EQUATIONS FOR CALCULATING THE CONSTANTS OF ENZYME INHIBITION/ACTIVATION BY n INHIBITORS/ACTIVATORS

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Based on the method of vector representation of enzyme reactions in three-dimensional $K'_m V'$ coordinates, equations for calculating the constants of enzyme inhibition $K_{i(n)}$ and activation $K_{a(n)}$ by two or more inhibitors/activatos were deduced. Inhibition of calf alkaline phosphatase by anions of molybdic (MoO_4^{2-}) and tungstic (WO_4^{2-}) acid was studied. The constants of enzyme inhibition by each of these anions and by their mixture in the same concentration were determined. The value of the phosphatase inhibition constant obtained experimentally was compared with that calculated using the appropriate equation. The prospects of using the equations obtained to analyze the kinetics of enzyme inhibition/activation by two or more simultaneous inhibitors/activators and to assess the contribution of each of these compounds are discussed.

The problem of the quantitative assessment of the simultaneous effect of two, three or more inhibitors or activators has always been of interest for enzymologists, since such situations are frequent in research practice, e.g., in studies of enzyme inhibition in the presence of the components of a buffer solution. It is no less important to know if such an interaction enhances or diminishes the effect of an inhibitor, etc.

Earlier papers[1-4] describe the three-dimensional coordinates and a method of vector representation of enzymic reactions in it. This paper analyzes the applicability of the method for processing the results of the effect on the enzyme of simultaneously two, three or more inhibitors or activators.

THEORETICAL

Associative* (Type IV_i or Competitive) Enzyme Inhibition

All the three-dimensional vectors $\boldsymbol{PIV_i}$ of reactions characterized by the relation-

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*   The classification of the types of enzymic reactions is described in refs[1-4].

** K'_m and V' are the values of effective Michaelis constants and maximum reaction rates determined in the presence of inhibitors (i) or activators (a); K_m^0 and V^0 are the values of the same parameters of the initial (neither inhibited nor activated) reaction.

ships** $K'_m > K^0_m$, $V' = V^0$, $i > 0$, will be located only in plane σ_{IVi} which is the locus of their existence[2−4] (Fig. 1).

Vector representation of the results of enzyme inhibition by two simultaneous different (not interacting) associative inhibitors at equal concentration $[i_1] = [i_2]$ but differing in their efficiency such that $2(K'_{m(i1)} - K^0_m) = (K'_{m(i2)} - K^0_m)$ in plane σ_{IVi} will be as shown in Fig. 2, where $\textbf{PIV}_{i1}$ is a threedimensional vector characterizing the intensity of inhibition by inhibitor i_1 and $\textbf{PIV}_{i2}$ is the intensity of inhibition by inhibitor i_2. The resulting effect (vector $\textbf{PIV}_{(i1+i2)}$) will be the sum of the components:

$$\textbf{PIV}_{(i1+i2)} = \textbf{PIV}_{i1} + \textbf{PIV}_{i2} \tag{1}$$

or, in their projection on semiaxis PK'_m,

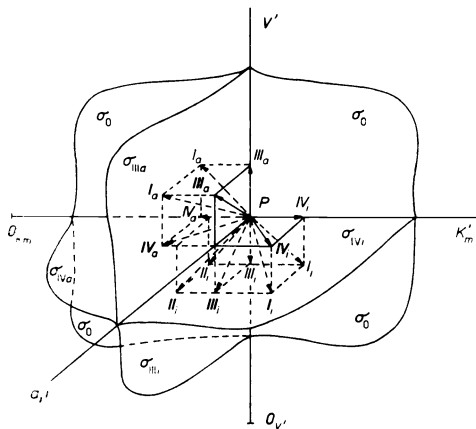$$PIV_{(i1+i2)} = PIV_{i1} + PIV_{i2} = \sum_{l=1}^{n} PIV_{i(l)} \tag{2}$$

FIG. 1

Illustration of the method of vector representation of enzymic reactions in three-dimensional $K'_m V'$ coordinates (with aligned semi-axis Pi of the molar concentrations of inhibitors (i) and activators (a)). Symbols: $\textbf{PIV}_i$, $\textbf{PI}_i$, $\textbf{PIII}_i$ etc., are the vectors characterizing the intensity of the associative, two-parameter matched, catalytic, etc., types of enzyme inhibition; PIV_i, PI_i, $PIII_i$, etc., (scalar values) are the projections of these vectors on base plane σ_0, P is the point of intersection of axes, its coordinates being $(K^0_m; V^0; 0)$
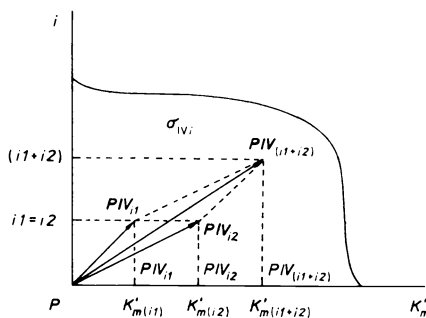
FIG. 2

Vector representation of data on the associative type of enzyme inhibition by two inhibitors at equal concentration (in plane σ_{IVi}, Fig. 1). Symbols: $\textbf{PIV}_{i1}$, $\textbf{PIV}_{i2}$, $\textbf{PIV}_{(i1+i2)}$ are the vectors characterizing the intensity of enzyme inhibition by inhibitors i_1 and i_2 and the mixture $(i_1 + i_2)$ at the same concentration; PIV_{i1}, PIV_{i2} and $PIV_{(i1+i2)}$ are the projections of these vectors on semi-axis PK'_m

and semi-axis Pi,

$$Proj_{Pi}\textbf{PIV}_{(i1+i2)} = (i_1 - 0) + (i_2 - 0) = \sum_{l=1}^{n} i(l),\qquad(3)$$

where $PIV_{(1+i2)} = Proj_{\sigma_0}\textbf{PIV}_{(i1+i2)}$; $PIV_{i1} = Proj_{\sigma_0}\textbf{PIV}_{i1}$, etc. are projections (scalar values) of the three-dimensional vectors $\textbf{PIV}_{(i1+i2)}$, $\textbf{PIV}_{i1}$, etc. on semi-axis PK'_m (Fig. 2) or, which is identical, on the base plane σ_0 (Fig. 1); n, the upper limit of summation (equal in this case to 2).

