SUBUNIT STRUCTURES OF AMP DEAMINASE ISOZYMES IN RAT

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SUMMARY

AMP deaminases A and B have been purified to apparent homogeneity from rat muscle and liver, respectively. The molecular weights of 286,000 and 351,000 were obtained for the native muscle and liver enzymes, respectively, by sedimentation equilibrium studies. On polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, the muscle preparation exhibited a single polypeptide band with a molecular weight of 72,000; the liver preparation, a molecular weight of 85,000. The data indicate that each enzyme has a tetrameric structure.

Although the physiological role of AMP deaminase (EC 3.5.4.6) remains obscure, it may be important to stabilize the adenylate energy charge (1), in the conversion of adenine nucleotide to inosine or guanine nucleotide (2-5) and furthermore as an enzyme in the purine nucleotide cycle (6-10). Previous studies demonstrated that there are three parental isozymes (A, B and C) in the rat (11). Isozyme A is the form found in muscle; B, the major form in liver; C, the form found in heart. They differed from one another with respect to their chromatographic behavior, their kinetic properties, and their reactions with specific antibodies (11).

Most of studies on molecular structure of AMP deaminase have been carried out on the muscle enzyme (12-17). In this paper, we report the subunit structures of isozymes A and B in the rat. The molecular weights of the native form and subunit of the muscle enzyme were found to differ significantly from those reported by Coffee and Kofke (16). Of particular interest is the finding that each isozyme is composed of four subunits with identical size, however the molecular weights of the subunits of two isozymes are significantly different; 72,000 for isozyme A and 85,000 for isozyme B.

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MATERIALS AND METHODS

Enzyme activity was determined colorimetrically as described previously (11). One unit of enzyme activity is defined as the amount of enzyme that yields 1 μmole of ammonia per min. Specific activity is defined as units per mg protein. Protein concentrations were determined by the method of Lowry *et al* (18) or by the fluorometric method of Böhlen *et al* (19), using bovine serum albumin as a reference protein.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed by the method of Weber and Osborn (20).

Sedimentation equilibrium measurements were carried out using a Beckman-Spinco Model E analytical ultracentrifuge equipped with Rayleigh interference optics and a temperature control unit. High speed equilibrium sedimentation (21) was carried out in a multichannel cell (22) with sapphire windows. The rat muscle and liver AMP deaminases were dialyzed against 0.02 M potassium phosphate (pH 7.0) containing 0.2 M NaCl and 0.1 % 2-mercaptoethanol. Samples were diluted with dialysate to 0.25, 0.4 and 0.5 mg/ml. The molecular weights were calculated from the plots of the logarithm of the fringe displacement *versus* the square of the radial distance, assuming a partial specific volume of 0.73 (12).

Purification of rat liver AMP deaminase: Wister rats (250-300g) were used, unless otherwise stated all steps were carried out at 4° C. The frozen livers (100g) were homogenized in a Waring Blender with 5 volumes of 0.3 M NaCl in buffer A which consisted of 20 mM potassium phosphate (pH 7.0) and 0.1 % 2-mercaptoethanol. The homogenate was centrifuged at 0° C for 30 min at 20,000 x g, and the supernatant was saved. To the extract were added 1.6 x 20 cm column volumes of phosphocellulose, which was previously equilibrated with extraction buffer. The suspension was stirred for 1 hr, and then transferred to a Buchner funnel where phosphocellulose was washed repeatedly with extraction buffer. The phosphocellulose was transferred to a column and the enzyme was eluted with 1.5 M NaCl in buffer A. The solution was then applied to the top of a column of Sephadex G-200 (2.0 x 90 cm) which was previously equilibrated with 0.3 M KCl in buffer A. The column was eluted with the same buffer and fractions, 3 ml each, were collected. The fractions containing the enzyme was pooled and solid ammonium sulfate added to yield 0.6 of saturation. The precipitated protein was dissolved in 7 ml of buffer B which consisted of 30 mM potassium phosphate (pH 7.0) and 0.1 % 2-mercaptoethanol, and the solution was dialyzed for 16 hrs against 500 ml of the same buffer. The enzyme was then applied to a DEAE-cellulose column (0.9 x 10 cm) which was previously equilibrated with buffer B. The column was washed with 20 ml of the same buffer, followed by elution with a linear gradient between 0 and 0.3 M NaCl in buffer B (75 ml each). The fractions containing the enzyme were pooled. The enzyme solution was then adsorbed onto a phosphocellulose column (0.9 x 5 cm) and a linear gradient of 0.3 to 1.2 M NaCl in buffer A (50 ml each) was applied to the column. Fractions comprising peaks were pooled and concentrated by ultrafiltration.

