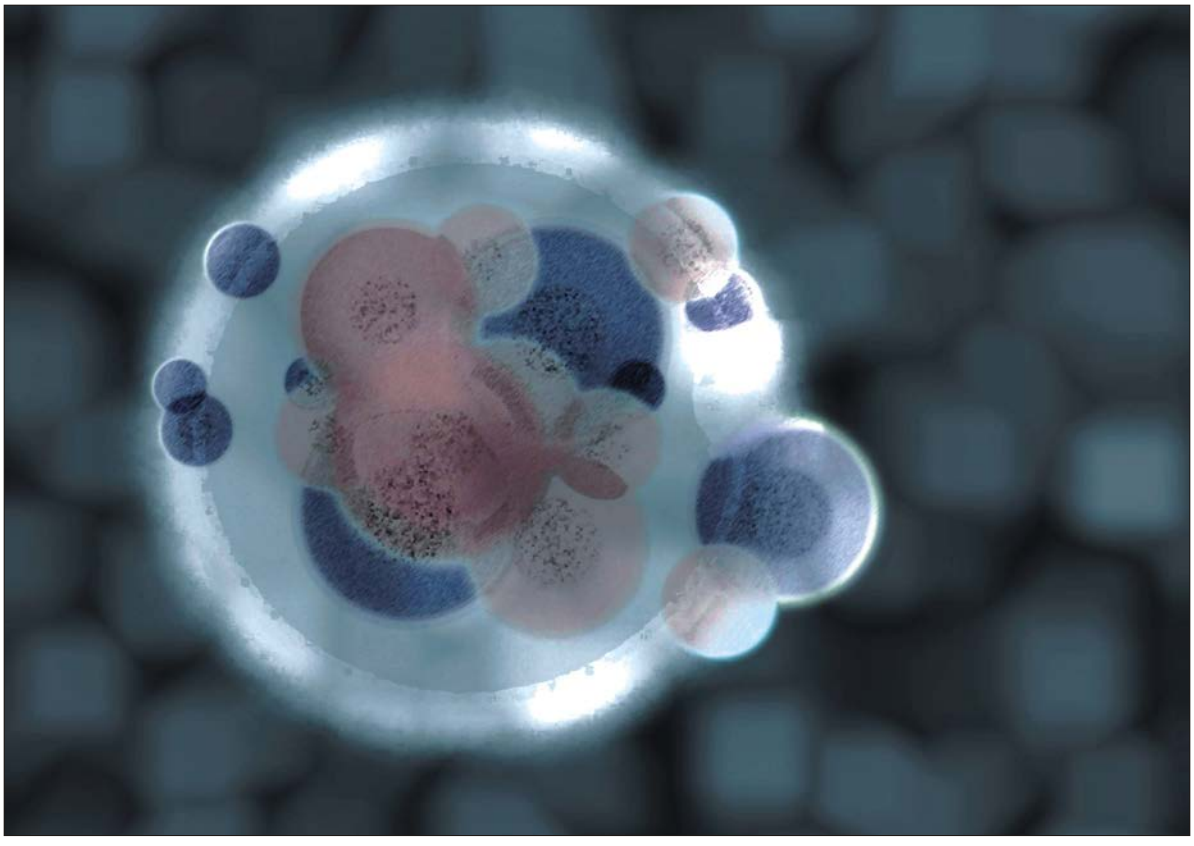

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 5 (1): 92-97, 2005
ISSN 1727-3048

Additional Possibility of Data Analysis of Enzyme Inhibition and Activation 2. Geometrical Portraits of Enzymatic Reactions for Data Processing in Enzyme Inhibition

[1]V.I. Krupyanko, [1,2]P.V. Krupyanko and [2]I.N. Dorokhov
[1]G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142290 Pushchino, Prospect Nauki 5, Moscow region, Russia
[2]D.I. Mendeleev Moscow State University, Moscow, Miusskaya 5, 121352, Russia