To derive the equations for calculating the constants $K(\text{IV}_{i(n)})$ of associative enzyme inhibition we shall use the property of the vector representation method which states that the ratio of kinetic parameters corresponding to the positive difference of the coordinates of the projection of the three-dimensional vector $\textbf{L}$ on semi-axis P_i to those corresponding to the positive difference of the coordinates of the projection of the same vector on base plane σ_0, multiplied by the kinetic parameters corresponding to the last member in the base difference of the coordinates — is the form of equation for calculating the constant of enzyme inhibition (K_i) or activation (K_a) depending on which effect is studied[2−4].

As seen in Fig. 2, the value of coordinate $K'_{m(i1+i2)}$ of projection $PIV_{(i1+i2)}$ on semi-axis PK'_m will be, due to the axes $0K'_m$ and $0V'$ intersecting point $P(K^0_m; V^0; 0)$ (Fig. 1), in the following dependence on coordinates K'_m and K^0_m of its projections PIV_{i1} and PIV_{i2}:

$$K'_{m(i1+i2)} = K^0_m + \sum_{l=1}^{n} (K'_{mi(l)} - K^0_m),\qquad(4)$$

where $n = 2$.

Therefore, the positive difference of the coordinates of projection $PIV_{(i1+i2)}$ on semi-axis PK'_m (Fig. 2) or, which is identical, on plane σ_0 (Fig. 1) will be

$$Proj_{\sigma_0}\textbf{PIV}_{(i1+i2)} = K'_{m(i(n))} - K^0_m\qquad(5)$$

or, taking into account Eq. (4),

$$Proj_{\sigma_0}\textbf{PIV}_{(i1+i2)} = \sum_{l=1}^{n} (K'_{m(l)} - K^0_m).\qquad(6)$$

Hence, since the expression of the positive difference of the coordinates of vector projection $\textbf{PIV}_{(i1+i2)}$ on semi-axis Pi is already known (Eq. (3)) as well as the last member in the base difference of the coordinates (Eq. (5)), the equation for calculating $K(\text{IV}_{(i1+i2)})$ for the given (associative) inhibition by inhibitors i_1 and i_2

will have the form:

$$K\big(\mathrm{IV}_{(i1+i2)}\big) = \frac{Proj_{P_i}\mathbf{PIV}_{(1+i2)}}{Proj_{\sigma_0}\mathbf{PIV}_{(i1+i2)}} K_m^0 \tag{7}$$

or, taking into consideration Eqs (3) and (6),

$$K\big(\mathrm{IV}_{(i1+i2)}\big) = \varrho \sum_{l=1}^{n} i(l) \frac{K_m^0}{\sum_{l=1}^{n}\big(K'_{m(l)} - K_m^0\big)}, \tag{8}$$

where n is the limit of summation and ϱ the coefficient characterizing the interaction of inhibitors. If such an interaction (formation of associates etc.) leads to the weakening of their efficiency, $\varrho < 1$; if it leads to their enhancement, $\varrho > 1$; in the absence of any interaction, $\varrho = 1$.

As can be easily seen $(\mathrm{Fig.}\ 2)$, in the case of three or more inhibitors the projection lengths of the resulting vectors $\mathbf{PIV}_{i(n)}$ on semi-axes PK'_m and Pi will be determined by the same Eqs (6) and (3), where $n = 3, 4, \ldots$; thus, the eqaution for calculating constants $K(\mathrm{IV})_{i(n)})$ of enzyme inhibition by n inhibitors will have the same form as Eq. (8), where $n = 3, 4, \ldots$.

On the other hand, analysis shows that at $n = 1$ Eq. (8) will be simplified to the known equation[5-7] for calculating constant $K(\mathrm{IV}_i)$ of associative (or competitive) enzyme inhibition by a single inhibitor:

$$K(\mathrm{IV}_i) = i\big(K_m^0/(K'_m - K_m^0)\big). \tag{9}$$

How will Eq. (8) simplify if $[i_1]$ and $[i_2]$ are the concentrations of the same inhibitor? Equal concentrations of the same inhibitor $[i_1] = [i_2]$ will lead to the equal increase of the reaction parameter K'_m. Vectors $\mathbf{PIV}_{i1}$, $\mathbf{PIV}_{i2}$ and $\mathbf{PIV}_{(i1+i2)}$ will spread along one and the same line in plane $\sigma_{\mathrm{IV}i}$. Eq. (6) will simplify to

$$\sum_{l=1}^{n}\big(K'_{m(l)} - K_m^0\big) = n\big(K'_m - K_m^0\big), \tag{10}$$

Eq. (3) to

$$\sum_{l=1}^{n} i(l) = ni \tag{11}$$

(where $n = 2$) and, thus, Eq. (8) will simplify to the form of Eq. (9).

Now, if $[i_1] = 2[i_2]$, then $\big(K_{m(i1)} - K_m^0\big) = 2\big(K'_{m(i2)} - K_m^0\big)$ and vectors $\mathbf{PIV}_{i1}$, $\mathbf{PIV}_{i2}$ and $\mathbf{PIV}_{(i1+i2)}$ will also spread along one line in plane $\sigma_{\mathrm{IV}i}$ and this, as we have already seen $\big(\mathrm{Eqs}\ (10), (11)\big)$, indicates that Eq. (8) will again simplify to the form of Eq. (9). Hence, Eq. (8) is a more general form of the equation for calculating

$K(IV_i)$ constants of enzyme inhibition valid both for n different inhibitors and for n concentrations of the same inhibitor.

