Developmental changes of chicken liver AMP deaminase*

Józef SPYCHAŁA, Krystian KALETHA and Wiesław MAKAREWICZ

Department of Biochemistry, Medical School, ul. Dębinki 1, 80–211 Gdańsk, Poland

1. The AMP deaminase activity measured in crude chicken liver extract did not change significantly during development. 2. The livers of 10- and 14-day chick embryos, 1-day, 5-, 10- and 16-week-old chickens and adult hens were examined for the existence of multiple forms of AMP deaminase. Phosphocellulose column chromatography revealed the existence of two peaks of enzyme activity in the liver of 10- and 16-week-old chickens and adult hens. 3. Kinetic studies with the preparations of AMP deaminase revealed sigmoid-shaped substrate-saturation curves at all developmental stages and hyperbolic-shaped saturation curves for the enzyme form appearing in 10-week-old chickens. 4. All AMP deaminases investigated were susceptible to activation by ATP and inhibition by P_i . 5. Kinetic and regulatory properties as well as pH optima of all the enzyme preparations tested indicate that AMP deaminase isolated from the embryos and from 1-day-old chicks was similar to the form I isolated from adult hens and differed significantly from the form II of this enzyme.

INTRODUCTION

The importance of AMP deaminase for the regulation of adenylate catabolism in mammalian liver makes this enzyme an interesting subject for comparative studies. The rate-limiting step in the degradation of adenine nucleotides in the mammalian liver is the conversion of AMP into IMP, catalysed by AMP deaminase (EC 3.5.4.6) (Hers & Van den Berghe, 1979; Van den Berghe *et al.*, 1977, 1980), and in ureotelic species this reaction seems to be concerned mainly with uric acid production. However, as far as uricotelic animals are concerned there is an open question as to what extent the activity of AMP deaminase and its regulatory properties relate to the high synthesis *de novo* of IMP in the liver.

Studying the developmental pattern of liver enzymes involved in AMP metabolism, Wegelin *et al.* (1978) showed that the activity of chicken liver AMP deaminase measured in the absence of ATP did not change significantly between days 9 and 20 of embryo development. They also presented evidence that ATP, although an activator at the early stages of pre-hatching development, becomes an inhibitor shortly before hatching. On the basis of this observation one may expect the appearance of a distinct molecular form of AMP deaminase at this stage. On the other hand, two separate molecular forms of AMP deaminase from adult hen liver have been isolated and partially characterized (Spychała & Makarewicz, 1983).

Therefore the purpose of the present paper was to investigate the appearance of multiple forms of AMP deaminase in the developing chicken liver and to compare the kinetic and regulatory properties of these forms.

MATERIALS AND METHODS

White Leghorn female chickens (18 months old), 1-day-old chicks and fertilized eggs at different stages of development were purchased from Gdańskie Zakłady Drobiarskie. Chickens up to ages of 5, 10 and 16 weeks were maintained on ordinary laboratory diet of 20–22% (w/w) protein content. Animals were killed by decapita-

tion and the livers were chilled immediately after removal. The homogenate was prepared and chromatographic procedure was performed as described previously (Spychała & Makarewicz, 1983). The 1:2 (w/v) homogenate in 0.089 M-potassium phosphate buffer, pH 6.5, containing 0.18 M-KCl and 2 mM-2-mercaptoethanol was centrifuged at 18000 g for 30 min. Batch adsorption of the supernatant with phosphocellulose P-11 from Whatman (manufactured before 1980) was performed at 4 °C for 30 min. Phosphocellulose column chromatography consisted of a step elution of contaminating proteins with 0.7 M-KCl, followed by a 0.7–1.5 M-KCl linear gradient. The fractions corresponding to the major portion of each peak were pooled, concentrated in polyethylene glycol and dialysed against 1.0 M-KCl, buffered with 20 mM-imidazole, pH 6.8.

AMP deaminase activity was determined from the amount of ammonia produced, by using the phenol-hypochlorite method (Chaney & Marbach, 1962). The reaction mixture contained, in 0.5 ml final volume, 50 mM-cacodylate buffer, pH 6.5, 100 mM-KCl, enzyme, AMP and other compounds at the concentrations indicated.

Protein concentration was measured spectrophotometrically (Layne, 1957).