Abstract: By studying the inhibitory effect of increasing concentration of sodium tungstate on calf and porcine alkaline phosphatase and that of sodium molybdate on calf alkaline phosphatase, the perspectiveness of using the geometrical portraits of enzymatic reactions was shown for analysis of the mechanism of proceeding of biotechnological, pilot-plant and laboratory processes catalyzed by enzymes under varying conditions, such as, increasing concentration of inhibitors, activators and other introduced reaction components; change in ionic strength of the medium; temperature increase etc. The new method permits to provide higher yield of the reaction product and select the enzyme most stable to the particular conditions of a certain process.

Key words: Alkaline phosphatases, L_i vectors of enzyme inhibition, intensity of inhibition, constants of inhibition, geometrical portraits of enzyme inhibition

INTRODUCTION

Success of any fermentation process at the biotechnological, pilot-plant and laboratory scale depends to much extent on selection of a stable enzyme, intensity of enzyme inhibition by reaction products, introduced inhibitors, activators, change in temperature and other conditions of proceeding of fermentation, which allow increase in the product yield[1-6]. A vector method of representation of enzymatic reactions[7-10] opens up additional possibilities for studying the mechanism of proceeding of such processes by construction of the geometric portraits of analyzed reactions at varying concentration of introduced components (inhibitors, activators etc.) or different conditions (temperature increase, change in the ionic strength of solution, etc.).

The study is devoted to comparative analysis of the experimental data on inhibitory effect of increasing concentration of WO_4^{2-} and MoO_4^{2-} amions on calf alkaline phosphatase and also of the efficiency of inhibitory effect of increasing concentration of WO_4^{2-} on porcine alkaline phosphatase by using such additional parameters of enzyme inhibition as:

a) Intensity of enzyme inhibition
b) Overall effect of enzyme inhibition
c) A geometric portrait of a developing process of enzyme inhibition.

MATERIALS AND METHODS

Chemicals: Calf intestinal alkaline phosphatase (EC 3.1.3.1)-a product of Fluka (Switzerland), porcine intestinal alkaline phosphatase (EC 3.1.3.1) - a product of Sigma (USA).

Substrate: p-nitrophenylphosphate 2CH-salt (pNPP) - a product of Serva (Germany).

Inhibitors: $Na_2WO_4 \cdot 2H_2O$ and $Na_2MoO_4 \cdot 2H_2O$, crystalline salts of high purity grade.

In case of calf phosphatase, the concentration of pNPP was changed within $0.98 \cdot 10^{-4}$-$0.294 \cdot 10^{-4}$ M and that of the enzyme was kept constant-1.13 μg mL^{-1}.

In case of porcine phosphatase, the concentration of pNPP was changed within $0.98 \cdot 10^{-4}$-$0.327 \cdot 10^{-4}$ M and that of the enzyme was kept constant-1.77 μg mL^{-1}. Selection of the substrates was stipulated by an interval of minimum error in the determination of K_m and V parameters[11,12].

The concentrations of inhibitors are indicated in Fig. 1-3. The process of pNPP cleavage was recorded by a double-beam CF-4DR spectrophotometer (Optica

Corresponding Author: Dr. V.I. Krupyanko, Leading Researcher, Department of Analytical Biochemistry, G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Russia Fax: 7+095-956-33-70 E-mail: krupyanko@ibpm.pushchino.ru