Catalytic (Type III_i or Noncompetitive) Enzyme Inhibition

Representation of the results of enzyme inhibition simultaneously by two different (not interacting) catalytic $(V' < V^0, K'_m = K^0_m, i > 0)$ inhibitors of equal concentrations $[i_1] = [i_2]$ but differing in their effect on the enzyme in such a way that $2(V^0 - V'_{i2}) = (V^0 - V'_{i1})$, will be in plane σ_{IIIi} as shown in Fig. 3. It can be seen that the length of projection $PIII_{(i1+i2)}$ of the resulting vector $\boldsymbol{PIII}_{(i1+i2)}$ on semi-axis $P0_{V'}$ will be determined by a similar sum (Eq. (2)) of the lengths of projections $PIII_{i1}$ and $PIII_{i2}$ of its components:

$$PIII_{(i1+i2)} = PIII_{i1} + PIII_{i2} = \sum_{l=1}^{n} PIII_{i(l)} . \qquad (12)$$

The positive difference of the coordinates of $PIII_{(i1+i2)}$ and each of its constituent projections, due to $V' < V^0$ here, will be

$$PIII_i = V^0 - V' \qquad (13)$$

and, therefore, the value of coordinate $V'_{(i1+i2)}$ of the end of projection $PIII_{(i1+i2)}$ on semi-axis $P0_{V'}$ (Fig. 3) will be determined by

$$V'_{(i1+i2)} = V^0 - \sum_{l=1}^{n} (V^0 - V'_l) \qquad (14)$$

whence the form of the positive difference of the coordinates of this projection will be
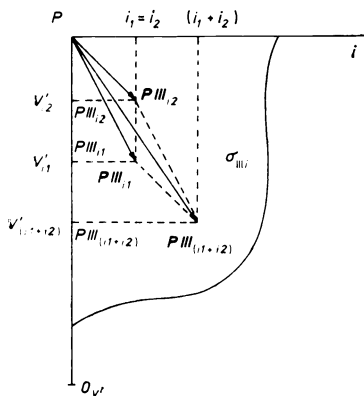

Fig. 3

Vector representation of data of catalytic inhibition by two inhibitors at equal concentration (in plane σ_{IIIi}, Fig. 1). Symbols: $\boldsymbol{PIII}_{i1}$, $\boldsymbol{PIII}_{i2}$ and $\boldsymbol{PIII}_{(i1+i2)}$ are the vectors characterizing the intensity of enzyme inhibition by inhibitors i_1 and i_2 and their mixture $(i_1 + i_2)$ at equal concentration. $PIII_{i2}$, $PIII_{i2}$ and $PIII_{(i1+i2)}$ are the projections of these vectors on semi-axis $P0_{V'}$

$$Proj_{P0(V')}\mathbf{PIII}_{(i1+i2)} = V^0 - V'_{(i1+i2)} = \sum_{l=1}^{n}(V^0 - V'_l). \tag{15}$$

The positive difference of the coordinates of the vector $\mathbf{PIII}_{(i1+i2)}$ projection on semiaxis Pi, due to the feature of plotting this axis (the value of concentrations $i = 0$ or $a = 0$ point P; Fig. 1), will be determined by the similar sum of components:

$$Proj_{Pa,i}\mathbf{PIII}_{(i1+i2)} = (i_1 - 0) + (i_2 - 0) = \sum_{l=1}^{n} i(l) \tag{16}$$

and, therefore, the equation for calculating constants $K(\text{III}_{i(n)})$ of (catalytic) enzyme inhibition by n inhibitors will have the form

$$K(\text{III}_{i(n)}) = \frac{Proj_{Pa,i}\mathbf{PIII}_{i(n)}}{Proj_{\sigma_0}\mathbf{PIII}_{i(n)}} V'_{i(n)} \tag{17}$$

or, taking into consideration Eqs (14), (15) and (16),

$$K(\text{III}_{i(n)}) = \varrho \sum_{l=1}^{n} i(l) \frac{V^0 - \sum_{l=1}^{n}(V^0 - V'_l)}{\sum_{l=1}^{n}(V^0 - V'_l)}. \tag{18}$$

A simple analysis shows that at $n = 1$ Eq. (18) will be simplified to the form of the known equation[5-7] for calculating constants $K(\text{III}_i)$ of catalytic (or noncompetitive) enzyme inhibition by a single inhibitor:

$$K(\text{III}_i) = i(V'/(V^0 - V')). \tag{19}$$

In the case of equal $[i_1] = [i_2]$ or different $[i_1] > [i_2]$ concentrations of the same inhibitor Eq. (18) will also be simplified to the form of Eq. (19).

When deriving other equations for calculating constants $K_{i(n)}$ and $K_{a(n)}$, we shall use an analytical technique so as not to resort to plotting the spatial vectors in the $K'_m V'$ coordinates (like the diagrams in Figs 2 and 3). It is easily seen that on substituting the expression of $K'_{m(i(n))}$ and $i(n)$ coordinates of the vector $\mathbf{PIV}_{(i1+i2)}$ projections (Eqs (4) and (3)) we obtain the same form of the equation for calculating the inhibition constant $K(\text{IV}_{i(n)})$ (Eq. (8)) as was deduced using the geometric representation (Fig. 2). Similarly, on substituting expressions (14) and (16) into Eq. (19) we obtain the same form as Eq. (18) for calculating constants $K(\text{III}_{i(n)})$. This technique is more convenient, since the equations for calculating constants K_a/K_i of enzyme activation/inhibition by one activator/inhibitor are already

