

## A VECTOR METHOD OF REPRESENTING INDIVIDUAL TYPES OF ENZYMIC REACTIONS IN $K'_m V'$ COORDINATES

Vladimir I. KRUPYANKO

*Institute of Biochemistry and Physiology of Microorganisms*

*U.S.S.R. Academy of Sciences, Pushchino, Moscow Region, 142292, U.S.S.R.*

Received November 24th, 1986

A vector method of representing individual types of enzymic reactions in  $K'_m V'$  coordinates is described. Equations for enzyme activation (and nontrivial inhibition) constants are deduced using the method. The inhibiting effect of  $\text{Na}_2\text{WO}_4$  on the initial rates of *p*-nitrophenyl phosphate decomposition by calf intestinal alkaline phosphatase was studied, and the constant of enzyme inhibition by this compound was determined. Other uses of the method are discussed.

Analysis of the effect of activators (*a*) and inhibitors (*i*) on the kinetic parameters ( $K_m^0$  and  $V^0$ ) of fundamental (neither activated nor inhibited) enzymic reactions shows the possibility of existence (within the framework of the simple single-substrate scheme by Michaelis–Menten) of five types of enzyme activation and five types of enzyme inhibition<sup>1–3</sup>. With respect to activation these are shown in Fig. 1a.

Type I<sub>a</sub>: The activator intensifies the binding of the enzyme to the substrate ( $K'_m < K_m^0$ ) and increases the maximum rate of reaction ( $V' > V^0$ ) ( $K'_m$ , effective Michaelis constant and  $V'$ , maximum reaction rate determined in the presence of an activator of this type).

Type II<sub>a</sub>: The activator increases the maximum rate of reaction ( $V' > V^0$ ) and decreases the affinity of the enzyme for the substrate ( $K'_m > K_m^0$ ).

Type III<sub>a</sub>: The activator increases the maximum rate of reaction ( $V' > V^0$ ) and does not affect the affinity of the enzyme for the substrate ( $K'_m = K_m^0$ ).

Type IV<sub>a</sub>: The activator increases the affinity of the enzyme for the substrate ( $K'_m < K_m^0$ ) and has no effect on the maximum rate of reaction ( $V' = V^0$ ).

Type V<sub>a</sub>: The activator increases the affinity of the enzyme for the substrate ( $K'_m < K_m^0$ ) and decreases the maximum reaction rate ( $V' < V^0$ ).

The inhibition patterns are similar (Fig. 1b), with the difference that the presence of inhibitors (*i*) in all these cases will lead to opposite results, viz. to the decrease of maximum rates of reactions ( $V' < V^0$ ) and to reduced binding of enzymes with substrates ( $K'_m > K_m^0$ ) as for instance in the case of two-parameter matched inhibition.

The eleven types of Table I can be presented as a two-dimensional coordinate system (Fig. 2a) (refs<sup>2,3</sup>) in which  $K'_m$  values (left to right) of inhibited or activated enzymic reactions are plotted on the x-axis and  $V'$  values (upwards) on the y-axis;

the axes intersect at a right angle at point  $P(K_m^0; V^0)$  which corresponds to the values of the basic parameters  $K_m^0$  and  $V^0$ .

Let us analyze the system. Plotting on both axes of  $K_{m1}'$  and  $V_1'$  parameters (e.g. of some enzymic reaction inhibited according to  $I_i$ :  $K_{m1}' > K_m^0$ ,  $V_1' < V^0$ ), followed by drawing from the points obtained ( $K_{m1}'$  and  $V_1'$ ) of mutually perpendicular lines and connecting the points of intersection of the axes and of the perpendiculars by line  $L$  (Fig. 2b) yields both the point ( $I_i$ ) and diagram (line  $PI_i$ ) representation of the three dimensional  $PI_i$  vector of this reaction (Fig. 2c). It should be noted that line  $PI_i$  is a scalar.

Only one point and one definite projection  $\hat{L}$  of the three dimensional vector  $L$  will correspond to any particular combination of  $K_m'$  and  $V'$  parameters in this system; any general relationship of kinetic parameters (see Table I) will have its place in the  $K_m'V'$  coordinates. Thus,  $PIV_i$  projections of vectors  $PIV_i$  of the one-parameter ( $K_m -$ ) or competitive inhibition will be positioned on the horizontal semi-axis  $PK_m'$ ; those of  $PI_i$  projections of mixed inhibition in the field of the fourth quadrant etc. (Fig. 2a, b).