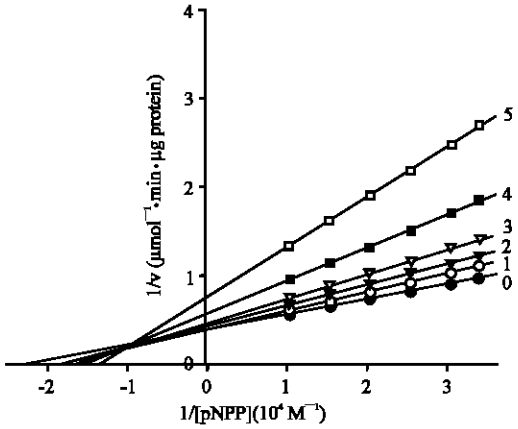

Fig. 1: Lineweaver-Burk's plots of inhibitory effect of WO_4^{2-} on calf alkaline phosphatase. Note: the concentration of WO_4^{2-} (10^{-4} M): line 1-0.0625, line 2-0.125, line 3-0.25, line 4-0.5 and line 5-1.0. Line 0-the inhibitor is absent

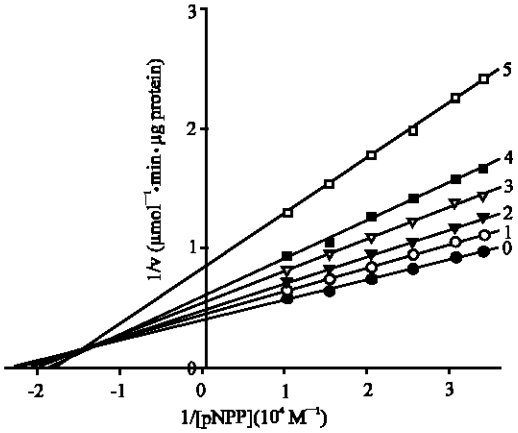

Fig. 2: Lineweaver-Burk's plots of inhibitory effect of MoO_4^{2-} on calf alkaline phosphatase. Note: the concentration of MoO_4^{2-} (10^{-4} M): line 1-0.0625, line 2-0.125, line 3-0.25, line 4-0.5 and line 5-1.0. Line 0-the inhibitor is absent

Milano, Italy). Reactions were carried out in 0.05 M Tris-HCl buffer, pH 9.0, with ionic strength 0.1 by NaCl of high purity at constant stirring by registering the absorption increment ($+\Delta D_{400}$) of a solution containing the substrate, inhibitor and enzyme versus a solution of the same concentration, but without the enzyme.

Determination of enzyme activity: The initial rates (v) of p-nitrophenylphosphate cleavage by calf and porcine alkaline phosphatases were calculated by a slope angle of tangents to the initial segments of curves representing the

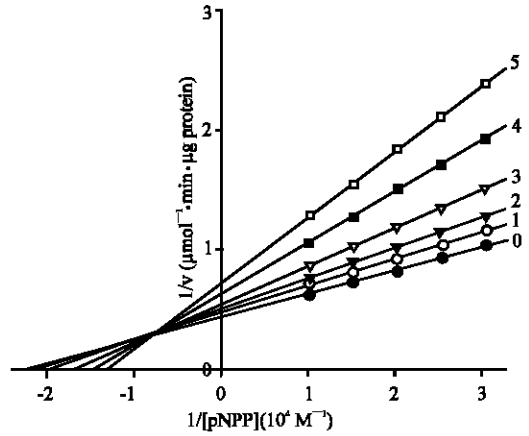

Fig. 3: Lineweaver-Burk's plots of inhibitory effect of WO_4^{2-} on porcine alkaline phosphatase. Note: the concentration of WO_4^{2-} (10^{-4} M): line 1-0.0625, line 2-0.125, line 3-0.25, line 4-0.5 and line 5-1.0. Line 0-the inhibitor is absent

