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Review Article

The Constants of Enzyme Inhibition and Activation Should not be Dependent on the Choice of a Coordinate System Used for Their Calculation

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Abstract

The choice of the secondary coordinate systems causes considerable difficulties when calculating the K_i (and K_a) constants of enzyme inhibition and activation. So, for instance, plots of uncompetitive inhibition in the (v^{-1} ; s^{-1}) and (1/ v_i; i) coordinate system intersect nowhere, but in the (S/ v_i; i) coordinate system they intersect at the point above x-axis. And conversely the plots of competitive inhibition show no point of intersection in the (S/ v_i; i) coordinate system. The secondary coordinates, free from mentioned difficulties, and examples of K_i and $K_a$constants calculation are printed.

Keywords: Enzyme; Coordinate System

Introduction

By comparison of Figure 2 and 4, (monograph)1 it is seen that when the (v^{-1}; i) coordinate systems is applied to calculate K_{III} constant of uncompetitive enzyme inhibition (Table 2, line 2) a series of parallel lines with no intersection is obtained. When the (S/ v; i) coordinate system is employed, the series of lines intersect at one point with the x-axis -i = K_{IIi}. And conversely when for calculation of K_{IVi} constant of competitive enzyme inhibition (Table 2, line 4) we use the (S/ v; i) coordinate system a series of parallel lines with no intersection is obtained, and a set of straight lines intersecting at one point with the x-axis -i = K_{IVi} is plotted in a case of the (v^{-1}; i) coordinate systems [1,2].

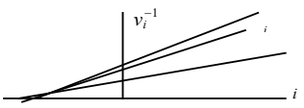
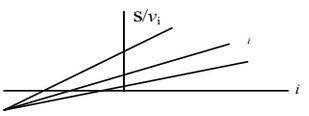
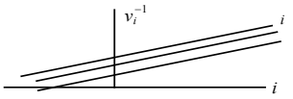
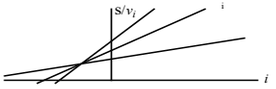
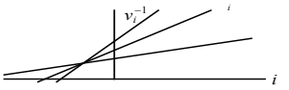
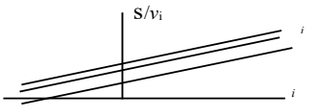
No	Effect	Type of effect	Graphs in (v_i^{-1}; i) coordinate	Graphs in S/v_i:i coordinate
1	Inhibition (i > 0)	I_i		
2		I_i		
3		III_i		
4		IV_i		
5		V_i		
6		V_i		
7		VII_i		
8	No effect (i = 0),(a = 0)	I_0		

Table 1: Position of graphs in v_i^{-1};i and S/v_i;i coordinate [1].

Similar situation is observed when Lineweaver-Burk plots are constructed in the other forms of the (v^{-1}; S^{-1}) coordinate system [3-7].

The considerable difficulties are arise by the necessity of treating experimental data with parallel lines or lines with no one intersection point (Examples 1 and 2).

Coming up against similar situations the authors:

- Either make attempts to characterize these plots by a course of change of $K_m^{'}$ and V' parameters of the reaction [8-10].
- Or calculate the value of one of the two[11-16] secondary K_{ii} –intercept and K_{is} –slope constants of enzyme inhibition.

It is also the same in a case of enzyme activation [12,16-19].

The analysis shows that the values of the effective $K_m^{'}$ Michaelis constant and V' maximum reaction rate determined in the presence of the inhibitor (i) or activator (a) may change in different directions or one of them may remain unchangeable at all [20].

Moreover, it is difficult to explain a situation when the plots constructed by the authors in the intercept and slope forms intersect at different points along the x-axis [14-19]. The result of estimation shows that the values of the K_{ii} and K_{is} constants of inhibition are not the same. So, new questions arise, for instance, as to what the value of the K_{ii} constant of inhibition characterize in this case, if the parallel to it value of the K_{is} constant of inhibition represents the strength of enzyme binding to the inhibitor (the value is obtained using data of the same experiment but applying another coordinate systems). It is obvious that the same inhibitor could not demonstrate simultaneously two different strength of enzyme binding.

