CORRECTION OF A DATA ANALYSIS EQUATION RELATING TO TWO-SUBSTRATE ENZYME CATALYZED REACTIONS

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Symmetric anti-directivity in the course of change in the ratios between K_A and K_B in the equation $v = VAB/(K_AK_B + K_AB + K_BA + AB)$ is suggested to be potential for applying when this equation is used to treat data on the mechanism of enzymatic action involving two ($A + B$) substrates.

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Introduction

An appreciation of Eqn. 1 and number of its variations

$$v = \frac{VAB}{K_A K_B + K_A B + K_B A + AB} \qquad (1)$$

where

v – initial reaction rate,

V – maximum reaction rate,

A – concentration of the first substrate,

B – concentration of the second substrate,

K_A – the Michaelis constant of the first substrate,

K_B – the Michaelis constant of the second substrate

are frequently used at data analysis of enzyme inhibition and activation of the two-substrate ($A + B$) enzyme catalyzed reactions in the conditions, when the proceeding of cleavage of the first A substrate (at excessive concentration of the 2nd substrate ($B >> A$) is studied and vice versa, when the first substrate ($A >> B$) is taken in excess or ($A = B$).[1 – 14]

The estimation of the Michaelis K_m constants and V_m maximum reaction rates by the 1st (and the 2nd) substrate is made by the Eqn. 2.[1,7,9,11,14]

$$v = \frac{V}{\frac{K_A}{A} \cdot + \cdot 1} \qquad (2)$$

The same approach is used to study the kinetics of the reaction proceeding in the presence of enzyme inhibitors (i) or activators (a).[5]

Thus, the values of parameters V and K_A acquire the sense of the effective K'_A and V' constants and maximum reaction rates determined with the attempt to calculate the respective K_i and K_a constants of enzyme inhibition and activation by the Eqn. (1) or its numerous modifications.[1 – 14]

Analysis of Eqn. (1) and its modifications shows that they are applicable only in the cases of (i) two monoparametrical associative (type IV_i, by the parametrical classification Table 1, line 4),[15] (ii) the same enzyme competitive inhibition (according to the traditional terminology),[16,17] (iii) catalytic (Table 1, line 3)[18–24] or (iv) the same enzyme non-competitive inhibition.[16, 17]

Other attempts to modify Eqn. (1) aimed to calculate the constants of mixed (K'_A)[10,11,12] and non-competitive enzyme inhibition[10,11,16] may lead to unacceptable values of such constants for practical use.

As for the attempts to use Eqn. (1) and its modifications for calculation of data on enzyme activation, the results may turn out the opposite, i.e. data treatment of enzyme activation is performed within enzyme inhibition using the terminology of inhibited enzyme catalyzed reactions and by taking into account the respective K_i constants of enzyme inhibition.[1–7, 14]

The difficulties in use of the Eqn. (1) and its modifications are in the fact that these modifications do not take into account the symmetric anti-directivity of effects of enzyme activation to effects of enzyme inhibition so evident at the level of changes in initial rates of these enzyme catalyzed reactions.

$$v_i < v_0; \qquad v_a > v_0 \qquad (3)$$

The following approach is used for calculation of the K_{IVi} constants of associative enzyme inhibition of type IV_i (Table 1, line 4) by authors in most cases.

(a) the Eqn. (1) is simplified to Eqn. (4)

$$v_i = \frac{VA}{K_A\left(1+\frac{i}{K_{is}}\right)+A} \qquad (4)$$

where K_A parameter acquires the sense of K^0_m, the Michaelis constant, and the K_{is} parameter i.e., the constant of competitive enzyme inhibition.