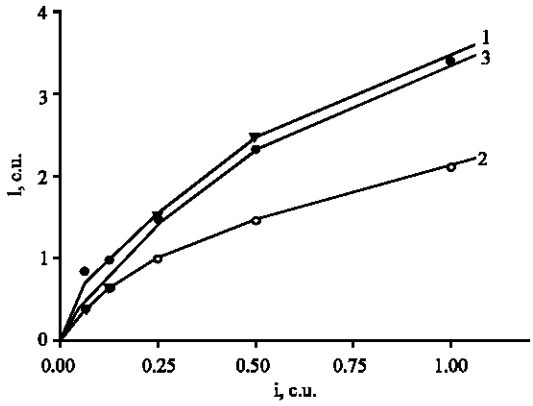

Fig. 4: Plots of the l length dependence of L_{li} vectors for: calf alkaline phosphatase on the concentration of WO_4^{2-} (curve 1), calf alkaline phosphatase on the concentration of MoO_4^{2-} (curve 2) and porcine alkaline phosphatase on the concentration of WO_4^{2-} (curve 3)

course of reactions determined in not less than five parallel experiments. The kinetic K'_m and V' parameters of enzyme inhibition were calculated by plots in the (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk, using the computer program Sigma Plot (Version 4.0) USA. The root-mean-square deviation at five measurements was as follows; v = ±2.5%, K_m and V = ±7.5%, l and S_t = ±10%.

It seemed necessary to introduce conventional units (c.u.) for processing of data on enzyme inhibition in the $K'_mV'I$ coordinate system (the l length of L_i vectors and

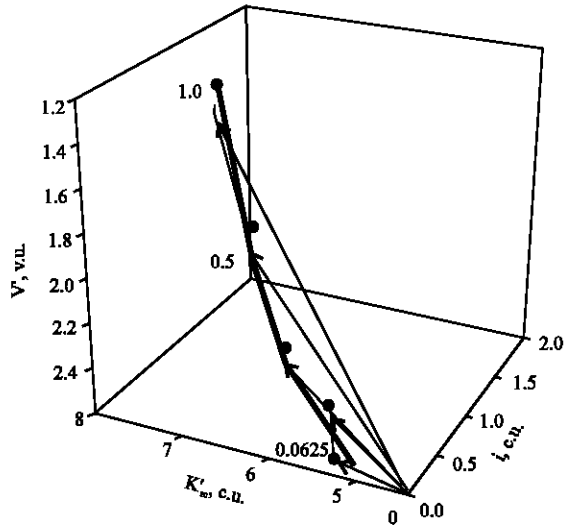

Fig. 5: Representation of the data (Fig. 1) in the three-dimensional K'_mV' I coordinate system. The position of the mobile end of L_{li} vectors of calf alkaline phosphatase inhibition by respective concentrations of WO_4^{2-} is denoted by digits 0.0625, 0.5, 1.0.The thick line represents a characteristic curve, the thin line is the experimental curve of reaction. Other designations are given in the text and Fig. 4

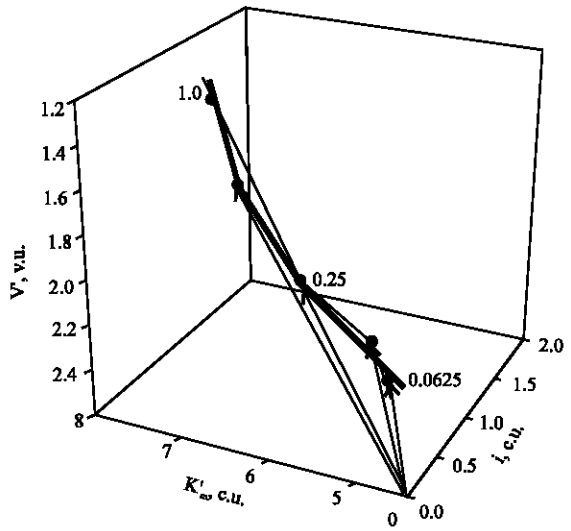

Fig. 6: Representation of the data (Fig. 2) in the three-dimensional K'_mV' I system of rectangular coordinates. The position of the mobile end of L_{li} vectors of calf alkaline phosphatase inhibition by respective concentrations of MoO_4^{2-} is indicated by digits 0.0625, 0.25, 1.0. Other designations are given in the text and Fig. 5