A summary of the purification is given in Table I. Preparations with specific activities of 900 to 1,000 units per mg have been obtained (about 15,000-fold purification) in about 14 % yield. The specific activities of the liver enzyme preparations have been consistently about 50 % of those of muscle enzyme preparations.

AMP deaminase from rat skeletal muscle: AMP deaminase was isolated from rat leg muscle as follows. Frozen muscles (80g) were homogenized, extracted, and the enzyme was adsorbed to phosphocellulose as described above for the liver enzyme. Elution was carried out with a linear salt gradient of 0.3 to 1.2 M NaCl in buffer A (200 ml each); the active fractions were pooled and solid

TABLE I

PURIFICATION OF LIVER AMP DEAMINASE

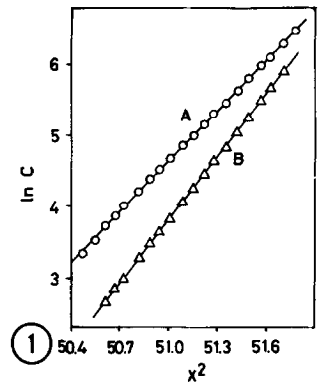
Fraction	Total Volume	Total Protein	Total Activity	Specific Activity	fold
	ml	mg	units		
Extract	500	11,460	695.0	0.061	1
P-cellulose	4.0	86.20	540.5	6.27	103
Sephadex G-200	39.0	15.06	396.8	26.35	432
Ammonium sulfate	7.2	6.06	372.5	61.47	1,008
DE-52	25.0	0.80	177.0	221.25	3,627
P-cellulose	0.4	0.10	94.2	942.00	15,443

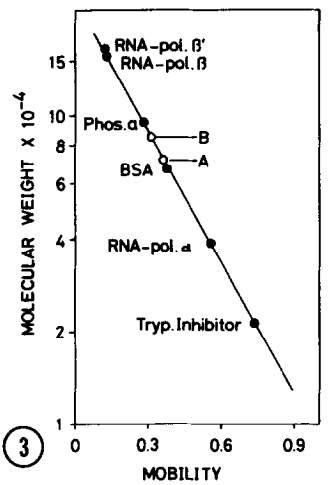
ammonium sulfate was added to yield 0.6 of saturation. The precipitated protein was dissolved in 2 ml of 0.3 M KCl in buffer A and the solution was dialyzed for 3 hrs against the same buffer. The enzyme solution was processed through the Sephadex G-200 gel filtration and DEAE-cellulose column chromatography steps as described for the liver enzyme. A preparation that exhibited a specific activity of 1,800 to 2,000 was obtained in about 30 % yield.

RESULTS AND DISCUSSION

Molecular weights of native AMP deaminases from rat muscle and liver were determined by sedimentation equilibrium analysis using the meniscus depletion technique (21). The results of a typical experiment are shown in Fig. 1. Graphs of ln C *versus* X^2 were linear indicating that the preparation were homogeneous with respect to molecular weight. Calculation of the molecular weight from these data gave weight average molecular weights of 286,000 and 351,000 for the muscle and liver enzymes, respectively.

The molecular weights of the monomeric units of the rat muscle and liver AMP deaminases were determined by electrophoresis in 7.5 % polyacrylamide gels carried out in the presence of 0.1 % sodium dodecyl sulfate. A set of representative electrophoresis patterns are illustrated in Fig. 2. Each deaminase migrated as a single electrophoresis species, indicating that each enzyme consisted of subunits of a single size class. The muscle deaminase subunits migrated more rapidly than those from the liver deaminase; molecular weights of 72,000 and 85,000 were calculated for the muscle and liver enzymes, respectively (Fig. 3).