AMP was supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.), and sodium cacodylate and 2-mercaptoethanol were from Loba Chem. (Vienna, Austria). ATP and all other reagents were of analytical grade and were purchased from POCH (Glinice, Poland).

RESULTS

In order to measure the AMP deaminase activity in crude liver extracts prepared from the chicken liver at various developmental stages, extracts were dialysed overnight against 1.0 M-KCl buffered with 20 mM-imidazole, pH 6.8. For 10- and 14-day embryos, 1-day-old chicks and adult hens the activities obtained were 1.05 ± 0.27 ($n = 5$), 1.49 ± 0.25 ($n = 4$), 1.22 ± 0.15 ($n = 3$) and 1.56 ± 0.32 ($n = 5$) $\mu\text{mol/min per g wet wt.}$

*Dedicated to Professor Mariusz M. Żydowo on the occasion of his 60th birthday.

Fig. 1. Phosphocellulose column chromatography of AMP deaminase from the liver of 10- and 14-day chick embryos and 1-day and 5-, 10- and 16-week-old chickens

The Pharmacia K 16/20 glass column was used, and for elution of the enzyme activity a linear 0.7–1.5 M-KCl gradient at a flow rate of 20 ml/h was employed (fraction volume was 4.0 ml). Both KCl solutions were adjusted to pH 7.0 with 1.0 M- K_2HPO_4 . The elution profiles are representative of at least two experiments.

respectively. For 10-day embryos and adult hens the AMP deaminase activity, recalculated per g dry wt., was 4.8 ± 0.8 ($n = 4$) and 4.6 ± 0.6 ($n = 3$) $\mu\text{mol/min per g dry wt.}$ respectively. The above values are means $\pm$ S.D. for the numbers of experiments in parentheses.

The elution profiles of liver AMP deaminase chromatographed on phosphocellulose are presented in Fig. 1. Elution with a linear 0.7–1.5 M-KCl gradient revealed the existence of two separate peaks of AMP deaminase activity in livers of 10- and 16-week-old chickens. When similar chromatography was carried out with the liver extracts of 10-, 14- and 17-day chicken embryos (results not shown) as well as of 1-day- and 5-week-old chickens, a single peak of enzyme activity was observed.

The occurrence of a second form of AMP deaminase

in the liver of 14-day chick embryo might have been suspected because the active fractions formed a rather broad peak and the elution profile was irregular (Fig. 1). This separation procedure has been repeated three times in separate experiments without further resolution of enzyme activity. However, at this developmental stage one cannot exclude a small contamination of the main peak of embryonic liver AMP deaminase by another form of this enzyme.

Kinetic properties of the two forms of AMP deaminase from adult hen liver presented in Fig. 2 indicate that, in the absence of added effectors, the substrate-saturation curve was hyperbolic for form II and sigmoidal for form I. Both enzyme forms were activated by ATP and inhibited by P_i (Fig. 2 and Table 1); however, the

Fig. 2. Influence of ATP and P_i on the kinetics of AMP deaminases isolated from the liver of 10-day chick embryo and adult hen

The activity of the enzyme was measured in the absence of effectors ($\circ$), or with addition of 3 mM-ATP ($\bullet$) or 4 mM- P_i ($\blacktriangle$). The curves are representative of three experiments.

regulatory effects of these compounds, particularly at low substrate concentrations, were more pronounced for form I. In the presence of both ATP and P_i the two enzyme forms displayed hyperbolic kinetics, with a Hill coefficient (h) of 1.0, but with different $s_{0.5}$ values (Table 1).

The effect of substrate concentration on the rate of the reaction catalysed by AMP deaminase purified from the liver of 10-day chick embryos is also shown in Fig. 2. The shape of the saturation curves resembles very much that of form I of the enzyme from adult hen liver.

The values of the ratio of the reaction velocities at high

and low substrate concentration presented in Table 1 indicate that the ratio was highest for the enzyme from the liver of 10-day chick embryos and diminished in the course of ontogenesis. However, the two adult forms differed in this respect considerably, form I being more like the embryonic enzyme. Thus the two molecular forms of the enzyme differ significantly in their substrate affinity. At each developmental stage, the presence of ATP caused a marked decrease in the ratio as well as of $s_{0.5}$ values, indicating an increase in the enzyme-substrate affinity. The effect of ADP was similar but less pronounced.