(b) For calculation of the K_{IIIi} constant of catalytic (type III_i) (Table 1, line 3) or non-competitive enzyme inhibition[16,17] the following modification of the equation (1) is used [1,2,9, 14]

$$v_i = \frac{VA}{K_A\left(1+\frac{i}{K_{is}}\right)+A\left(1+\frac{i}{K_{ii}}\right)} \qquad (5)$$

where K_A is Michaelis constant (K^0_m) and K_{is} and K_{ii} parameters acquire the sense of the K_i constant of non-competitive enzyme inhibition.

Use of the Eqns. (4) and (5) after simple transformations, when the symbol K_{is} slope constant of enzyme inhibition (Eqn. 4) acquires the sense of the K_{IVi} constant of competitive (i.e. associative enzyme inhibition, Table 1, line 4) and the symbols K_{is} slope constants and K_{ii} intercept constants of enzyme inhibition (which is the same i.e. K_{is} = K_{ii}, because the (K'_mV^0/K^0_mV'; i) coordinates of slopes at $K'_m = K^0_m$ are simplified to the coordinates of intercepts ($1/V'$;i)[23] acquire the symbol of the K_{IIIi} constant of catalytic (Table 1, line 3) or non-competitive[16,17] enzyme inhibition.

After substitution of the K_{is} parameter in Eqn. (4) by K_{IVi} and re-grouping of denominator Eqn. (5) with transfer of the co-multiplayer

$$1+\frac{i}{K_{IIIi}} = 1+\frac{i}{K_{is}} = 1+\frac{i}{K_{ii}}$$

in the denominator at V, a possibility opens up to take into account symmetric anti-directivity of effects of enzyme activation to effects of enzyme inhibition, because then Eqn. (4) is simplified to Eqn. (6) and Eqn. (5) to Eqn. (7).

$$v_{IVi} = \frac{V^0}{1+\frac{K^0_m}{S}\left(1+\frac{i}{K_{IVi}}\right)} \qquad (6)$$

$$V_{IIIi} = \frac{V^0\left(\frac{1}{1+\frac{i}{K_{IIIi}}}\right)}{1+\frac{K^0_m}{S}} \qquad (7)$$

These equations are widely used and for a long time in enzyme kinetics for calculation of the constants K_{IVi} and K_{IIIi} of competitive and non-competitive enzyme inhibition, respectively.[16,17]

To enhance the possibility of applying the approach described in Eqns. (1–5) for data treatment of enzyme activation, it is necessary to write Eqns. 6 and 7 in the form of Eqns. 8 - 10.

$$v_{IVi} = \frac{V^0}{1+\frac{K^0_m}{S}\cdot F} \qquad (8)$$

$$v_{IIIi} = \frac{V^0\left(\frac{1}{F}\right)}{1+\frac{K^0_m}{S}} \qquad (9)$$

$$F = 1+\frac{e}{K_e} \qquad (10)$$

where F is the factor (always positive) of multiplicity of increase of the kinetic parameters K^0_m and V^0 of initial (uninhibited i = 0 and non-activated a = 0) reaction determined in the presence of effectors (e): i, a, $B >> A$ and etc.

By comparison of the dependence between the position of factor F in Eqns. (8) and (9) and the position of lines IV and III relative to line 0 of initial uninhibited and non-activated enzyme catalyzed reaction (Table 1, Figure 3 and 4) it is easy to see that if the presence of the 3rd component (i, a, $B >> A$ and etc.) in the reacting system leads to increase of the K^0_m reaction parameter ($K'_m > K^0_m$) (Table 1, Figure 4, line IV) then the F factor will be in numerator at K^0_m, (Table 1, Eqn. 4a) thus, demonstrating increase of this parameter by $1+e/K_e$ times (Eqn. 6 in the text or Eqn. 4a, in Table 1), to demonstrate a decrease of the V^0 parameter ($V'<V^0$), as one might expect, the F factor will be in denominator at V^0, showing the fact of decrease of this parameter by $1+e/K_e$ times (Eqn. 7 in the text), (Eqn. 3a in Table 1). It is analogous to other equations of Table 1.