Due to the development of a vector method representation of the enzymatic reactions [21-27], taking into account the position of L vectors in the three-dimensional (branched) $K_m^{''}V^{''}I$ coordinate system (Figure 1a, 1b) and their L projections on the basic σ_0 plane of (Figure 1), namely in scalar two-dimensional $K_m^{''}V^{''}$ coordinate system (Figure 2).

The symbols of kinetic parameters the same: K_m', V', $K_m^{\,0}$, as in Figure 1a and 1b. The symbols of (L_{Ii}, L_{IVi}... L_{Ia} ...) projections of all three-dimensional L vectors (many of which absent in Figure 1a and 1b): are placed completely (14 L projections), I_o, II_o ... quadrants of this plane, the magnitude of φ angle about 3400.

It became possible to:

- Group all type of inhibition and activation of the enzyme according to unified, parameter classification (Table 2);
- Derive the equations of calculation of the K_i (and K_a) constants of enzyme inhibition (and activation (Table 2) and
- Suggest unified forms of the secondary coordinates of the estimation of these constants (Table 3) that help avoid complications indicated in Introduction.

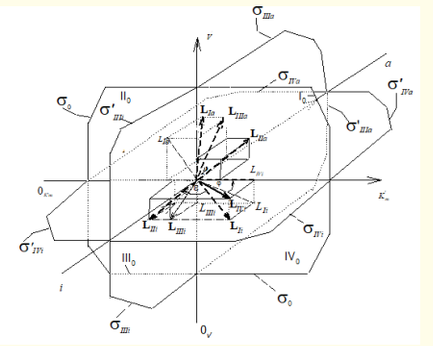


Figure 1a: Three dimensional (branched, incomplete) $K_m'V'I$ system of rectangular coordinate with separately P_i and P_a semi-axes of molar concentrations of [i] inhibitor and [a] activator. Only 8 **L** vectors of enzymatic reactions (the symbols: L_{Ii}, L_{IVi}, L_{IIIi}, L_{IIi}, L_{Ia}, L_{IIIa}, L_{IIa} 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.

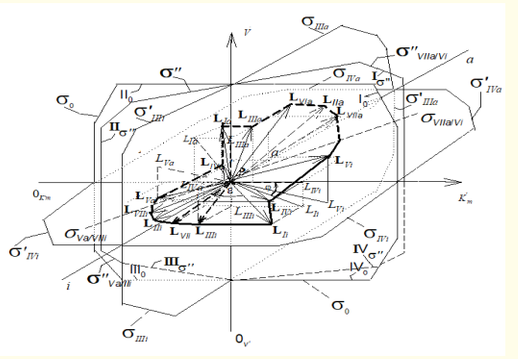


Figure 1b: Three dimensional (complete) $K_m'V'I$ coordinate system, (the same as Figure 1a), bat with all 15 L vectors (7 type of additional L vectors placed without of appropriate parallelepipeds). The ends of all mobile L vectors are joined by dash line (broken part – activated, unbroken part– L vectors 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 of this figure, are placed completely in (Fig. 2). σ''IIa/Vi – first ($I_{\sigma'}$) and σ''$_{Va/Ii}$ – third ($III_{\sigma''}$) quadrants of transient σ'' plane, $\sigma_{VIIa/Vi}$ and $\sigma_{Va/VIIi}$ – beginning and finishing ends of the line of orthogonal σ'' transient plane projection on basic σ_0 plane (in Figure 1b and 2, market by broken lines), the magnitude of φ angle about 3400.

