ISOZYMES OF RAT BRAIN AMP DEAMINASE: DEVELOPMENTAL CHANGES AND CHARACTERIZATIONS OF FIVE FORMS

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1. Introduction

AMP deaminase is known to be physiologically important in the stabilization of energy charge [1], interconversion of adenine, inosine and guanine nucleotides [2–5] and furthermore as a key enzyme in the purine nucleotide cycle [6–8]. The deaminase exists in multiple molecular forms in different rat tissues [9–12]. On the basis of chromatographic, immunological, and kinetic properties, three parental forms (Types A, B and C) have been detected [11,12]. Type A AMP deaminase is the only form found in skeletal muscle; Type B, the major isozyme of kidney and liver; Type C, the form found in heart.

Previous studies demonstrated that there are five different chromatographic forms of AMP deaminase in the adult rat brain; they have been designated as isozymes I through V on the basis of order of elution from the column [12]. Isozymes I and V correspond in elution position to the only isozyme found in cardiac tissue and to the major component found in kidney and liver, respectively.

In this paper we report the changes of AMP deaminase isozymes in developing rat brain and also the studies on the interrelationship between the properties of the isozymes found in adult rat brain.

2. Materials and methods

2.1. *Materials*

Adenosine monosulfate and adenosine phosphoramidate were purchased from Sigma Chemical Co. AMP, dAMP and ATP were obtained from Boehringer, Mannheim. Phosphocellulose was the product of Brown Co. Other reagents were commercial preparations of the highest purity available.

2.2. *Enzyme assay and protein determination*

Enzyme activity was determined colorimetrically by estimating production of ammonia by phenol-hypochlorite reagents [13]. Typical reaction mixture contained 30 mM AMP, 20 mM potassium phosphate (pH 7.0), 150 mM NaCl, 0.02% 2-mercaptoethanol, and 0.05% bovine serum albumin in a final volume of 0.25 ml. The reaction was usually carried out at 37°C for 10 min and 1 unit of enzyme activity is defined as the amount of enzyme that yields 1 μmole of ammonia per min. Protein concentrations were determined by the method of Lowry et al. [14] using bovine serum albumin as a reference protein.

2.3. *Animals and enzyme extraction*

Wister rats at various stages were used. Embryonic age was determined by observation of vaginal plugs, and embryos were considered to be zero day old on the date of plug discovery. Sex was disregarded until 17th postnatal day, after which only male rats were used. Cerebra (cerebral hemispheres dissected free of cerebellum and brain stem) were removed and homogenized in a Waring blender in 5 vol. of a cold solution containing 0.02 M potassium phosphate (pH 7.0), 0.05 M NaCl and 0.1% 2-mercaptoethanol. The homogenates were centrifuged at 20 000 *g* for 20 min and the clear supernatants were used for the phosphocellulose chromatography.

2.4. *Immunological procedures*

AMP deaminases A, B and C were prepared from rat skeletal muscle, kidney and heart, respectively, and antisera against these isozymes were prepared by weekly injection into the rabbit as described elsewhere [12]. The antisera used in the present studies are the preparations used in earlier studies [12]. The studies on the effects of the antisera on the precipitation of the different AMP deaminase isozymes were carried out in the following manner. The same amount of isozyme, based on unit of activity (0.1 unit), was mixed with increasing vol. of antisera in a final vol. of 0.4 ml. After incubation for 18 h at 4°C, the samples were then centrifuged for 15 min at 20 000 *g* and the supernatants were assayed for enzyme activity. Non-immunized rabbit sera were used in place of the antisera in control experiments.

3. Results

3.1. *Developmental changes*

On wet weight basis, the total AMP deaminase activity of late fetal brain was 30% lower than the enzyme level of adults, and steady increase of enzyme activity occurred with increasing age. When the enzyme activity was expressed per mg of protein, no change was observed during development.