Now, this distribution of projections leads to some interesting results:

1) Combining all types of reactions, the  $K_m'V'$  coordinates (Fig. 2a) make it possible to reveal the interrelations between their particular types, i.e., the directions of probable changes in their mechanisms during consecutive alterations of  $K_m'$  or  $V'$ , the alterations being introduced in any direction and starting with any reaction. Other plots<sup>4-6</sup> are very limited in this respect.

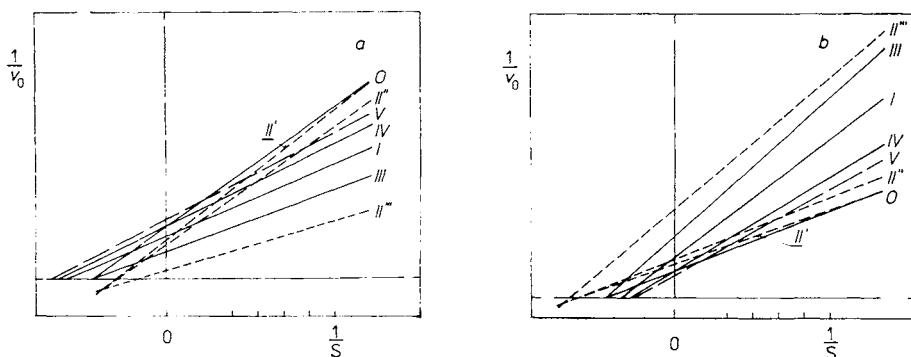


FIG. 1

Various types of enzyme activation (a) and inhibition (b). a: type  $I_a$  (lines I and O); type  $II_a$  (any of the lines,  $II'$ ,  $II''$  or  $II'''$ , and O); type  $III_a$  (lines III and O); type  $IV_a$  (lines IV and O); type  $V_a$  (lines V and O). b: type  $I_i$  (lines I and O); type  $II_i$  (any of the lines  $II'$ ,  $II''$  or  $II'''$  and O); type  $III_i$  (lines III and O); type  $IV_i$  (lines IV and O) and type  $V_i$  (lines V and O)

TABLE I  
Types of activation ( $a$ ) and inhibition ( $i$ ) of enzymes

Type of effect	Correlation between $K_m$ and $V$ parameters	Tendencies for "improving" parameters	New name	Traditional name
			Activation	
I <sub>a</sub>	$V' > V^0$ $K'_m < K_m^0$	$V+$ $K_m+$	two-parameter matched activation	
II <sub>a</sub>	$V' > V^0$ $K'_m > K_m^0$	$V+$ $K_m-$	two-parameter mismatched activation	
III <sub>a</sub>	$V' > V^0$ $K'_m = K_m^0$	$V+$	catalytical activation	
IV <sub>a</sub>	$V' = V^0$ $K'_m < K_m^0$	$K_m+$	associative activation	
V <sub>a</sub>	$V' < V^0$ $K'_m < K_m^0$	$V-$ $K_m+$	pseudoactivation	
			No effect	
	$V' = V^0$ $K'_m = K_m^0$		initial (neither inhibited nor activated) reaction	
			Inhibition	
V <sub>i</sub>	$V' > V^0$ $K'_m > K_m^0$	$V+$ $K_m-$	pseudoinhibition	competitive
IV <sub>i</sub>	$V' = V^0$ $K'_m > K_m^0$	$K_m-$	associative inhibition	noncompetitive
III <sub>i</sub>	$V' < V^0$ $K'_m = K_m^0$	$V-$	catalytical inhibition	uncompetitive
II <sub>i</sub>	$V' < V^0$ $K'_m < K_m^0$	$V-$ $K_m+$	two-parameter mismatched inhibition	
I <sub>i</sub>	$V' < V^0$ $K'_m > K_m^0$	$V-$ $K_m-$	two-parameter matched inhibition	mixed

Thus, for instance, if in the pseudoinhibition reaction (type  $V_i$ :  $V' > V^0$ ,  $K'_m > K_m^0$ ) one would decrease  $V'$  (by introducing a noncompetitive inhibitor), the vector

