ON LINEAR CONCENTRATION DEPENDENCES OF K'_m, V' AND $1/v_i$ ENZYME KINETIC PARAMETERS

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The most frequently occurring incorrect uses of the plots of intercepts, slopes and other coordinates for the determination of constants of enzyme activation K_a and of nontrivial inhibition $K(II_i)$, $K(V_i)$, $K(I_i)$, were analyzed. The correct plots are shown. Calculations of the $K(I_i)$ and $K(II_i)$ constants of nontrivial types of enzyme inhibition using the corrected plots of the intercepts are given.

In analyzing the kinetic properties of enzymes intercepts* and slopes** of plots are often determined. The same applies to the determination of the inhibition (K_i) and activation (K_a) constants[1-7]. In plotting such dependences, authors often resort to their modifications. Thus, for example, of the two simultaneously changing parameters $1/K'_m$ and $1/V'$ one is preferred more often $1/V'$ (refs[3,5-9]). In the case of activation this parameter is also plotted versus the inverse concentrations of activator[3,5,7,9-13]. For the two-parameter matched and mismatched types[14-16] of enzyme inhibition and activation this is inadmissible. Also inadmissible is to plot the dependences of slopes on inhibitor or activator concentrations in the two-parameter mismatched types of inhibition (II_i and V_i) (refs[3,6,7]) and activation (V_a and II_a) (refs[3,11,13]), since the equations of the respective constants do not include the ratio K'_m/V' (Table I). Some other arbitrary modifications are also used[4,10].

Prior to the derivation of the equations of enzyme activation and nontrivial $K(II_i)$, $K(V_i)$ inhibition constants[14-16] it was not always clear which combinations of K'_m and V' parameters can be used for constructing the linear dependences on the plots

* E.g., the values of $1/K'_m$ or $1/V'$ on a Lineweaver–Burk plot, where K'_m and V' are the effective Michaelis constant and maximum reaction rate determined in presence of inhibitor (i) or activator (a); K^0_m and V^0 are the same parameters of the control, neither inhibited nor activated.

** E.g., the dependences of slopes (tg w') on the molar concentrations of inhibitor (i) or activator (a), where tg w' is the slope determined by the K'_m/V' ratio and tg $w^0 = K^0_m/V^0$.

TABLE I

Characteristics of the possible types of enzyme inhibition and activation

Effect	Type	Relationship between K_m and V parameters	Designation	Equation for calculating K_i and K_a constants	Eq.; Fig.
Inhibition ($i > 0$)	I_i	$K'_m > K^0_m$, $V' < V^0$	Two-parameter matched inhibition	$K(I_i) = \frac{i}{K'_m V^0 / K^0_m V' - 1}$	(1); 1a
	II_i	$K'_m < K^0_m$, $V^0 < V^0$	Two-parameter mismatched inhibition	$K(II_i) = \frac{i}{K^0_m V^0 / K'_m V' - 1}$	(2); 1b
	III_i	$K'_m = K^0_m$, $V' < V^0$	Catalytic inhibition	$K(III_i) = \frac{i}{V^0 / V' - 1}$	(3); 1c
	IV_i	$K'_m > K^0_m$, $V' = V^0$	Associative inhibition	$K(IV_i) = \frac{i}{K'_m / K^0_m - 1}$	(4); 1d
	V_i	$K'_m > K^0_m$, $V' > V^0$	Pseudo-inhibition	$K(V_i) = \frac{i}{K'_m V' / K^0_m V^0 - 1}$	(5); 1e

No		$K'_m = K^0_m,\ V' = V^0$	Control (neither inhibited nor activated) enzymic reaction		
Activation ($a > 0$)	V_a	$K'_m < K^0_m,\ V' < V^0$	Pseudo-activation	$K(V_a) = \dfrac{a}{K^0_m V^0 / K'_m V' - 1}$	(6); 2a
	IV_a	$K'_m < K^0_m,\ V' = V^0$	Associative activation	$K(IV_a) = \dfrac{a}{K^0_m / K'_m - 1}$	(7); 2b
	III_a	$K'_m = K^0_m,\ V' > V^0$	Catalytic activation	$K(III_a) = \dfrac{a}{V' / V^0 - 1}$	(8); 2c
	II_a	$K'_m > K^0_m,\ V' > V^0$	Two-parameter mismatched activation	$K(II_a) = \dfrac{a}{K'_m V' / K^0_m V^0 - 1}$	(9); 2d
	I_a	$K'_m < K^0_m,\ V' > V^0$	Two-parameter matched activation	$K(I_a) = \dfrac{a}{K^0_m V' / K'_m V^0 - 1}$	(10); 2e

of intercepts or slopes. The deduction of these equations[14-16] (Table I) makes it possible to introduce certain corrections into the practice of using these coordinates.

This work deals with the analysis of using the plots of intercepts and slopes as well as the Dixon plot in the determination of the K_a and K_i constants.

Plots of Intercepts and Slopes

To make the present calculation more comprehensible, the plots are analyzed using the types of enzyme inhibition and activation described earlier[14-16].