known[1-4] (cf. Fig. 4). Now we need to obtain the expression for interrelation of coordinates $K'_{m(a(n))}$, $V'_{a(n)}$ and $a(n)$ of the **PIV**$_{a(n)}$ and **PIII**$_{a(n)}$ vector projections on semi-axes $P0_{K'm}$, PV' and Pa,i (Fig. 1). It would be convenient here to use the symmetry of the positions of projections PIV_i and PIV_a as well as $PIII_i$ and $PIII_a$ of the respective three-dimensional vectors **PIV**$_i$, **PIV**$_a$, **PIII**$_i$ and **PIII**$_a$ (Fig. 1) on semi-axes PK'_m, $P0_{Km'}$, $P0_{V'}$, PV' of base plane σ_0 (Fig. 5) and also to utilize the fact that if the numerical value of the coordinate of the end of projection is greater than that of the beginning (as, e.g. $K'_m > K^0_m$ on semi-axes PK'_m, Fig. 2), then its interrelation with the coordinates of the constituent projections will be determined by the sum (Eq. (4)); if it is smaller (as, e.g. $V' < V^0$ on semiaxes $P0_{V'}$, Figs 1, 3), by the difference (Eq. (14)). But then in Fig. 1 (and Fig. 5, which is the projection of Fig. 1 on plane σ_0) it can be seen that coordinates $K'_{m(a(n))}$ of the vector **PIV**$_{a(n)}$ projection of associative (type IV$_a$, refs[1-4]) enzyme activation will be on semi-axis $P0_{K'm}$, where $K'_m < K^0_m$ and thus their interconnection with the coordinates of the constituent projections will be expressed by the difference

$$K'_{m(a(n))} = K^0_m - \sum_{l=1}^{n}\left(K^0_m - K'_{m(l)}\right) \tag{20}$$
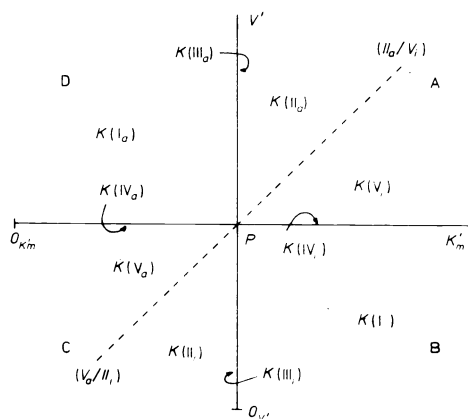
Equations in quadrants A—D:
A: $K(II_a) = a/(K'_m V'/K^0_m V^0 - 1)$; $K(V_i) = i/(K'_m V'/K^0_m V^0 - 1)$
B: $K(IV_i) = i/(K'_m/K^0_m - 1)$; $K(I_i) = i/(K'_m V^0/K^0_m V' - 1)$; $K(III_i) = i/(V^0/V' - 1)$
C: $K(II_i) = i/(K^0_m V^0/K'_m V' - 1)$; $K(V_a) = a/(K^0_m V^0/K'_m V' - 1)$
D: $K(IV_a) = a/(K^0_m/K'_m - 1)$; $K(I_a) = a/(K^0_m V'/K'_m V^0 - 1)$; $K(III_a) = a/(V'/V^0 - 1)$

FIG. 4

Distribution of the equations for calculating constants K_i/K_a of enzyme inhibition/activation by one inhibitor/activator by the elements of the two-dimensional $K'_m V'$ coordinates. Arrows indicate the applicability (depending on the positions of the respective projections) of the equations for calculating constants $K(IV_i)$, $K(III_i)$, $K(IV_a)$, and $K(III_a)$. The applicability region of the other equations are the areas of the respective squares or their parts (cf. the position of the respective equations)

and coordinates $V'_{a(n)}$ of the vector $\boldsymbol{PIII}_{a(n)}$ projection for the catalytic (type III_a) enzyme activation on semiaxis PV' (Figs 1 and 5), where $V' > V^0$, will be determined by the sum

$$V'_{a(n)} = V^0 + \sum_{l=1}^{n} (V'_l - V^0) \tag{21}$$

which, for making it more obvious, would be more convenient to tie in with the respective semi-axes of the two-dimensional $K'_m V'$ coordinates (Fig. 6).

Now, comparing the data of Figs 4 and 5 we can obtain all the other equations for calculating constants $K_{i(n)}$ and $K_{a(n)}$. Thus, for instance, substituting the expressions for coordinates $K'_{m(i(n))}$ (Eq. (4)) and $V'_{i(n)}$ (Eq. (14)) (Fig. 6) in the equation for calculating constants $K(I_i)$ of the two-parameter matched I_i type of enzyme inhibition (cf. Fig. 4, the equation for $K(I_i)$) we will obtain the following form of equation for calculating constants $K(I_{i(n)})$:

$$K(I_{i(n)}) = \varrho \sum_{l=1}^{n} i(l) \frac{K_m^0 [V^0 - \sum_{l=1}^{n} (V^0 - V'_l)]}{[K_m^0 + \sum_{l=1}^{n} (K'_{m(l)} - K_m^0)] V^0 - K_m^0 [V^0 - \sum_{l=1}^{n} (V^0 - V'_l)]}. \tag{22}$$

Substitution of the expression of parameters $K'_{m(a(n))}$ and $V'_{a(n)}$ (Eqs (20), (21)) in the equation for calculating inhibition constants $K(I_i)$ (Fig. 4) is not permissible, because projections PI_i (Figs 1 and 5) of vectors $\boldsymbol{PI}_i$ in this type of inhibition characterized by the $K'_m > K_m^0$, $V' < V^0$, $i > 0$ relationships[1,2,4] are only in the fourth quadrant of plane σ_0 (Fig. 5). This plane is the geometrical locus of their existence where its coordinates K'_m and V' will be determined by Eqs (4) and (14).