We can summarize all the above discussion concerning Eqns. (1 – 10) in the form of two rules.

(1) The necessity of taking into account the symmetric anti-directivity in the course of change of ratios between (K_A and K_B) parameters in Eqn. (1) when this equation is used to calculate ν_i and ν_a of the initial velocities of the enzyme catalyzed reactions inhibition and activation and

2) To validate the applicability of the above rule using the practical examples.

Experimental

We studied the influence of 5-fluorouracil (5-Fur) on initial rates of p-nitrophenylphosphate (pNPP) cleavage in the reaction catalyzed by porcine alkaline phosphatase (E.C. 3.1.3.1). The reagents obtained from Sigma (USA) were employed. The plots of a course of pNPP cleavage were recorded by a CF-4 DR two-beam spectrophotometer (Optica Milano, Italy). Reactions were performed in 0.05 M Tris-HCl buffer (pH 9.0) at ionic strength 0.1 using NaCl of high purity at constant mixing[15] in a thermostat (37 ^{0}C) by monitoring the increase of absorbance ($+\Delta A_{400}$) of solution containing the substrate, enzyme and activator against the solution of the same composition but without the enzyme. The concentration of pNPP was changed from 0.294 x 10^{-4} – 0.98 x 10^{-4} M, the concentration of porcine alkaline phosphatase was 2.1 μg mL^{-1}.

The initial reaction rates (ν) were defined by the angle of slope of tangents to initial segments of curves of a course of enzyme catalyzed reactions in not less that five parallel experiments. The kinetic parameters K_m and V parameters were calculated by plots in the (ν^{-1}; S^{-1}) coordinates of Lineweaver-Burk using the program Sigma Plot, version 2000 (USA). Root-mean-square deviation of determination: $\nu = \pm 2.5$ %, K_m and $V = \pm 7.5$ %, $K_{Va} = \pm 10$ %.

Results and Discussion

Determination of the intersection points of straight lines.

The results show (Figure 1) that a course of change of initial rates of activated enzyme catalyzed reactions (ν_a) obtained in the presence of 5-Fur (his symbol a_V) goes above the plot drawn in the absence of ($a_V = 0$) activator, i.e. it is an activated enzymatic reaction ($\nu_a > \nu_0$). Increment of the ordinates between the points ($\Delta\nu = \nu_a - \nu_0$) at increasing concentration of the substrate cleaved enlarges – obviously, line 1 at further increase in substrate concentration will intersect line 0, i.e. enzyme activated will be replaced by enzyme inhibition ($\nu_a < \nu_0$). What type of enzyme activation is there in the range of used substrate concentrations? The experimental data (Figure 1) are enough to determine the values of (K'_m, K^0_m, V', V^0) parameters to employ any computing program as for example, given in literature.[21] According to the ratio of the parameters, $K'_m < K^0_m$, $V' < V^0$, $a_V > 0$, one can establish (as seen from Table 1, line 11) that this enzyme catalyzed reaction corresponds to type (V_a) of enzyme activation, hence, the equation (11a) of Table 1 can be used for calculation of ν_{Va} rates of this enzyme catalyzed reaction.

However, it is more convenient to plot the dependence of ν_{Va} and ν_0 parameters of Figure 1 in the coordinates of Lineweaver-Burk (Figure 2), which results in the same values of K^0_m = 5.45 x 10^{-5} M, V^0 = 9.363 μmol· min^{-1} μg protein^{-1} parameters of initial reaction and K'_m = 3.47 x 10^{-5} M, V'= 8.803 μmol·min^{-1} μg protein^{-1}, which can also be obtained using the parameters of Figure 1 and 2.