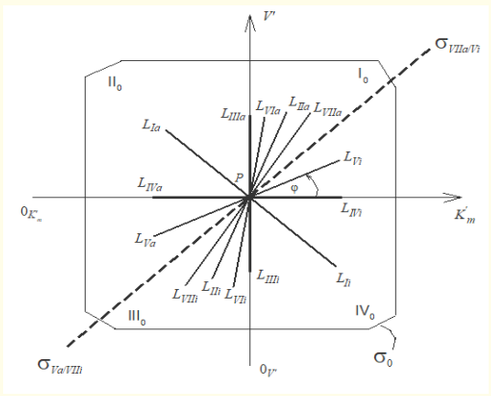


Figure 2: Two-dimensional (scalar) K_m', V' coordinate system.

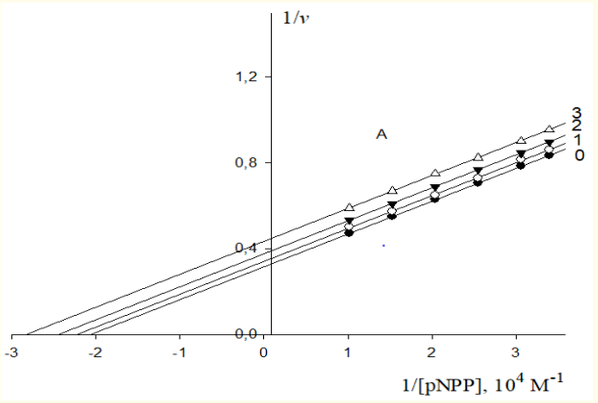


Figure 3A: The inhibitory effect of iso-propanol (i) on the initial rates of of pNPP cleavage by eel alkaline phosphatase. Note: inhibitor concentration (M)): 0.0002 – straight line 1; 0.0005 – straight 2 and 0.001 – straight 3. Straight line 0 – no inhibitor, , μmol/(min·μg of protein).

And also by considering the symmetry of the position of $\mathbf{L_a}$ vectors activated relatively (towards) → $\mathbf{L_i}$ vectors of the (same type) inhibited reactions (the symmetry by octants) (Figure 1a, 1b) and projections of these vectors on the basic σ_0 plane of figures Figure 1a, 1b (more convenient see in Figure 2) it was found out that instead of the forms:

$$(tg\omega'; \frac{1}{a}) \text{ slope and } (\frac{1}{V'}; \frac{1}{a}) \text{ intersect (1)}$$

The dependencies of slope angles (K_m'/V' = tg ω') of the lines on the reciprocal value of concentration of the activator (1/a), it is necessary to apply a slope form (unconverted form relatively the concentration of activator a) may be use:

$$(\frac{tg\omega^0}{tg\omega'}; a), \text{ (2)}$$

Not only in a case of (III_a type) catalytic activation (Table 3, line 13),

$$(V'; a) \text{ (3)}$$

But also in all other cases (Table 3) which are actually a simplified version of the slope form [20,26,27].

The practical examples of calculation of constants in more difficult cases: K_{IIi} constant (parallel lines, Figure 3A) and K_{VIi} constant (lines intersect at different points, Figure 4A), are given.

Examples of constant calculation

Example 1: Treating data using the plots with parallel lines we used the results of the study of the inhibitory effect of the increasing concentrations of iso-propanol (i-Prop) on the initial rate of pNPP cleavage by eel alkaline phosphatase (EC 3.1.3.1) – an enzyme of Sigma (USA).

The results of the study (Figure 3A).

Show that the presence of the inhibitor in concentration of 0.0002 M is a result of the following change in the parameters of substrate cleavage by eel phosphatase:

V' = 2.927 μmol/(min·μg of protein), K_m'= 4.47 ·10^{-5} M,
V^0 = 1.62 μmol/(min·μg of protein), K_m^0= 4.824·10^{-5} M). At inhibitor concentration, 0.0005 M the values of parameters became: V' = 2.66 μmol/(min·μg of protein),
K_m' = 4.071 ·10^{-5} M. At inhibitor concentration, 0.001 M the values of parameters became
V' = 2.307·10^{-5} μmol/(min·μg of protein). = 3.525·10^{-5} M.

