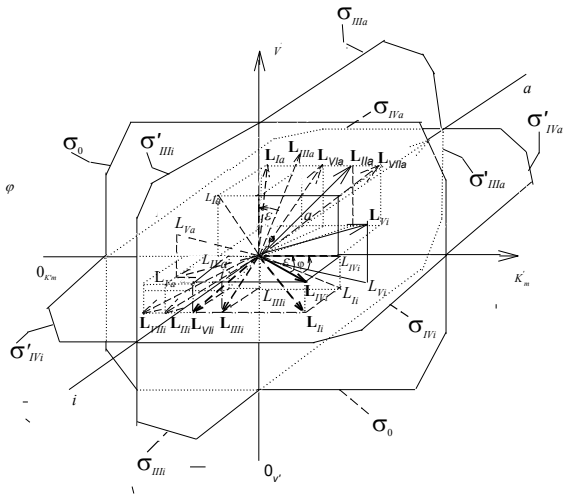


Figure 1a: Three dimensional (complete) $K_m V I$ coordinate system, (the same as Fig. 1), with all 14 **L** vectors (7 type of $\boldsymbol{L}_i$ inhibited (i), 7 type of $\boldsymbol{L}_a$ activated (a) enzymatic reactions. The 15th $\boldsymbol{L}_0$ vector of initial reaction (and it $\boldsymbol{L}_0$ 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 (Fig. 2). The broken line $\sigma_{VIIa/Vi}$ – first (I_σ) to $\sigma_{Va/VIIi}$ i – third (III_σ) quadrants of σ_0 plane are denote transient station between: $VII_a \leftrightarrow V_i$ *and* $V_a \leftrightarrow VII_i$ type of enzymatic reactions, The magnitude of φ angle about 340^0.

as well as that scalar ***L*** projections of these vectors on basic σ_0 plane were also oppositely directed (in the same Figs 1, 1a), or one can use (Fig. 2), convenient for scalar vector representations. of system shown in Figs 1, 1a)

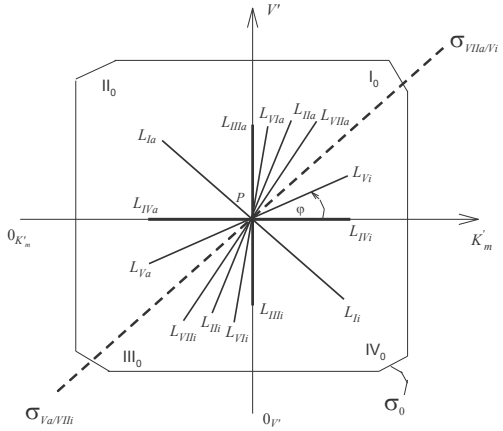


Figure 2: Two-dimensional (scalar) $K_m V$ coordinate system.

The symbols of kinetic parameters: such as, the same K_m, *V*, $K^0 m$ are identical to those as in Fig. 1. The symbols of (L_{Ii}, L_{IVi}... L_{Ia} …) projections of all three-dimensional L vectors (many of which are absent in Fig. 1) and placed completely in Fig. 2, (14 L projections). The broken line $\sigma_{VIIa/Vi}$ – from first (I_σ) to σVa/VIIi i – third (III_σ) quadrants of σ_0 plane are denote transient station between: $VII_a \leftrightarrow V_i$ and $V_a \leftrightarrow VII_i$ type of enzymatic reactions. The magnitude of φ angle in this Figure is about 30^0.

It was possible to:

A. Complete a creation of unified (symmetrical) "Parameter-based classification" of the types of enzymatic reactions. It includes 15 individual types of catalyzed reactions. Among them there are 7 inhibited enzymatic reactions, 7 activated enzymatic reactions and one zero-order (I_0 type) initial (uninhibited, $i = 0$ and non-activated $a = 0$) enzymatic reactions characterized by the position of zero-order L_0 vector at point P (K^0_m, V^0, *0)* of the origin of coordinates (Fig. 1).

B. Derive equations that can be used for calculation of rate constants, They are 7 equations for calculations of rate constants of enzyme activation, K_a (Eqs: 9 - 15, Table 1) and new 5 equations for calculation of rate constants of enzyme inhibition (Eqs: 1, 2, and 5 - 7, Table 1), a total amount of these equations accounts for 14 equations (of these, 12 were newly derived); and (Eqs: 3 and 4, Table 1) have long been known (11-14).

However, there are some questions that need answers. One of these questions which could seem insignificant at first glance can be formulated as follows:

It is incorrect the use any other equations (Eq.: 2 - 7, Table 1) to calculate the value of the rate of KLi constant, especially (Eqs.: 3 and 4, Table 1). Such examples in publication are numerous.