Type I_i: two-parameter matched (or traditionally called mixed) inhibition. This is characterized by $K'_m > K^0_m$, $V' < V^0$, $i > 0$ (Table I, Fig. 1*a*). As seen from Eq. (*1*) of Table I, to calculate the $K(I_i)$ constants of this type of inhibition, use can be made of both the $(K'_m V^0/K^0_m V'; i)$ and $(K'_m/V'; i)$ normalized plots of intercepts where line *2* (Fig. 3*b*)

$$K'_m V^0/K^0_m V' = i/K(I_i) + 1 \qquad (11)$$

and second line *1* (Fig. 3*b*)

$$K'_m/V' = (K^0_m/V^0)\,(i/K(I_i)) + K^0_m/V^0 \qquad (12)$$

will intersect the abscissa (molar concentrations of inhibitor) of the same point, viz. $-i = K(I_i)$.

Taking into consideration that $\mathrm{tg}\, w' = K'_m/V'$ where w' is the slope of line *1* (Fig. 1*a*) to the abscissa and w^0 is the slope of line *0* to this axis ($\mathrm{tg}\, w^0 = K^0_m/V^0$) (Fig. 1), Eqs (*11*) and (*12*) make it possible to use the equivalent plots of slopes $(\mathrm{tg}\, w'/\mathrm{tg}\, w^0; i)$ and $(\mathrm{tg}\, w'; i)$ where line

$$\mathrm{tg}\, w'/\mathrm{tg}\, w^0 = i/K(I_i) + 1 \qquad (13)$$

and second line

$$\mathrm{tg}\, w' = (\mathrm{tg}\, w^0)\,(i/K(I_i)) + \mathrm{tg}\, w^0 \qquad (14)$$

and will also intersect the abscissa at a single point, viz. $-i = K(I_i)$.

Examples of using the plot of slopes to determine the inhibition constants are available in the literature[1-3]. Examples of using the plot of intercepts (in the form of Eqs (*11*), (*12*)) are absent which is explainable by the absence in the publications (until 1986) (ref.[14]) of Eq. (*1*) on the basis of which they could have been suggested. The system of two equations for the calculation of the $K(I_i)$ constants which existed up to that time (cf. Eqs 8. 56 in ref.[17]) did not make this possible.

The processing of the experimental data of calf alkaline phosphatase inhibition by WO_4^{2-} anions (Fig. 3*a*) demonstrates the convenience of using both the first (Eq. (*11*)) and the second (Eq. (*12*)) plots of intercepts for the calculation of the $K(I_i)$

inhibition constants (Fig. 3*b*). However, there are no objections against the plots of slopes to be used here (Eqs (*13*) and (*14*)).

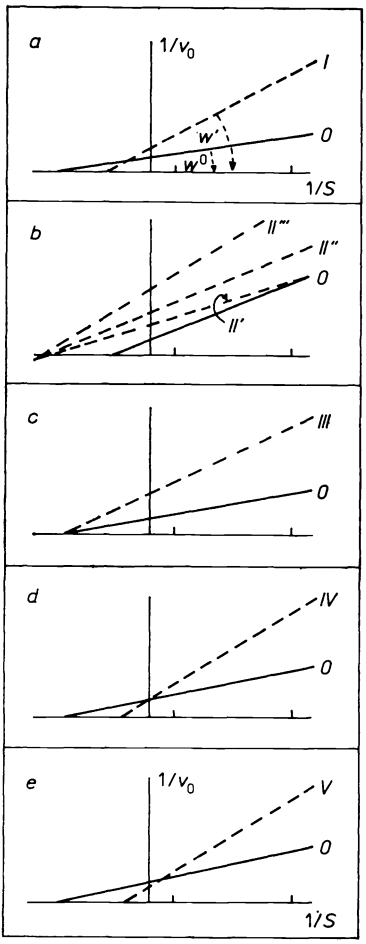

FIG. 1

Positions of the lines of inhibited reactions (dashed lines) relative to line *0* of the non-inhibited reaction in $(1/v; 1/S)$ coordinates. *a* Type I_i of enzyme inhibition — line *I*; *b* type II_i (subtype II'_i of inhibition — line *II'*, subtype II''_i — line *II''*, subtype II'''_i — line *II'''*); *c* type III_i inhibition — line *III*; *d* type IV_i — line *IV*; e type V_i inhibition — line *V*

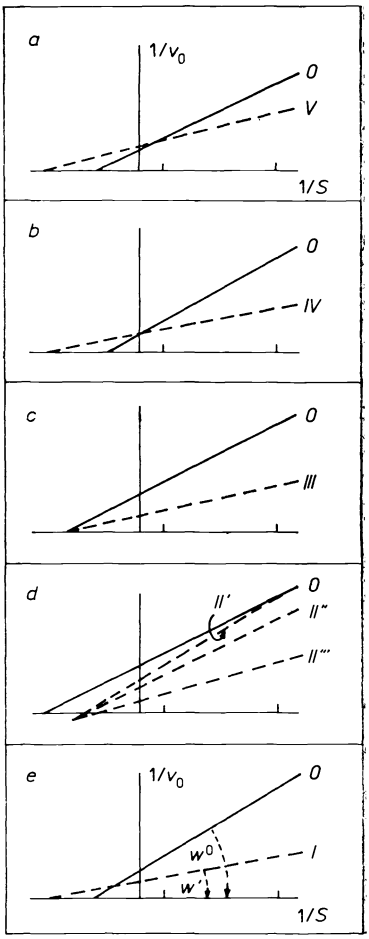

FIG. 2

Positions of lines of activated reactions (dashed lines) relative to line *0* of the non-activated reaction in $(1/v; 1/S)$ coordinates. *a* Type V_a of enzyme activation — line *V*; *b* type IV_a activation — line *IV*; *c* type III_a — line *III*; *d* type II_a (subtype II'_a of activation — line *II'*, subtype II''_a — line *II''*, subtype II'''_a — line *II'''*); e type I_a activation — line *I*

