AN INVESTIGATION OF THE SUBUNIT STRUCTURE AND AMP-DEAMINASES FROM RABBIT AND CHICKEN MUSCLE

Albert Boosman, David Sammons and Oscar Chilson

Department of Biology and Committee on Molecular Biology

Washington University, St. Louis, Mo. 63130

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Summary

Native rabbit and chicken muscle AMP-deaminases have been compared by gel filtration chromatography. These studies, along with previously-reported ultracentrifugal analyses, suggest that these enzymes are virtually identical in size and shape. Chemical and physical evidence is presented which shows that: (a) each enzyme has four subunits; (b) the molecular weights of these subunits are indistinguishable (69,000 Daltons and 73,000 Daltons, as determined by gel filtration chromatography in guanidine hydrochloride and electrophoresis in SDS-containing polyacrylamide gels, respectively; and (c) the native enzymes are not stabilized by disulfide bridges. Although previous studies suggest that these enzymes share certain structural features, the results presented here provide direct evidence, at the molecular level, that the adenylate deaminases from these sources have similar quaternary structures.

Muscle adenylate deaminases from several organisms exhibit close similarities in catalytic (1,2), as well as immunological (1,3), properties; however the molecular bases of the implied interspecies structural resemblances are not known. Studies of the catalytic (1,4,5), physical (6,7), and immunological (1) properties of rabbit muscle AMP-deaminase have provided indirect evidence consistent with a multichain structure for this enzyme. In a recent investigation of the molecular weights of the polypeptide chains of various sarcoplasmic proteins the size of the subunit of rabbit muscle adenylate deaminase was reported to be one-fourth that of the native enzyme (8).

The investigations reported here have allowed us to: compare certain chemical properties of the deaminases from chicken and rabbit muscle, demonstrate that the adenylate deaminases from these sources have virtually identical Stokes radii,

show that these proteins can be dissociated to produce polypeptide chains of a single size class, and conclude that the structure of the native enzymes does not involve disulfide bridges.

Methods and Materials

Adenylic acid deaminases were isolated from chicken breast muscle and rabbit skeletal muscle by previously-described procedures (3,5). Rabbit IgG was prepared as described by Keckwick (9). Bio-Gel A 5m (200-400 mesh) and 15m (100-200 mesh) were obtained from Bio-Rad Laboratories; 5'-AMP, ovalbumin, bovine alpha-chymotrypsinogen A, bovine serum albumin, bovine hemoglobin, bovine ribonuclease, bovine liver catalase, bovine heart lactic dehydrogenase, equine cytochrome c, rabbit muscle aldolase, rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, insulin, transferrin, Tris, DNP-beta-alanine, 2-mercaptoethanol, iodoacetic acid, and p-mercuribenzoate were from the Sigma Chemical Co. Blue dextran 2000 was from Pharmacia; pig heart malic dehydrogenase from Boheringer and Soehne, and apoferritin from Cal Biochem.

Electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate was performed by a modification of the method of Shapiro et al. (10) as described by Weber and Osborn (11). Gels were stained for protein by a combination and modification of the methods described by Weber and Osborn (11) and by Marchesi, et al. (12).

Native deaminases and various standards were chromatographed on a column of Bio-Gel A 5m at room temperature in 0.25 M potassium chloride + 0.1 M potassium phosphate (pH 6.5). Relative concentrations of eluted solutes were determined by measuring absorbancies at appropriate wavelengths and or by assays of enzyme activities. Distribution coefficients (K_D's) were determined as described by Fish, Mann, and Tanford (13) and Stokes radii were determined graphically as suggested by Ackers (14). Carboxymethylated derivatives of appropriate standards, as well as the deaminases were subjected to gel filtration chromatography on Bio-Gel 15m columns equilibrated with 6 M guanidine hydrochloride (pH 5.0); the experimental conditions and calculation of molecular

weights were performed as described by Fish, Mann, and Tanford (13).

Tyrosine to tryptophan ratios were determined by ultraviolet spectroscopy on a Beckman Acta V recording spectrophotometer. Baseline corrections and calculations were performed as described by Beaven and Holiday (15). The sulfhydryl contents of the denatured proteins were determined in 8 M urea as described by Boyer, (16).