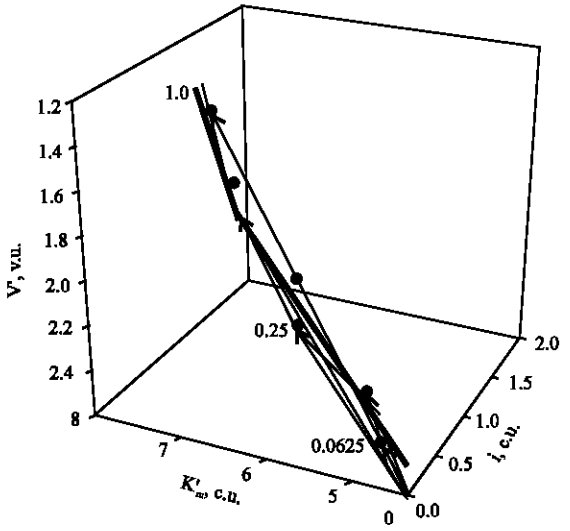

Fig. 7: Representation of the data (Fig. 3) in the three-dimensional K'_mV' I system of rectangular coordinates. The position of the mobile end of L_{li} vectors of porcine alkaline phosphatase inhibition by respective concentrations of WO_4^{2-} is indicated by digits 0.0625, 0.25, 1.0. Other designations are given in the text and Fig. 5

the S_t areas overlapped by vectors at shift due to increasing concentration of inhibitors) (Fig. 5-7). For that purpose, the following intervals of parameters were taken: on the abscissa axis $i = 1 \cdot 10^{-5}$ M = 1 c.u., on the ordinate axis $K_m = 1 \cdot 10^{-5}$ M = 1 c.u. and on the applicate axis V = 1 μmol pNPP/min·μg protein = 1 c.u. given in concrete coordinate values (Table 1, the 2nd line). This allowed calculation of the length (l) of L_i vectors of enzyme inhibition in conventional units (l, c.u.) by the equation:

$$l_i = \left(\left(K_m^{'} - K_m^{0}\right)^2 + \left(V^{'} - V^{0}\right)^2 + \left(i - 0\right)^2\right)^{0,5} \qquad (1)$$

and determination of the areas overlapped by the mobile end of L_i vectors at increasing concentration of WO_4^{2-} and MoO_4^{2-}: S_t, c.u.$^{2-}$ using the equation (2). Hence, it follows from Table 1 that to calculate the $l_{0.0625}$ length of $L_{0.0625}$ vector of calf alkaline phosphatase inhibition at a concentration of WO_4^{2-} anions $0.0625 \cdot 10^{-4}$ M, the following values of parameters ought to be used: i = 0.0625 c.u., K_m = 5.28 c.u., V = 2.51 c.u. and the value of $l_{0.0625}$ length of $L_{0.625}$ vector will be 0.834 c.u. By analogy, to calculate the S_t area overlapped by $L_{0.625}$ vector of calf alkaline phosphatase at increase in the concentration of WO_4^{2-} from $0.0625 \cdot 10^{-4}$ M to $0.125 \cdot 10^{-4}$ M, the 2nd and the 3rd lines of Table 1 and the equation (2) must be used.

RESULTS AND DISCUSSION

Conventional data analysis of enzyme inhibition: A gradual increase in the concentration of WO_4^{2-} and MoO_4^{2-} was exhibited by enhancement of the coordinated I_i type of enzyme inhibition ($K'_m > K^0_m$, $V' < V^0$, $i > 0$)[7-10,13] of calf alkaline phosphatase by anions of wolframic (Fig. 1) and tungstic acids (Fig. 2). This follows from the enlargement of slope angles of respective plots in the double reciprocal coordinates (v^{-1}; S^{-1}) and the increase in K'_m values and decrease of V' parameters in both cases (Table 1 and 2).

