Effect of temperature on the activity of AMP deaminase from chicken heart and skeletal muscle at different stages of development

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Summary. The effect of temperature on purified 1-day-old chicken and adult hen heart muscle AMP deaminase was studied and compared to previous studies on this enzyme from skeletal muscle. The temperature-induced changes in the kinetic parameters of the reaction were shown to be different at these 2 stages of development. This suggests the possibility of developmental changes in the isozymic pattern of AMP deaminase in the heart tissue as has already been shown for skeletal muscle.

The studies of Ogasawara et al.[2] on the rat demonstrated that AMP deaminase exists in multiple molecular forms in different tissues, which differ from one another with respect to their chromatographic, kinetic and immunological properties. The forms of this enzyme found in skeletal muscle, liver and cardiac muscle have been designated as isozymes A, B and C respectively. In addition to these Raggi et al.[3] have further fractionated rat and rabbit skeletal muscle AMP deaminase into 2 forms which differ in both their chromatographic and kinetic properties.

The kinetic properties of skeletal muscle AMP deaminase have been examined in several laboratories and there is general agreement that the activity of the enzyme is sensitive to a number of low molecular weight metabolites. Potassium ion is the most potent activator and inorganic phosphate a potent inhibitor of the enzyme[4–7].

Heart muscle AMP deaminase has been less extensively studied. The most important allosteric effectors of the heart enzyme are sodium ions, ATP, ADP and orthophosphate[8–10]

The influence of temperature on the activity of AMP deaminase from skeletal and heart muscle of several animal species has been investigated previously in our laboratory[11–13]. For chicken and rat skeletal muscle enzyme, developmental differences in thermal sensitivity have been observed[14,15], suggesting the possibility of a change in the isozyme pattern during development. Recently Sammons and Chilson[16] proved the isozymic nature of chicken skeletal muscle AMP deaminase and characterized the kind of developmental changes. The changes in thermal sensitivity of heart muscle AMP deaminase during chicken development described in this paper suggest that an isozymic transition may also occur in this tissue.

Materials and methods. Materials. White Leghorn fertilized eggs, chickens and hens were used for experiments. Eggs were received on the 12th day of their development and further incubation took place in the laboratory. Hens were bought from commercial sources.

Purification of the enzyme. AMP deaminase was prepared from leg muscles and heart by chromatography on

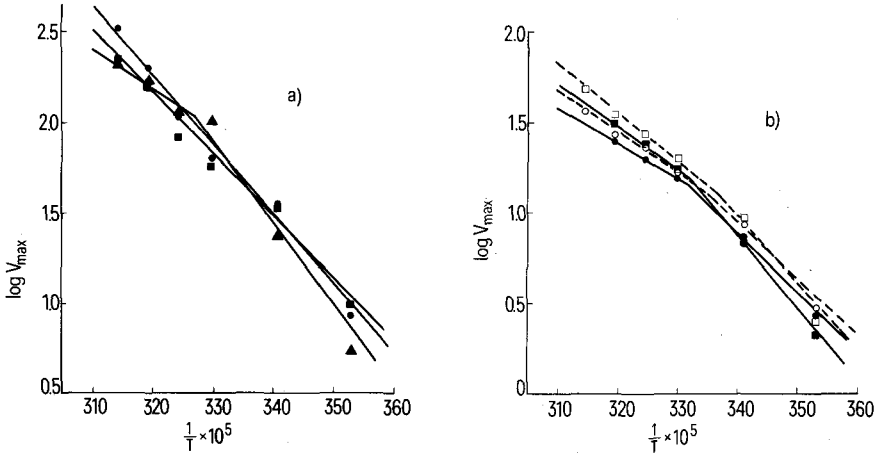

Fig. 1. Arrhenius plot for the reaction catalyzed by *a* 14 day embryo (▲), 1 day chicken (■) and adult hen (●) skeletal muscle AMP deaminase and *b* 1 day chicken (■) or adult hen (●) heart muscle AMP deaminase. Dotted lines represent Arrhenius plots for the reaction catalyzed by 1 mM ATP-activated heart muscle enzyme. The straight lines were fitted by the method of linear regression.

