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

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Abstract: Possible use of a vector method of representation of enzymatic reactions is considered in the four and five-dimensional \((K'_nV,V',A'_n)\) and \((K'_nV',A'_n)\) coordinate systems for data processing of a simultaneous course of change in four \(K'_n\) \(V\), \(V'\), \(A\), and five \(K'_n\) \(V\), \(E'_n\), \(A\), \(n\) parameters of inhibition of the mutant forms of \(E. coli\) 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 \((K'_nV,V',A'_n)\) and \((K'_nV',A'_n)\) coordinate systems 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 \((K'_nV',E'_n,A'_n)\) coordinate system, the four-dimensional \((K'_nV',E'_n,A'_n)\) coordinate system, the introduction of only one parameter \((E'_n)\) - which represents a course of change in the energy barriers of pNPP cleavage catalyzed by the mutant forms of \(E. coli\) E15 alkaline phosphatase distinguished in the position of charged (Lys, Lys or Arg, Arg) amino acid residues in N-terminal peptides - affects all the parameters \(K'_n\) \(V\), \(V'\), \(E'_n\). This leads to a complicated procedure in the determination of the \(S_4\) areas overlapped by \(L_4\) vectors in the four-dimensional \((K'_nV',E'_n,A'_n)\) 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:

\[
S_4 = \sqrt{(p-1)(p-l_{AB})(p-l_{AC})(p-l_{BC})} \quad (1)
\]

where, \(p\) - a half value of the triangle’s perimeter formed by the \(L_{AB}\), \(L_{AC}\) and \(L_{BC}\) vectors of the coordinate system, while \(l_{AB}\), \(l_{AC}\) and \(l_{BC}\) 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 \((K'_nV',E'_n,0)\) coordinate system to the point \(K'_n\) \(V\), \(E'_n\), \(n\) at the 1st substitution \((n_{AB})\) 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 in each peptide and the coordinates of the above vector point may be expressed as a function:

\[
l_{AC} = (K'_nV'_n)^2 + (V'_n-V)^2 + (E'_n-E)^2 + (n_{AB}U)^2 \quad (2)
\]

According to Table 1 and the adopted unification of the coordinates:

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where, \( K'_m \) - a value of the Michaelis effective constant determined at substitution of neutral amino acid residues by lysine (or arginine) ones, \( K'_m \) - the Michaelis constant of initial (unmodified \( n = 0 \)) enzyme form, \( V' \) - the maximum rate of pNPP cleavage catalyzed by modified alkaline phosphatase, \( V'_o \) - the reaction rate of pNPP cleavage catalyzed by the unmodified alkaline phosphatase. It is by analogy for \( E'_p \) and \( E'_o \) 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[2-3] 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 \( K'_m, V', E'_p, n _{i} \), and \( A' \) parameters dependent on \( n_{i} \) (the position of substitution) fails to give a picture of the 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.[8] and Nesmeyanova et al.[9]

**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[10] by increase in the absorption (+ΔAbs) 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 0.294×10−6 to 0.98×10−3 M, an interval of minimum error in the \( V \) in \( K'_m \) 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.).

\[
I_{o} = \frac{(1.0-0.3)^{2} + (2.03-2.85)^{2} + (0.63-0.74)^{2}}{(0.2-0.6)^{2}} - 1.84 \text{ c.u.}
\]

\[
3
\]

**Table 1:** A course of change in the \( K'_m, V, E'_p, I_{d} \) and \( S_{i} \) parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the four-dimensional \((K'_m, V, E'_o, I_{d}) \) coordinate system