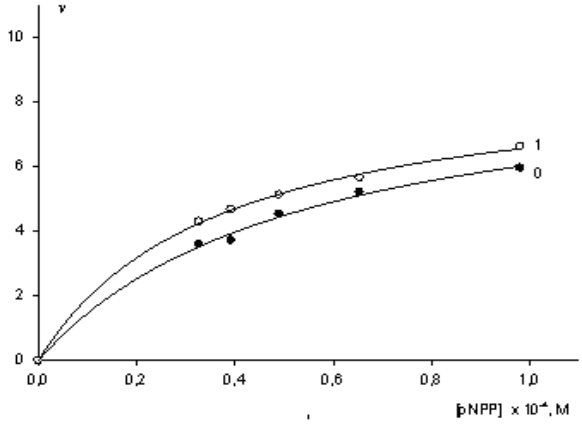


Figure 1. Plots of activating effect of 5-Fur on initial rates of pNPP cleavage catalyzed by porcine alkaline phosphatase represented in the (v; S) coordinates. Note: line 1 – the concentration of activator 0.001 M, line 0 – the activator is absent; v μmol·min^{-1} μg protein^{-1}

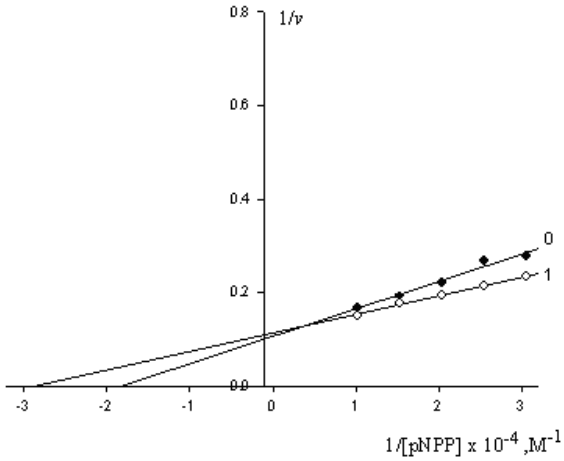


Figure 2. Plots of activating effect of 5Fur on initial rates (ν) of pNPP cleavage catalyzed by porcine alkaline phosphatase represented in the (ν^{-1}; S^{-1}) coordinates. Note: line 1 – the concentration of activator 0.001 M, line 0 – the activator is absent; v μmol·min^{-1} μg enzyme^{-1}

It is seen from Table 1 (Figure 11) that when the position of the experimental line V of activated reaction (Figure 2 of the text and figure 11 of Table 1) goes below the line 0 of initial reaction in the 1st quadrant of the coordinates of Lineweaver-Burk plot and crosses it at a point located to the left of a scale of reverse concentrations of substrate cleaved, but to the right of the ordinate axis. This point of the coordinates can be approximately determined from the plot in Figure 2, using the projections of the point on the coordinate axes. For more exact determination of the coordinates one needs to use a general solution of Eqns. 11a and 8a of Table 1 in (ν^{-1}; S^{-1}) coordinates.

$$\frac{1}{v'} = \frac{K'_m}{V'} \cdot \frac{1}{S} + \frac{1}{V'} \qquad (11)$$

and

$$= \qquad \frac{1}{v_0} = \frac{K^0_m}{V^0} \cdot \frac{1}{S} + \frac{1}{V^0} \qquad (12)$$

where: K'_m and V' are concrete values of parameters of Eqn. (11).

By making equal the right parts of Eqns. (11) and (12) one gets Eqn. (13)

$$\frac{1}{S_{int}} = \frac{V' - V^0}{K'_m V^0 - K^0_m V'} \qquad (13)$$

and

$$\frac{1}{v_{int}} = \frac{K^0_m - K'_m}{K^0_m V' - K'_m V^0} \qquad (14)$$

By substituting the values of all necessary parameters obtained earlier (Figure 2 in text) in the deduced equations, it can be established that the plots of this figure intersect in the point with the coordinates $1/S_{int}$ and $1/v_{int}$ at $0.375 \cdot 10^4$ M^{-1} and 0.128 $\mu mol^{-1} \cdot min \cdot \mu g$ protein, respectively.