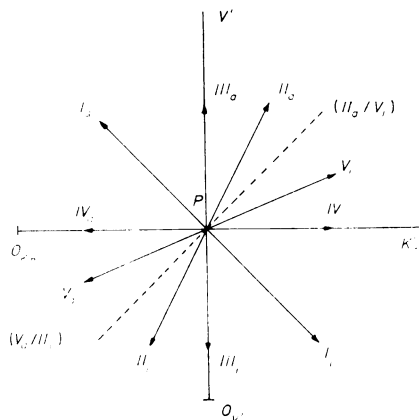FIG. 5

Distribution of the projections PIV_i, PI_i, $PIII_i$, etc., of the respective three-dimensional vectors $\boldsymbol{PIV}_i$, $\boldsymbol{PI}_i$, $\boldsymbol{PIII}_i$, etc., (Fig. 1) on its base plane σ_0

Since these (more general) forms of equations for calculating constants $K_{a(n)}$ and $K_{i(n)}$ can find application in the analysis of complicated cases (effect of two, three or more inhibitors or activators on the enzyme), it seems to be expedient to adduce them all in this work so as prevent errors due to their derivation.

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Inhibition

The equation for calculating inhibition constants $K(IV_{i(n)})$, $K(III_{i(n)})$ and $K(I_{i(n)})$ were already obtained above (Eqs (8), (18), (22)). Here we need only to derive the equation for calculating constants $K(II_{i(n)})$ of two-parameter mismatched (type II_i) enzyme inhibition characterized by the relationships $K'_m < K^0_m$, $V' < V^0$, $i > 0$) (refs[1,2,4]) (projection PII_i, Fig. 5):

$$K(II_{i(n)}) = \varrho \sum_{l=1}^{n} i(l) \frac{\left[K^0_m - \sum_{l=1}^{n}(K^0_m - K'_{m(l)})\right]\left[V^0 - \sum_{l=1}^{n}(V^0 - V'_l)\right]}{K^0_m V^0 - \left[K^0_m - \sum_{l=1}^{n}(K^0_m - K'_{m(l)})\right]\left[V^0 - \sum_{l=1}^{n}(V^0 - V'_l)\right]} \quad (23)$$
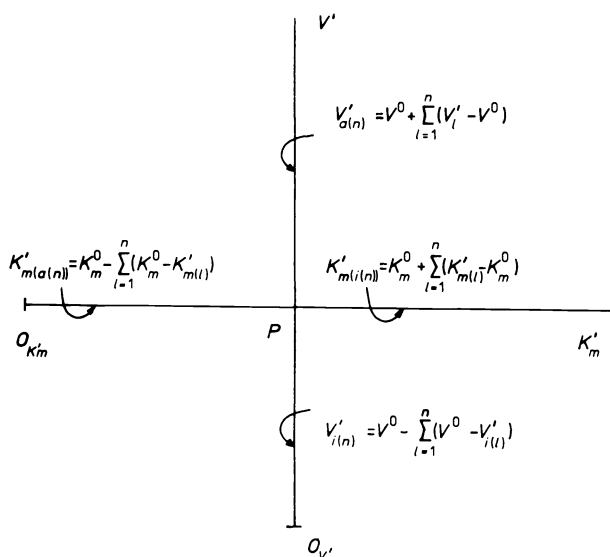

FIG. 6

Distribution of the equations of the interrelation of the resulting projections (designated by ') and coordinates $K'_{m(i(n))}$, $V'_{i(n)}$, $K'_{m(a)(n)}$ and $V'_{a(n)}$ with those of their constituent segments on axes PK'_m, $PO_{V'}$, $PO_{K_m'}$, and PV' (Fig. 1) within positive values of their differences

and the equation for calculating constants $K(V_{i(n)})$ of enzyme pseudoinhibition (type V_i) characterized by the relationships $K'_m > K^0_m$, $V' > V^0$, $i > 0$ (projection PV_i, Fig. 5):

$$K(V_{i(n)}) = \varrho \sum_{l=1}^{n} i(l) \frac{K^0_m V^0}{\left[K^0_m + \sum_{l=1}^{n} (K'_{m(l)} - K^0_m)\right] \left[V^0 + \sum_{l=1}^{n} (V'_l - V^0)\right] - K^0_m V^0}. \qquad (24)$$

Activation

The equation for calculating constants $K(I_{a(n)})$ of two-parameter matched (type I_a) enzyme activation characterized by the relationships $K'_m < K^0_m$, $V' > V^0$, $a > 0$ (projection PI_a, Fig. 5) can be obtained by substituting the expressions for parameters $K'_{m(a(n))}$ and $V'_{a(n)}$ (Eqs (20), (21)) in the $K(I_a)$ equation (Fig. 4):

$$K(I_{a(n)}) = \varrho \sum_{l=1}^{n} a(l) \frac{\left[K^0_m - \sum_{l=1}^{n} (K^0_m - K'_{m(l)})\right] V^0}{K^0_m \left[V^0 + \sum_{l=1}^{n} (V'_l - V^0)\right] - \left[K^0_m - \sum_{l=1}^{n} (K^0_m - K'_{m(l)})\right] V^0}. \qquad (25)$$

The other equations will be obtained similarly.

