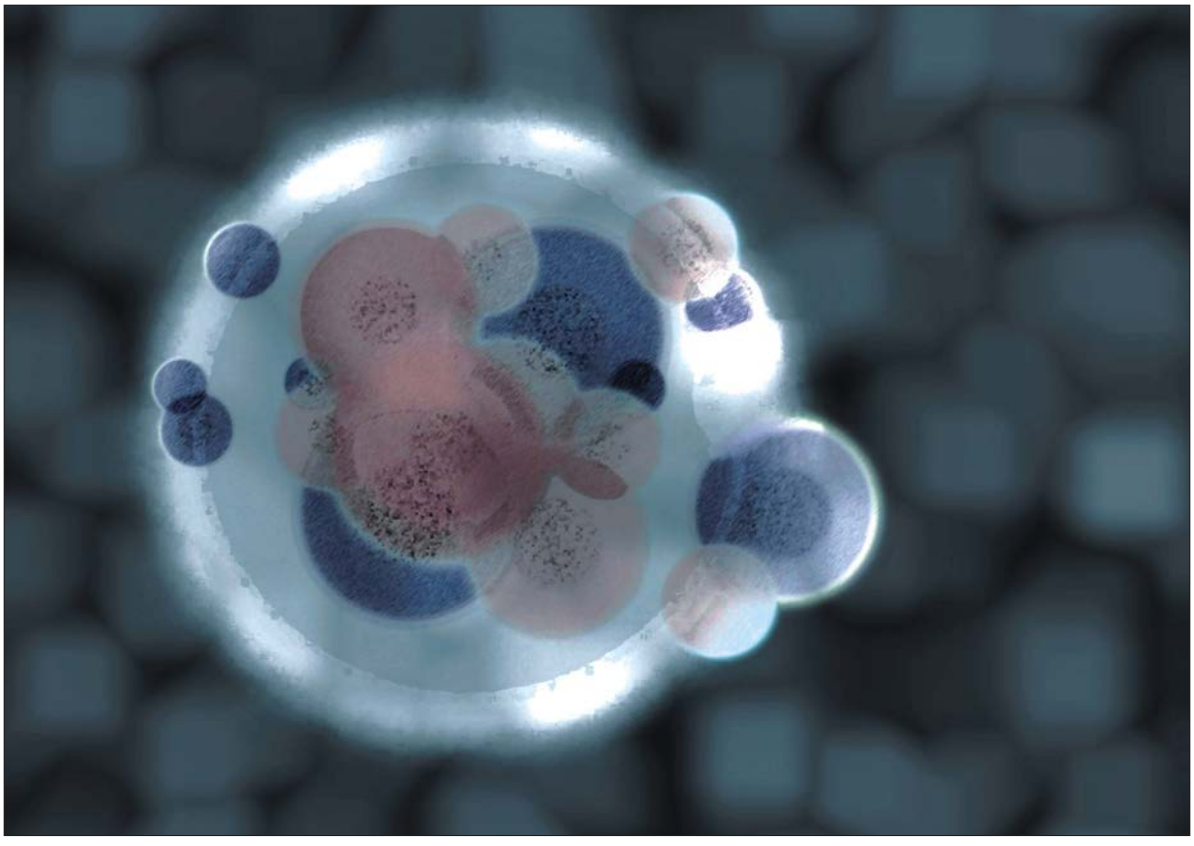

Journal of Biological Sciences

ISSN 1727-3048

science alert

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

Journal of Biological Sciences 5 (5): 633-636, 2005
ISSN 1727-3048

Additional Possibility of Data Analysis of Enzyme Inhibition and Activation. 7. Analysis of Multiparameter Data in Enzyme Kinetics Using N-dimensional Coordinate Systems

V.I. Krupyanko
G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms,
Russian Academy of Sciences, 142290 Pushchino, Prospect Nauki 5, Moscow Region, Russia

