



Journal of Biological Sciences

ISSN 1727-3048

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Additional Possibility of Data Analysis of Enzyme Inhibition and Activation

3. Geometrical Portraits of Enzymatic Reactions for Data Processing in Enzyme Temperature Activation

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Abstract: When calculating enzyme energy activation (E_a), researchers tend to only plot a temperature dependence of the course of change in the logarithm ($\lg V$) of maximum reaction rate upon ($1/T^\circ\text{K}$) and determine these thermodynamic parameters by the slope angle ($\text{tg } \alpha$) of experimental lines in the ($\lg V; 1/T$) coordinates of Arrhenius. Yet, the course of change in the Michaelis constant (K_m), the second important parameter of enzyme activation, is not taken into consideration as there is no this parameter in the Arrhenius equation. In practice, simultaneous account of temperature dependence in the course of change of both V and K_m parameters allows additional analysis of the dynamics of enzyme temperature activation. A vector method of representation of such data in the three-dimensional $K_m Vt$ coordinate system is proposed. Examples of data processing in temperature activation of bovine pyrimidine-specific RNases A and B using conventional methods and the proposed one are given.

Key words: RNase A, RNase B, energy activation, intensity activation, geometrical portrait of enzyme temperature activation

INTRODUCTION

Enzyme energy activation (E_a) is one of essential factors in the selection of protein catalysts for use in biotechnology and under laboratory conditions. Its values are also important for studying the mechanism of enzyme action. Thus, researchers who start working with enzymes have to determine these thermodynamic parameters^[1-17] When estimating E_a , they plot temperature dependencies of the course of change in maximum reaction rate V (or k_{cat}) and calculate E_a by respective graphs in the ($\lg V; 1/T$) coordinates of Arrhenius. Out of two equally important and simultaneously determined kinetic parameters of enzyme activation temperature dependence of the course of change in the Michaelis constant (K_m) is not taken into consideration, though this parameter also changes with temperature as V (Table 1 and Fig. 1-3). In practice, simultaneous account of the course of change V and K_m permits to obtain additional data on the dynamics of enzyme activation. Since there is no K_m parameter in the Arrhenius coordinates (Eq. 2), the dynamics of its temperature dependence is not presented in most pertinent publications^[1-17].

A vector method of representation of enzymatic reactions^[18,19] allows simultaneous account of the course of change in V and K_m parameters of enzyme temperature activation using the three-dimensional $K_m Vt$ coordinate system (Fig. 4 and 5). It also provides some new techniques for analysis of the mechanism of enzyme action by using such auxiliary parameters of enzyme temperature activation as:

- Intensity of enzyme temperature activation
- Overall effect of enzyme temperature activation
- A geometrical portrait of enzyme temperature activation.

This study presents data processing in enzyme temperature activation by taking as an example pyrimidine-specific Rnase A and RNase B.

MATERIALS AND METHODS

Chemicals: Bovine pyrimidine-specific RNase A (EC 3.1.27.5) and RNase B (EC 3.1.27.5). Substrate: cytidine-2',3'-monophosphate, sodium salt (C>p). The reagents are products of Sigma (USA).

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Table 1: Temperature dependencies of the kinetic parameters (K_m , 10^{-4} M and V , $\mu\text{mol}/\text{min } \mu\text{g-protein}$) of C>p cleavage catalyzed by RNase A and RNase B (l_i , c.u.), (S_i , c.u.²)

Temperature (°C)	RNase A				RNase B			
	K_m	V	l_i	S_i	K_m	V	l_i	S_i
15 (1.5 c.u.)	5.61 (5.61 c.u.)	7.32 (7.32 c.u.)	9.34		5,5	4.91	7.52	
20	4.59	8.33	9.72	6.997	5.23	6.07	8.26	4.17
25	3.87	8.43	9.61	4.224	6.27	7.80	10.32	1.41
30	3.53	8.87	10.01	3.096	6.06	8.87	11.15	4.60
35	3.14	9.44	10.55	3.390	6.38	10.09	12.44	2.53
37	3.75	10.87	12.08	1.844	6.72	11.49	13.82	3.11
				$\Sigma 19.56$				$\Sigma 16.44$

The course of substrate cleavage catalyzed by ribonucleases was recorded on a double-beam CF-4DR spectrophotometer (Optica Milano, Italy).

Reactions were carried out in 0.01 M MES buffer (pH 6.0) with ionic strength of 0.1 (by NaCl of high purity) at constant stirring. Dependences were plotted by increase in the absorption ($+\Delta A_{236}$) of a solution containing the substrate and the enzyme against a solution of the same composition, but without the enzyme.