This according to the parametric classification (Table 2) corresponds to type ($K_m' < K_m^0$, $V' < V^0$, $i > 0$) of bi parametrical dis coordinated (or uncompetitive [2-4] enzyme inhibition; L_{IIi} vectors of this reaction, accordingly values of K_m', K_m^0, V', V^0 parameters will placed in III[rd] octant of (Figure 1a and 1b) and L_{IIi} projections of these vectors в – in III[rd] guardant of (Figure 2). Consequently. for calculation of K_{IIi} constant of enzyme inhibition it is necessary to use an Equation (2, Table 2). where the length of orthogonal projection of L_{IIi} vectors of this reaction on the basic σ_0 plane of the three-dimensional $K_m'V'I$ coordinate system (Figure 1a and 1b) is taken into account:

$$K_{IIi} = i / \left(\left(\frac{K_m^0 - K_m'}{K_m'} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5} \text{ (4)}$$

Substitution of the necessary parameters obtained after data treatment (Figure 3A) into this equation gives the following values of the K_{IIi} constants (10^{-3} M): 1.77; 1.89 and 1.91 at the first. second and third concentration of iso-propanol.

Equation (4) opens another, more desirable possibility of calculation of these constants – construction of the plots depicting the dependencies of the change in the denominator value (A) in the equation on the increasing concentrations of the inhibitor in the (A; i) coordinate systems (Figure 3B). This Figure shows that

K_{IIi} = 1/tg a. (5)

Where tg a is an slope angle of the experimental line toward the x-axis.

Having treated data from Figure 3b in this coordinates (using a computer program Sigma Plot version 10) it was shown that the average value of the constant is K_{IIi} = 1.83·10^{-3} M.

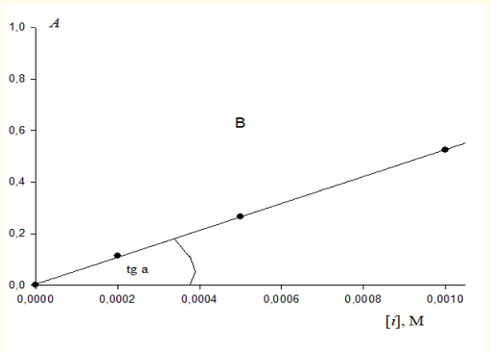


Figure 3B: The dependence of the change in A parameters obtained using data of Figure 3A in equation (4) on increasing concentration of i-Prop in the (A;) coordinates. A is a denominator of equation (4).

It points to the weak binding (K_{IIi}/ K_m^0 = 183/4.8 = 31) of isopropanol to the enzyme. It is true: the molecule of i-Prop that bears no negative charge provides an access of negatively charged orthophosphate residues of pNPP to ionogenic, positively charged amino acid residues of the active center of the enzyme.

The experiment practice represents numerous examples relating to the use of the secondary (1/*V*';*i*) and (1/*K*';*i*) uncorrected coordinates in the intersection form for the treatment of data of such type of enzyme inhibition (Figure 3A). in the first case it is for calculation of the K_{ii} -intercept constant and in the second case for calculation of the K_{is} slope constant of enzyme inhibition [11-16].

Example 2

For data treatment using the plots depicting straight lines with no intersection the results of study on the activating effect of arginine-containing activator (ArgA) on the initial rates of cleavage of P_9 –polysphosphate by vacuolar Mg^{2+}-independent polyphosphatehydrolase from the fungus *Neurospora crassa* (EC3.6.11) were used. The conditions of the enzyme isolation and the exploration of its activity are given in the work [28].

The results of this experiment which are depicted in (Figure 4A) show that cleavage of P9 -polyphosphate by the studied polyphosphatase in the presence of 1.1 μM ArgA is represented by the parameters: $K_m^{'}$ = 3.810 ·10^{-4} M.