Results and Discussion

Several properties of the rabbit and chicken enzymes are summarized in Table 1. The ratios of the absorbancies at 280 nm and 260 nm indicate the virtual absence of bound nucleotides. There are no published values for the amino acid analysis of the avian enzyme, but the Tyr/Try ratio which we observed for the rabbit deaminase is in excellent agreement with the ratio obtained by

Table 1

Comparison of the Chemical and Physical Properties of AMP-deaminases from Chicken and Rabbit Skeletal Muscle

Enzyme Source	280/260	Tyr/Trp	Moles PMB per Mole Enzyme	Subunit Molecular Weight SDS-Gels	Gel Filtration in Guanidine HCl	Stokes Radius
Rabbit	1.9	6.2	31.8	73,000 Daltons	69,000 Daltons	59 Å
Chicken	2.0	5.6	28.4	"	"	"

Wolfenden, Tomozawa, and Bamman (17); our determination of the number of thiol groups per molecule of rabbit deaminase which react with p-mercuribenzoate (PMB) in 8 M urea are also in quantitative agreement with the cysteine content of this protein (17). The latter observation supports the hypothesis that muscle deaminase does not contain disulfide bridges.

We have previously reported that the sedimentation constants of rabbit and chicken adenylate deaminases are virtually identical (determined at 6 mg per ml) (3). More recently it has been shown that these enzymes sediment at

equal rates in sucrose velocity gradients at protein concentrations of less than 1 mg/ml. Additional evidence for similarities in physical properties is reflected in their Stokes radii (Table 1). The value obtained for native rabbit deaminase by gel filtration, 59 Å compares well with the value of 56 Å obtained by hydrodynamic methods (17). Taken together, these results suggest that rabbit and chicken muscle AMP-deaminases have similar, if not identical molecular weights.

Various proteins having polypeptide chains of known size and the two deaminases were denatured and electrophoresed on SDS-gels as described in the Methods and Materials section. Mobilities of the polypeptides were determined on samples electrophoresed individually, on separate gels, as well as in various combinations; calculations were performed as described by Weber and Osborn (11). A set of representative electrophoresis patterns are illustrated in Figure 1. Both deaminases ran as a single electrophoretic species, indicating that each enzyme consists of subunits of a single size class. Furthermore, the subunits

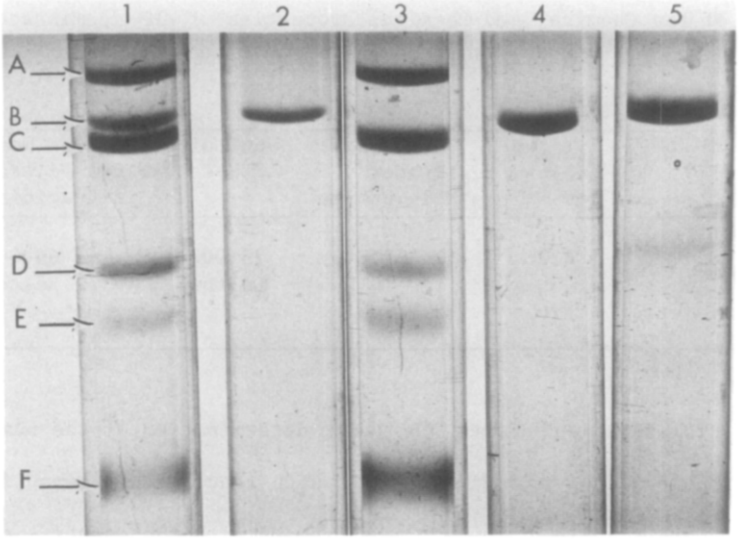

Figure 1. Electrophoresis Patterns.
Various standards and adenylate deaminases were electrophoresed in SDS-polyacrylamide gels as described in the text. A, Phosphorylase a; B, Adenylate deaminase; C, Catalase; D, Glyceraldehyde 3-phosphate dehydrogenase; E, alpha-chymotrypsinogen; F, Hemoglobin. All standards plus chicken muscle adenylate deaminase were applied to gel number 1. Chicken muscle deaminase; Standards alone; rabbit muscle deaminase; and chicken and rabbit muscle deaminases plus glyceraldehyde 3-phosphate dehydrogenase were applied to gels 2,3,4, and 5, respectively.

from the rabbit and chicken AMP-deaminases coelectrophoresed, suggesting that these enzymes have subunits of identical size. Quantitative data from several experiments are summarized in Figure 2. The molecular weight of the subunits as determined by this method is 73,000 Daltons.