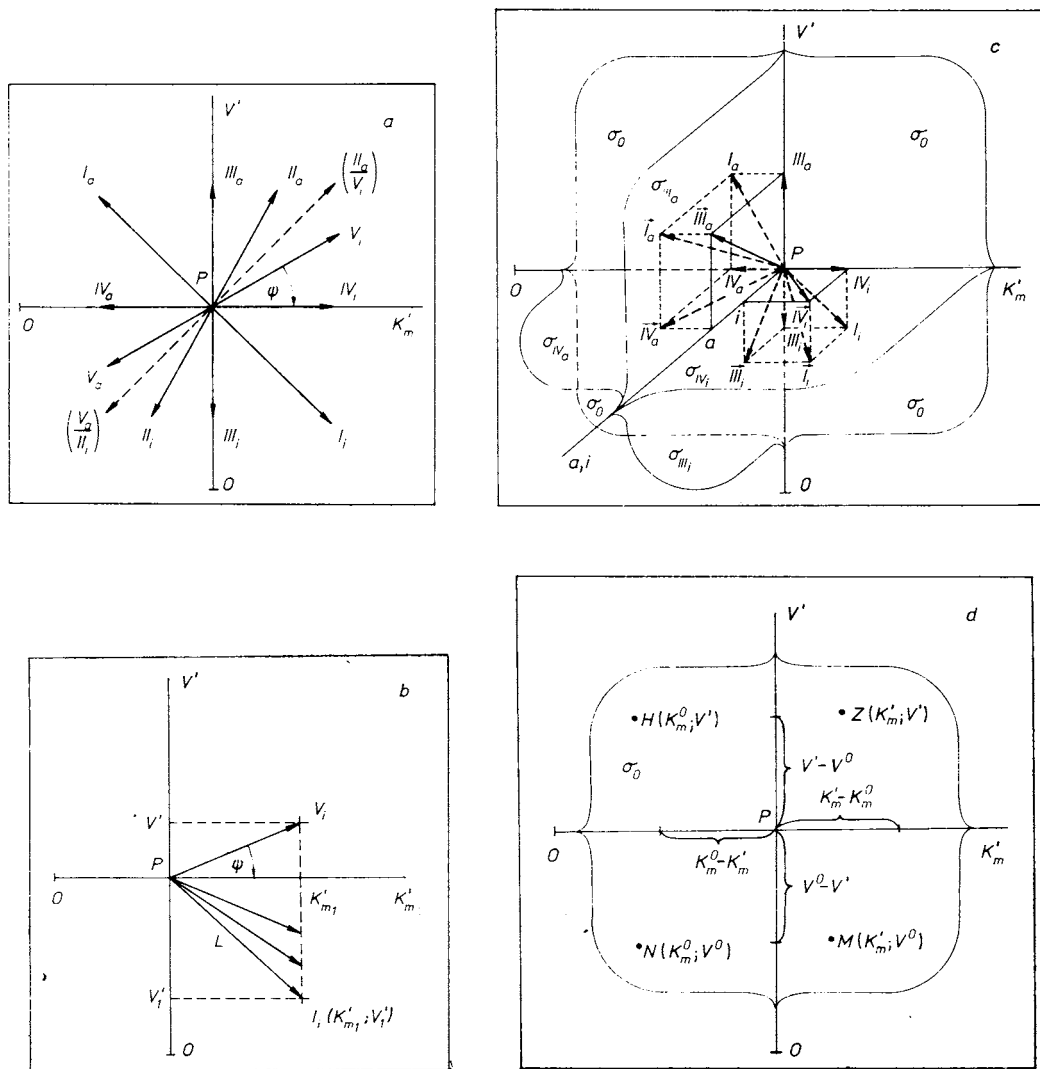


FIG. 2

Enzyme reaction representations. *a* Illustration of enzymic reactions in the two-dimensional  $K'_m V'$  coordinates. *b* Illustration of changing parameter  $V'$  in  $K'_m V'$  coordinates (with  $K'_m$  constant). *c* Illustration of the three-dimensional  $K'_m V'$  coordinates. *d* The coordinates of the vector projection ends on plane  $\sigma_0$  of the three-dimensional  $K'_m V'$  coordinates (in terms of their positive differences)

projection  $PV_i$  moving clockwise would first (at  $V' = V^0$ ) occupy the position of the projection  $PIV_i$  on the semi-axis  $PK'_m$  (Fig. 2a), and then (at  $V' < V^0$ ) would shift over to the fourth quadrant. It would indicate the following sequence of changes in the reaction types:  $V_i \rightarrow IV_i \rightarrow I_i$ . Now, as seen from Fig. 2a, b, vector projections of the families  $PV_i$  and  $PI_i$  have two "degrees of freedom": the possibility to change both the length (i.e. reaction intensity) and the slope (i.e. reaction mechanism)

$$\operatorname{tg} \psi = \frac{V' - V^0}{K'_m - K_m^0}, \quad (1)$$

whereas vector projections  $PIV_i$  (competitive inhibition) have only one, viz. the possibility to change the length.