Let us consider the following examples.

Example 1. Determination of the type of inhibited reaction: The study (Vi) of pNPP cleavage catalyzed by porcine alkaline phosphatase revealed that initial rates in the presence of 1.10^{-5} M WO^{2-}_4 decreased $V_i < V_0$ within the whole interval of concentrations of substrate cleaved (Fig. 3).

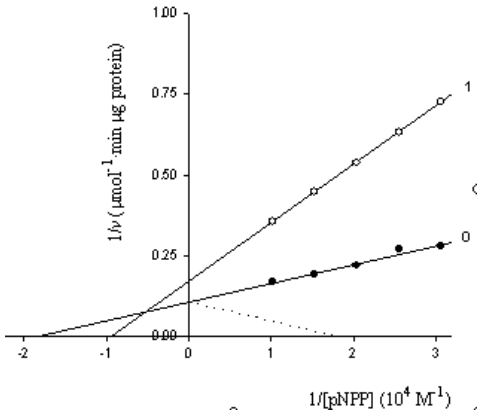


Figure 3: Graphs of inhibitory effect of Na_2WO_4 on the initial rates (V_i) of pNPP cleavage catalyzed by porcine alkaline phosphatase in the coordinates of Lineweaver-Burk. Line 1 – the concentration Na_2WO_4 is 1.10^{-5} M, line (0) – the inhibitor is absent.

i. e. this is inhibited reaction. The construct plots in the (V^{-1}; S^{-1}) double reciprocal coordinates (Fig. 3), permitted to establish that: $K^0_m = 5.45\ 10^{-5}$ M, V0= 9.36 μmol/(min.μg protein) and $K_m = 10.62.10^{-5}$ M, V = 5.86 μmol/(min.μg protein) experimental lines intersect the coordinate axes in the point: $K_m > K^0_m$, $V < V^0$ which correspond to all the features of the biparametrically coordinated

(Ii type) of enzyme inhibition by WO^{2-}_4 anions (Tables 1, line 1) and one should use (Eq. 1, Table 1) to calculate of K_{Ii}. constant of enzyme inhibition. Substitution of all the appropriate parameters in this equation

$$K_I = i / \left(\left(\frac{K'_m - K^0_m}{K^0_m} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5} = 8.92 \cdot 10^{-6} \text{ M}, \quad (7)$$

Indicates a more strong binding of the enzyme to WO^{2-}_4 (K^0_m / K_{Ii} = 5.45/0.892 = 6.11) than to the cleaved substrate.

But all attempts to calculate the constant of this type of enzyme inhibition using when V = V^0, would yield (K_{Ivi}) = $10.54 \cdot 10^{-6}$ M, or when $K_m = K^0_m$, would yield (K_{IIIi}) = $16.74 \cdot 10^{-6}$ M which would differ: in first case (K_{Ivi} / K_{Ii} = 10.54/8.92) more than 1.2, in the second case more than (16,7/8.92 = 1.87) times from the K_{Ii} calculated by Eq. (1, Table 1). This happens, because a ratio of the (V and V^0) in the first and K_m and K^0_m parameters of this reaction is not taken into consideration.

Obviously no one another equation (Eqs. 2, 5 – 7 of Table 1), don't may to be used for construction and data treatment of (Fig. 3), such as: by the choice of Eq. 2 in Table 1 (instead Eq. 1) – it needs to take into account, that in this case should be: $K_m < K^0_m$ and (Fig. 2 in Table 1), and so on:
by the choice of Eq. 3 – that should be: $K_m = K^0_m$,
by the choice of Eq. 4 – that should be: $V = V^0$,
by the choice of Eq. 5 – that should be: $V > V^0$,
by the choice of Eq. 6 – that should be: $K_m < K^0_m$ and $tgw < tgw^0$.
by the choice of Eq. 7 – that should be: $K_m < K^0_m$ and $tgw < tgw^0$.
In experimental practice the examples of using the Eqs. 4 and 3 (and other equations of Table 1) for the treatment of data analogous to Fig. 3 (or he same Fig. 1, Table 1) numerous (15-22).

Example 2. Determination of the type of activated reaction: It was shown that initial rates V_a of pNPP cleavage catalyzed by canine alkaline phosphatase in the presence of $1 \cdot 10^{-3}$ M Guo increased Va > V0within the whole interval of concentrations of the substrate cleaved (Fig. 3).