Abstract: Possible use of a vector method of representation of enzymatic reactions is considered in the four and five-dimensional ($K'_mV'E'_an_i$) and ($K'_mV'E'_aA'n_i$) coordinate systems for data processing of a simultaneous course of change in four K'_m, V', E'_a, n_i and five K'_m, V', E'_a, A', n_i) parameters of inhibition of the mutant forms of *Escherichia coli* E15 alkaline phosphatase modified by substitution of neutral amino acid residues in two N-terminal peptides by positively charged lysine residues (Lys, Lys) in the positions (+2+3), (+5+6), (+13+14), (+19+20) and arginine ones (Arg, Arg) in the positions (+5+6), (+13+14), (+19+20). It is established that at transfer of positive charges to the enzyme active centre the maximum inhibitory effect of enzyme activity occurs at substitution in the position (+13+14) from the N-terminus. This effect intensifies with increasing charge of amino acid residues (Lys→Arg).

Key words: Mutant forms of *E. coli* E15 alkaline phosphatase, a vector method of data processing in the four- and five-dimensional coordinate systems

INTRODUCTION

Study of a simultaneous course of change in multiparameter data, for example, representing four and more kinetic parameters reveals that the earlier developed three-dimensional ($K'_mV'T$) and ($K'_mV't$) coordinate systems[1-5] cannot be applied as their use is restricted by taking into account only three parameters.

However, in enzyme kinetics some cases require to simultaneously take into account four (Table 1 and 2), five (Table 3 and 4) and more parameters. This can be done by use of the four and five-dimensional coordinate systems and those of higher order, which is connected with complicated accounting and graphic methods of data processing. If to consider instead of the three-dimensional ($K'_mV'E'_an_i$) coordinate system, the four-dimensional ($K'_mV'n_i$) coordinate system, the introduction of only one parameter (E'_a) - which represents a course of change in the energy barriers of pNPP cleavage catalyzed by the mutant forms of *E. coli* E15 alkaline phosphatase[6] distinguished in the position of charged (Lys, Lys or Arg, Arg) amino acid residues in N-terminal peptides - affects all the parameters K'_m, V' and E'_a. This leads to a complicated procedure in the determination of the S_4 areas overlapped by L_4 vectors in the four-dimensional ($K'_mV'E'_an_i$) coordinate system and also eliminates visual representation of these vectors in such a coordinate system.

To simplify the calculation of the S_4 areas in the four-dimensional coordinate system and the coordinate systems of higher order, one may use the formula of Hero:

$$S_4 = (p\cdot(p-l_{AB})(p-l_{AC})(p-l_{CB}))^{0.5} \quad (1)$$

where, p – a half value of the triangle's perimeter formed by the L_{AB}, L_{AC} and L_{CB} vectors of the coordinate system, while l_{AB}, l_{AC} and l_{CB} are their lengths determined using the sums of remainders' squares in the same coordinates by the subradical expression of their connection with respective vectors. For example, a length of the four-dimensional L_{AB} vector coming out from the origin of the four-dimensional (K^0_m;V^0;E^0_a;0) coordinate system to the point K'_m, V', E'_a, n_i at the 1st substitution (n_{+2+3}) of neutral amino acid residues by lysine ones in the position (+2+3) of the N-terminal peptides, i.e., simultaneous substitution of two amino acid residues[6] in each peptide and the coordinates of the above vector point may be expressed as a function:

$$l_{AC} = ((K'_m-K^0_m)^2+(V'-V^0)^2+(E'_a-E^0_a)^2+(n_{0.2}-0)^2)^{0.5} \quad (2)$$

or according to Table 1 and the adopted unification of the coordinates:

Corresponding Author: Fax: 7+095 956 33 70

$$l_{AC} = ((1.0-0.3)^2+(2.03-2.85)^2+(0.63-0.74)^2 +(0.2-0)^2)^{0.5} = 1.84 \text{ c.u.} \quad (3)$$

where, K'_m - a value of the Michaelis effective constant determined at substitution of neutral amino acid residues by lysine (or arginine) ones, K^0_m - the Michaelis constant of initial (unmodified $n_i = 0$) enzyme form, V' - the maximum rate of pNPP cleavage catalyzed by modified alkaline phosphatase, V^0 - the reaction rate of pNPP cleavage catalyzed by the unmodified alkaline phosphatase. It is by analogy for E'_a and E^0_a, which are energy parameters of the process.