Besides, as follows from the positions of the projections  $PII_a$  and  $PV_i$ , as well as  $PV_a$  and  $PII_i$  (Fig. 2a), these pairs of reactions are characterized by transition states with coincident values of  $K$  and  $V'$  parameters; this is indicated by the appearance of transient vector projections  $P(II_a/V_i)$  and  $P(V_a/II_i)$  in the first and third quadrants of the  $K'_m V'$  coordinates.

2) Analysis of vector projections in the two-dimensional  $K'_m V'$  coordinates establishes proportionality between the projection lengths  $PIV_i$  and  $PIII_i$  for the inhibited reactions and the reciprocals of the respective inhibition constants. As seen from Fig. 2a, the projection length  $PIV_i$  is determined by the following difference of parameters:

$$PIV_i = K'_m - K_m^0. \quad (2)$$

The same difference, however, is a part of the well-known<sup>4,5</sup> equation for the competitive inhibition constant ( $K(IV_i)$ ):

$$K(IV_i) = i \cdot K_m^0 / (K'_m - K_m^0). \quad (3)$$

From this, taking  $A = i \cdot K_m^0$ , we get

$$PIV_i = A / K(IV_i). \quad (4)$$

With the differences of kinetic parameters ( $K'_m - K_m^0$  and  $V' - V^0$ ) constant, the vector projections  $PIV_a$  and  $PIII_i$  are negative in sign, which reflects their anti-directionality with respect to other vector projections of the same types ( $PIV_i$  and  $PIII_a$ ).

This property of the coordinates is of use, for instance, in deducing the equations for associative  $K(IV_a)$  and catalytic  $K(III_a)$  enzyme activation constants.

As seen from Fig. 2a, the projection length  $PIII_i$  of the noncompetitive inhibition

vector will be expressed as

$$-PIII_i = V' - V^0 \quad (5)$$

or (in terms of the positive difference coordinates)

$$PIII_i = V^0 - V'. \quad (6)$$

On the other hand, activity of the same type of inhibition can be characterized also by the value of the constant  $K(III_i)$  (refs<sup>4,5</sup>)

$$K(III_i) = i \cdot V' / (V^0 - V'). \quad (7)$$

With  $B = i \cdot V'$  we have

$$PIII_i = B / K(III_i). \quad (8)$$

From this it follows that the projection length  $PIII_i$  is proportional to the reciprocal value of the inhibition constant  $K(III_i)$ . Extending the property to the vector projections  $PIV_a$  and  $PIII_a$  for activated reactions and considering their antidirectionality (Fig. 2a) to vector projections for inhibited reactions of the same type ( $PIII_a$  and  $-PIII_i$ ;  $-PIV_a$  and  $PIV_i$ ) we can derive equations for the respective activation constants. Namely, if the relationships

$$PIV_a = C / K(IV_a) \quad (9)$$

and

$$PIII_a = D / K(III_a) \quad (10)$$

are correct then by finding, for instance, the expression of constant  $C$  of Eq. (9) one can derive the equation for calculating constants  $K(IV_a)$ . What this constant should be is suggested by deriving a similar relationship between projection  $PIV_i$  (symmetrical to  $PIV_a$ ) and  $K(IV_i)$  in Eq. (4). In that case, to establish the relationship, the constant ( $A = i \cdot K_m^0$ ) should be the product of the inhibitor molar concentration by the last term in the positive difference  $K'_m - K_m^0$ .

Substituting the activator  $a$  for the inhibitor and multiplying it by  $K'_m$  (since in this case  $PIV_a = K_m^0 - K'_m$ ) we can find that  $C = a \cdot K'_m$ ; then

$$K(IV_a) = \frac{a}{(K_m^0 / K'_m) - 1}. \quad (11)$$

Finding in a similar manner the expression of constant  $D$  in Eq. (10) ( $D = a \cdot V^0$ ).

makes it possible to derive the equation for calculating activation constants  $K(\text{III}_a)$ :

$$K(\text{III}_a) = \frac{a}{(V'/V^0) - 1}. \quad (12)$$

No other coordinate system<sup>4-6</sup> makes this possible.