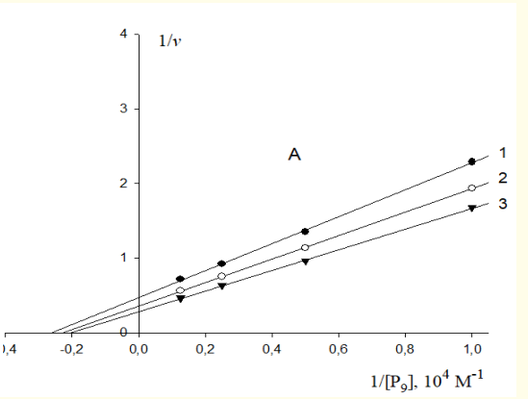


Figure 4A: Activating effect of ArgA on the initial rate (v_{VIIa}) of cleavage of P_9-polyphosphate by vacuolar polyphosphate from Neurospora crassa: straight line 1 – concentration of ArgA is 1.1 μM; straight line 2 – 2.2 μM; straight line 3 – 3.3μM. Symbols: μE/min.

$V^{'}$ = 2.110·μE/min;

At 2.2 μM – the parameters: $K_m^{'}$ = 4.359·10^{-4} M, $V^{'}$ = 2.773·μE/min;

At 3.3 μM – the parameters: $K_m^{'}$ = 4.836·10^{-4} M, $V^{'}$ = 3.494·μE/min.

It satisfies VI_a type of enzyme activation ($K_m^{'} > K_m^0, V^{'} > V^0, a > 0$). (Table 2, line 10).

Calculation of the coordinates of interception points at the plots in Figure 4A by equations:

$$-\frac{1}{S_c} = \frac{V^{'} - V^0}{K_m^0 V^{'} - K_m^{'} V^0} \text{ and } \frac{1}{v_c} = \frac{K_m^0 - K_m^{'}}{K_m^0 V^{'} - K_m^{'} V^0}. \quad (6)$$

Demonstrate that straight lines 1 and 2 intersect at the point having the coordinates (-0.486 104 M^{-1}; -0.403 μE^{-1} min) and straight lines 1 and 3 have the point of intersection with the coordinates (-0.446 104 M^{-1}; -0.3301 μE^{-1}·min).

For calculation of the K_{VIa} constant of enzyme activation we used the equation (10), (Table 2, line 10).

$$K_{VIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5} \quad (7)$$

Substitution of the parameters (Figure 4A) in this equation gives the following values of the constants of polyphosphatase activation: K_{VIa} = 3.182 (μM) – on ArgA concentration interval (2.2 – 1.1 = 1.1) μM and K_{VIa} = 3.103 μM – ArgA concentration interval (3.3 – 1.1 = 2.2) μM (for both cases K_m^0 = 3.810 ·10^{-4} M).

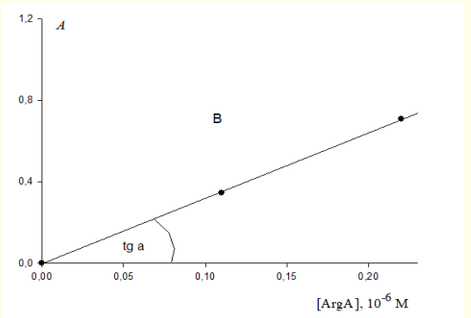


Figure 4B: The dependence of the change in the A parameters in equation 7 on the increasing concentrations of ArgA in the coordinates (A;). Symbols: the intervals of the ArgA concentrations used are 1.1 μM and 2.2 μM.

