Effect of temperature on the activity of AMP-deaminase from rat heart

K. Kaletha, A. Skladanowski and St. Bogdanowicz*

Department of Biochemistry and Department of Physiology, Medical School, ul. Debinki 1, PL-80-211 Gdańsk (Poland),*
25 September 1978

Summary. In the presence of 1 mM ATP, the plots of Michaelis dependence of the reaction catalysed by AMP-deaminase from rat heart were hyperbolic at all temperatures between 10 and 40 °C. Calculation of the energy of activation for ATP-activated enzyme and of the enzyme-substrate complex formation is presented.

AMP-deaminase (E.C. 3.5.4.6) exists in multiple molecular forms in different rat tissues[1-4]. There are 3 parental, homogenic forms of the enzyme, and only one of them (type C) was detected in the heart muscle tissue of the rat[5]. The rat heart AMP-deaminase is an oligomeric, probably tetrameric enzyme protein under allosteric control of few low molecular metabolites, particularly of potassium and sodium ions, ATP and ADP. In the absence of these nucleotides and ions, the kinetics of adenylic acid deamination exhibit a sigmoid-shaped profile which changes to hyperbolic when ATP, ADP or sodium are present in the incubation mixture[4,6]. A sigmoid-shaped curve on the Michaelis plot was still observed, however, when the reaction was carried out in the presence even high concentrations of KCl alone, both with rat[6], beef[7] and pig[8] heart AMP-deaminase.

It has been found recently, for the skeletal muscle enzyme, that the extent of modifying effect exerted by the effectors of the activity of AMP-deaminase also depends on temperature[9]. No information is available about the influence of temperature on the kinetic properties of AMP-deaminase isolated from heart tissue in the presence and in the absence of ATP - the main effector of heart enzyme. Investigation of the effect of temperature on $S_{0.5}$ and V_{max} parameters, as well as calculation of activation energy for this catalytical process, is the main aim of the present paper.

Materials and methods. AMP-deaminase was prepared from rat hearts by the procedure described by Smiley et al.[10]. The enzyme adsorbed on phosphocellulose column was eluted with 1 M KCl solution, pH 7, containing 1 mM mercaptoethanol. The enzyme preparation obtained was purified about 80 times and its sp. act. was 0.8 μmoles/min/mg of protein as estimated at 6 mM concentration of the substrate and 30 °C.

The incubation medium in the final volume of 0.5 ml contained 0.1 M succinate-KOH buffer, pH 6.5, 100 mM KCl and different concentration of the substrate (AMP) and effector (ATP) as indicated in figure 1 and the table. After equilibration of temperature, 15 μl of appropriately diluted enzyme, containing 21.9 μg of the enzyme protein was added to start the reaction. The incubation was carried out for 10 min at 4 different temperatures and the initial velocity of the reaction was determined from the mean amount of ammonia estimated by the phenol-hypochlorite method according to Chaney and Marbach[11]. 2 parallel incubations were carried out.

To obtain the necessary measure of precision, the statistical method of Wilkinson[12] was used to calculate the substrate concentration giving the half-maximum velocity ($S_{0.5}$) and maximum velocity of the reaction (V_{max}).

In the absence of ATP, the kinetics of AMP deamination was sigmoid-shaped and the method of Endrenyi et al.[13] for calculation of maximum velocity of the reaction was used. On plotting log $[v_0/(V_{max}-v_0)]$ versus log [S], the concentration of the substrate at which log $[v_0/(V_{max}-v_0)]=0$ allows us to determine the $S_{0.5}$ value.