The equation for calculating constants $K(II_{a(n)})$ of two-parameter mismatched (type II_a) enzyme activation characterized by the relationships $K'_m > K^0_m$, $V' > V^0$, $a > 0$ (projection PII_a, Fig. 5) will be

$$K(II_{a(n)}) = \varrho \sum_{l=1}^{n} a(l) \frac{K^0_m V^0}{\left[K^0_m + \sum_{l=1}^{n} (K'_{m(l)} - K^0_m)\right] \left[V^0 + \sum_{l=1}^{n} (V'_l - V^0)\right] - K^0_m V^0}. \qquad (26)$$

The equation for calculating constants $K(III_{a(n)})$ of catalytic (type III_a) enzyme activation characterized by the relationships $K'_m = K^0_m$, $V' > V^0$, $a > 0$ (projection $PIII_a$, Fig. 5) will have the form

$$K(III_{a(n)}) = \varrho \sum_{l=1}^{n} a(l) \frac{V^0}{\sum_{l=1}^{n} (V'_l - V^0)}. \qquad (27)$$

The equation for calculating constants $K(IV_{a(n)})$ of associative (type IV_a) activation characterized by the relationships $K'_m < K^0_m$, $V' = V^0$, $a > 0$ (projection PIV_a, Fig. 5) will be

$$K(\mathrm{IV}_{a(n)}) = \varrho \sum_{l=1}^{n} a(l) \frac{K_m^0 - \sum_{l=1}^{n}(K_m^0 - K'_{m(l)})}{\sum_{l=1}^{n}(K_m^0 - K'_{m(l)})} \; . \tag{28}$$

The equation for calculating constants $K(\mathrm{V}_{a(n)})$ of enzyme pseudoactivation (type V_a) characterized by the relationships $K'_m < K_m^0$, $V' < V^0$, $a > 0$ (projection PV_a, Fig. 5) will be

$$K(\mathrm{V}_{a(n)}) = \varrho \sum_{l=1}^{n} a(l) \frac{[K_m^0 - \sum_{l=1}^{n}(K_m^0 - K'_{m(l)})][V^0 - \sum_{l=1}^{n}(V^0 - V'_l)]}{K_m^0 V^0 - [K_m^0 - \sum_{l=1}^{n}(K_m^0 - K'_{m(l)})][V^0 - \sum_{l=1}^{n}(V^0 - V'_l)]} \; . \tag{29}$$

Analysis of Eq. (22) shows that at $V' = V^0$ it will simplify to the form of Eq. (8); at $K'_m = K_m^0$, to that of Eq. (18); at $n = 1$, to the equation for constants $K(\mathrm{I}_i)$ of enzyme inhibition by a single inhibitor[1-4]

$$K(\mathrm{I}_i) = i \frac{K_m^0 V'}{K'_m V^0 - K_m^0 V'} \; . \tag{30}$$

Hence, Eqs (9) and (19) are particular cases of Eqs (8) and (18) which, in turn, are particular cases of Eq. (22). This reflects the circumstance that both type IV_i (associative) and III_i (catalytic) enzyme inhibition are particular (or borderline) cases (when either $V' = V^0$ or $K'_m = K_m^0$) of two-parameter matched (type I_i) enzyme inhibition which is well illustrated by the vector representation of these reactions. Thus, for example, in Fig. 1 (and its two-dimensional variant, Fig. 5) one can see that at $K'_m \to K_m^0$, vectors $\textbf{\textit{PI}}_i$ (or, which is the same, their projection PI_i on plane σ_0; Fig. 5) will be shifted towards plane $\sigma_{\mathrm{III}\,i}$ (semi-axis $PO_{v'}$; Fig. 5) and at $K'_m = K_m^0$ it will be located on it. At $V' \to V^0$, vectors $\textbf{\textit{PI}}_i$ will shift to plane $\sigma_{\mathrm{IV}i}$ and at $V' = V^0$ they will transfer into it (as vectors $\textbf{\textit{PIV}}_i$ characterizing associative enzyme inhibition, $K'_m > K_m^0$, $V' = V^0$, $i > 0$).

Further, in the same Fig. 1 (and Fig. 5) one can see that vectors $\textbf{\textit{PV}}_i$ (projection PV_i, Fig. 5) will also shift towards $\sigma_{\mathrm{IV}i}$ (semi-axis PK'_m; Fig. 5) and at $V' = V^0$ will be located on it. Hence it follows that plane $\sigma_{\mathrm{IV}i}$ is the geometrical locus of existence of vectors $\textbf{\textit{PIV}}_i$ occupying an intermediate (transitory) position between vectors $\textbf{\textit{PI}}_i$ and $\textbf{\textit{PV}}_i$ of two-parameter matched (type I_i) and two-parameter mismatched (type V_i) enzyme inhbition. It is not unexpected, then, that at $V' = V^0$ Eq. (24) for calculating pseudoinhibition constants $K(\mathrm{V}_{i(n)})$ will also be simplified to the form of Eq. (8) for inhibition constants $K(\mathrm{IV}_{i(n)})$.

It is similar for other "transitory" planes σ_{IIIi}, σ_{IVa} and σ_{IIIa} (Fig. 1). As could be seen above, at $K'_m = K^0_m$, Eq. (22) for calculating constants $K(I_{i(n)})$ will simplify to Eq. (18). It is easily seen that another equation, Eq. (23), for calculating inhibition constants $K(II_{i(n)})$ at $K'_m = K^0_m$ will also simplify to Eq. (18) for calculating catalytic inhibition constants $K(III_{i(n)})$. The same holds for Eqs (25) and (26) which at $K'_m = K^0_m$ will simplify to Eqs (27), etc. This important consequence justifies one to compare inhibition (or activation) constants even when the type of an inhibited (or activated) enzymic reaction changes during the study.

The use of equations for calculating constants $K_{i(n)}$ and $K_{a(n)}$ makes it possible to answer one more significant question in enzymology: whether particular inhibitors or activators interact or not. It can be judged by the coincidence (or non-coincidence) of the values of constants $K_{i(n)}$ or $K_{a(n)}$ obtained experimentally with those calculated using one of these equations.