The activation constant (K_a) for ATP was 0.09 ± 0.01 mM and 0.17 ± 0.04 mM respectively for forms I and II of AMP deaminase purified from the liver of adult hen. The K_a value for the enzyme from 10-day chick embryo was 0.13 ± 0.03 mM (Fig. 3).

We have also investigated the effect of pH on the activity of AMP deaminase isolated at various developmental stages. For all the preparations investigated except adult enzyme II, the highest activity was observed at pH 6.5. Optimum pH for form II was 6.75. During ontogenesis the pH-dependence of the enzyme activity underwent substantial changes. At the early developmental stage (12-day embryo) relatively high activity was observed at pH 5.75 (41% of the optimal) and low at pH 7.5 (17%). For form I from adult hen the respective values at pH 5.75 and 7.5 were 39 and 29%, but for form II 24 and 74%. Thus form I resembles rather the embryonic form of the enzyme also in its pH-dependence.

Table 1 presents collected values of $s_{0.5}$ and h measured in the absence of the effectors and in the presence of ATP and/or P_i for all AMP deaminases tested. In control experiments h for all the enzyme preparations investigated was in the range 1.6–1.9, except for form II of the enzyme from adult hen liver, and similarly in the presence of 4 mM- P_i it was 2.0–2.3 for most of the enzymes tested, but was 1.7 for form II from adult hen liver. The $s_{0.5}$ parameter for forms I and II was 4.2 and 1.4 mM respectively in the control experiments, and the embryonic AMP deaminases displayed intermediate values, whereas in the presence of 4 mM- P_i the $s_{0.5}$ value of AMP deaminase I from adult hen was very similar to the respective values of embryonic enzymes and was fairly different from the value of 1.7 calculated for form II from adult hen. The concomitant presence of P_i and ATP did not cause any change in the $s_{0.5}$ value of form II of adult hen liver enzyme as compared with the effect of ATP alone. However, the $s_{0.5}$ value for the form I increased about 10-fold if P_i was present with ATP, as compared with the presence of ATP alone.

DISCUSSION

Chicken liver AMP deaminase appears to follow a different developmental pattern from AMP deaminases from several other avian tissues. The data present in this paper confirm the observation by Wegelin *et al.* (1978) and clearly prove that the activity of chicken liver enzyme does not change significantly during ontogenesis. On the other hand, in rat liver the activity of this enzyme was highest in the 18-day fetus, then decreased, being lowest in the 10-day-old rat, and afterwards increased slowly, reaching the adult value in 1-month-old animals (Andres & Machado, 1982).

Table 1. Kinetic properties of chicken liver AMP deaminase at different developmental stages in the absence and in the presence of effectors

As most of the reactions showed sigmoid-shaped kinetics within the 0.2–20.0 mM-AMP concentration range, the $V_{\max}$ values were calculated by using the method of linearization of the Hill equation described by Endrenyi *et al.* (1975). The intersection point of the Hill plot with the abscissa at $\log[v/(V-v)] = 0$ allowed calculation of the $s_{0.5}$ value. The kinetic data are representative of two experiments and the ratio values are mean of three measurements.

Developmental stage	Control		3 mM-ATP		4 mM-P _i		ATP and P _i		Ratio $\left(\frac{v \text{ at } 10.0 \text{ mM-AMP}}{v \text{ at } 0.3 \text{ mM-AMP}}\right)$		
	$s_{0.5}$	h	$s_{0.5}$	h	$s_{0.5}$	h	$s_{0.5}$	h	No effector	With 3 mM-ATP	With 0.5 mM-ADP
	(mM)		(mM)		(mM)		(mM)				
10-day embryo	3.0	1.8	0.2	1.0	4.7	2.0	—	—	156	26	34
14-day embryo	2.1	1.9	0.6	1.0	5.9	2.1	—	—	102	16	33
1-day chicken	2.2	1.9	0.3	1.0	4.5	2.3	—	—	38	9	20
Adult hen, Form I	4.2	1.6	0.2	1.0	5.9	2.2	1.9	1.0	38	5	6
Adult hen, Form II	1.4	1.0	0.4	1.0	1.7	1.5	0.5	1.0	6	2	6

Fig. 3. Influence of ATP on the activity of AMP deaminase isolated from the liver of 10-day chick embryo and adult hen

AMP concentration was 0.4 mM. The curves are representative of two experiments.