To deduce equations for calculation of the coordinates of the intersection point of Figure 1, one must act analogously, i.e. by making equal the right parts of Eqn. (8a) and Eqn. (11a) (Table 1) and re-writing them to the form of Eqn. (15).

$$v' = \frac{V'}{1 + \frac{K'_m}{S}} \qquad (15)$$

It can be established that curvilinear plots of figure 1 shall intersect in a point with the coordinates:

$$S_{int} = \frac{K'_m V^0 - K^0_m V'}{V' - V^0} \qquad (16)$$

and

$$v_{int} = \frac{K^0_m V' - K'_m V^0}{K^0_m - K'_m} \qquad (17)$$

By having substituted the values of necessary parameters (Figure 1 or 2) in Eqns. (16) and (17), one can show that plots of this figure shall intersect in a point with the coordinates S_{int} and v_{int} at $2.67 \cdot 10^{-4}$ M and 7.813 $\mu mol \cdot min^{-1}$ μg $protein^{-1}$ respectively. This would be much more difficult to establish by using non-linear regression of curves 1 and 0 (Figure 1).

Analysis of the parallelism positions of plots of a course of change in initial reaction rates in the (v^{-1}; S^{-1}) coordinates.

In experimental practice a necessity often arises to state the presence of parallelism of lines at data treatment of the non-trivial II_i and II_a types of enzyme inhibition and activation (Figures 2 and 14, Table 1).

As an example, let us use the results of a study of the inhibitory effect of increasing concentrations of isopropanol on initial rates of pNPP cleavage catalyzed by eel alkaline phosphatase (EC 3.1.3.1). Enzyme is a Sigma (USA) product. A range of pNPP concentration used and a technique of recording the initial rates of substrate cleavage have been given above. The concentration of enzyme was 2.46 μg mL^{-1}. The results are shown in Figure 3.

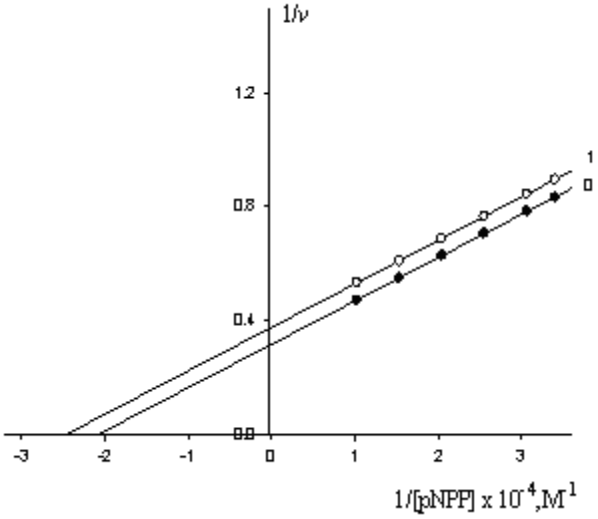
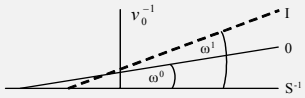
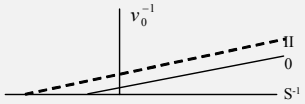
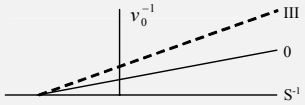
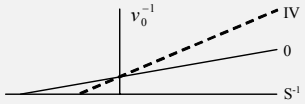
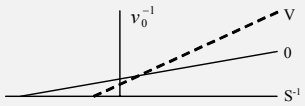
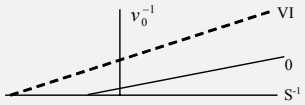
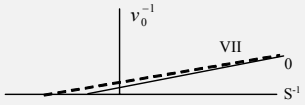
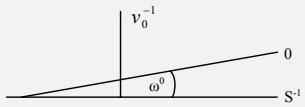
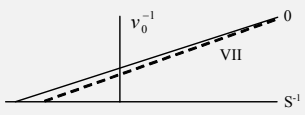
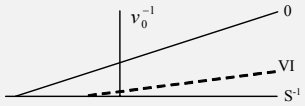
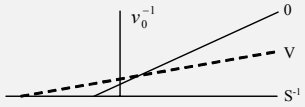
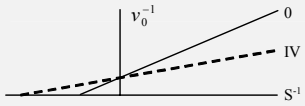
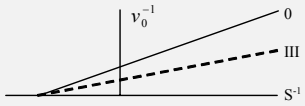
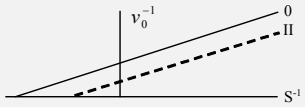
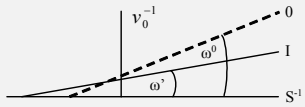