According to equation (7) rewritten as:

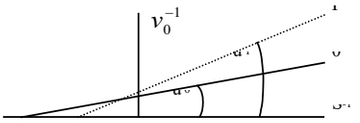
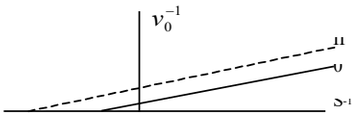
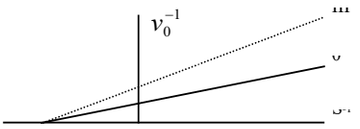
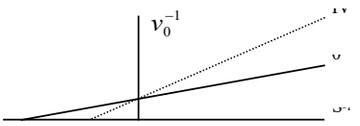
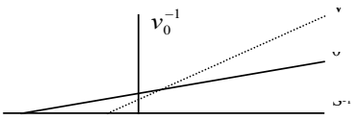
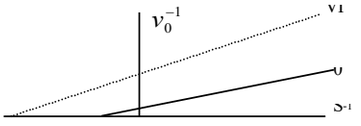
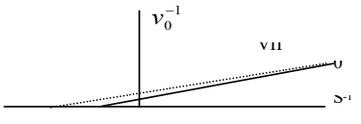
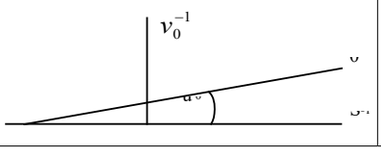
K_{VIa} = 1/tg a = $1/(A/a) = a/A$ (8)

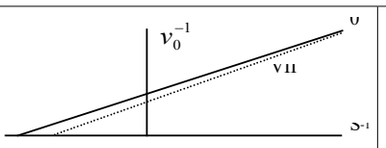
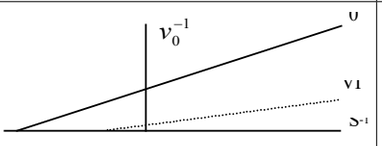
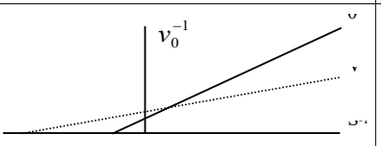
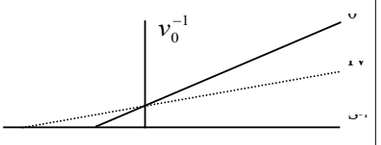
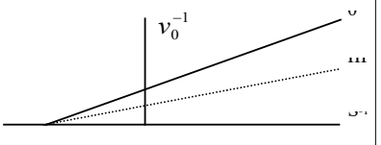
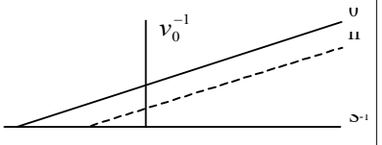
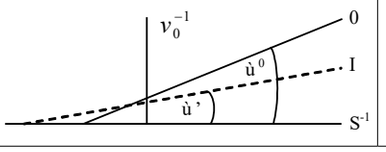
With numerical values of A-denominators in the equation and the intervals of the [ArgA] activator concentrations used it follows from Figure 4B.

That

K_{VIa} = 1/b(1) = 1/0,3223 (μM)$^{-1}$ = 3,1027 μM , (8)

Where b(1) = 3.2227 is a parameter of the Statistics program in Sigma Plot 10, USA. It analogously for all similar type of enzyme activation.

№	Effect	Type of effect	Plots in the (V_o^{-1} ; S^{-1}) coordinate systems	A ratio between $K_m^{'}$ and $V^{'}$ parameters	New name of reaction types	Traditional name
1	Inhibition (i >0)	I_i		$K_m^{'} > K_m^0$, $V^{'} < V^0$	Bi parametrically dis coordinated inhibition	Mixed inhibition
2		I_i		$K_m^{'} < K_m^0$, $V^{'} < V^0$ ($g\,\omega' = g\,\omega^0$)	Un associative inhibition	Uncompetitive inhibition
3		III_i		$K_m^{'} = K_m^0$, $V^{'} < V^0$	Catalytic inhibition	Noncompeti-tive inhibition
4		IV_i		$K_m^{'} > K_m^0$, $V^{'} = V^0$	Associative inhibition	Competitive inhibition
5		V_i		$K_m^{'} > K_m^0$, $V^{'} > V^0$	Pseudo inhibition	
6		V_i		$K_m^{'} < K_m^0$, $V^{'} < V^0$ ($g\,\omega' > g\,\omega^0$)	Dis coordinated inhibition	
7		VII_i		$K_m^{'} < K_m^0$, $V^{'} < V^0$ ($g\,\omega' < g\,\omega^0$)	Transient inhibition	
8	No effect	I_0		$K_m^{'} = K_m^0$, $V^{'} = V^0$	Initial reaction	