Analysis of Eq. (*1*) (and Fig. 1*a*) as well as Eqs (*11*) and (*12*) shows that the use of the dependences of only $1/V'$ on i or K'_m on i to determine the $K(I_i)$ constants[3,4,11] would be incorrect since in this case both K'_m and V' change simultaneously.

Type II_i: two-parameter mismatched inhibition[14-16]. As seen from Eq. (*2*) (Table I), the inhibition constants $K(II_i)$ (in all three subtypes II'_i, II''_i and II'''_i of type II_i (refs[14-16]); cf. Fig. 1*b*, lines *II'*, *II''* and *II'''*, respectively) can be calculated using the dependence of only the $(K^0_m V^0/K'_m V'; i)$ plot of intercepts or of the normalized plot $(1/K'_m V'; i)$ where line *1* (Fig. 4*b*)

$$K^0_m V^0/K'_m V' = i/K(II_i) + 1 \qquad (15)$$

and second line 2 (Fig. 4*b*)

$$1/K'_m V' = (1/K^0_m V^0)(i/K(II_i)) + 1/K^0_m V^0 \qquad (16)$$

will intersect the abscissa at point $-i = K(II_i)$. This can serve as a convenient criterion of reliability of the results obtained (Fig. 4*b*). At the same time, as seen from Eq. (*2*) (and Eqs (*15*), (*16*)), no combination of slopes of the lines in such cases can yield a linear dependence of their change on inhibitor concentration; also inadmissible here is the separate use of the $(1/V'; i)$ or $(1/K'_m; i)$ plots of intercepts for calculating

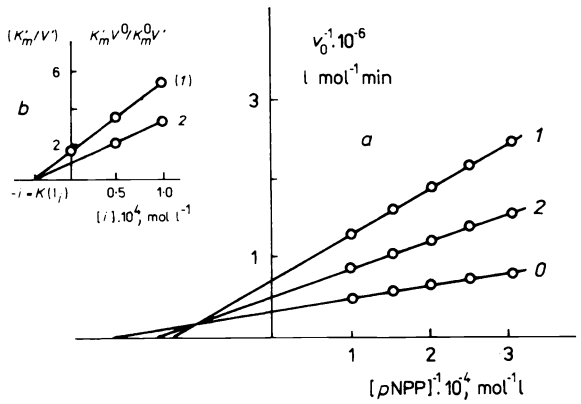

FIG. 3

Inhibitory effect of Na_2WO_4: *a* On the initial rates of decomposition of pNPP by calf alkaline phosphatase. Conditions: 0·05M Tris-HCl buffer, pH 9·0; ionic strength 0·1 mol l^{-1} (NaCl), 37°C. Concentration: pNPP, 32·7 μmol l^{-1} — 98·0 μmol l^{-1}; phosphatase, 0·98 μg/ml; Na_2WO_4, 50 μmol l^{-1} (line 2); 0·1 mmol l^{-1} (line *1*); line *0*, no inhibitor. *b* On the $(K'_m V^0/K^0_m V'; i)$ plot (line 2), and on the $(K'_m/V'; i)$ plot (line *1*) (dimensions of the ordinate for line *1* in min)

the inhibition constants $K(\mathrm{II}_i)$ (ref.[5]) and for analyzing the mechanisms of these reactions[3,7,8], since in each of these cases both K'_m and V' parameters change simultaneously.

Type III_i: catalytic[14-16] (traditionally called "non-competitive") inhibition. Analysis of the position of the lines (Fig. 1c, Table I) and the known equation (Eq. (3)) for calculating the inhibition constants $K(\mathrm{III}_i)$ have long ago suggested[17] the possibility of using both the $(V^0/V'; i)$ and $(1/V'; i)$ plots of intercepts where the straight line for

$$V^0/V' = i/K(\mathrm{III}_i) + 1 \qquad (17)$$

as well as for

$$1/V' = (1/V^0)\,(i/K(\mathrm{III}_i)) + 1/V^0 \qquad (18)$$

will intersect the abscissa at a single point, viz. $-i = K(\mathrm{III}_i)$.