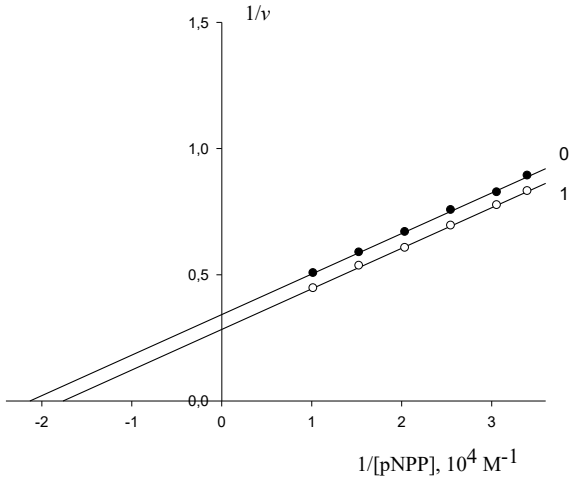


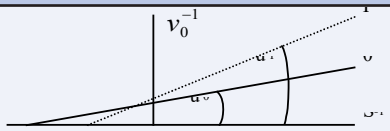
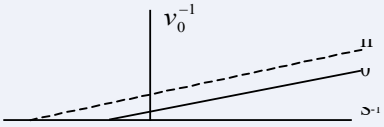
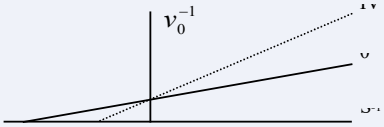
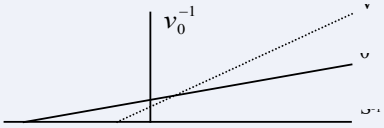
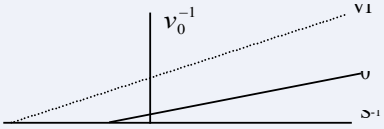
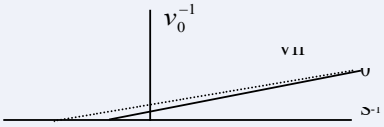
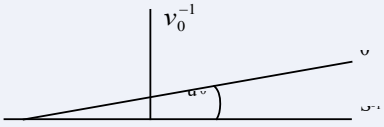
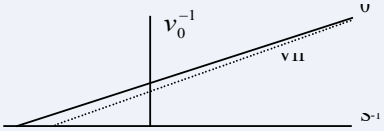
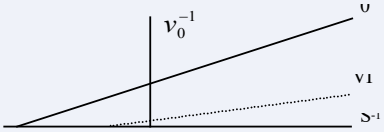
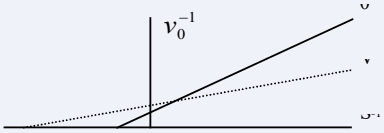
Figure 4: Activating effect of guanosine (Guo) on the initial rates (V_a) of pNPP cleavage catalyzed by canine alkaline phosphatase in the coordinates of Lineweaver-Burk. Line 1 – the concentration of Guo is 1 10^{-3} M, line (0) – the activator is absent. Which have all the features of the unassociative activation, ll_a type, of enzyme (Table 1, line 14).

The study allowed to establish that $K^0_m = 4.69 \cdot 10^{-5}$ *M*, V^0 = 2.921 μmol/(min·μg protein), K^0_m = 5.67·10-5 *M*, V = 3.527 μmol/(min·μg protein). Plotting of dependencies in the above coordinates revealed that the experimental line 1 of activated reaction is located below and parallel to the line (0) of initial (nonactivated, a = 0) reaction at the ratio of parameters: $K_m > K^0_m$, $V > V^0$ (with correlation $Km / V = K^0_m / V^0$) i.e., these lines would never intersect (Fig. 4). As is easily seen from Table 1 (line 14), this corresponds to all the features of the unassociative, lla type, of activation and to calculate a course of change in Vll_a as (a) function of S, (Eq. 14, Table 1) must be used for calculation of the Kll_a constant of activation. Substitution of the obtained from (Fig. 3) parameters in Eq. (14) yields to:

$$K_{IIa} = a / \left(\left(\frac{K'_m - K^0_m}{K^0_m} \right)^2 + \left(\frac{V' - V^0}{V^0} \right)^2 \right)^{0.5} = 9.07 \cdot 10^{-4} \text{ M}, \quad (8)$$

That shows that the binding of this enzyme to guanosine (K_{lla} / K^0_m = 90.7/4.69 = 19) is by 19.3 times lower than to the substrate. But attempt to calculate the constant of this type of enzyme activation using when V = V^0, would yield (K_{lla}) = $5.79 \cdot 10^{-3}$ M, or when Km = K^0_m, would yield (Klla) = $4.48 \cdot 10^{-3}$ M. (K_{lla} / K_{lla} = 57.9/9.07) more than 6.37, in the second case more than (44.8,7/9.07 = 4.94) times from the K_{lla} calculated by Eq. (14, Table 1). This happens, because a ratio of the (V and V^0) in the first and K_m and K^0_m parameters of this reaction is not taken into consideration.