All the parameters given in Eq. 1 and 2 as well as in the three-dimensional coordinate system[1-5] must be expressed in conventional units (c.u.) (Materials and methods).

Let us study how transfer of the positive charges along the N-terminal peptide in the direction to the enzyme active centre affects a change in the catalytic activity of mutant phosphatases and how this may be represented as a function, because separate analysis of a simultaneous course of change in each of the individual K'_m, V', E'_a, n_i and A' parameters dependent on n_i (the position of substitution) fails to give a picture of overall inhibitory effect and the positions of substitution as a function, i. e. how charge transfer inside the molecule influences the activity of phosphatases in both cases: (Lys, Lys) and (Arg, Arg) (Table 1-4).

MATERIALS AND METHODS

The technique of obtaining *E. coli* E15 mutant forms of alkaline phosphatase is described by Kononova *et al.*[6] and Nesmeyanova *et al.*[7]

Substrate: Para-nitrophenylphosphate 2CHA salt (pNPP) is a product of Serva (Germany).

The cleavage of pNPP was recorded on a CF-4 DR double-beam spectrophotometer (Optica Milano, Italy). The reaction was carried out in 0.05 M Tris-HCl buffer, pH 9.0, at ionic strength of 0.1 using NaCl of high purity in a thermostat (37°C) under constant stirring[1] by increase in the absorption ($+\Delta A_{400}$) of a solution containing the substrate and the enzyme against a solution of the same composition, but without the enzyme.

The concentration of pNPP was determined within 0.294×10^{-4} to 0.98×10^{-4} M, an interval of minimum error in the V и K_m values[8]. The concentration of unmodified *E. coli* phosphatase was 0.67 μg mL^{-1}. The concentration of the mutant forms of enzyme was determined by cleavage pNPP and refer to arbitrary units (arb.u.). 1 arb.u. = $0.1A_{400}$/min in optimal conditions.

Table 1: A course of change in the K'_m, V', E'_a, l and S_4 parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the four-dimensional ($K'_mV'E'_an_i$) coordinate system

Enzyme, Lys, Lys substitution	n_i (c.u.)	K'_m (c.u.)	V' (c.u.)	E'_a (c.u.)	l/c.u.	S_4 (c.u.^{2})
wild type	0.0	0.30	2.85	0.6.3		
n(+2+3)	0.2	1.00	2.03	0.747	1.102	
n(+5+6)	0.5	1.20	1.79	0.80	1.487	0.1297
n(+13+14)	1.3	1.35	1.45	0.83	2.189	0.476
n(+19+20)	1.9	1.47	1.12	0.86	2.833	0.328

Table 2: A course of change in the K'_m, V', E'_a, l and S_4 parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the four-dimensional ($K'_mV'E'_an_i$) coordinate system

Enzyme, Arg Arg, substitution	n_i (c.u.)	K'_m (c.u.)	V' (c.u.)	E'_a (c.u.)	l/c.u.	S_4 (c.u.^{2})
wild type	0.0	0.30	2.85	0.63		
n(+5+6)	0.5	0.52	1.83	0.72	1.157	
n(+13+14)	1.3	0.56	1.22	0.75	2.101	0.274
n(+19+20)	1.9	0.66	0.734	0.78	2.867	0.1669

Table 3: A course of change in the K'_m, V', E'_a, A', l and S_5 parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the five-dimensional ($K'_mV'E'_aA'n_i$) coordinate system