We employed chromatographic procedures for quantitation of rat brain isozymes during development. The isozymic patterns of AMP deaminase activity found when crude extracts of adult and fetal brains were subjected to phosphocellulose column chromatography are shown in fig.1. Five peaks of activity are found in adult brain. On the basis of order of elution from the column, these isozymes have been numbered I through V consecutively, I being eluted at the first and V at the last. The distribution of activity of the five isozymes in Peaks I–V was 25, 17, 12, 13 and 33%, respectively. In fetal brain, isozyme V was the most predominant form; 3 days before birth, the deaminases I, II and III accounted for only 6% of the total AMP deaminase activity (fig.1). Most developmental changes of AMP deaminase in rat brain, as detected by chromatography on phosphocellulose column, occurred during 3 weeks in postnatal life. The activity of Peaks I, II and III increased gradually after birth to a level of about 50% by the age of

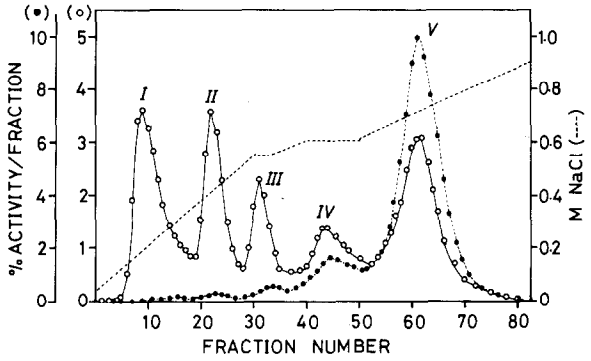

Fig.1. Phosphocellulose column chromatography of AMP deaminase from adult (○) and fetal (•) rat brain extracts. Frozen preparations (13 g and 11 g for adult and 18th day fetal brains, respectively) were homogenized with 5 vol. of cold solution containing 0.05 M NaCl and buffer A, which consisted of 0.02 M potassium phosphate (pH 7.0) and 0.1% 2-mercaptoethanol. After centrifugation at 20 000 *g* for 20 min, the supernatant was placed on a phosphocellulose column (0.9 × 10 cm) which was previously equilibrated with the extraction buffer and the column was washed with 30 ml of the same buffer. A linear gradient, 0.05–0.55 M NaCl in buffer A, 100 ml for each of the two vessels, was then applied. At the end of first gradient, the column was washed with 20 ml of 0.55 M NaCl in buffer A. Then, a second gradient, 0.55–0.9 M NaCl in buffer A, 150 ml each, was applied. After 50 ml of the second gradient had passed onto the column, the gradient was stopped to keep the NaCl concentration constant and further 70 ml elution was carried out at that concentration of NaCl. Then, a third gradient, 0.61–0.9 M NaCl again in buffer A, 100 ml per vessel, was applied. Fractions, about 7 ml/30 min/tube, were collected and assayed for enzyme activity.

20–30 days and remained at this level thereafter (fig.2). Developmental changes of brain AMP deaminase activity are thus characterized by changes in distribution of isozymes.

3.2. *Characterization of five forms*

The fractions corresponding to the major portion of each individual peak obtained from adult rat brain (fig.1), were pooled and concentrated by ultrafiltration. These fractions were used in subsequent studies as the individual isozymes from brain.

The substrate affinities of each of the AMP deaminase isozyme were examined. Under the standard assay condition, the K_m values for AMP of 3, 4.5, 6, 8 and 13 mM were obtained for isozymes

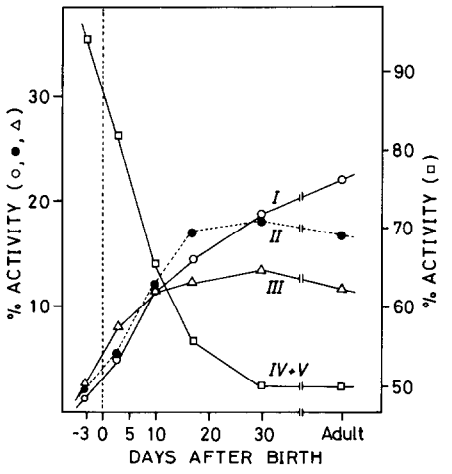

Fig.2. Developmental changes of brain AMP deaminase isozymes. Frozen preparations of brain from various ages were homogenized and the extracts were applied to phosphocellulose column as described under the legend to fig.1. The data are presented as percentages of activities in isozymes I, II, III and IV + V, since in some experiments the resolution of isozyme IV from V was insufficient. For each time point, the results presented are means of two or three separate chromatographic experiments. Each brain sample was a pool of 10 to 30 brains.

I, II, III, IV and V, respectively. Isozymes II, III and IV thus appear to possess kinetic properties somewhere within the limits defined by isozymes I and V. The substrate specificity of the resolved isozymes from brain was also investigated. Zielke and Suelter [15] have shown, from their studies on the deaminase form rabbit muscle, that the same molecule catalyzes the deamination of AMP, dAMP, adenosine monosulfate and adenosine phosphoramidate. The activity ratios, expressed as a fraction of the rate obtained with AMP as substrate, of the resolved isozymes from rat brain fall in an ordered series from isozymes I through V (table 1). Furthermore, the activity ratios of isozymes I and V are comparable with those of C and B type isozymes, respectively [12].