In conclusion we would like to draw the attention to the possibility of writing these equations in matrix forms. Thus, for instance, from Eq. (22) for calculating inhibition constants $K(I_{i(n)})$ one can see that the denominator here is the determinant of the second-order matrix composed of the end (main diagonal) and of the beginning (secondary diagonal) of the respective $\boldsymbol{PI}_{i(n)}$ vector in $K'_m V'$ coordinates (Fig. 1); the numerator is the product of the sum of molar concentrations of inhibitors used by the determinant of the diagonal second-order matrix composed of the coordinates of the beginning of the same vector (within the positive value of their differences):

$$K(I_{i(n)}) = \frac{\varrho \sum\limits_{l=1}^{n} i(l)}{\begin{vmatrix} K^0_m + \sum\limits_{l=1}^{n}(K'_{m(l)} - K^0_m) & V^0 - \sum\limits_{l=1}^{n}(V^0 - V'_l) \\ K^0_m & V^0 \end{vmatrix}} \cdot \begin{vmatrix} K^m_0 & 0 \\ 0 & V^0 - \sum\limits_{l=1}^{n}(V^0 - V'_l) \end{vmatrix} \cdot$$

$$(31)$$

The situation is similar with Eq. (18) for calculating inhibition constants $K(III_{i(n)})$, with the difference that here, due to $K'_m \to K^0_m$ at $K'_m = K^0_m$,

$$K(III_{i(n)}) = \frac{\varrho \sum\limits_{l=1}^{n} i(l)}{\begin{vmatrix} K^0_m & V^0 - \sum\limits_{l=1}^{n}(V^0 - V'_l) \\ K^0_m & V^0 \end{vmatrix}} \cdot \begin{vmatrix} K^0_m & 0 \\ 0 & V^0 - \sum\limits_{l=1}^{n}(V^0 - V'_l) \end{vmatrix} \cdot \qquad (32)$$

Likewise then for all the other Eqs (8), (23)−(29), as well as Eqs (9), (19), (30) and equations of Fig. 4 (ref.[2]).

Representation of the equations for calculating constants K_i and K_a in matrix form is connected with the geometrical positions of the respective vectors in the $K_m' V'$ coordinates (Fig. 1). This is more convenient for analysis of the mechanisms of reactions studied.

EXPERIMENTAL

Rabbit alkaline phosphatase (EC 3.1.3.1), a homogeneous preparation of Sigma (U.S.A.). Substrate p-nitrophenylphosphate (pNPP), a crystalline preparation (sodium salt) from the same company. Inhibitors: sodium tungstate $Na_2WO_4.2\,H_2O$ and sodium molybdate Na_2MoO_4. $.2\,H_2O$, both crystalline.

Decomposition of pNPP was observed spectrophotometrically (an Optica Milano CF-4DR double-beam spectrophotometer) by the increase of absorption at 405 nm $(+\Delta A_{405})$ of a solution, containing substrate, enzyme and inhibitor, against the solution of the same composition but without enzyme. The reaction was carried out in $0{\cdot}05$M Tris-HCl buffer, pH $8{\cdot}9$; in the presence of $0{\cdot}1$M-NaCl, 37°C. Final concentrations of substrate were $32{\cdot}7{-}98\,\mu mol\,l^{-1}$; of the enzyme, $1{\cdot}96\,\mu g\,ml^{-1}$; inhibitors, $12{\cdot}5\,\mu mol\,l^{-1}$ (Na_2WO_4) and $0{\cdot}125\,mmol\,l^{-1}$ (Na_2MoO_4).

Initial reaction rates (v_0) were determined by the slope of the initial (straight) sections of the substrate decomposition curves $\Delta A_{405} = f(t)$ at $t \rightarrow 2{\cdot}5$ s in 5 or more parallel experiments.

The root-mean-square deviation in the five-fold determination was $v_0 = \pm 2{\cdot}5\%$; K_m, V, $K_i =$ $= \pm 10\%$.

RESULTS AND DISCUSSION

The results are shown in Figs 7 and 8. In Fig. 7 (lines 2 and 0) shows that tungstate anions (WO_4^{2-}) at concentration of $12{\cdot}5\,\mu mol\,l^{-1}$ exhibit all the features of the two-parameter matched $(K_m' > K_m^0,\; V' < V^0,\; i > 0)$ inhibition of phosphatase. Their presence in solution results in the following change of the parameters of pNPP decomposition: $K_m' = 181{\cdot}7\,\mu mol\,l^{-1}; V' = 3{\cdot}25\,\mu mol\,l^{-1}\,min^{-1}\,(K_m^0 = 47{\cdot}6\,\mu mol\,l^{-1};$ $V^0 = 5{\cdot}3\,\mu mol\,l^{-1}\,min^{-1})$. Substitution of these data into Eq. (30) makes it possible to calculate the value of $K(I_{i(Na_2WO_4)})$ to be $2{\cdot}39\,\mu mol\,l^{-1}$.

Anions of molybdenic acid (MoO_4^{2-}) also exhibit all the features of the two-parameter matched $(K_m' > K_m^0,\; V' < V^0,\; i > 0)$ inhibition of this enzyme (Fig. 7, lines 3 and 0). Their presence in solution at a concentration of $0{\cdot}125\,mmol\,l^{-1}$ leads to the following change of parameters of pNPP decomposition: $K_m' = 93{\cdot}5$ $\mu mol\,l^{-1};\; V' = 4{\cdot}2\,\mu mol\,l^{-1}\,min^{-1}$. Substitution of these parameters in Eq. (30) gives the following values of inhibition constants $K(I_{i(Na_2MoO_4)}) = 84{\cdot}6\,\mu mol\,l^{-1}$.

Studies of phosphatase inhibition by a mixture of the salts at equal concentrations $(12{\cdot}5\,\mu mol\,l^{-1}$ Na_2WO_4 and $0{\cdot}125\,mmol\,l^{-1}$ $Na_2MoO_4)$ show (Fig. 7, lines 1 and 0) that the effect of two-parameter matched inhibition $(K_m' > K_m^0,\; V' < V^0,$ $i > 0)$ is still there but it is not a mere algebraic sum $(K_m' = 0{\cdot}226\,mmol\,l^{-1};\; V' =$ $= 2{\cdot}09\,\mu mol\,l^{-1}\,min^{-1})$ of its constituent effects (cf. above). The value $1/v_i$ does not add up either; in this case within the entire concentration range of the decomposed substrate $1/v_{i1} + 1/v_{i2} < 1/v_{(i1+i2)}$.