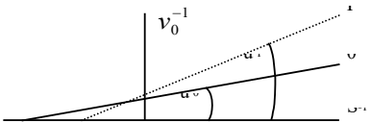
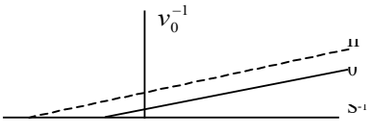
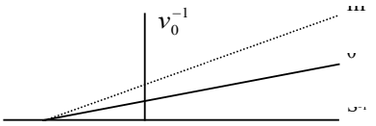
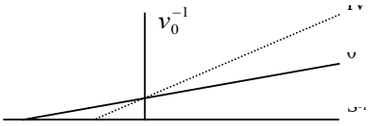
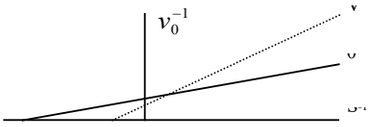
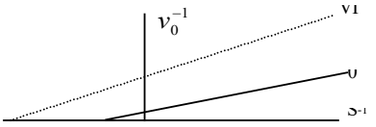
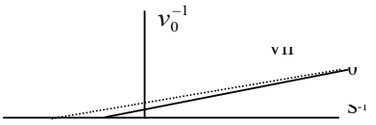
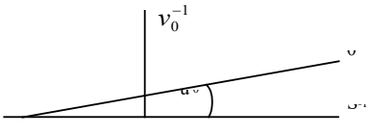
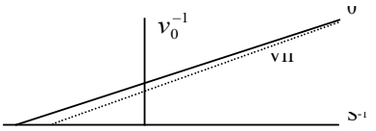
9	Activation ($a > 0$)	VII_a		$K_m' > K_m^0,\ V' > V^0$ $(g\ \omega' > g\ \omega^0)$	Transient activation	
10		V_a		$K_m' > K_m^0,\ V' > V^0$ $(g\ \omega' < g\ \omega^0)$	Dis coordinated activation	
11		V_a		$K_m' < K_m^0,\ V' < V^0$	Pseudo-activation	
12		IV_a		$K_m' < K_m^0,\ V' = V^0$	Associative activation	Competitive activation
13		III_a		$K_m' = K_m^0,\ V' > V^0$	Catalytic activation	Noncompetitive activation
14		I_a		$K_m' > K_m^0,\ V' > V^0$ $(g\ \omega' = g\ \omega^0)$	Un associative activation	Uncompetitive activation
* 15		I_a		$K_m' < K_m^0,\ V' > V^0$	Bi parametrically coordinated activation	Mixed activation

Type of effect	An equation of calculation of the K_i and K_a constants
I_i	$K_{Ii} = i \Big/ \left(\left(\frac{K_m' - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5}$
I_i	$K_{IIi} = i \Big/ \left(\left(\frac{K_m^0 - K_m'}{K_m'} \right)^2 + \left(\frac{V^0 - V'}{V'} \right)^2 \right)^{0.5}$
III_i	$K_{IIIi} = \frac{i}{V^0 / V' - 1} = \frac{i}{\frac{V^0 - V'}{V'}}$
IV_i	$K_{IVi} = \frac{i}{K_m' / K_m^0 - 1} = \frac{i}{\frac{K_m' - K_m^0}{K_m^0}}$