Making use of this property (of symmetry and proportionality) for  $PI_i$  and  $PI_a$  vector projections (Fig. 2a) and also equation for inhibition constants<sup>4</sup>

$$K(I_i) = \frac{i}{(K'_m V^0)/(K_m^0 V') - 1} \quad (13)$$

we can derive the equation (symmetrical to Eq. (13) by  $K'_m$  and  $V'$ ) to calculate the constant  $K(I_a)$  for two-parameter-matched activation (type  $I_a$ ):

$$K(I_a) = \frac{a}{(K_m^0 V')/(K'_m V^0) - 1} \quad (14)$$

from which one can see, that Eqs (11) and (12) are only particular cases of Eq. (14) which, at  $V' = V^0$ , simplifies to Eq. (11) and, at  $K'_m = K_m^0$ , to Eq. (12).

The same follows from the diagram analysis (Fig. 2a): at  $V' = V^0$  the vector projection  $PI_a$  takes the position of the projection  $PIV_a$ , and at  $K'_m = K_m^0$  the position of the projection  $PIII_a$  and similarly for the  $PI_i$  and other projections<sup>2,3</sup>.

3) The above method of constructing coordinates with axes intersecting at a point which corresponds to the initial  $K_m^0$  and  $V^0$  kinetic parameters (Fig. 2a) allows for its variations; for instance, the inversion of one ( $(1/K'_m) V'$ ) or both ( $(1/K'_m)(1/V')$ ) kinetic parameters, 180° turn of one of the axes, etc. The analysis shows, however, that no single variation has an advantage over the above  $K'_m V'$  coordinates which are the most simple and convenient.

## EXPERIMENTAL

Alkaline phosphatase from calf intestine (EC 3.1.3.1.), its substrate *p*-nitrophenyl phosphate (*p*NPP) homogeneous preparation from Sigma (U.S.A.).  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  crystalline salt, chemically pure.

Decomposition of *p*-nitrophenyl phosphate (*p*NPP) was observed spectrophotometrically (an "Optica Milano" CF-4 double beam spectrophotometer) by an increase in the optical density ( $+\Delta A_{405}$ ) of a solution consisting of the substrate, enzyme, and inhibitor (see legends to Fig. 3a) as compared with that of the same composition but without the enzyme. The reaction proceeded at 37°C with constant stirring.

The initial reaction rates ( $v_0$ ) were determined from the tangents to the initial straight sections

of the substrate decomposition curves  $+\Delta A_{405} = f(t)$ . No less than five consecutive assays were performed.

The root-mean-square deviation in five assays was  $v_0 = \pm 3.5\%$ ,  $K_m$  and  $V$ , and  $K_i = \pm 10\%$ .

## RESULTS AND DISCUSSION

a) As seen from Fig. 3a,  $\text{Na}_2\text{WO}_4$  at concentrations of  $5 \cdot 10^{-5} \text{ mol l}^{-1}$  and  $1 \cdot 10^{-4} \text{ mol l}^{-1}$  shows an inhibiting effect on phosphatase, which is characterized