Taking into account the equality $K'_m = K^0_m$ (Fig. 1c and Table I) this is equivalent to the construction of the dependences in the $(\mathrm{tg}\ w'/\mathrm{tg}\ w^0; i)$ and $(\mathrm{tg}\ w'; i)$ plots of slopes where straight lines

$$\mathrm{tg}\ w'/\mathrm{tg}\ w^0 = i/K(\mathrm{III}_i) + 1 \qquad (19)$$

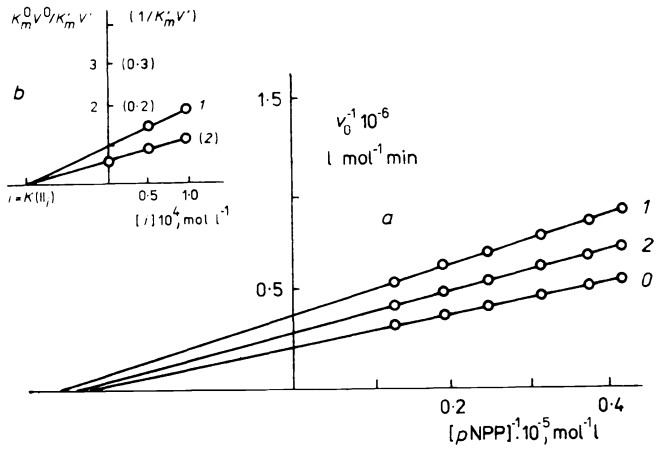

FIG. 4

Inhibitory effect of *p*NP: *a* On the initial rates of *p*NPP by *Pseudomonas* neutral phosphatase. Conditions: 0·05M Tris-acetate buffer, pH 6·5; ionic strength 0·1 (NaCl); 37°C. Concentration: pNPP, 23 μmol l^{-1} — 78·4 μmol l^{-1}; enzyme, 1·82 μg/ml; *p*NP, 50 μmol l^{-1} (line 2); 0·1 mmol l^{-1} (line 1); line 0, no inhibitor. *b* On the $(K^0_m V^0/K'_m V'; i)$ plot (line 1); and on the $(1/K'_m V'; i)$ plot (line 2) (dimensions of the ordinate axis in this case in mol^{-2} l^2 min)

and

$$\text{tg } w' = (\text{tg } w^0)\,(i/K(\text{III}_i)) + \text{tg } w^0 \qquad (20)$$

will intersect the abscissa at $-i = K(\text{III}_i)$, with the only difference that the line of Eq. (*20*) will intersect the ordinate at tg w^0 and the line of Eq. (*18*), at $1/V^0$, which is more convenient for checking the results.

Examples of the simultaneous use of the plots of intercepts in the form of $(1/V'; i)$ and of slopes in the form of $(\text{tg } w'; i)$ for the determination of the inhibition constants $K(\text{III}_i)$ are available in the literature[3,7].

Type IV_i: associative[14–16] (traditionally called "competitive") inhibition of enzymes (Fig. 1*d*). Analysis of the second known equation (Eq. (*4*), Table I) for calculating the inhibition constants $K(\text{IV}_i)$ has long ago suggested[17] the possibility of using both the $(K'_m/K^0_m; i)$ and $(K'_m; i)$ plots of intercepts where line 2 (Fig. 5*b*)

$$K'_m/K^0_m = i/K(\text{IV}_i) + 1 \qquad (21)$$

as well as line *1* (Fig. 5*b*)

$$K'_m = K^0_m(i/K(\text{IV}_i)) + K^0_m \qquad (22)$$

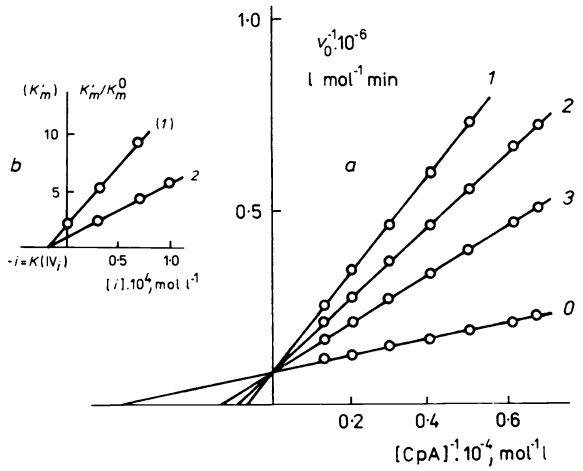

FIG. 5

Inhibitory effect of 3′ AMP: *a* On the initial rates of decomposition of CpA by *P. brevicompactum* nonspecific RNase. Conditions: 0·05M Na-acetate buffer, pH 4·7; ionic strength 0·1 mol l^{-1} (NaCl), 24°C. Concentration: CpA, 0·144 mmol l^{-1} — 0·736 mmol l^{-1}; RNase *P. brevicompactum*, 0·147 μmol l^{-1}; 3′AMP, 30 μmol l^{-1} (line *3*); 70 μmol l^{-1} (line 2) and 0·1 mmol l^{-1} (line *1*). *b* On the $(K'_m/K^0_m; i)$ plot (line 2) and on the $(K'_m; i)$ plot (line *1*) (dimensions of the ordinate in this case in mol l^{-1}

will intersect the abscissa at $-i = K(\mathrm{IV}_i)$ or the equivalent plots of slopes $(\mathrm{tg}\ w'/\mathrm{tg}\ w^0; i)$ and $(\mathrm{tg}\ w'; i)$ where

$$\mathrm{tg}\ w'/\mathrm{tg}\ w^0 = i/K(\mathrm{IV}_i) + 1 \qquad (23)$$

as well as

$$\mathrm{tg}\ w' = \mathrm{tg}\ w^0(i/K(\mathrm{IV}_i)) + \mathrm{tg}\ w^0 \qquad (24)$$

will intersect the abscissa at $-i = K(\mathrm{IV}_i)$.