Results and discussion. Figure 1 presents Michaelis plots of the reaction catalyzed by rat heart muscle AMP-deaminase

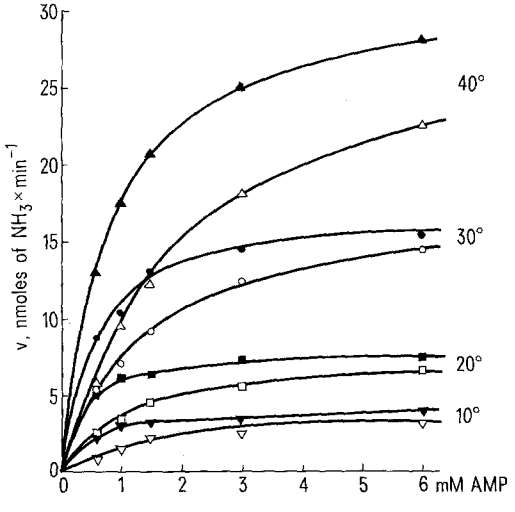

Fig. 1. The dependence on temperature of the reaction catalyzed by purified rat heart AMP-deaminase in the absence (open symbols) and in the presence (closed symbols) of 1 mM ATP. For experimental conditions see text.

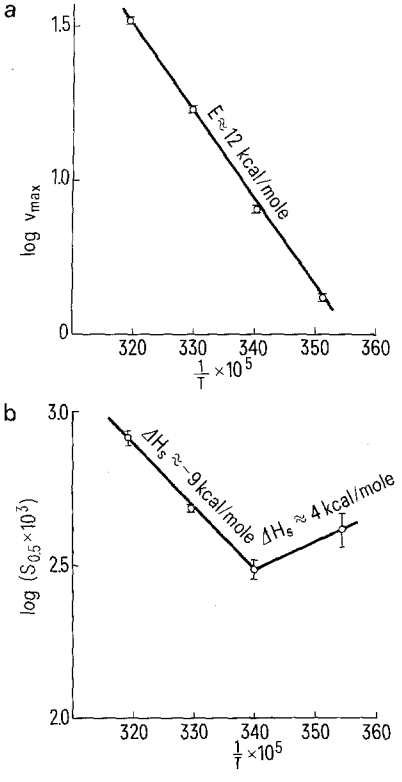

Fig. 2. Arrhenius plot (a) and the dependence of log $S_{0.5}$ on reciprocal of temperature (b) for the reaction catalyzed by 1 mM ATP-activated rat heart AMP-deaminase. The straight lines were fitted by the method of linear regression. The bars represent SD.

at 4 different temperatures in the presence and in the absence of 1 mM ATP. As may be seen from the figure, the shape of the plots depended in a visible way on the temperature and on the composition of the incubation mixture. In the presence of 1 mM ATP, the plots were hyperbolic at all temperatures tested. In the absence of ATP, the reaction velocities were much lower, particularly in the region of low substrate concentration. The calculated value of cooperativity coefficient was about 1.3 for temperature 30 °C and did not change significantly when the temperature was changed. It may be also noticed from this figure that the range of the substrate concentration in which the activatory effect of ATP was most distinct ('the regulatory region' of the enzyme activity) spreads with the increase of temperature. The table presents calculated values of the substrate concentrations giving the half-maximum velocity ($S_{0.5}$) and maximum velocities (V_{max}) of the reaction catalyzed by rat heart muscle AMP-deaminase for all temperatures tested. As may be seen from this table, both in the presence and in the absence of ATP the increase of temperature caused a rise in V_{max}. The presence of 1 mM ATP did not influence these values significantly. This fact confirms our earlier suggestions that the rat heart AMP-deaminase displays the K_m-type allosteric regulation. On the other hand, at all temperatures tested the values of $S_{0.5}$ calculated for ATP-activated enzyme were much (3–4 times) lower than these calculated for unactivated enzyme. Under both conditions, the $S_{0.5}$ values were lowest at 20 °C and changed only little in the region of 10–30 °C. The rise of temperature from 30 to 40 °C caused a distinct increase of $S_{0.5}$-value for the reaction carried out in the absence of ATP.

The dependence of $S_{0.5}$ and V_{max} on temperature for the reaction catalyzed by purified rat heart AMP-deaminase

Effector added	Temperature (°C)	$S_{0.5}$ (mM)	V_{max} (nmoles/min)
None	10	1.79 (0.30)*	3.69 (0.38)*
	20	1.55 (0.21)*	7.70 (0.53)*
	30	2.85 (0.08)*	17.19 (0.26)*
	40	3.14 (0.28)*	29.75 (0.72)*
1 mM ATP	10	0.42 (0.09)	4.23 (0.21)
	20	0.31 (0.03)	8.12 (0.40)
	30	0.49 (0.01)	17.12 (0.42)
	40	0.86 (0.05)	32.92 (0.66)

The values in the brackets represent SEM or SD*.