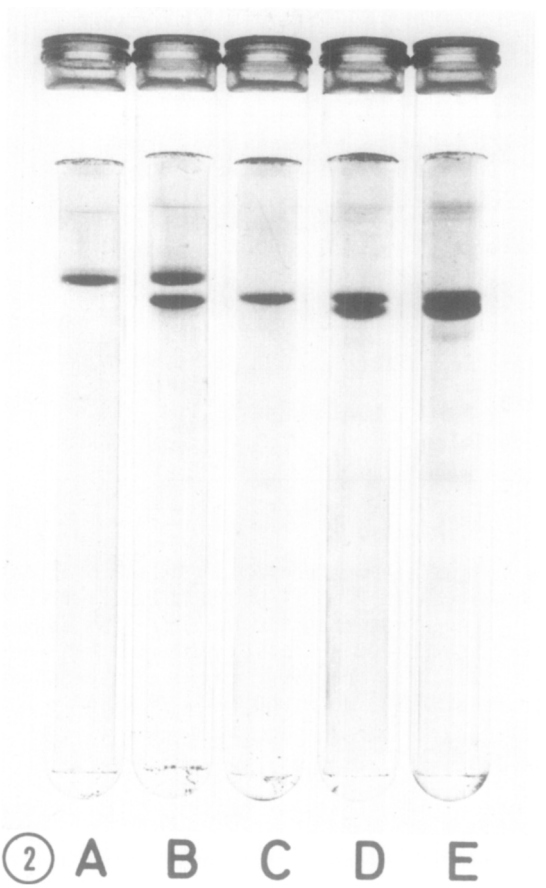


Fig. 1. High speed sedimentation equilibrium analysis of rat muscle and liver AMP deaminases.
A; the muscle enzyme (0.25 mg/ml) was centrifuged at 11,920 rpm at 21.5° C using AN-D rotor in 0.02 M potassium phosphate (pH 7.0) containing 0.2 M NaCl and 0.1 % 2-mercaptoethanol.
B; the liver enzyme (0.25 mg/ml) was centrifuged at 10,927 rpm at 20.7° C under the same condition as in A.
The data were plotted in terms of the logarithm of the fringe displacement versus the square of the radical distance.

Fig. 2. Sodium dodecyl sulfate gel electrophoresis of muscle and liver AMP deaminases.
The samples, prior to electrophoresis, were incubated for 3 min in boiling water in 0.01 M sodium phosphate (pH 7.0) containing 1 % sodium dodecyl sulfate and 0.5 % 2-mercaptoethanol. A. liver enzyme; B, liver enzyme + muscle enzyme; C, muscle enzyme; D, muscle enzyme + bovine serum albumin (1 μg); E, muscle enzyme + bovine serum albumin (2.5 μg).

Fig. 3. Molecular weight calibration curve for 7.5 % acrylamide gels run in 0.1 % sodium dodecyl sulfate.
The protein standards were: RNA polymerase, α, β, and β' subunits; phosphorylase a; bovine serum albumin; and trypsin inhibitor. A, muscle enzyme; B, liver enzyme.

These results indicated that each enzyme is a tetramer composed of four identical, or very similar subunits. Coffee and Kofke purified the muscle enzyme to apparent homogeneity from rat and showed that the native enzyme has a tetrameric structure consisting of four identical subunits having a molecular weight of 60,000 (16). The molecular weight of 238,000 found in their studies on the native enzyme is significantly lower than that of 286,000 found in these studies or 290,000 reported by Ronca-Testoni et al (13). In our gel electrophoresis in the presence of sodium dodecyl sulfate, a molecular weight of 72,000 was obtained instead of 60,000. The reasons for these differences are not obvious, however repeated experiments showed that the subunits of the muscle enzyme migrated more slowly than bovine serum albumin in electrophoresis (Fig.2).

On the structure of the heart enzyme, the studies are in progress, but the existence of five isozymes in brain, where both B and C subunits exist (23), strongly supports the tetrameric structure of isozyme C.

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