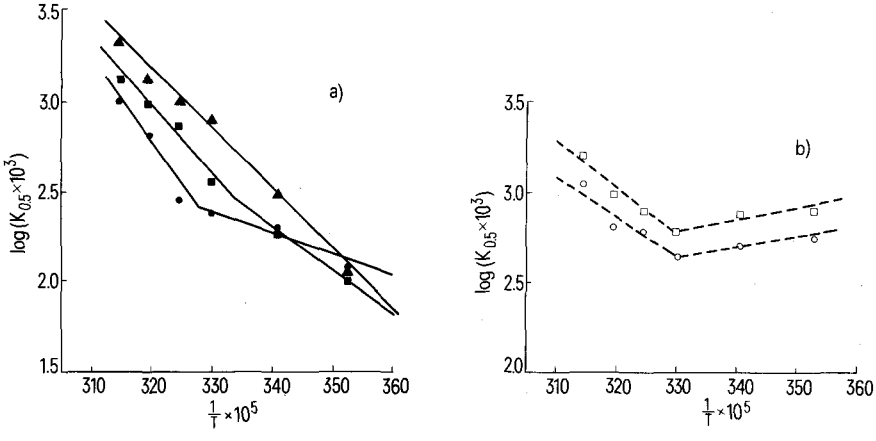

Fig. 2. The dependence of log $K_{0.5}$ on reciprocal of temperature for the reaction catalyzed by *a* 14 day embryo (▲). 1 day chicken (■) and adult hen (●) skeletal muscle AMP deaminase and *b* 1 day chicken (□) or adult hen (○) 1 mM ATP-activated heart muscle enzyme. The straight lines were fitted by the method of linear regression.

phosphocellulose essentially according to the procedure of Smiley et al.[17]. While preparing the enzyme from embryonic skeletal muscle and 1-day-old chicken heart, a gradient up to 2 M KCl was necessary to elute the enzyme from the column. About 200 embryos or 100 1-day-old chickens were routinely used for one preparative run. The specific activity of the heart specimen was 2.4 and 0.5 µmoles of ammonia liberated per min at 6 mM substrate concentration for adult hen and 1-day-old chicken respectively. The specific activity of appropriate skeletal muscle enzyme preparations was measured at 60 µM substrate concentration and was 100-250-fold higher[14].

Enzyme assay. Skeletal muscle AMP deaminase activity was determined spectrophotometrically with the aid of a Unicam SP-800 spectrophotometer fitted with a constant temperature cell housing, by measuring the changes of absorbancy at 265 and 285 nm. The initial velocity of the reaction expressed in µmoles of AMP decomposed per minute was calculated from the initial, linear part of the curve lasting no longer than 1 min, using the molar coefficients determined by Smiley and Suelter[5]. For the initial velocity calculations, suggestions of Lee and Wilson[18] were taken into account.

When the enzyme from heart was investigated, the incubation was carried out for 10 min and the ammonia formed was estimated by the phenol-hypochlorite method[19]. Three parallel incubations were carried out routinely.

The incubation mixture in a final volume of 3 ml (for skeletal muscle enzyme) or 0.5 ml (for heart enzyme) contained 0.1 M K-succinate buffer (pH 6.6 for skeletal muscle enzyme or 6.5 for heart enzyme), 0.1 M KCl and different concentrations of AMP. After equilibration of temperature, 10-20 µl of appropriately diluted enzyme was added to the incubation mixture to start the reaction.

Calculation of kinetic parameters. When the reaction exhibited hyperbolic kinetics, the statistical method of Wilkinson[20] was used to calculate the ligand concentration giving half-maximum velocity ($K_{0.5}$) and maximum velocity (V_{max}). When kinetics of the reaction showed a sigmoid-shaped profile (heart muscle AMP deaminase in the absence of ATP), the method of Endrenyi et al.[21], based on linearization of the Hill equation, was used to calculate these parameters.