In other avian tissues the activity of AMP deaminase undergoes substantial changes in the course of development. In chick-embryo heart the activity of this enzyme is high at days 7–17 of incubation; it decreases about 4-fold at hatching and remains low for 18 days of postnatal development. Afterwards the activity increases slowly, reaching its original embryonic value in the heart of adult hens (Purzycka-Preis *et al.*, 1975). AMP deaminase activity in the chick brain rises gradually during the pre-hatching period and continues to rise after hatching, reaching the value observed in adult hen brain homogenate at the end of the first month (Doemel & Chilson, 1969). Similarly, Kruckenberg *et al.* (1978) showed that in chick erythrocytes the activity of AMP deaminase increases steadily during maturation, reaching 95% of the adult value at 16 weeks after hatching. However, studies on the AMP deaminase activity in chick

leg and breast muscle in the course of ontogenesis showed a rather rapid rise of activity during week 3 of the pre-hatching period, attaining the adult value by the time of hatching (Kendrick-Jones & Perry, 1967; Sammons & Chilson, 1978), and no further increase in the activity was observed.

So far, liver AMP deaminase appears to be the only chicken AMP deaminase the activity of which remains virtually unchanged during development. In terms of the classification introduced by Greengard & Thorndike (1974), this enzyme should be placed in cluster I, i.e. among the enzymes that attain 80% of its mature activity at day 10 of incubation. This may indicate that its physiological function in the liver is mandatory to life throughout development.

More than one form of AMP deaminase was found in the liver of fetal rat only, whereas in the liver of adult rat and man a single form of this enzyme was detected (Ogasawara *et al.*, 1978, 1982; Smith *et al.*, 1977; Spychała, 1984). In a previous paper we demonstrated the existence of two separate molecular forms of AMP deaminase in the liver of adult hen (Spychała & Makarewicz, 1983). However, the embryonic livers and the livers of 1-day-old and 5-week-old chickens contained only one form of this enzyme (Fig. 1). Form II appeared in the liver only between weeks 5 and 10 of post-hatching development. In the light of this finding, the assumption could be made that the appearance of the second form of AMP deaminase in the adult chicken liver might be of similar significance to the increase in enzyme amount during development reported for other tissues. This assumption is justified by the fact that form II, displaying a lower $s_{0.5}$ value, and being less ATP-dependent and particularly less P_i-dependent, is catalysing the reaction more efficiently, especially at low substrate concentration. Thus one could propose another mechanism of adaptation of the enzyme activity to the changing cellular environment, i.e. the emergence of a kinetically distinct form which could fulfil the changing cellular demand for higher activity.

The kinetic properties of all AMP deaminases tested, presented in Fig. 2 and summarized in Table 1, provide

the evidence that the embryonic and post-hatching chicken liver enzyme is generally similar to the form I of adult hen. This conclusion particularly concerns the enzyme isolated from the liver of 10-day embryos. However, one should also notice that the gradual diminution of the susceptibility of the AMP deaminase to the concentration of adenine nucleotides occurs in the course of development (Table 1). This finding seems to be in accord with the decrease in the concentration of ATP and in the ATP/ADP ratio during embryogenesis, as reported by Manzoli & Wegelin (1970). The significance of these findings remains to be elucidated.

The generally similar kinetic and regulatory properties of chicken embryonic and early post-hatching liver AMP deaminases suggest that there is no rapid change of either isoenzyme pattern or the total enzyme activity during the pre-hatching development. It should be pointed out that there is a marked re-arrangement of metabolism in chicken liver between day 10 of incubation and the day of hatching. According to Romanoff (1967), proteins provide the major source of energy to the chicken embryo during the second week of pre-hatching development, and the fat during the last week. Unfortunately, there are no data on the extent of uric acid production in the embryonic chicken liver, and thus it is difficult to relate the AMP deaminase activity to the nitrogen metabolism. Nevertheless, one may suppose that the main metabolic function of AMP deaminase in the avian liver may be concerned with the massive uric acid production.

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