Figure 3. Plot of inhibitory effect of i-PrOH on initial rates of pNPP cleavage by eel alkaline phosphatase represented in the (v^{-1}; S^{-1}) coordinates. Note: line 1 – the concentration of inhibitor 0.0005 M, line 0 –the inhibitor is absent; v $\mu mol \cdot min^{-1}$ μg $protein^{-1}$ that the initial parameters V^0 = 3.162 $\mu mol \cdot min^{-1}$ μg $protein^{-1}$ and K^0_m= $4.824 \cdot 10^{-5}$ M of pNPP cleavage in the presence of 0.0005 M inhibitor change as follows: V' = 2.66 $\mu mol \cdot min^{-1}$ μg $protein^{-1}$ and K' = $4.071 \cdot 10^{-5}$ M, which characterizes the II_i type ($K'_m < K^0_m$, $V' < V^0$, $i > 0$) of unassociative enzyme inhibition (Table 1, line 2).

The similarity of the slope angles of plots 1 and 0, K'_m/V' = 1.530 conventional units (c. u.) and K^0_m/V^0 = 1.526 c.u. (conventional unit) respectively is quite satisfactory, considering the deviations in values of K'_m, K^0_m, V' and V^0.

Table 1. Equations for calculation of the v_i and v_a initial rates of enzyme catalyzed reactions

No	Effect	Type of effect	Correlation between the K'_m and V' parameters	Plots in the (v_0^{-1}; S^{-1}) coordinates	Equations for calculation of v_i and v_a (see continuation.)
1	Inhibition ($i > 0$)	I_i	$K'_m > K^0_m$; $V' < V^0$		v_{Ii}= (Eq. 1a)
2		II_i	$K'_m < K^0_m$; $V' < V^0$ $tg\omega' = tg\omega^0$		v_{IIi}= (Eq. 2a)
3		III_i	$K'_m = K^0_m$; $V' < V^0$		v_{IIIi}= (Eq. 3a)
4		IV_i	$K'_m > K^0_m$; $V' = V^0$		v_{IVi}= (Eq. 4a)
5		V_i	$K'_m > K^0_m$; $V' > V^0$		v_{Vi}= (Eq. 5a)
6		VI_i	$K'_m < K^0_m$; $V' < V^0$ $tg\omega' > tg\omega^0$		v_{VIi}= (Eq. 6a)
7		VII_i	$K'_m < K^0_m$; $V' < V^0$ $tg\omega' < tg\omega^0$		v_{VIIi}= (Eq. 7a)
8	No effect	I_0	$K'_m = K^0_m$; $V' = V^0$		v_0= (Eq. 8a)