<table>
<thead>
<tr>
<th>Enzyme, Lys, Arg substitution</th>
<th>n (_{i})</th>
<th>( K'_m ) (c.u.)</th>
<th>V (c.u.)</th>
<th>E'_p (c.u.)</th>
<th>I_{d} (c.u.)</th>
<th>S_{i} (c.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>0.0</td>
<td>0.30</td>
<td>2.85</td>
<td>0.63</td>
<td>1.40</td>
<td>1.10</td>
</tr>
<tr>
<td>n(+5+6)</td>
<td>0.2</td>
<td>1.60</td>
<td>2.03</td>
<td>0.74</td>
<td>1.102</td>
<td>0.129</td>
</tr>
<tr>
<td>n(+13+14)</td>
<td>1.3</td>
<td>1.35</td>
<td>1.45</td>
<td>0.83</td>
<td>2.189</td>
<td>0.476</td>
</tr>
<tr>
<td>n(+19+20)</td>
<td>1.9</td>
<td>1.47</td>
<td>1.12</td>
<td>0.86</td>
<td>2.833</td>
<td>0.328</td>
</tr>
</tbody>
</table>

**Table 2:** A course of change in the \( K'_m, V, E'_p, I_{d} \) and \( S_{i} \) parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the four-dimensional \((K'_m, V, E'_o, I_{d}) \) coordinate system

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**Table 3:** A course of change in the \( K'_m, V, E'_p, A', I_{d} \) and \( S_{i} \) parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the five-dimensional \((K'_m, V, E'_o, A', I_{d}) \) coordinate system

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<td>0.264</td>
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**Table 4:** A course of change in the \( K'_m, V, E'_p, A', I_{d} \) and \( S_{i} \) parameters of pNPP cleavage catalyzed by mutant forms of *E. coli* E15 alkaline phosphatase in the five-dimensional \((K'_m, V, E'_o, A', I_{d}) \) coordinate system

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**Enzyme activity assay:** The initial reaction rates (\( v_{i} \) and \( v_{j} \)) 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 \( (v_{1}^{-1}, S^{-1}) \) coordinates of Lineweaver-Burk using the computer programs Sigma Plot Version 2000 and Bureca (USA).

Root mean square deviation at five measurements was as follows: \( v_{1} = \pm 2.5\% \), \( K'_m \) and \( V' \) = \( \pm 7.5\% \), I and \( S_{i} = \pm 10\% \).

Energy barriers (\( E'_p \)) of catalyzed reactions were determined in the \( (\log V'_{1}/T) \) coordinates of Arrhenius[9-12] using the same computer programs.

For equiprobable taking into account all the parameters used in the experiment \( K'_m, V' \), \( E'_o \), \( A' \), and \( n_{i} \), they were unified to simple univalent conventional units (c.u.)[13-15] as follows: \( K'_m = 1 \times 10^{-5} \text{ M} = 1 \text{ c.u.} \).
V' = 1 μmol/min, μg protein = 1 c.u., E' = 1 Kcal/mol = 1 c.u. and N' = 100 mE/μg protein of cell = 0.1 c.u. The place of a substituted amino acid residues in the N-terminal peptide was expressed as n = 13 = 1.3 c.u.

Since two neutral amino acid residues were simultaneously substituted by positively charged ones (Lys, Arg 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 arginine 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.[3] The closer amino acid residues to the active enzyme centre the lesser their overall inhibitory effect on the enzyme determined by the S c.u. areas overlapped by four-dimensional Ln vectors in the (K'V'E'A)n 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 areas overlapped at shift of the mobile ends of Ln 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 to make such a conclusion by the traditional (separate) data processing (Table 1).

Fig. 1: Dependence of the overall inhibitory effect (S, c.u.) 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'nV'E'A) coordinate system. Curve 1-(Lys, Lys) substitution, line 2-(Arg, Arg) substitution.

Fig. 2: Dependence of the overall inhibitory effect (S, c.u.) 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'nV'E'A)n coordinate system. Curve 1-(Lys, Lys) substitution, line 2-(Arg, Arg) substitution.

Use of the four-dimensional (K'nV'E'A) 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 \((K'_e, V'_e, A'_e)\) coordinate system (Table 3, 4 and Fig. 2) shows that at increasing positive charge of amino acid residues (Lys\(+\rightarrow\)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. 2) that use of the four and five-dimensional coordinate systems permits to represent as a function (S, and S_r) 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'_e, V'_e, and A'_e each differently changing due to the position of substitution \((a_i)\) in the N-terminal peptides such assay would have been impossible.

REFERENCES