Results and discussion. Table 1 presents the values of $K_{0.5}$ and V_{max} calculated for AMP deaminase from chicken

Table 1. The dependence of $K_{0.5}$ and V_{max} on temperature for the reaction catalyzed by chicken skeletal muscle AMP deaminase at 3 different stages of development

Temperature °C	14-day embryo $K_{0.5}$ (mM)	14-day embryo V_{max} (µM/min)	1-day chicken $K_{0.5}$ (mM)	1-day chicken V_{max} (µM/min)	Adult hen $K_{0.5}$ (mM)	Adult hen V_{max} (µM/min)
10	0.11	5.51	0.10	10.02	0.12	9.81
	(0.01)	(0.97)	(0.02)	(2.55)	(0.01)	(2.08)
20	0.30	23.58	0.18	33.87	0.20	35.94
	(0.05)	(2.67)	(0.03)	(4.09)	(0.01)	(8.06)
30	0.80	104.62	0.35	55.86	0.24	63.25
	(0.11)	(10.56)	(0.11)	(7.84)	(0.04)	(10.38)
35	0.98	131.81	0.73	90.16	0.28	107.58
	(0.04)	(11.12)	(0.05)	(3.61)	(0.03)	(12.20)
40	1.31	173.47	0.94	157.80	0.65	206.08
	(0.07)	(8.19)	(0.14)	(6.68)	(0.07)	(13.25)
45	2.08	212.41	1.28	245.67	1.00	333.67
	(0.12)	(27.21)	(0.10)	(37.56)	(0.17)	(59.38)

Values in the brackets represent SD.

Table 2. The dependence of $K_{0.5}$ and V_{max} on temperature for the reaction catalyzed by chicken heart muscle AMP deaminase at 2 different stages of development

Effectors added	Temperature °C	1-day chicken $K_{0.5}$ (mM)	1-day chicken V_{max} (nM/min)	Adult hen $K_{0.5}$ (mM)	Adult hen V_{max} (nM/min)
None	10	1.85	2.15	2.36	2.73
		(0.25)	(0.37)	(0.35)	(0.29)
	20	2.32	6.94	2.31	7.39
		(0.24)	(0.72)	(0.21)	(1.12)
	30	2.52	17.53	2.37	15.68
		(0.29)	(1.57)	(0.22)	(1.22)
	35	2.82	23.39	2.72	19.88
		(0.15)	(1.87)	(0.35)	(2.02)
	40	3.74	31.11	3.22	24.72
		(0.29)	(1.89)	(0.36)	(1.62)
ATP (1 mM)	10	0.79	2.59	0.56	3.08
		(0.16)	(0.18)	(0.14)	(0.21)
	20	0.73	9.52	0.51	8.83
		(0.09)	(0.11)	(0.10)	(0.38)
	30	0.60	19.97	0.44	16.81
		(0.03)	(0.20)	(0.10)	(0.78)
	35	0.78	27.37	0.61	23.20
		(0.13)	(1.10)	(0.08)	(0.75)
	40	0.96	34.75	0.64	27.17
		(0.10)	(1.04)	(0.09)	(0.99)
	45	1.60	47.81	1.12	36.57
		(0.29)	(2.69)	(0.08)	(0.75)

Values in the brackets represent SD.

Table 3. Values of energy of activation (E_a) and enthalpy of the enzyme-substrate complex formation (ΔH_s) for the reaction catalyzed by chicken skeletal and heart muscle AMP deaminase at different stages of development

Stage of development	Source of enzyme		E_a (kcal/mole)		ΔH_s (kcal/mole)
14-day embryo	Skeletal muscle	a)	22.07 (2.17)		
		b)	9.81 (1.60)		−14.72 (1.05)
1-day chicken	Skeletal muscle		15.45 (1.98)	a)	−10.67 (1.23)
				b)	−15.92 (2.24)
	Heart muscle	a)	17.91 (0.93)		-
		a)*	17.46 (0.31)	a)*	2.33 (0.39)
		b)	13.77 (1.54)		-
		b)*	11.78 (0.97)	b)*	−12.04 (1.52)
Adult hen	Skeletal muscle		17.87 (0.35)	a)	− 5.60 (0.86)
				b)	−19.59 (2.35)
	Heart muscle	a)	14.92 (0.95)		-
		a)*	14.50 (0.49)	a)*	2.05 (0.32)
		b)	11.12 (0.74)		-
		b)*	10.25 (0.19)	b)*	−10.89 (1.25)

For biphasic type of temperature dependence on figures 1 and 2, the values of these parameters were calculated for lower (a) and upper (b) range of temperatures. * Values in the presence of 1 mM ATP. Values in the brackets represent SD.

skeletal muscle at 3 different stages of development. It is apparent from this table that the influence of temperature on the maximum velocity is different for embryo, 1-day-old chicken and adult hen muscle enzymes. In contrast to the chicken and hen, the maximum velocity of the reaction catalyzed by embryo skeletal muscle AMP deaminase rises sharply up to 30 °C but the further increase of this parameter is diminished at higher temperatures. Also the value of $K_{0.5}$ rises with temperature in a way dependent on the kind of tissue from which the enzyme was extracted. As has been found previously[14], in contrast to the enzyme from embryo tissue, the $K_{0.5}$-value for the enzyme extracted from adult hen muscle does not change significantly with temperature between 10 and 35 °C, but increases sharply at higher temperatures.