9	Activation ($a > 0$)	VII_a	$K'_m > K^0_m$; $V' > V^0$ $tg\omega' > tg\omega^0$		ν_{VIIa}= (Eq. 9a)
10		VI_a	$K'_m > K^0_m$; $V' > V^0$ $tg\omega' < tg\omega^0$		ν_{VIa}= (Eq. 10a)
11		V_a	$K'_m < K^0_m$; $V' < V^0$		ν_{Va}= (Eq. 11a)
12		IV_a	$K'_m < K^0_m$; $V' = V^0$		ν_{IVa}= (Eq. 12a)
13		III_a	$K'_m = K^0_m$; $V' > V^0$		ν_{IIIa}= (Eq. 13a)
14		II_a	$K'_m > K^0_m$; $V' > V^0$ $tg\omega' = tg\omega^0$		ν_{IIa}= (Eq. 14a)
15		I_a	$K'_m < K^0_m$; $V' > V^0$		ν_{Ia}= (Eq. 15a)

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

№ 1. (*type I_i, biparametrically coordinated inhibition*)

$$\upsilon_{Ii} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{yi}}\right)}}{1+\dfrac{K^0_m}{S}\cdot\left(1+\dfrac{i}{K_{xi}}\right)} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{IIIi}}\right)}}{1+\dfrac{K^0_m}{S}\cdot\left(1+\dfrac{i}{K_{IVi}}\right)} \qquad (1a)$$

№ 2. (*type II*$_i$, *unassociative inhibition*)

$$\upsilon_{IIi} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{yi}}\right)}}{1+\dfrac{K_m^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{xa}}\right)}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{IIIi}}\right)}}{1+\dfrac{K_m^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{IVa}}\right)}} \quad (2a)$$

№ 3. (*type III*$_i$, *catalytic inhibition*)

$$\upsilon_{IIIi} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{yi}}\right)}}{1+\dfrac{K_m^0}{S}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{IIIi}}\right)}}{1+\dfrac{K_m^0}{S}} \quad (3a)$$

№ 4. (*type IV*$_i$, *associative inhibition*)

$$v_{IVi} = \frac{V^0}{1+\dfrac{K_m^0}{S}\cdot\left(1+\dfrac{i}{K_{xi}}\right)} = \frac{V^0}{1+\dfrac{K_m^0}{S}\cdot\left(1+\dfrac{i}{K_{IVi}}\right)} \quad (4a)$$

№ 5. (*type V*$_i$, *pseudoinhibition*)

$$v_{Vi} = \frac{V^0 \cdot \left(1+\dfrac{i}{K_{ya}}\right)}{1+\dfrac{K_m^0}{S}\cdot\left(1+\dfrac{i}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1+\dfrac{i}{K_{IIIa}}\right)}{1+\dfrac{K_m^0}{S}\cdot\left(1+\dfrac{i}{K_{IVi}}\right)} \quad (5a)$$

№ 6. (*type VI*$_i$, *discoordinated inhibition*)

$$v_{VIi} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{yi}}\right)}}{1+\dfrac{K_m^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{xa}}\right)}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{IIIi}}\right)}}{1+\dfrac{K_m^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{IVa}}\right)}} \quad (6a)$$

№ 7. (*type VII*$_i$, *transient inhibition*)

$$v_{\mathrm{VIIi}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{\mathrm{yi}}}\right)}}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{\mathrm{xa}}}\right)}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{i}{K_{\mathrm{IIIi}}}\right)}}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{i}{K_{\mathrm{IVa}}}\right)}} \tag{7a}$$

№ 8. *Initial* (*uninhibited and nonactivated*) *reaction*

$$v_0 = \frac{V^0}{1+\dfrac{K_{\mathrm{m}}^0}{S}} \tag{8a}$$

№ 9. (*type VII*$_a$, *transient activation*)

$$v_{\mathrm{VIIa}} = \frac{V^0 \cdot \left(1+\dfrac{a}{K_{\mathrm{ya}}}\right)}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\left(1+\dfrac{a}{K_{\mathrm{xi}}}\right)} = \frac{V^0 \cdot \left(1+\dfrac{a}{K_{\mathrm{IIIa}}}\right)}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\left(1+\dfrac{a}{K_{\mathrm{IVi}}}\right)} \tag{9a}$$