Analysis of the values of K_{Ii} constants[7-10,13] of calf phosphatase inhibition by WO_4^{2-} (Fig. 1) and MoO_4^{2-} (Fig. 2) reveals that in both cases the strength of binding of anions to calf alkaline phosphatase weakens at increasing concentration, which may be due to the effect of saturation of the enzyme with these inhibitors (Table 1 and 2). By comparing these data, one may also conclude that anions of wolframic acid bind stronger to the studied phosphatase than those of tungstic acid: $K_{Ii}(WO_4^{2-}) < K_{Ii}(MoO_4^{2-})$. The effect of WO_4^{2-} on calf and porcine alkaline phosphatases shows that in this case it is the classical type of difficulty in binding of the enzyme to the substrate and a decrease occurs in the maximum rate of substrate cleavage at increasing concentration of WO_4^{2-}, which corresponds to one and the same coordinated I_i type of inhibition of these enzymes (Fig. 1 and 3). In both cases the Michaelis-Menten constants gradually increase, while the maximum reaction rate decreases, i.e., they change in the opposite direction as was in the first case (Fig. 1 and 2).

Additional analysis: Conventional analysis of these data gives no answer as to the dynamics of inhibitory effect of WO_4^{2-} and MoO_4^{2-} on the enzymes. The answer seems even more difficult, since the values of K'_m and V' parameters of enzyme inhibition changed in the opposite direction in all the experiments (Table 1-3). To solve the problem, an approach was needed that would allow simultaneous representation of the dynamics of change in the K'_m and V' parameters of enzyme inhibition. A proposed vector method of representation of enzymatic reactions in the $K'_mV'I$ coordinate system makes it possible to obtain such data by comparing the geometrical portraits of studied reactions.

Let as take as an example data processing of inhibition of calf and porcine alkaline phosphatases by WO_4^{2-} and MoO_4^{2-}.

By using the equation (1) and the adopted intervals of shift to c.u., one can easily see (Fig. 4, curves 1 and 2)

Table 1: Inhibitory effect of increasing concentration of WO_4^{2-} on calf alkaline phosphatase

Inhibitor (10^{-4} M)	K'_m (10^{-5} M)	V' (μmol/min μg protein)	K_{Ii} (10^{-5} M)	l (c.u.)	S_i (c.u.^{2})
0.00	4.45	2.56			
0.0625	5.28	2.51	2.94	0.834	
(0.0625 c.u.)	(5.28 c.u.)	(2.51 c.u.)			
0.125	5.39	2.30	3.59	0.983	0.716
0.250	5.97	2.15	4.17	1.59	1.123
0.50	6.56	1.74	4.27	2.32	2.002
1.0	7.43	1.32	4.45	3.36	2.513

$\Delta I = 2.53$ c.u. $\Sigma = 6.354$ c.u.^{2}

Table 2: Inhibitory effect of increasing concentration of MoO_4^{2-} on calf alkaline phosphatase

Inhibitor (10^{-4} M)	K'_m (10^{-5} M)	V' (μmol/min μg protein)	K_{Ii} (10^{-5} M)	l (c.u.)	S_i (c.u.^{2})
0.00	4.45	2.56			
0.0625	4.75	2.33	3.65	0.383	
0.125	4.91	2.13	3.85	0.642	0.6814
0.250	5.14	1.89	4.44	0.994	0.8936
0.5	5.50	1.71	5.84	1.44	1.0337
1.0	5.69	1.22	5.94	2.08	2.0844

$\Delta I = 1.7$ c.u. $\Sigma = 4.69$ c.u.^{2}

Table 3: Inhibitory effect of increasing concentration of WO_4^{2-} on porcine alkaline phosphatase

Inhibitor (10^{-4} M)	K'_m (10^{-5} M)	V' (μmol/min μg protein)	K_{Ii} (10^{-5} M)	l (c.u.)	S_i (c.u.^{2})
0.00	4.355	2.244			
0.0625	4.704	2.100	4.020	0.3808	
0.125	4.923	1.972	4.340	0.6421	0.5536
0.250	5.778	1.833	4.003	1.5020	1.2190
0.5	6.690	1.574	4.205	2.4800	1.7170
1.0	7.445	1.373	5.574	3.3625	2.0010