In earlier work it was shown that the antisera to the skeletal muscle, kidney and heart isozymes precipitated the corresponding isozyme, however no cross-reaction was observed [12]. Therefore, the effects of these antisera upon the activity of the isozymes from brain were determined in order to gain further understanding of their structural interrelationships. Antisera to the muscle enzyme had no effect on any of five isozymes. The effects of the antisera to the heart and kidney enzymes on the activity of the brain isozymes are shown in fig.3. The antisera to the

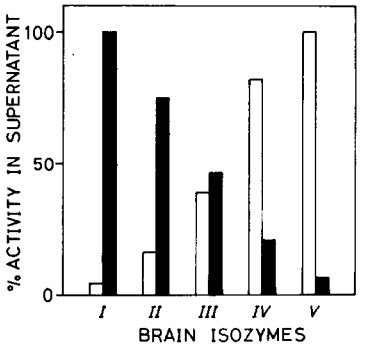

Fig.3. Precipitations of five isozymes from brain extracts by antisera. The active fractions under each peak in fig.1 were pooled, concentrated by ultrafiltration, and used for antisera precipitations. The data given are the results obtained by the addition of 10 μl of antisera to the heart enzyme (open bar) and 75 μl of antisera to the kidney enzyme (closed bar).

Table 1
Relative substrate specificity of five isozymes from rat brain

	Isozymes				
Ratio	I	II	III	IV	V
dAMP/AMP	0.160	0.125	0.067	0.060	0.032
AMS/AMP	0.270	0.232	0.126	0.080	0.031
APA/AMP	0.172	0.146	0.103	0.105	0.093

Assays were carried out in the presence of 30 mM substrate, 2 mM ATP and 4 mM $MgCl_2$ [1]. The value given is the ratio of activity observed with dAMP, adenosine monosulfate (AMS) or adenosine phosphoramidate (APA) to that observed with AMP.

heart enzyme completely removed the activity of isozyme I and had a decreasing effect on isozymes II through IV, but no effect on the activity of isozyme V. Antisera prepared against the kidney enzyme was employed in an analogous fashion. The results obtained were the reverse of those observed with the antisera to the heart enzyme, e.g., the greatest precipitation was observed with isozyme V, progressively less precipitation was found for isozymes IV through II, and no precipitation was observed with isozyme I (fig.3). Thus, all the activity in brain extracts was sensitive to either the antisera to the heart enzyme or those to the kidney enzyme.

4. Discussion

A purpose of the present study was to elucidate the relationships between the parental isozymes of AMP deaminase and those present in the brain. The results of the present work, along with previous results [12], demonstrate that the chromatographic, kinetic and immunological properties of isozymes I and V are similar to the isozyme in heart (Type C) and to the major isozyme in kidney and liver (Type B), respectively.

When the resolved isozymes from rat brain were subjected to gel filtration on Sepharose 6B column, all isozymes are eluted as a single peak of activity with an elution vol. identical to that found for the heart or kidney enzyme. Similar results have been obtained with crude brain preparations containing all five isozymes. These results indicate that five isozymes from brain have the same mol. wts of about 300 000, and therefore the possibility can be ruled out that some forms are indeed an aggregated form of others. In view of the tetrameric structure of muscle enzyme (mol. wt 270 000) of rat and other species [16,17], brain isozymes are also assumed to be tetramers.

Isozymes II, III and IV are eluted from phosphocellulose at the intermediate positions between those of two parental types, and furthermore, from a monotonic increase in K_m values for AMP in isozymes I through V, and also from the data of relative substrate specificity, isozymes II, III and IV appear to possess kinetic properties somewhere within the limits defined by two parental isozymes. These results, as well as the immunological properties of the intermediate three isozymes, would seem to leave little or no doubt that isozymes I through V from brain are tetramers with subunit compositions of C4, C3B, C2B2, CB3 and B4, respectively.

Developmental studies showed that fetal brain contained isozyme V as the predominant form and little isozymes I, II and III, which increased up to an adult level during 3 weeks in postnatal life. These shifts in isozyme patterns are in agreement with the concept that in fetal brain only AMP deaminase B subunits are synthesized and C subunit synthesis is not taking place yet. After birth, the synthesis of subunit C continues to increase to a level of adult during 3 weeks in postnatal life.

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