№ 10. (*type VI*$_a$, *discoordinated activation*)

$$v_{\mathrm{VIa}} = \frac{V^0 \cdot \left(1+\dfrac{a}{K_{\mathrm{ya}}}\right)}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\left(1+\dfrac{a}{K_{\mathrm{xi}}}\right)} = \frac{V^0 \cdot \left(1+\dfrac{a}{K_{\mathrm{IIIa}}}\right)}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\left(1+\dfrac{a}{K_{\mathrm{IVi}}}\right)} \tag{10a}$$

№ 11. (*type V*$_a$, *pseudoactivation*)

$$v_{\mathrm{Va}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{a}{K_{\mathrm{ya}}}\right)}}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{a}{K_{\mathrm{xa}}}\right)}} = \frac{V^0 \cdot \dfrac{1}{\left(1+\dfrac{a}{K_{\mathrm{IIIi}}}\right)}}{1+\dfrac{K_{\mathrm{m}}^0}{S}\cdot\dfrac{1}{\left(1+\dfrac{a}{K_{\mathrm{IVa}}}\right)}} \tag{11a}$$

№ 12. (*type IV*$_a$, *associative activation*)

$$v_{IVa} = \frac{V^0}{1 + \frac{K_m^0}{S} \cdot \frac{1}{\left(1 + \frac{a}{K_{xa}}\right)}} = \frac{V^0}{1 + \frac{K_m^0}{S} \cdot \frac{1}{\left(1 + \frac{a}{K_{IVa}}\right)}} \quad (12a)$$

№ 13. (*type III*$_a$, *catalytic activation*)

$$v_{IIIa} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{ya}}\right)}{1 + \frac{K_m^0}{S}} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{IIIa}}\right)}{1 + \frac{K_m^0}{S}} \quad (13a)$$

№ 14. (*type II*$_a$, *unassociative activation*)

$$v_{IIa} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{ya}}\right)}{1 + \frac{K_m^0}{S} \cdot \left(1 + \frac{a}{K_{xi}}\right)} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{IIIa}}\right)}{1 + \frac{K_m^0}{S} \cdot \left(1 + \frac{a}{K_{IVi}}\right)} \quad (14a)$$

№ 15. (*type I*$_a$, *biparametrically coordinated activation*)

$$v_{Ia} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{ya}}\right)}{1 + \frac{K_m^0}{S} \cdot \frac{1}{\left(1 + \frac{a}{K_{xa}}\right)}} = \frac{V^0 \cdot \left(1 + \frac{a}{K_{IIIa}}\right)}{1 + \frac{K_m^0}{S} \cdot \frac{1}{\left(1 + \frac{a}{K_{IVa}}\right)}} \quad (15a)$$

There is another possibility to characterize parallelism of such plots (Figure 3). Data analysis shows that a system of linear Eqns. (2a) and (8a) will not have a general solution if the determinant of this system is equal to zero, and it may occur in the case of parallelism of the positions of respective lines (like the lines of Figure 3). Substituting the experimental data of Figure 3 in Eqn. (18) yields a value of 0.004 c.u. for the determinant (K′m/V′ – K0m/V0), which is a satisfactory feature of parallelism of these lines.

$$\begin{vmatrix} \frac{K_m^0}{V^0} & -1 \\ \frac{K'_m}{V'} & -1 \end{vmatrix} = 0 \quad (18)$$

Examples of data of the IIi and IIa types of biparametrically discoordinated enzyme inhibition and activation15 are rather often found in practical enzymology, 10–14 but the technique employed to determine parallelism of the positions of lines of the like type (see Eqns. 11 and 12) by estimating the value of the determinant is not used for data treatment in studies of analogous types of enzyme

activation and inhibition. Probably, it may be explained by the absence of Eqns. 2a and 14a of Table 1 for calculation of initial reaction rates of such types of enzyme inhibition and activation in practical enzymology.

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