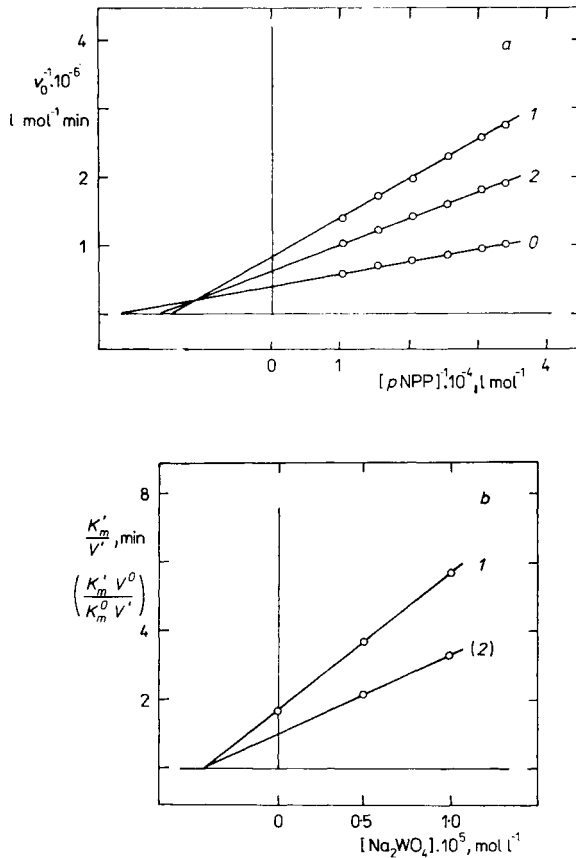


FIG. 3

The inhibiting effect of  $\text{Na}_2\text{WO}_4$  on initial rates of  $p\text{NPP}$  decomposition by calf intestine alkaline phosphatase. *a* Conditions:  $0.05 \text{ mol l}^{-1}$  Tris-HCl buffer, pH 9.0; ionic strength  $0.1 \text{ mol l}^{-1}$  (NaCl),  $37^\circ$ . Final concentration of phosphatase  $0.98 \mu\text{g ml}^{-1}$ ;  $p\text{NPP}$   $2.94 \cdot 10^{-5} - 9.8 \cdot 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ .  $\text{Na}_2\text{WO}_4$   $1 \cdot 10^{-4} \text{ mol l}^{-1}$  (line 1),  $5 \cdot 10^{-5} \text{ mol l}^{-1}$  (line 2). Line 0, no inhibitor. *b* These data presented in the  $(K_m'/V'; a)$  coordinates (line 1), in  $(K_m^0 V^0 / K_m' V')$ ; *a* coordinates (line 2)



by the following change in *p*NPP decomposition parameters:  $V' = 1.72 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1} \text{ min}^{-1}$  ( $V^0 = 2.63 \cdot 10^{-5} \text{ mol l}^{-1} \text{ min}^{-1}$ ) and  $K'_m = 6.25 \cdot 10^{-5} \text{ mol l}^{-1}$  ( $K_m^0 = 4.43 \cdot 10^{-5} \text{ mol l}^{-1}$ ) in the first case and  $V' = 1.28 \cdot 10^{-6} \text{ mol l}^{-1} \text{ min}^{-1}$ ,  $K'_m = 7.3 \cdot 10^{-5} \text{ mol l}^{-1}$  in the second case, which satisfies all the features of type  $I_i$  ( $K'_m > K_m^0$ ,  $V' < V^0$ ) of the two-parameter-matched enzyme inhibition (see Table I and Fig. 1b, lines *I* and *O*). For calculating the enzyme inhibition constant one can use Eq. 13. The substitution into this equation of the above data gives  $K(I_i) = 4.32 \cdot 10^{-5} \text{ mol l}^{-1}$  for the first case ( $5 \cdot 10^{-5} \text{ mol l}^{-1} \text{ Na}_2\text{WO}_4$ ) and  $K(I_i) = 4.19 \cdot 10^{-5} \text{ mol l}^{-1}$  for the second case ( $1 \cdot 10^{-4} \text{ mol l}^{-1} \text{ Na}_2\text{WO}_4$ ). The difference between the values does not exceed 10% and they can be considered equally close to the true value.

Two other, the most desirable, techniques of type I inhibition constants follow from Eq. (13). The first technique is by plotting the change in the ratio of  $(K'_m V^0) / (K_m^0 V')$  parameters as dependable on *i*, where the line

$$\frac{K'_m V^0}{K_m^0 V'} = \frac{1}{K(I_i)} i + 1 \quad (13a)$$

will cross the axis of molar concentrations of the inhibitor ( $\text{Na}_2\text{WO}_4$ ) at point  $-i = K(I_i)$  (Fig. 3b, line 2). The second one is by plotting the change of only efficiently manifested  $K'_m/V'$  parameters as dependable on *i*, where the line

$$\frac{K'_m}{V'} = \frac{K_m^0}{V^0} \frac{1}{K(I_i)} i + \frac{K_m^0}{V^0} \quad (13b)$$

will also cross the abscissa axis at point  $-i = K(I_i)$  (Fig. 3b, line 1). Both techniques are equally suitable for estimating errors occurring in experiments. The value of the phosphatase inhibition constant calculated using the data of Fig. 3b is  $K(I_i) = 4.24 \cdot 10^{-5} \text{ mol l}^{-1}$ .

Similar techniques of determining  $K_i$  and  $K_a$  are acceptable also in all the other cases. Thus, it can be seen from Eqs (11)–(18) that for calculating type I activation constants one can make use of  $(K'_m V') / (K_m^0 V^0)$ ; *a*) and  $V' / K'_m$ ; *a*) parameters (see Eq. (14)); for calculating type II activation constants,  $(K'_m V') / (K_m^0 V^0)$ ; *a*) and  $(K'_m V')$ ; *a*) coordinates (see Eq. (15)); for calculating type IV activation constants,  $(K_m^0 / K'_m)$ ; *a*) and  $(1 / K'_m)$ ; *a*) coordinates (Eq. (11)), etc.