Similarly to the previous case (type III_i) there are many examples of using the $(K'_m/K^0_m; i)$ and $(\mathrm{tg}\ w'; i)$ plots for the determination of inhibition constants $K(\mathrm{IV}_i)$, and in studies of the mechanisms of these reactions[2,3,5,-7,18]. Considerably less frequent is the use of $(K'_m; i)$ plots[1,4] although their certain advantage (the use of control point K^0_m known beforehand) is evident.

Type V_i: enzyme pseudo-inhibition[14-16]. Analysis of Eq. (5) (Table I) and Fig. 1e shows that to calculate the $K(\mathrm{V}_i)$ constants of this type of inhibition, use can be made of the linear dependences in only the $(K'_mV'/K^0_mV^0; i)$ plot of intercepts or their normalized variant $(K'_mV'; i)$ where line

$$K'_mV'/K^0_mV^0 = i/K(\mathrm{V}_i) + 1 \qquad (25)$$

and line

$$K'_mV' = K^0_mV^0(i/K(\mathrm{V}_i)) + K^0_mV^0 \qquad (26)$$

will intersect the abscissa at $-i = K(\mathrm{V}_i)$.

At the same time, Eq. (5) (as well as Eqs (25), (26)) do not make it possible to plot here linear dependences in slopes. However, such attempts are available in the literature[8,19].

Activation. Comparison of the equations of the activation constants (Eqs (6)–(10), Table I) and the positions of the plots of the activated reactions (Fig. 2a–2e) with the respective inhibition equations (Eqs (1)–(5)) and plots with respect to line 0 shows the presence of opposite tendencies of changing the K'_m and V' parameters in each of the following cases: I_a and I_i; II_a and II_i; III_a and III_i; IV_a and IV_i; V_a and V_i of activation/inhibition. This antidirectivity can be used to discuss the applicability of similar plots of slopes and intercepts, symmetrically opposite by their combinations of K'_m and V' (as well as $\mathrm{tg}\ w'$) in caculations of K_a constants (Table II).

As can be easily seen from Eqs (6) and (9) as well as Eq. (10) (Table I) in the case of enzyme activation it is also inadmissible to separately use the $1/K'_m$ or $1/V'$ parameters to plot their linear dependences on the concentrations of activators (a) or, especially, their inverse values $1/a$. Besides, it follows from Eqs (6) and (9) that in the two-parameter mismatched activation types V_a and II_a it is also inadmissible to

TABLE II

Distribution of the plots of intercepts and slopes by the types of enzyme inhibition (*i*) and activation (*a*)

Effect	Type	Plots for calculating K_i			
		intercepts		slopes	
		full	normalized	full	normalized
Inhibition	I_i	$\frac{K'_m V^0}{K^0_m V'}$; *i*	$\frac{K'_m}{V'}$; *i*	$\frac{\text{tg } w'}{\text{tg } w^0}$; *i*	tg w'; *i*
	II_i	$\frac{K^0_m V^0}{K'_m V'}$; *i*	$\frac{1}{K'_m V'}$; *i*		
	III_i	$\frac{V^0}{V'}$; *i*	$\frac{1}{V'}$; *i*	$\frac{\text{tg } w'}{\text{tg } w^0}$; *i*	tg w'; *i*
	IV_i	$\frac{K'_m}{K^0_m}$; *i*	K'_m; *i*	$\frac{\text{tg } w'}{\text{tg } w^0}$; *i*	tg w'; *i*
	V_i	$\frac{K'_m V'}{K^0_m V^0}$; *i*	$K'_m V'$; *i*		

Effect	Type	Plots for calculating K_a			
		intercepts		slopes	
		full	normalized	full	normalizcd
Activation	I_a	$\frac{K^0_m V'}{K'_m V^0}$; *a*	$\frac{V'}{K'_m}$; *a*	$\frac{\text{tg } w^0}{\text{tg } w'}$; *a*	$\frac{1}{\text{tg } w'}$; *a*
	II_a	$\frac{K'_m V'}{K^0_m V^0}$; *a*	$K'_m V'$; *a*		
	III_a	$\frac{V'}{V^0}$; *a*	V'; *a*	$\frac{\text{tg } w^0}{\text{tg } w'}$; *a*	$\frac{1}{\text{tg } w'}$; *a*
	IV_a	$\frac{K^0_m}{K'_m}$; *a*	$\frac{1}{K'_m}$; *a*	$\frac{\text{tg } w^0}{\text{tg } w'}$; *a*	$\frac{1}{\text{tg } w'}$; *a*
	V_a	$\frac{K^0_m V^0}{K'_m V'}$; *a*	$\frac{1}{K'_m V'}$; *a*		

plot linear dependences on the slopes. Such attempts occur in the practice of processing the results of enzyme activation[3,7,8-13]; even without any information on Eqs (*1*), (*2*), (*5*), (*6*)–(*10*) (Table I) (ref.[14]) they made numerous attempts to linearize these data.