Table 1: Parametric classification of the types of enzymatic reactions

No	Effect	Type of effect	Correlation between the and parameters	Graphs in the (v^{-1};S^{-1}) coordinates
1	Inhibition (i > 0)	I_i	$K'_m > K^o_m$, $V' < V^O$	
2		II_i	$K'_m > K^o_m$, $V' < V^O$ $tgw' < tgw^0$	
3		III_i	$K'_m > K^o_m$, $V' < V^O$	
4		IV_i	$K'_m > K^o_m$, $V' < V^O$	
5		V_i	$K'_m > K^o_m$, $V' < V^O$	
6		VI_i	$K'_m > K^o_m$, $V' < V^O$	
7		VII_i	$K'_m > K^o_m$, $V' < V^O$ $tgw' < tgw^0$	
8	None	I_0	$K'_m > K^o_m$, $V' < V^O$	
9	Activation (a > 0)	VII_a	$K'_m > K^o_m$, $V' < V^O$ $tgw' < tgw^0$	
10		VI_a	$K'_m > K^o_m$, $V' < V^O$ $tgw' < tgw^0$	

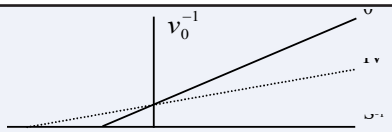
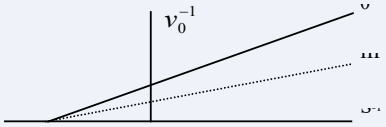
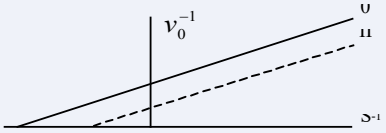
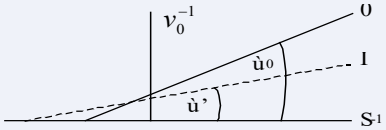
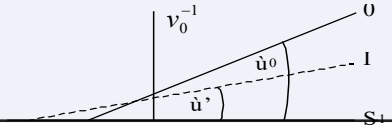
11		V_a	$K'_m > K^o_m$, $V' < V^O$	
12		IV_a	$K'_m > K^o_m$, $V' < V^O$	
13		III_a	$K'_m > K^o_m$, $V' < V^O$	
14		II_a	$K'_m > K^o_m$, $V' < V^O$ $tgw' < tgw^0$	
15		I_a	$K'_m > K^o_m$, $V' < V^O$	

Table 2: Equations for calculation of the Ki and Ka constants

Type of effect	New name of the types of enzymatic reactions	Traditional name	Corrected equation for calculation of the K_i and K_a constants
I_i	biparametrically coordinated inhibition	mixed inhibition	$K_I = i/\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
II_i	unassociative inhibition	uncompetitive inhibition	$K_{IIi} = i/\left(\left(\frac{K^0_m - K'_m}{K'_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$
III_i	catalytic inhibition	noncompetiti-ve inhibition	$K_{IIIi} = \frac{i}{V^0/V' - 1}$
IV_i	associative inhibition	competitive inhibition	$K_{IVi} = \frac{i}{K'_m/K^0_m - 1}$
V_i	pseudoinhibition		$K_V = i/\left(\left(\frac{K'_m - K^0_m}{K^0_m}\right)^2 + \left(\frac{V' - V^0}{V^0}\right)^2\right)^{0.5}$
VI_i	discoordinated inhibition		$K_{VIi} = i/\left(\left(\frac{K^0_m - K'_m}{K'_m}\right)^2 + \left(\frac{V^0 - V'}{V'}\right)^2\right)^{0.5}$

VII_i	transient inhibition		$K_{VIIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$
I_0	initial (uninhibited i = 0 and non-activated) enzymatic reaction		
VII_a	transient activation		$K_{VIIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
VI_a	discoordinated activation		$K_{VIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
V_a	pseudoactivation		$K_{V} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$
IV_a	associative activation	competitive activation	$K_{IVa} = \frac{a}{K_m^0 / K_m^{'} - 1}$
III_a	catalytic activation	noncompetitive activation	$K_{IIIa} = \frac{a}{V^{'} / V^0 - 1}$
II_a	unassociative activation	uncompetitive activation	$K_{IIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
I_a	biparametrically coordinated activation *	mixed activation	$K_{I} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$

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