$\Delta I = 2.98$ c.u. $\Sigma = 5.491$ c.u.^{2}

that the dynamics of intensity of calf alkaline phosphatase inhibition by WO_4^{2-} and MoO_4^{2-} and that of porcine alkaline phosphatase by WO_4^{2-} (Fig. 4, curve 3) develop at significant shift from linearity to more delay at higher concentration of anions. This is in good accord with the above-described effect of enzyme saturation with anions as well as the effect of enhancing competition between the increasing concentration of molecules of the substrate and the above anions for the substrate-binding site of the active center of alkaline phosphatases in all the cases.

The attention should also be paid to coincidence of the position of curves representing the process of intensity of the inhibitory effect of WO_4^{2-} on calf and porcine alkaline phosphatases (Fig. 4, curves 1 and 3). This gives grounds to assume similarity in the structure of the active center of each phosphatase. With the aim to confirm this, the vector method of representation of enzymatic reactions in the three-dimensional $K'_mV'I$ coordinate system provides another possibility - to compare the geometrical portraits of reactions under study.

In a coordinate system obtained by intersection of the horizontal $0K'_m$ abscissa of numerical values of effective Michaelis-Menten constants K'_m determined in the presence of i (or a) and the ordinate axis of numerical values of maximum reaction rate V′ in the point $P(K^0_m; V^0; 0)$, which corresponds to the values of K^0_m and V^0 parameters of initial uminhibited (i = 0) and nonactivated (a = 0) enzymatic reaction, each inhibited or activated enzymatic reaction will have its own vector representation-a concrete three-dimensional L vector of this reaction in accordance with K'_m, V′ and i (or a) parameters (Fig. 1 in[13], vectors: L_{Ii}, L_{IIIi}, ...L_{Ia}, etc.). Hence, it might be noted that:

a) the length (l) of such vector characterizes the intensity of proceeding of inhibited (i>0) or activated (a>0) enzymatic reaction (Eq. 1)
b) the position of L vector characterizes the type of reaction
c) the trajectory made by the mobile end of L vector represents an individual curve characteristic of each reaction
d) the area overlapped by L vectors at their shift due to increasing concentration of inhibitor or activator, etc:

$$S_i\ (c.u.^2) = 0{,}5 \cdot \left(\begin{vmatrix} K_{m1} & V_1 \\ K_{m2} & V_2 \end{vmatrix}^2 + \begin{vmatrix} V_1 & i_1 \\ V_2 & i_2 \end{vmatrix}^2 + \begin{vmatrix} i_1 & K_{m1} \\ i_2 & K_{m2} \end{vmatrix}^2 \right)^{0,5}, \quad (2)$$

is a measure of overall effect of the inhibitor on the enzyme in a certain interval of used concentrations. Evidently, a change of any additional or the conventional K'_m, V′ and i parameters of enzyme inhibition influences not only the configuration of a geometrical portrait of reaction (Fig. 5-7), but also all other parameters (Eqs. 1-3), whose comparison permits to get new information about the mechanism of developing interaction between inhibitors and enzymes. This technique is convenient in data processing of enzyme inhibition and activation. Based on the comparison of parameters (Table 1-3), two assumptions can be made: first, as there is much similarity in the configuration of geometrical portraits depicting the inhibitiory effect of WO_4^{2-} on calf and porcine alkaline phosphatases, and which is more important, second, the coincidence of values of the overall inhibitory effect S_i (Eq. 2) of WO_4^{2-} on these enzymes and Δl values of developing intensity of inhibitory effect of anions on both enzymes, the values independent of the outline of portraits (Table 1 and 3):

$$\Delta l = l_{max} - l_{min}, \quad (3)$$

one may conclude on similarity in the structure of active centres of these phosphatases.