It should be noted that neither the term “slope” nor the term “intercept” are acceptable to the full degree for characterizing these coordinates, since no combination of line slopes can express the coordinates in the case of two-parameter-mismatched types of enzyme inhibition (Eqs (17), (18)) or activation (Eqs (15), (16)). There are no intercept features in the  $(V'; a)$  coordinates for calculating the type III activation constants (see Eq. (12)), etc.

b) Introduction into practice of the  $K'_m V'$  coordinates would facilitate both the determination of activation and/or inhibition types and the calculation of respective  $K_a$  and/or  $K_i$  constants.

The following simple rule for the choice of a necessary equation would be of use.

If the experimental data are identical with the positions of lines *IV* and *O* (Fig. 1a), Eq. (11) is to be used; if they are identical with lines *III* and *O* (Fig. 1a), Eq. (12) is to be used; if they are identical with lines *II* (any of the three: *II'*, *II''*, or *II'''*) and *O* (Fig. 1a), we shall use the following equation:

$$K(II_a) = \frac{a}{(K'_m V') / (K^o_m V^o) - 1} \quad (15)$$

If the data fit lines *I* and *O* (Fig. 1a), Eq. (14), if they fit lines *V* and *O* (Fig. 1a), then the following equation:

$$K(V_a) = \frac{a}{(K^o_m V^o) / (K'_m V') - 1} \quad (16)$$

If the experimental lines for inhibition are identical with lines *I* and *O* (Fig. 1b), Eq. (13) should be used; if they are identical with lines *II* (any of the three: *II'*, *II''*, *II'''*, Fig. 1b) and *O*, then the equation:

$$K(II_i) = \frac{i}{(K^o_m V^o) / (K'_m V') - 1} \quad (17)$$

If they are identical with lines *V* and *O* (Fig. 1b), the equation

$$K(V_i) = \frac{i}{(K'_m V') / (K^o_m V^o) - 1} \quad (18)$$

is applicable.

If the experimental data are identical with lines *IV* and *O* (Fig. 1b), we shall use the widely known Eq. (3) (refs<sup>4,5</sup>); if with lines *III* and *O* (Fig. 1b), another known Eq. (7) (refs<sup>4,5</sup>) is to be used.

When deriving Eq. (15) to (18), we used the method of vector representation of enzymic reactions in the three-dimensional  $K'_m V'$  coordinates (Fig. 2c).

If from point *P* of the two-dimensional  $K'_m V'$  coordinates (Fig. 2a) we erect the semi-axis  $P_{a,i}$  of molar concentrations of activator (*a*), or inhibitor (*i*) (superimposed semi-axes) and pass the planes  $\sigma_{IV}$ ,  $\sigma_{III}$ ,  $\sigma_{V_a/II}$ ,  $\sigma_{V_a}$ , etc., through the semi-axis

\* The derivation of Eqs (15)–(18) see below.

$P_{a,i}$  and each of the semi-axes  $PK'_m$ ,  $PO_{V'}$ ,  $P(V_a/II_i)$ ,  $PO_{K'_m}$  etc. (Fig. 2a), we obtain the convenient three-dimensional  $K'_m V'$  coordinates (Fig. 2c).

As seen from Fig. 2c, any concrete combination of  $K'_m$ ,  $V'$ , and  $i$  (or  $a$ ) in such coordinates will correspond to only one strict position  $\mathbf{L}$  of the three-dimensional vector of a given inhibited (or activated) enzymic reaction. Thus, the three-dimensional vectors  $\mathbf{PIV}_i$  (of the competitive inhibition reactions) will be positioned only on plane  $\sigma_{IV_i}$ , vectors  $\mathbf{PIII}_i$  on plane  $\sigma_{III_i}$ , vectors  $\mathbf{PI}_a$  (of the two-parameter-matched activation reactions) in the space of the second octant limited by planes  $\sigma_{IV_a}$ ,  $\sigma_{III_a}$  and  $\sigma_0$ ; vectors  $\mathbf{PII}_a$  in the subspace limited by planes  $\sigma_{III_a}$ ,  $\sigma_{II_a/V_i}$  and  $\sigma_0$  etc.