Enzyme, Lys Lys, substitution	n_i (c.u.)	K'_m (c.u.)	V' (c.u.)	E'_a (c.u.)	A' (c.u.)	l (c.u.)	S_5 (c.u.^{2})
wild type	0.0	0.30	2.85	0.63	0.68		
n(+2+3)	0.2	1.00	2.03	0.747	0.26	1.179	
n(+5+6)	0.5	1.20	1.79	0.80	0.39	1.515	0.205
n(+13+14)	1.3	1.35	1.45	0.83	0.49	2.197	0.512
n(+19+20)	1.9	1.47	1.12	0.86	0.64	2.833	0.393

Table 4: A course of change in the K'_m, V', E'_a, A', l and S_5 parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the five-dimensional ($K'_mV'E'_aA'n_i$) coordinate system

Enzyme, Arg Arg, substitution	n_i (c.u.)	K'_m (c.u.)	V' (c.u.)	E'_a (c.u.)	A' (c.u.)	l (c.u.)	S_5 (c.u.^{2})
wild type	0.0	0.30	2.85	0.63	0.68		
n(+5+6)	0.5	0.52	1.83	0.72	0.17	1.268	
n(+13+14)	1.3	0.56	1.22	0.75	0.34	2.132	0.443
n(+19+20)	1.9	0.66	0.734	0.78	0.43	2.881	0.2824

Enzyme activity assay: The initial reaction rates (v_0 and v_i) were calculated by a slope angle of tangents to the initial segments of curves representing substrate cleavage in not less than five parallel experiments.

The kinetic K'_m and V' parameters were estimated by plots in the (v^{-1}; S^{-1}) coordinates of Lineweaver-Burk using the computer programs Sigma Plot Version 2000 and Eureca (USA).

Root mean square deviation at five measurements was as follows: $v_i = \pm2.5\%$, K'_m, V' and $E'_a = \pm7.5\%$, l and $S_i = \pm10\%$.

Energy barriers (E'_a) of catalyzed reactions were determined in the (lg V':1/T) coordinates of Arrhenius[9-12] using the same computer programs.

For equiprobable taking into account all the parameters used in the experiment K'_m, V', E'_a, A' and n_i, they were unified to simple univalent conventional units (c.u.)[2-5] as follows: $K'_m = 1\times10^{-5}$ M = 1 c.u.,

V′= 1×μmol/min, μg protein = 1 c.u., E'_a = 1 Kcal/mol = 1 c.u. and A′ = 100 mE/μg protein of cell = 0.1 c.u. The place of a substituted amino acid residue in the N-terminal peptide was expressed as n_i = 13 = 1.3 c.u.

Since two neutral amino acid residues were simultaneously substituted by positively charged ones (Lys, Lys or Arg, Arg) in each N-terminal peptide, the positions of the first pair of substituted residues were used for construction of the abscissa axis (Fig. 1 and 2). Plotting the dependencies for the second pair of substituted amino acid residues would have shifted the curves to the right in Fig. 1 and 2 by a unit.

RESULTS AND DISCUSSION

As seen from Table 1, 2 and Fig. 1, a gradual transfer of lysine (Table 1) and arguinine positive charges (Table 2) from the N-terminal peptide to the active enzyme centre presents a saturation function (Fig. 1). The X-ray structural analysis of high resolution revealed that each peptide is located near the enzyme active centre with the N-terminus directed outside[13]. The closer amino acid residues to the active enzyme centre the lesser their overall inhibitory effect on the enzyme determined by the S_i, c.u.^{2} areas overlapped by four-dimensional L_4 vectors in the ($K'_mV'E'_an_i$) coordinate system. This allows to take into account a simultaneous change in all the parameters, which are K'_m, V′ and E'_a in this case. It should be noted that the effect intensifies with increase in the charge attained by substitution using arginine amino acid residues instead of lysine ones (Lys→Arg) (Table 2).