WO_4^{2-} anions are better potential inhibitors than MoO_4^{2-}: A weaker action of MoO_4^{2-} on calf alkaline phosphatase than of WO_4^{2-} on the same enzyme (Table 1-3) might be due to chemical properties of these anions. Thus, tungsten anions are more capable of saturating their insufficient electron density at the expense of surrounding oxygen atoms in the structure of $=WO_4$ than molybdenum atoms in the analogues structure of $=MoO_4$, because tungsten has greater energy expenditure (higher ionization potentials) for removal of the same number of electrons than molybdenum[14]. Hence, the structure of $=WO_4$ will more effectively interact with electron donor groups of the enzyme active centre, for example, nitrogen tertiary imidasole atoms, which have the undivided pair of electrons on the 2s orbitale accessible for such interaction.

The functioning of histidine amino acids in the active centre of alkaline phosphatases has been proved many times[15].

One might suggest that all analogous calculations may also be used for data processing in enzyme activation.

REFERENCES

1. Handbook of Enzyme Biotechnology. 2nd Edn. (Wiseman, A., Ed.), 1986. New York., J. Wiley, pp: 12-457.

2. Biochemical Engineering and Biotechnology Handbook, (Atkinson, B. and F. Mavituna, Eds.), 1983. New York, The Nature Press, pp: 5-1119.
3. Arora, D.S. and D.K. Shandu, 1985. Laccase production and wood degradation by white-rot *Doedolea flavida*. Enzyme Microb. Technol., 7: 405-408.
4. Lobarzewsky, J. and A. Paszezyarski, 1985. Lignocellulose biodegradation with immobilized cellulase, d-glucose oxidase and fungal peroxidase. Enzyme Microb. Technol., 7: 564-566.
5. Ge, Y., H. Zhou, Y. Tong and W. Li, 1999. Coimmobilization of glycoamilase and glucose isomerase for one-step conversion of dextran to fructose. J. Biotechnol., 67: 33-40.
6. Jiandlang, L., S.M. Lee and Y.M. Koo, 2001. Hydrolysis of paper moll sludge using an improved enzyme system. J. Microbiol. Biotechnol., 11: 362-368.
7. Krupyanko, V.I., 1990. A Vector Method of Representation of Enzymatic Reactions. Moscow, Nauka, pp: 3-142 (In Russian).
8. Krupyanko, V.I., 1986. A vector method of representing individual types of enzymatic reactions in K'_mV' coordinates. Collect. Czech. Chem. Comm., 53: 161-72.
9. Krupyanko, V.I., 1995. Coordinate correction for calculating the constants of enzyme activation and inhibition. Appl. Biochem. Microbiol. (Moscow, Interperiodica Publishing), 31: 408-420.
10. Krupyanko, V.I., 1996. Applicability of vector presentation of enzymatic reactions to the analysis of enzyme activation and inhibition. Appl. Biochem. Microbiol. (Moscow, Interperiodica Publishing), 32: 144-152.
11. Krupyanko, V.I. and P.V. Krupyanko, 1999. Selection of substrate concentrations for determining the Michaelis constant and maximum rate of an enzymatic reaction. Appl. Biochem. Microbiol., (Moscow, Interperiodica Publishing), 35: 116 119.
12. Irvin, H. Segel., 1975. Enzyme Kinetics. New York, John Wiley and Sons, pp: 47-100.
13. Krupyanko, V.I., 2005. Additional possibility of data analysis of enzyme inhibition and activiation. 1: Equations for calculation of the K_a and K_i constants of enzyme activation and nontrivial types of enzyme inhibition. J. Biol. Sci., 5: 82-91.
14. Chemist's Handbook, 1971. Vol. 1. Leningrad: Khimiya (Chemistry), pp: 325 (in Russian).
15. Robert, B. McComb, George N. Bowers and Solomon Posen, 1979. Alkaline Phosphatase. New York, Plenum Press, pp: 5-986.