The concentration (C>p) was changed within $9.8 \cdot 10^{-4}$ - $1.96 \cdot 10^{-4}$ M. Selection of the substrate was stipulated by an interval of minimum error in the determination of K_m and V parameters^[20,21]. The concentration of RNase A and RNase B was 1.48 and 1.32 $\mu\text{g mL}^{-1}$.

Determination of enzyme activity: The kinetic V and K_m parameters were calculated by plots in the ($v^{-1}; s^{-1}$) coordinates of Lineweaver-Burk by the computer program Sigma Plot Version 4.0 (USA).

The root-mean-square deviation at five measurements was as follows: $v = \pm 2.5\%$; K_m , V and $E_a = \pm 7.5\%$; l and $S_i = \pm 10\%$.

Enzyme energy activation (E_a) was estimated by plots in the ($\lg V; 1/T$) coordinates of Arrhenius (Fig. 3) using the same computer program.

In order to calculate the length of vectors in enzyme activation (Eq. 3) and S_i areas overlapped by L_i vectors (Eq. 4) at temperature increase (Fig. 4-6), it was necessary to unify the parameters by use of conventional units (c.u.). For this purpose, the following intervals were taken: $t = 10^\circ\text{C} = 1$ c.u.-on the 0t absciss, $K_m = 1 \cdot 10^{-4}$ M = 1 c.u.-on the 0 K_m ordinate and $V = 1 \cdot \mu\text{mol C>p}/\text{min} \cdot \mu\text{g protein} = 1$ c.u.-on the 0V applicate.

This allowed construction of spatial vectors of enzyme temperature activation in the three-dimensional $K_m V t$ coordinate system (Fig. 4 and 5) for determination of the length of L_i vectors and calculation of the S_i areas overlapped by L_i vectors at their shift due to temperature increase.

For example, to calculate the l vector length of RNase A activation at 15°C (Eq. 3), the following values must be expressed in conventional units (Table 1, line 1): $t = 1.5$ c.u., $K_m = 5.61$ c.u., $V = 7.32$ c.u. (given in brackets), from where: $l_{15} = 9.34$ c.u. (Fig. 4 and 6). Accordingly, to l_n in order to calculate the S_i area at shift of the L_{15} vector for RNase A temperature activation to the position L_{20} , the first two lines in Table 1 are used.

RESULTS AND DISCUSSION

Conventional data analysis in enzyme temperature activation: The maximum reaction rate (V) of cytidine-2',3'-monophosphate cleavage catalyzed by both RNase A and RNase B increased in the whole temperature interval, not chieving the denaturation range ($>50^\circ\text{C}$) (Fig. 1-3 and Table 1).

For data processing, temperature dependencies of the course of change in the logarithm of maximum reaction rate of substrate hydrolysis were plotted in the ($\lg V; 1/T$) coordinates of Arrhenius by the slope angle ($\text{tg } \alpha$):

$$E_a = 2.303R \text{ tg } \alpha \quad (1)$$

of experimental lines (Fig. 3):

$$\lg V = \frac{E_a}{2,303 \cdot R} \cdot \frac{1}{T} + A, \quad (2)$$

where, R -the gas constant $1.987 \text{ cal mol}^{-1}$, T -temperature by the Kelvin scale ($^\circ\text{K}$), A - constant of integration. It was established that E_a was 5.63 and 6.41 Kcal mol^{-1} for RNase A and RNase B, respectively, which is in good accord with data on other ribonucleases^[22].

The main difference between the enzymes is as follows: RNase B has a carbohydrate residue comprising six molecules of mannose, two molecules of acetylglucosamine and a peptide of four amino acids^[23]. These enzymes are identical in other respects and RNase B can be considered as a glycoprotein analog of RNase A.

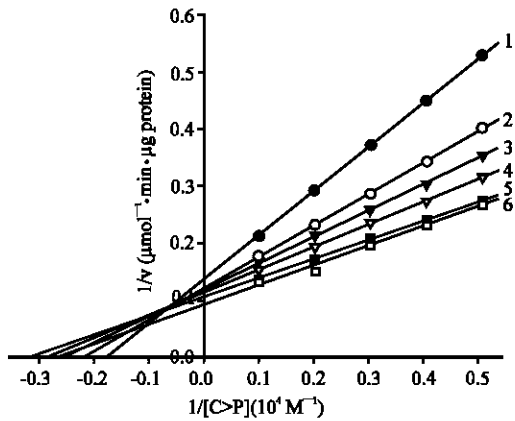


Fig. 1: Temperature dependencies of initial reaction rate (v) of C>p cleavage catalyzed by RNase A in the (v^{-1} ; S^{-1}) coordinates of Lineweaver-Burk. Note: line 1-experimental data obtained at 15°C, line 2-20°C, line 3-25°C, line 4-30°C; line 5-35°C and line 6-37°C