Dixon plots. Construction of the dependences in the $(1/v_i; i)$ plots for the determination of K_i, in studies of the mechanisms of enzymic reactions is also a widely used technique in enzyme kinetics[20,21]. Dixon[22] showed that in the $(1/v_i; i)$ plots the experimental lines of the catalytic enzyme inhibition (type III_i) intersect the abscissa at $-i = K(\mathrm{III}_i)$; and those of the associative type (IV_i), in the second quadrant (abscissa $-i = K(\mathrm{IV}_i)$). This phenomenon is used for the determination of both $K(\mathrm{III}_i)$ (ref.[20]) and $K(\mathrm{IV}_i)$ (ref.[21]) inhibition constants. However, the use of these plots for the calculation of $K(\mathrm{I}_i)$ constants of mixed enzyme inhibition (I_i type) can be objected. From the formula of the known[23] equation of v_i vs i:

$$v_i = V^0 \frac{(\alpha K(\mathrm{III}_i) + \beta i)/(\alpha K(\mathrm{III}_i) + i)}{1 + (K_m^0/S)\left[(\alpha K(\mathrm{IV}_i) + \alpha i)/(\alpha K(\mathrm{IV}_i) + i)\right]}, \tag{27}$$

where α and β are the coefficients characterizing the change of the enzyme-to-substrate affinity under the action of the inhibitor and that of the maximum rate of its transformation. It is seen that in the case of $\alpha > 1$, $\beta < 1$ (and this type of inhibition is characterized by just this relationship of the coefficients: cf. Table I) this equation is reduced to

$$v_i = V^0 \frac{1/(1 + i/K(\mathrm{III}_i))}{1 + (K_m^0/S)(1 + i/K(\mathrm{IV}_i))} \tag{28}$$

which in the $(1/v_i; i)$ plots will be a squared trinomial[15]

$$1/v_i = 1/V^0 + K_m^0/V^0S + \left[1/V^0K(\mathrm{III}_i) + K_m^0/V^0SK(\mathrm{III}_i) + K_m^0/V^0SK(\mathrm{IV}_i)\right] i + \\ + K_m^0 i^2/\left[V^0SK(\mathrm{III}_i)\,K(\mathrm{IV}_i)\right] \tag{29}$$

i.e. a parabola with the abscissa at the apex

$$-i = 0{\cdot}5K(\mathrm{IV}_i)\left[1 + S/K_m^0 + K(\mathrm{III}_i)/K(\mathrm{IV}_i)\right] \tag{30}$$

and intersecting the ordinate at

$$1/v_i = 1/V^0 + K_m^0/V^0S\,. \tag{31}$$

The right branch of the parabola will be located in the first quadrant of the plot

$(1/v_i; i)$ (Fig. 6) and, therefore, without a proper manifestation of the curvature[24] such dependences can be taken for straight lines. It can be easily seen that in the $(S/v_i; i)$ plot Eq. (*29*) will also describe the squared dependence of S/v_i on i. Meanwhile, there are examples of using the $(1/v_i; i)$ and $(S/v_i; i)$ plots to determine the $K(\mathrm{I}_i)$ enzyme inhibition constants, to prove the linearity of such dependences, etc.[21,25].

The use of Eqs (*32*) (refs[17,26])

$$v_i = V^0 \frac{1/(1 + i/K_i')}{1 + \frac{[K_m^0(1 + i/K_i)]}{[S(1 + i/K_i')]}} \qquad (32)$$

for calculating $K(\mathrm{I}_i)$ inhibition constants, as equations making it possible to linearize these dependences in the $(1/v_i; i)$ plot as well as allowing not only an increase of the Michaelis constants $(K_m' > K_m^0)$ but also their decrease $(K_m' < K_m^0)$ at $K_i > K_i'$ (refs[17,26]), i.e. equations permitting one also to analyze cases where the lines intersect in the third quadrant of the Lineweaver–Burk plot, as a mixed type (I_i) of enzyme inhibition, can be objected to in the following way. As shown by the method of vector representations of these reactions in the three-dimensional $K_m'V'$ coordinates[15,16], the $\boldsymbol{PI}_i$ vectors of the reactions of mixed (type I_i) enzyme, inhibition characterized by the relationship $K_m' > K_m^0$, $V' < V^0$, $i > 0$ (Table I) can only occupy the fourth sector space of this plot[15,16]. If the relationships of these parameters are changed to $K_m' < K_m^0$, $V' < V^0$, $i > 0$, these vectors will shift to the third sector space of the coordinates where, at $K_m' < K_m^0$, $V' < V^0$, $i > 0$, they will characterize the occurrence of reactions according to the II_i (two-para-

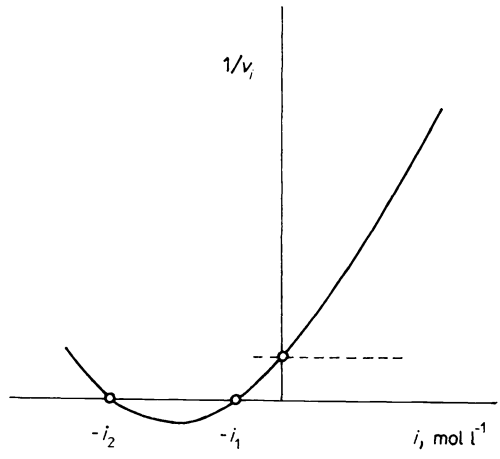

FIG. 6
Illustration of the change of $1/v_i$ vs i described by Eq. (*29*)

meter mismatched) type of inhibition; besides, at $K'_m = K^0_m$, $V' < V^0$, $i > 0$, i.e. during the transition from the fourth to the third sector space, they will characterize the occurrence of reactions according to the III_i (noncompetitive) type of inhibition. To calculate K_i constants, its concrete (Table I) equation (Eqs (*2*) and (*3*), respectively) shall be used in each of these cases.