A stepwise analysis of the results permits to get a representation of a course of change in each of the individual parameters of the process. As seen from Table 1 and 2, along with charge transfer inside the molecule the strength of binding (K'_m) of the mutant forms to cleaved substrate weakened, the maximum reaction rate (V′) decreased, while energy barriers (E'_a) of catalyzed reactions increased.

Calculation of the S_i areas overlapped at shift of the mobile ends of L_4 vectors in each of such substitutions revealed that the maximum inhibitory effect of positive charges (Lys, Lys) on mutant phosphatases at charge transfer to the active enzyme centre occurs in the substituted position (+13+14) followed by weakening in the position (+19+20) (Fig. 1). Based on the above, one may conclude that positively charged amino acid residues, which are the closest to the active enzyme centre, interact with negatively charged phosphate fragments of cleaved substrate, thus attracting them to the enzyme active centre. It would have been impossible

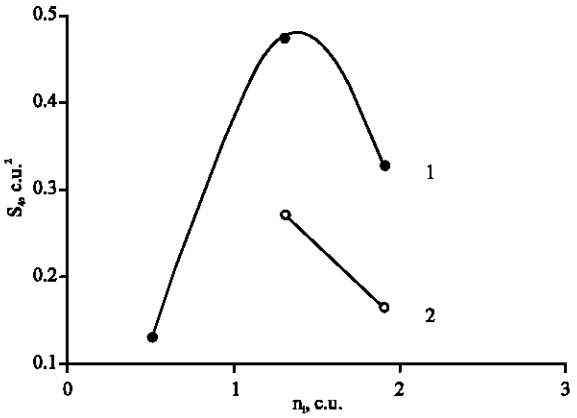

Fig. 1: Dependence of the overall inhibitory effect (S_4,c.u.^{2}) of positively charged amino acid residues on their position in the N-terminal peptide of the mutant forms of *E. coli* E15 alkaline phosphatase (Tables 1 and 2) in the four-dimensional ($K'_mV'E'_an_i$) coordinate system. Curve 1-(Lys, Lys) substitution, line 2-(Arg, Arg) substitution

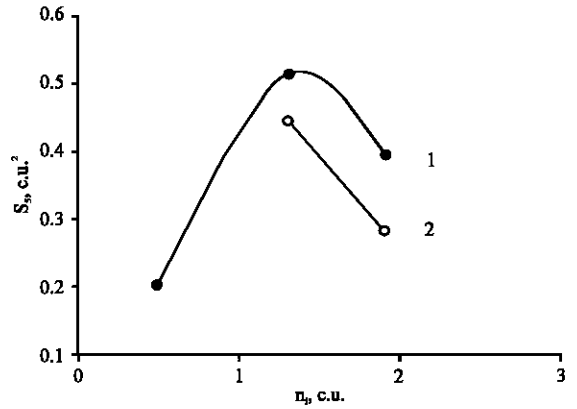

Fig. 2: Dependence of the overal inhibitory effect (S_5,c.u.^{2}) of positively charged amino acid residues on their position in the N-terminal peptide of the mutant forms of *E. coli* E15 alkaline phosphatase (Tables 3 and 4) in the five-dimensional ($K'_mV'E'_aA'n_i$) coordinate system. Curve 1-(Lys, Lys) substitution, line 2-(Arg, Arg) substitution

to make such a conclusion by the traditional (separate) data processing (Table 1).