To answer the question whether these inhibitors interact, we compared the values of phosphatase inhibition constants $K(I_{i(n)})$ obtained experimentally (Fig. 7, lines *1* and *0*).

$$K(I_{(i1 + i2)} = \frac{137 \cdot 5 \ \mu mol \ l^{-1}}{\dfrac{226 \cdot 5 \cdot 3}{47 \cdot 6 \cdot 2 \cdot 09} - 1} = 12 \cdot 5 \ \mu mol \ l^{-1}$$

FIG. 7

The change of initial rates of *p*-nitrophenyl-phosphate decomposition by rabbit alkaline phosphatase in the presence of 0·125 mmol l^{-1} Na_2MoO_4 (line *3*); 12·5 μmol l^{-1} Na_2WO_4 (line *2*) and the mixture of these salts at the same concentration (line *1*). Line *0*, no inhibitor. The other conditions are described in the text
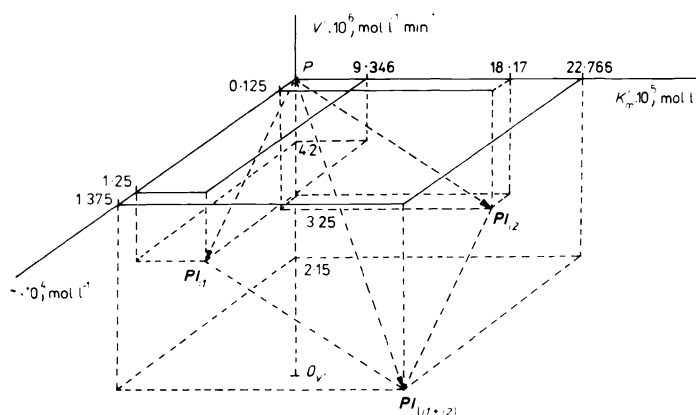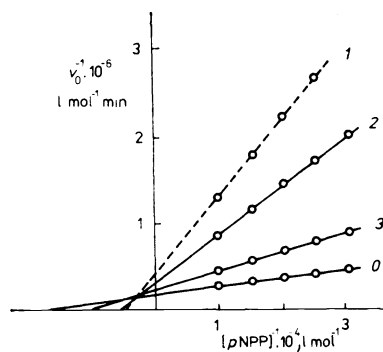

FIG. 8

Vector analysis of the data of Fig. 7 using their representation in three-dimensional coordinates. Symbols: **PI**$_{i1}$, is a vector characterizing the intensity of phosphatase inhibition by MoO_4^{2-} anions at a concentration of 0·125 mmol l^{-1}; **PI**$_{i2}$ is a vector of phosphatase inhibition by WO_4^{2-} anions at a concentration of 12·5 μmol l^{-1}; **PI**$_{(i1 + i2)}$ is a vector of phosphatase inhibition by the mixture of anions at the same concentrations

with that calculated using Eq. (22)

$$K\left(I_{(i1+i2)}\right) = 137 \cdot 5 \; \mu\text{mol } l^{-1} \; \frac{47 \cdot 6\left[5 \cdot 3 - (5 \cdot 3 - 3 \cdot 25) - (5 \cdot 3 - 4 \cdot 2)\right]}{5 \cdot 3\left[47 \cdot 6 + (181 \cdot 7 - 47 \cdot 6) + (93 \cdot 46 - 47 \cdot 6)\right] - A} =$$

$$= 12 \cdot 7 \; \mu\text{mol } l^{-1} \, ,$$

where A is the polynomial equal to the numerator of this fraction.

The discrepancy between the values of the calculated constant $K(I_{i(n)}) = 12 \cdot 7$ $\mu\text{mol } l^{-1}$ and that obtained experimentally $K(I_{i(n)}) = 12 \cdot 5 \; \mu\text{mol } l^{-1}$ does not exceed the experimental error. Hence, a conclusion can be made that the inhibitors mixed $(Na_2WO_4$ and $Na_2MoO_4)$ do not interact.

One more advantage of using the vector representation in the analysis of complex systems (the cases of simultaneous effect of two or more inhibitors on the enzyme) is an inverse result — the resolution of such vectors to their components. Thus, from Fig. 8 it can be seen that, knowing the coordinates of vector $\boldsymbol{PI}_{(i1+i2)}$ of the total effect of two inhibitors and the coordinates of one of its components (for instance, vector $\boldsymbol{PI}_{i1}$) it will be possible, using the parallelogram rule, to find the coordinates of the second vector $\boldsymbol{PI}_{i2}$ and, therefore, to calculate the value of the inhibition constant $K(I_{i2})$, which is of great importance in enzymological practice.

REFERENCES

1. Krupyanko V. I.: Prikl. Biokhim. Microbiol. (U.S.S.R.) *22*, 440 (1986).
2. Krupyanko V. I. in: *A Method of* $K'_m V'$ *Coordinate in the Fermentative Kinetics* (E. A. Burstein, Ed.), p. 6. ONTI Biological Research Center, Pushchino 1987.
3. Krupyanko V. I.: *Abstracts of Papers of XIIth Conference on Isoprenoids* (L. Novotný, Ed.), p. 46. Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 1987.
4. Krupyanko V. I.: Collect. Czech. Chem. Commun. *53*, 161, (1988).
5. Dixon M., Webb E. in: *Enzymes* (A. E. Braunstein and V. K. Antonov, Eds), Vol. 2, p. 481 Mir, Moscow 1982.
6. Kornish-Bowden A. in: *Principles of Enzyme Kinetics* (B. I. Kurganov, Ed.), p. 43. Mir, Moscow 1979.
7. Webb L. in: *Enzyme and Metabolic Inhibitors* (W. A. Yakowlev, Ed.), p. 163. Mir, Moscow 1966.