The deduction of the equations of the dependence v_a vs a in the case of catalytic (type III_a)

$$v_a = V^0 \frac{1 + a/K(III_a)}{1 + K^0_m/S} \tag{33}$$

and associative (type IV_a)

$$v_a = \frac{V^0}{1 + K^0_m/S(1 + a/K(IV_a))} \tag{34}$$

enzyme activation[15] makes it possible to suggest here some other plots for constructing the linear dependences of changing v_a vs a.

Thus, as can be easily seen from Eq. (*33*), the results of the studies of such (III_a) type of activation will fall on line

$$v_a = V^0/(1 + K^0_m/S) + V^0\{a/[(1 + K^0_m S)\, K(III_a)]\} \tag{35}$$

intersecting the abscissa at $-a = K(III_a)$ in the plot (v_a; a); in the case of type IV_a (Eq. (*34*)), in the plot $\{[v_a/(V^0 - v_a)]; a\}$, where line

$$v_a/(V^0 - v_a) = S/K^0_m + S[a/(K^0_m K(IV_a))] \tag{36}$$

will also intersect the abscissa at $-a = K(IV_a)$.

All the above-suggested linearized forms of equations for calculating the inhibition constants (Eqs (*11*)–(*16*), (*25*), (*26*)) as well as Eqs (*17*)–(*24*) also permit the use of slopes (tg τ) of these lines to the inhibitor molar concentration axis to calculate K_i. Thus, for instance, it can be seen from Eq. (*11*) that the slope of this line to the abscissa will have the following relationship to the inhibition constant: $K(I_i) = 1/\text{tg}\,\tau$. Analogously then for activations.

Examples of Calculations of Some Constants

A) Inhibition of calf alkaline phosphatase with Na_2WO_4 anions shows[16] that in the presence of 50 μmol l^{-1} of Na_2WO_4 the value of the Michaelis constant increases from $K^0_m = 44{\cdot}3$ μmol l^{-1} to $K'_m = 62{\cdot}5$ μmol l^{-1} and the maximum rate of reaction decreases from $V^0 = 2{\cdot}63$ μmol l^{-1} min^{-1} to $V' = 1{\cdot}72$ μmol l^{-1} min^{-1}. In the presence of 0·1 mmol l^{-1} Na_2WO_4 this process is amplified: $K'_m = 73$ μmol l^{-1},

$V' = 1{\cdot}28$ μmol l^{-1} min^{-1} which satisfies all the features of type I_i inhibition ($K'_m > K^0_m$, $V' < V^0$, $i > 0$) (Fig. 3*a*).

Plotting the dependences on the ($K'_m V^0/K^0_m V'$; i) coordinates, Fig. 3*b*, line *2*; and on the (K'_m/V'; i) coordinates, line *1*, makes it possible to determine the value of the inhibition constant: ($K(I_i) = 42{\cdot}4$ μmol l^{-1}. This is preferable to the uses of Eq. (*1*) $K(I_i) = 43{\cdot}2$ μmol l^{-1} and $K(I_i) = 41{\cdot}9$ μmol l^{-1} for 50 μmol l^{-1} and 0·1 mmol l^{-1} Na_2WO_4, respectively) since it gives the possibility to take into consideration the effect of random deviations.

B) Inhibition of *Pseudomonas* neutral phosphatase (pH_{opt} 6·5) (the reactions were carried out in 50 mmol l^{-1} Tris-acetate buffer, pH 6·5, ionic strength 0·1 (NaCl) at 37°C; final concentrations of pNPP varied from 23·3 μmol l^{-1} to 78·4 μmol l^{-1}; phosphatase 1·82 μg/ml) shows that the initial parameters ($K^0_m = 34{\cdot}7$ μmol l^{-1}; $V^0 = 4{\cdot}55$ μmol l^{-1} min^{-1}) of pNPP decomposition by the enzyme in the presence of 50 μmol l^{-1} *p*-nitrophenol (*p*-NP) take on the following values: $K'_m = 34{\cdot}1$ μmol l^{-1}; $V' = 3{\cdot}38$ μmol l^{-1} min^{-1} in the presence of 0·1 mmol l^{-1} *p*-NP; $K'_m = 32{\cdot}8$ μmol l^{-1}; $V' = 2{\cdot}65$ μmol l^{-1} min^{-1} (Fig. 4*a*). This satisfies all the features of type II_i inhibition ($K'_m < K^0_m$, $V' < V^0$) (subtype II'''_i to be more correct, because tg $w' >$ tg w^0) (refs[15,16]). As follows from Eq. (*2*) (Table I), to calculate the $K(II_i)$ inhibition constants, use can be made here of the ($K^0_m V^0/K'_m V'$; i) plot of intercept (Fig. 4*b*, line *1*) and the ($1/K'_m V'$; i) plot (Fig. 4*b*, line *2*). From the point of intersection of both lines with the abscissa (Fig. 4*b*) the constant will be calculated as $K(II_i) = 0{\cdot}127$ mmol l^{-1}.