Figure 2a presents the Arrhenius plot for the reaction examined in the presence of 1 mM ATP. The value of activation energy (E) calculated from the slope of this dependence being as high as 12140 ± 100 cal/mole of substrate represents the energy which is required for activation of the enzyme-substrate complex[14]. The value of the heat of formation for such complex (ΔH_s) may be calculated from the slopes of the dependence presented in figure 2b. As may be seen from the figure, the line of this dependence is biphasic. If one assumes that $S_{0.5}$ for the ATP-activated reaction represents true thermodynamic dissociation constant K_s, the value of 4280 ± 800 and -9450 ± 300 cal/mole may be obtained from this plot for temperatures below and above 20 °C respectively. This means that at lower temperatures the heat is absorbed during the formation of enzyme-substrate complex, whereas at higher temperatures (above 20 °C) the formation of this complex is evolving the heat[14]. One possible explanation of this peculiarity may be that there are 2 different forms of the enzyme which differs in their thermodynamic properties[15].

1 N. Ogasawara, M. Yoshino and Y. Kawamura, Biochim. biophys. Acta *258*, 650 (1972).
2 N. Ogasawara, H. Goto, T. Watanabe, Y. Kawamura and M. Yoshino, FEBS Lett. *44*, 63 (1974).
3 N. Ogasawara, H. Goto and T. Watanabe, FEBS Lett. *58*, 245 (1975).
4 N. Ogasawara, H. Goto and T. Watanabe, Biochim. biophys. Acta *403*, 530 (1975).
5 N. Ogasawara, H. Goto and T. Watanabe, in: Purine Metabolism in Man, p. 212. Ed. M.M. Muller, E. Kaiser and J.E. Seegmiller. Plenum Publishing Corporation, New York 1977.
6 K. Kaletha and A. Składanowski, Biochim. biophys. Acta, in press.
7 A. Składanowski, K. Kaletha and M. Żydowo, Int. J. Biochem. *10*, 177 (1979).
8 J. Purzycka-Preiss, E. Prus, M. Woźniak and M. Żydowo, Biochem. J. *175*, 607 (1978).
9 K. Kaletha, J. Thermal Biol. *1*, 157 (1976).
10 K.L. Smiley, A.J. Berry and C.H. Suelter, J. biol. Chem. *242*, 2502 (1967).
11 A.L. Chaney and E.P. Marbach, Clin. Chem. *8*, 130 (1962).
12 B.N. Wilkinson, Biochem. J. *80*, 324 (1961).
13 L. Endrenyi, C. Fajszi and F.H.F. Kwong, Eur. J. Biochem. *51*, 317 (1975).
14 W. Bladergroen, Einführung in die Energetik und Kinetik biologischer Vorgänge, p. 219. Wepf, Basel 1955.
15 M. Dixon and E.C. Webb, Enzymes, p. 150. Longman, London 1971.

Recovery of renal lactate dehydrogenase (LDH) isoenzyme pattern after obstruction relief in experimental hydronephrosis[1]

G. Emanuelli, G. Anfossi, G. Camussi, G. Calcamuggi, G. Cestonaro and G. Gatti

Institute of Internal Medicine, University of Turin, Corso Polonia 14, I-10100 Torino (Italy), 13 September 1978

Summary. The release of ureteral occlusion leads to a progressive recovery in LDH isoenzyme pattern with gradual increase of anodic fractions and decrease of middle and cathodic ones. Our findings demonstrate that the recovery is accomplished on the 10–14th day, in agreement with morphological and metabolic observation.

Histochemical and biochemical investigations performed in obstructive nephropathy have demonstrated several alterations in kidney enzyme content[2–4]. In particular, significant changes of renal lactate dehydrogenase (LDH) isoenzymes, as characterized by an increase in middle and cathodic fractions, have been demonstrated after experimental ureteral obstruction[5–7]. Such metabolic alterations have been related both to renal hypoxia and to the presence of a less differentiated cell population as they occur in obstructive nephropathy[5,6].