The influence of temperature on $K_{0.5}$ and V_{max} for the reaction catalyzed by 1-day-old chicken and adult hen heart AMP deaminase is presented in table 2. It may be seen from this table that the presence of 1 mM ATP in the reaction medium causes a distinct decrease in the $K_{0.5}$-value, but has negligible effect on the maximum velocity of the reaction. At each temperature tested, the values of V_{max} in the absence of ATP were only slightly smaller, in comparison to those calculated for the reaction performed in the presence of this nucleotide. However, in the absence of ATP, enzyme-protein denaturation was observed after 5–6 min of the course of the reaction. The plot $v \times t$ (time) vs time (not shown here) was not a straight line after this time under these conditions. The values of $K_{0.5}$, especially for the reaction catalyzed by hen heart enzyme, do not change significantly with temperature between 10 and 30–35 °C either in the presence or absence of ATP; however, they rise significantly with a further increase of temperature.

Figures 1, a and b present Arrhenius plots for skeletal (a) and heart (b) muscle AMP deaminase. As may be seen from these figures the plot is biphasic for embryonic skeletal muscle and for 1-day-old chicken and adult hen heart muscle enzymes. There is a visible break in the plot at temperatures of about 30 °C and 35 °C for heart and skeletal muscle enzymes respectively. The presence of ATP in the reaction mixtures of heart AMP deaminase had no effect on the shape of this plot.

Also in the plot of log $K_{0.5}$ vs temperature (figure 2,b), the biphasic type of temperature dependence, with a break at a temperature of about 30 °C, is observed for heart AMP deaminase. For embryo skeletal muscle enzyme, a straight line dependence in this plot is observed over the entire range of temperatures investigated, but it changes to a biphasic line during the development of chicken (figure 2,a). The break point which is only visible at a temperature of about 25 °C for the enzyme extracted from the muscle of 1-day-old chicken, is quite distinct for the muscle enzyme from adult hen at a temperature of 35 °C (figure 2,a).

The values of activation energy (E_a) - the energy required for activation of the enzyme-substrate complex, calculated from the slopes of appropriate tangents in figure 1, a and b, are presented in table 3. It may be seen from this table that there is a visible change in the value of E_a during the development of the chicken. For a physiological range of temperatures (35–40 °C) the calculated value of this parameter is as high as about 10 kcal/mole for embryo skeletal muscle and is increased to 18 kcal/mole for adult hen skeletal muscle enzyme. In spite of this, the value of the activation energy for heart muscle AMP deaminase seems to decrease during the development of the chicken; the value of E_a, which is as high as 14 kcal/mole of substrate for 1-day-old chicken AMP deaminase, decreases to the value about 11 kcal/mole for adult hen heart enzyme in the physiological range of temperatures.

Assuming that $K_{0.5}$ for AMP deaminase catalyzed reaction represents the true dissociation constant (K_s) the values of heat energy change during formation of the enzyme-substrate complex (ΔH_s) were calculated from the slopes of the lines in figure 2, a and b[22]. As may be seen from table 3, at the range of physiological temperatures both the skeletal and heart muscle AMP deaminase shows negative values of ΔH_s parameter, which means that at temperatures above 30 °C heat is evolved in the formation of the enzyme-substrate complex. In contrast to the skeletal muscle enzyme, however, at lower temperatures the enthalpy of binding of the substrate to heart enzyme is a slightly endothermic process.

The fact that a single enzyme could show a biphasic temperature dependence was originally demonstrated by Sizer[23] and there are several reasons for which Arrhenius plots may become nonlinear[24]. One possible explanation of this peculiarity may be that there are interconvertible forms of the enzyme which differ in their catalytic properties. In view of recent findings of Sammons and Chilson[16] it may be possible that the observed differences in thermal sensibility of AMP deaminase extracted from heart muscle illustrate the developmental changes in the isozymic pattern of this tissue.

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