Use of the four-dimensional ($K'_mV'E'_an_i$) coordinate system also fails to take into account the effect of another parameter, i. e., the specific activity of the studied mutant forms (A′, mE/mg protein of cell), which increased at positive charge transfer to the enzyme active centre (Table 1 and 2). Data processing that allows to take into

account a course of change in the specific activity of mutant phosphatases using the five-dimensional ($K'_mV'E'_aA'n_i$) coordinate system (Table 3, 4 and Fig. 2) shows that at increasing positive charge of amino acid residues (Lys→Arg) in the direction to the enzyme active center a total decrease in the enzyme activity coincides with the maximum inhibitory effect of substitutions in the same (+13+14) segment with a more appreciable effect. Thus, line 2 is located higher (Fig. 2) and has a larger slope angle to the abscissa axis than line 2 (Fig. 1).

The results of data processing demonstrate (Table 1-4 and Fig. 1, 2) that use of the four and five-dimensional coordinate systems permits to represent as a function (S_4 and S_5) the effect of positive charges on the catalytic activity of mutant phosphatases at charge transfer to the enzyme active centre.

Based on a separate analysis of a course of change in the individual parameters K'_m, V', E'_a and A' each differently changing due to the position of substitution (n_i) in the N-terminal peptides such assay would have been impossible.

REFERENCES

1. Krupyanko, V.I., 1990. A Vector Method of Representation of Enzymatic Reactions. Moscow. Nauka, pp: 3-142.
2. Krupyanko, V.I., 2004. A Vector Method of Representation of Enzymatic Reactions. 3. Three-dimensional system of the ($K'_mV'I$) coordinates convenient for representation of enzyme inhibition and activation. Process Biochem., 39: 825-832.
3. Krupyanko, V.I., 2005. Additional possibility of data analysis of enzyme inhibition and activation. 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.
4. Krupyanko, V.I., P.V. Krupyanko and I.N. Dorokhov, 2005. Additional possibility of data analysis of enzyme inhibition and activation. 2. Geometrical portraits of enzymatic reactions for data processing in enzyme inhibition. J. Biol. Sci., 5: 98-102.
5. Krupyanko, P.V., V.I. Krupyanko and I.N. Dorokhov, 2005. Additional possibility of data analysis of enzyme inhibition and activation. 3. Geometrical portraits of enzymatic reactions for data processing in enzyme temperature activation. J. Biol. Sci., 5: 98-102.
6. Kononova, S.V., C.N. Zolov, V.I. Krupyanko and M.A. Nesmeyanova, 2000. The primary structure of the N-terminal region of mature alkaline phosphatase is important for secretion and functioning of the enzyme. J. Applied Biochem. Microbiol., 65: 1270-1277.
7. Nesmeyanova, M.A., A.E. Kalinin, A.L. Karamyshev, N.I. Michaleva and V.I. Krupyanko, 1997. Overproduction, secretion, isolation and properties of recombinant alkaline phosphatase encoded in *Escherichia coli*. Process Biochem., 32: 1-7.
8. Krupyanko, V.I. and P.V. Krupyanko, 1999. Selection of substrate concentrations for determining the Michaelis constant and maximum rate of an enzymatic reaction. Applied Biochem. Microbiol., 35: 116-119.
9. Robert B. McComb, G.N. Bowers and S. Posen, 1979. Alkaline Phosphatase. New York, Plenum Press, pp: 5-986.
10. Lazdunski, C. and M. Lazdunski, 1966. Etude cinetigue du mecamsme d'action catalytigue de la phosphatase alcaline d'*Escherichia coli*. Biochim. Biophys. Acta, 113: 551-66.
11. Lazdunski, M., C. Petitclerc, D. Chappelet and C. Lazdunski, 1971. Flip-flop mechanisms in enzymology. A model: The alkaline phosphatase *Escherichia coli*. Eur. J. Biochem., 20: 124-139.
12. Garen, A. and C. Levinthal, 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. Biochim. Biophys. Acta, 38: 470-483.
13. Sowadzky, J.M., M.D. Handschumacher, H.M.K. Murthy, B.A. Foster and H.W. Wyckoff, 1985. Refined structure of alkaline phosphatase from *Escherichia coli* at 2.8 resolution. J. Mol. Biol., 186: 417-433.