V_i	$K_{Vi} = i / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
$V_{\ i}$	$K_{VIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$
VII_i	$K_{VIIi} = i / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$
No effect	
VII_a	$K_{VIIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
$V_{\ a}$	$K_{VIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
V_a	$K_{Va} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$
IV_a	$K_{IVa} = \frac{a}{K_m^0 / K_m^{'} - 1} = \frac{a}{\frac{K_m^0 - K_m^{'}}{K_m^{'}}}$
III_a	$K_{IIIa} = \frac{a}{V^{'} / V^0 - 1} = \frac{a}{\frac{V^{'} - V^0}{V^0}}$
$I_{\ a}$	$K_{IIa} = a / \left(\left(\frac{K_m^{'} - K_m^0}{K_m^0} \right)^2 + \left(\frac{V^{'} - V^0}{V^0} \right)^2 \right)^{0.5}$
I_a	$K_{Ia} = a / \left(\left(\frac{K_m^0 - K_m^{'}}{K_m^{'}} \right)^2 + \left(\frac{V^0 - V^{'}}{V^{'}} \right)^2 \right)^{0.5}$

Table 2. Parametrical classification and an equation for calculation of the K_i and K_a constants.

*Symbol of plots in Figures 1 – 15 correspond to the type of the characterized reaction. So, for instance, in Fig. 15 line 0 characterizes the position of the plot of the initial (not activated) reaction, line I is a position of the plot of the enzymatic reaction activated by I_a type etc.

No	Type of the effect	Plots in the (V_o^{-1} ; S^{-1}) coordinate systems	Coordinates for calculation of K_i slope and K_a slope constants
1	I_i		($A;i$)
2	I_i		($A;i$) (Fig. 3A, in text)
3	III_i		$\left(\frac{K_m^{'}}{V^{'}};i\right)$ and $\left(\frac{1}{V^{'}};i\right)$ or $\left(\frac{g\,\omega^{'}}{g\,\omega^{0}};i\right)$
4	IV_i		$\left(\frac{K_m^{'}}{V^{'}};i\right)$ and $\left(K_m^{'};i\right)$
5	V_i		($A;i$)
6	V_i		($A;i$)
7	VII_i		($A;i$)
8	I_0		Initial ($i=0$ and $a=0$) reaction
9	VII_a		($A;a$)

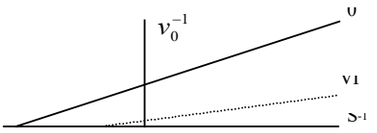
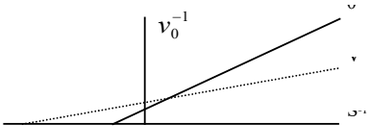
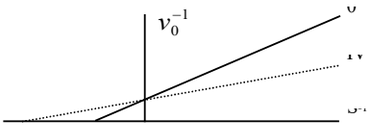
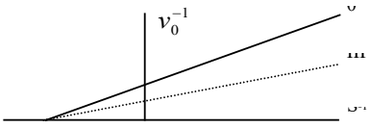
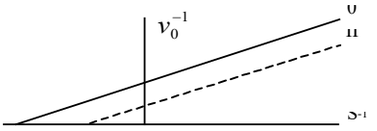
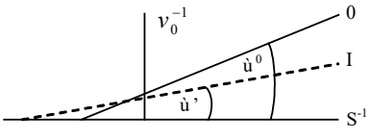
10	V_a		$(A;a)$ (Fig. 4A, in text)
11	V_a		$(A;a)$
12	IV_a		$\left(\frac{V'}{K_m'};a\right)$ and $\left(\frac{1}{K_m'};a\right)$ and $\left(\frac{\text{g}\,\omega^0}{\text{g}\,\omega'};a\right)$ $\left(\frac{1}{\text{g}\,\omega'};a\right)$
13	III_a		$\left(\frac{V'}{K_m'};a\right), \left(V';a\right), \left(\frac{\text{g}\,\omega^0}{\text{g}\,\omega'};a\right) \left(\frac{1}{\text{g}\,\omega'};a\right)$
14	I_a		$(A;a)$
15	I_a		$(A;a)$

Table 3: Secondary coordinates for calculation of K_i and K_a constants.
Notes: A is a denominator of the corresponding equation for calculation of K_i, or K_a constants (Table 2).

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