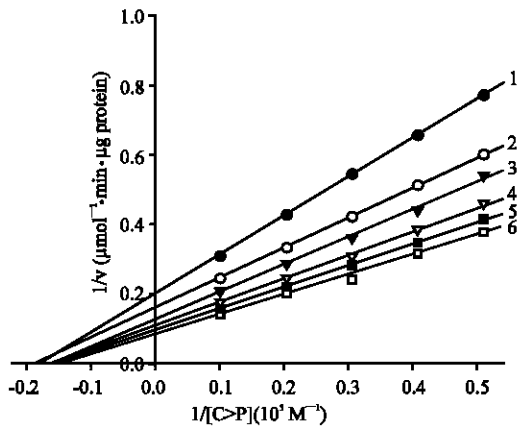


Fig. 2: Temperature dependencies of initial reaction rate (v) of C>p cleavage catalyzed by RNase B in the (v^{-1} ; S^{-1}) coordinates of Lineweaver-Burk. Note: line 1-experimental data obtained at 15°C, line 2-20°C, line 3-25°C, line 4-30°C; line 5-35°C and line 6-37°C

Glycoprotein has a molecular mass of 1350 Da and is strongly bound to the polypeptide chain of RNase B. A weaker of binding of RNase B to the substrate than of RNase A to the same substrate ($K_{m(B)} > K_{m(A)}$), accompanied by decrease in the maximum reaction rate ($V_{(B)} < V_{(A)}$) and increase of the energetic boundary of catalyzed reaction ($E_{a(B)} > E_{a(A)}$) can be explained by the presence of glycoprotein in the molecule of RNase B.

It is difficult to get information about the dynamics of enzyme temperature activation by conventional data processing of the course of change in the kinetic K_m and V parameters for both ribonucleases: researcher wants to

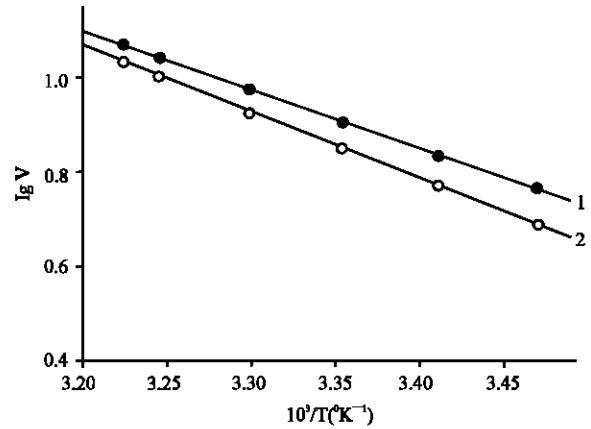


Fig. 3: Logarithmic dependence of maximum reaction rate ($\lg V$) of C>p cleavage catalyzed by RNase A (line 1) and RNase B (line 2) on reverse temperature ($1/T$, °K)

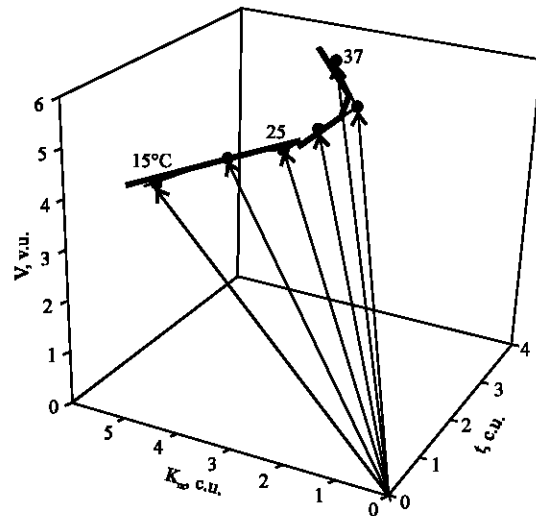


Fig. 4: Temperature dependence of the course of change in the position of L_t vectors of RNase A activation in the $K_m V t$ coordinate system. Note: the L_t vectors are indicated with by thin lines, the mobile end of L_t vectors of enzyme temperature activation-with a broken curve, the smoothed (characteristic) curve of the dynamics in enzyme temperature activation is marked with a thick line

know if the presence of the glycoprotein complex in the molecule of RNase B has a stabilizing effect on that enzyme or not and in what temperature interval and also if the mechanism of enzyme activation remains stable at temperature increase or not.