As seen from the same Eq. (*2*) (Table I), construction of the dependences on the plots of slopes would not be correct here (cf. the text).

C) Processing of the results of adenosine-3'-monophosphate (3'AMP) inhibition of the initial rates of cytidyl-3' → 5'-adenosine (CpA) decomposition by nonspecific *P. brevicompactum* RNase (ref.[27]) shows that upon increasing the 3'AMP concentration the following tendency of increasing the values of efficient Michaelis constants is observed: $K'_m = 0{\cdot}543$ mmol l^{-1} at 30 μmol l^{-1} 3'AMP; $K'_m = 1{\cdot}00$ mmol l^{-1} at 70 μmol l^{-1} 3'AMP and $K'_m = 1{\cdot}45$ mmol l^{-1} at 0·1 mmol l^{-1} 3'AMP ($K^0_m = 0{\cdot}238$ mmol l^{-1}, $V' = V^0$, Fig. 5*a*), *i.e.* this is an example of associative (type IV_i) inhibition.

Plotting the dependences on the (K'_m/K^0_m; i) coordinates of intercepts (Fig. 5*b*, line *2*); and on the (K'_m; i) coordinates, line *1*, makes it possible (by the point of intersection with the abscissa, Fig. 5*b* to determine the value of the inhibition constants of *P. brevicompactum* RNAse as $K(IV_i) = 20{\cdot}6$ μmol l^{-1}.

Examples of using the (K'_m/K^0_m; i) plots to determine the $K(IV_i)$ constants of associative enzyme inhibition are many. Those of using the (K'_m; i) plots are considerably fewer. As for their simultaneous use for these purposes, they are practically nil,

though a certain advantage of such an approach (the use of the control point K_m^0 known beforehand) is obvious (Fig. 5*b*).

REFERENCES

1. Terra W. R., Ferreira C., Blonchi A. G.: Biochim. Biophys. Acta *524*, 131 (1978).
2. Karen M. L., Glew R. H.: Biochem. J. *237*, 469 (1986).
3. Seubert P. A., Renosto F., Knudson P., Segel I.: Arch. Biochem. Biophys. *240*, 509 (1985).
4. Whitman M., Kaplan D., Roberts T., Kantley L.: Biochem. J. *247*, 165 (1987).
5. Garbers D. L.: Biochim. Biophys. Acta *523*, 82 (1978).
6. Vincenzini M. T., Vanni P., Giachetti E., Hanozet G. M., Penzauti G.: J. Biochem. *99*, 375 (1986).
7. Smith D. F., Kosow D. P., Wu C., Jamieson G. A.: Biochim. Biophys. Acta, *483*, 263 (1977).
8. Arche R., Estrada P., Acebal C.: Arch. Biochem. Biophys. *257*, 131 (1987).
9. Falany C. N., Green M. D., Tephly T. R.: J. Biol. Chem. *262*, 1218 (1987).
10. Cassels R., Fears R., Smith R. A. G.: Biochem. J. *247*, 395 (1987).
11. Kato N., Sahm M., Wagner P.: Biochim. Biophys. Acta *566*, 12 (1979).
12. Rose Z. B., Grove D. S., Seal S. N.: J. Biol. Chem. *261*, 1099 (1986).
13. Olson J. S., Ballow D. P., Palmer G., Massey V.: J. Biol. Chem. *249*, 4350 (1974).
14. Krupyanko V. I.: Prikl. Biokhim. Microbiol. *22*, 440 (1986).
15. Krupyanko V. I. in: *A Method of* K'_mV'*-Coordinates in Enzyme Kinetics* (E. A. Burschtein, Ed.), p. 3. ONTI Biological Research Center, Pushchino 1987.
16. Krupyanko V. I.: Collect. Czech. Chem. Commun. *53*, 161 (1988).
17. Dixon M., Webb E. in: *The Enzymes*, Vol. 2, p. 481. Mir, Moscow 1982.
18. Martinek K., Varfolomeyev S. D., Levashov A. V., Berezin I. V.: Mol. Biol. *5*, 351 (1971).
19. Meigs R. A.: Biochem. Biophys. Res. Commun. *145*, 1012 (1987).
20. Filippova I. Yu., Dukhovich A. F., Sokolova N. I., Ugarova N. N.: Biokhimiya *49*, 585 (1984).
21. Okuda M., Arakawa K.: J. Biochem. *98*, 621 (1985).
22. Dixon M.: Biochem. J. *55*, 170 (1953).
23. Webb E. in: *Enzymic and Metabolic Inhibitors*, p. 71. Mir, Moscow 1966.
24. Preudlove M. O., Biechy R. B., Moore A. L.: Biochem. J. *247*, 441 (1987).
25. Samoshina N. M., Metmentseva E. Yu., Borisova V. N., Nakhapetyan L. A.: Prikl. Biokhim. Microbiol. *21*, 741 (1985).
26. Palmer T. in: *Understanding Enzymes*, p. 158. Wiley, New York 1985.
27. Krupyanko V. I., Bezbordova S. I.: Biokhimiya *41*, 1442 (1976).