Now, comparing the known equations (refs<sup>4,5</sup>) for calculating the constants  $K(IV_i)$  (Eq. (3)),  $K(III_i)$  (Eq. (7)),  $K(I_i)$  (Eq. (13)), and  $K(I_a)$  (Eq. (14)) with positions of the respective vectors  $\mathbf{PIV}_i$ ,  $\mathbf{PIII}_i$ ,  $\mathbf{PI}_i$ , and  $\mathbf{PI}_a$  in the three-dimensional  $K'_m V'$  coordinates (Fig. 2c), we can draw the following conclusion:

Equations for inhibition/activation constants of enzymes are the ratio of the kinetic parameters corresponding to the positive differences of the end and origin coordinates of the respective three-dimensional vector projection on semi-axis  $P_{a,i}$  and the positive difference of the end and origin coordinates of these projections on plane  $\sigma_0$ , the ratio being multiplied by the last term in the positive difference of the base projection coordinates.

Let us consider the position of vector  $\mathbf{PIV}_i$  in the three dimensional coordinates. As seen from Fig. 2c, the ratio of the positive difference of the coordinates for the projection of the vector on semi-axis  $P_{a,i}$ ,  $i-O$ , and its projection on plane  $\sigma_0$ ,  $K'_m - K_m^0$ , multiplied by  $K_m^0$

$$\frac{i - o}{K'_m - K_m^0} \cdot K = K(IV)_i \quad (19)$$

will be the known equation for  $K(IV_i)$  (Eq. (3)) (refs<sup>4,5</sup>).

Let us now consider the position of vector  $\mathbf{PI}_i$ . As seen from Fig. 2a (which is the projection of the three-dimensional coordinates, Fig. 2c, on plane  $\sigma_0$ ) the positive difference of the coordinates for the vector projection on semi-axis  $PK'_m$  will be  $K'_m - K_m^0$  and on semi-axis  $PO_{V'}$ ,  $V^0 - V'$ . From this it follows that the positive difference of the coordinates for the vector projection on plane  $\sigma_0$ , will be:  $K'_m V^0 - K_m^0 V'$ . The projection of this vector on semi-axis  $P_{a,i}$  will be  $i-O$ , hence their ratio multiplied by  $K_m^0 V'$ :

$$\frac{i - O}{K'_m V^0 - K_m^0 V'} K_m^0 V' = K(I)_i \quad (20)$$

will just be the equation for  $K(I_i)$  constants of mixed enzyme inhibition (see Eq. (13))

Now, since the positive difference of parameters of three-dimensional vector projections on semi-axis  $PO_{K'_m}$  will be  $K_m^0 - K'_m$  (since here  $K'_m < K_m^0$ , see Table I

and Figs 1a, b), it is easy to obtain the expression for the positive difference of the coordinates of the three-dimensional vector projections on the third quadrant of plane  $\sigma_0$ . It will be  $K_m^0 V^0 - K_m' V'$ , Fig. 2d. Hence, the projections of the respective  $PV_a$  three-dimensional vectors of the activated reactions will be expressed through the difference of coordinates  $a - O$ , and of  $PII_i$  vectors of the inhibited reactions through the difference of coordinates  $i - O$ , the ratio of parameters in the first case will be  $(a - O)/(K_m^0 V^0 - K_m' V')$  (see Eq. (16)), in the second case  $(i - O)/(K_m^0 V^0 - K_m' V')$  (see Eq. (17)).

Finally, since the positive differences of the coordinates of the three dimensional vector projection on semi-axis  $PV'$  will be  $V' - V^0$  and on semi-axis  $PK_m'$ ,  $K_m' - K_m^0$ , the positive differences of the coordinates of  $PII_a$  and  $PV_i$  three-dimensional vector projections on the first quadrant  $\sigma_0$  will be  $K_m' V' - K_m^0 V^0$  (see Eqs (15) and (18)).

#### REFERENCES

1. Krupyanko V. I. in the book: *The Third International Conference on Chemistry and Biotechnology of Biologically Active Natural Products*, Vol. 4, pp. 250–253. Sofia, 1985.
2. Krupyanko V. I.: *A Method of  $K_m' V'$  Coordinates in Enzyme Kinetics*, p. 3. Preprint, Pushchino 1985.
3. Krupyanko V. I.: *Prikl. Biokhim. Microbiol. (U.S.S.R)* 22, 440 (1986).
4. Webb L. in the book: *Enzyme and Metabolic Inhibitors* (V. A. Jakowlev, Ed.), p. 156, Mir Publishers, Moscow 1966.
5. Dixon M., Webb E. in the book: *Enzymes* (A. E. Braunsten and V. K. Antonov, Eds), Vol. 2, p. 481. Mir Publishers, Moscow 1982.
6. Cornish-Bowden A.: *Principles of Enzyme Kinetics* (in Russian), p. 43. Mir Publishers, Moscow, 1979.