Additional analysis: Data analysis reveals that simultaneous account of the course of change in V and K_m

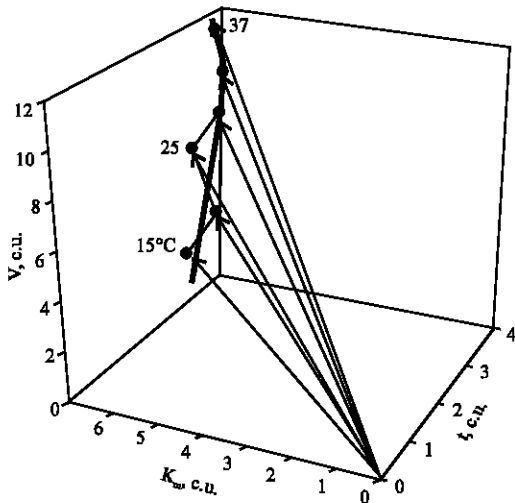


Fig. 5: Vector method of data representation of RNase B temperature activation in the \$K_m V_t\$ coordinate system. Note: other designations are as in Fig. 4

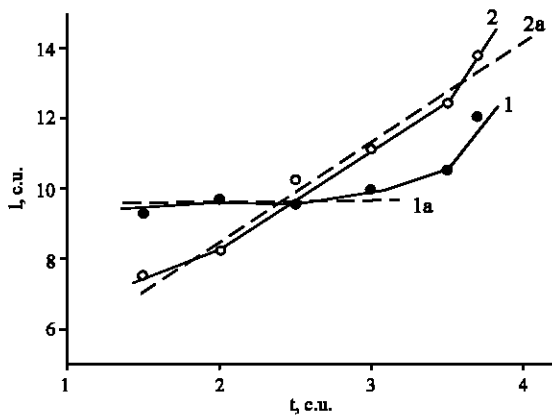


Fig. 6: Temperature dependence of the vector length of RNase A (line 1) and RNase B (line 2) activation. Note: the intervals of stability in the dynamics of enzyme temperature activation are marked with straight lines (1a and 2a)

parameters permits to answer some of the questions. For this purpose, a vector method of data representation on enzyme temperature activation in the three-dimensional \$K_m V_t\$ coordinate system (Fig. 4 and 5) is used, where each particular temperature °C as well as \$V\$ and \$K_m\$ kinetic parameter has its own vector representation of enzyme activity strictly definite by the length and position in the coordinate system—a concrete three-dimensional \$L_t\$ vector of enzyme temperature activation. Thus, \$l\$ – the length of such vector:

$$l = \sqrt{(V - 0)^2 + (K_m - 0)^2 + (t - 0)^2} \quad (3)$$

will characterize intensity of enzyme temperature activation, the area \$S_t\$ (c.u.)² overlapped by the vector due to temperature increase:

$$S_t = 0,5 \cdot \left(\left| \begin{matrix} K_{m1} & V_1 \\ K_{m2} & V_2 \end{matrix} \right|^2 + \left| \begin{matrix} V_1 & t_1 \\ V_2 & t_2 \end{matrix} \right|^2 + \left| \begin{matrix} t_1 & K_{m1} \\ t_2 & K_{m2} \end{matrix} \right|^2 \right)^{0,5} \quad (4)$$

will be a measure of overall effect of enzyme temperature activation, the trajectory made by the mobile end of \$L_t\$ vector will be a characteristic curve which is individual for each enzyme. On the whole, all these will represent a geometrical portrait of enzyme temperature activation. Such portraits demonstrate the rectangular segments characterizing stability of the mechanism of enzyme temperature activation are found only in the initial temperature interval (15-25°C) in the case of RNase A (Fig. 4), while in the case of RNase B constant strengthening of enzyme activation is observed in the whole temperature interval (Fig. 6, line 2a) - glycoprotein stabilizes the temperature of RNase B activation. This is also confirmed by the auxiliary (\$l_t\$ and \$S_t\$) parameters of enzyme temperature activation.

Linearity of plots representing change in the length of \$L_t\$ vectors of temperature activation by RNase A can only be found in the initial temperature interval (15-25°C), in the case of RNase B it retains within the whole temperature range (Fig. 6, dotted lines 1a and 2a). Besides, in the 1st case the length of \$L_t\$ vectors actually remains the same, while in the 2nd case (RNase B) it proportionally increases.

A comparative study of the \$S_t\$ areas overlapped by \$L_t\$ vectors in enzyme activation indicates that the overall effect of RNase A temperature activation proved higher (19.56/16.44 = 1.2) than of RNase B (Table 1, Fig. 4 and 5).

It may conclude that in biotechnology and under laboratory conditions at higher temperatures the use of RNase B is preferable than of RNase A in all respects.

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