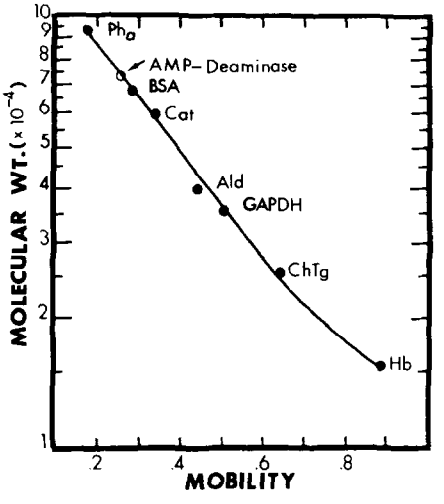

Figure 2. Relationship Between Electrophoretic Mobilities and Subunit Molecular Weights.
Carboxymethylated derivatives of various standards and the two deaminases were electrophoresed in SDS-polyacrylamide gels; mobilities were calculated as described in the text. Ph_a, Phosphorylase a; BSA, bovine serum albumin; Cat, Catalase; Ald, Aldolase; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; ChTg, alphachymotrypsinogen; Hb, Hemoglobin.

Polypeptides of a single size class were also indicated when reduced, carboxymethylated rabbit and chicken AMP-deaminases were chromatographed on 4 percent agarose equilibrated with 6 M guanidine hydrochloride, as described in the Methods and Materials section. Results of several experiments are summarized in Figure 3, this procedure yielded an apparent molecular weight of 69,000 Daltons.

Assuming a common molecular weight of 278,000 Daltons (7) for the native enzymes and the reported uncertainty (± 10 percent; 11) in the accuracy with which the molecular weights of polypeptide chains can be determined by electrophoresis in SDS-polyacrylamide gels, the data in Table 1 are consistent with a

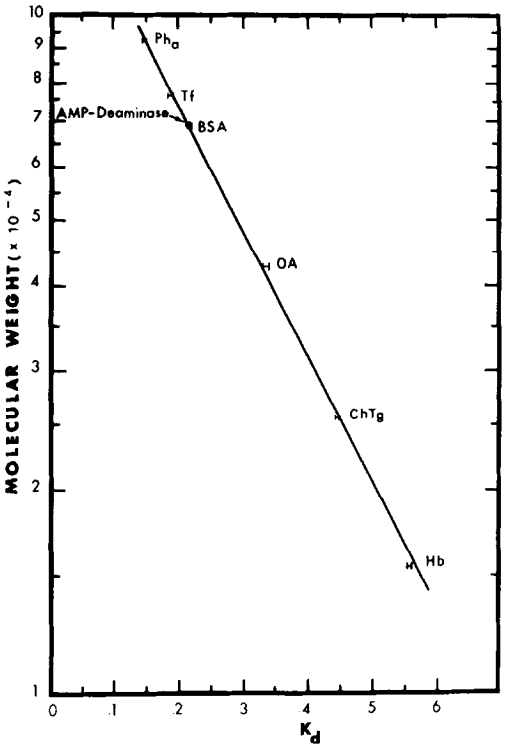

Figure 3. Relationship Between K_d's and Subunit Molecular Weights as Determined by Gel Filtration Chromatography in Guanidine Hydrochloride.
Various standards and the deaminases were chromatographed as described in the text. The distribution coefficient (K_d) for each sample was determined at least three times; the horizontal bars indicate the range of values obtained. Tf, Transferrin; OA, Ovalbumin; all other abbreviations are the same as given in the legend to Figure 2.

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structure that contains four subunits per molecule of native adenylate deaminase. Although quaternary structure is not invariably associated with non-hyperbolic kinetics, the results presented here are consistent with the sigmoid character of the substrate saturation curves obtained when these enzymes are assayed in the absence of modifiers as well as recently-reported equilibrium binding studies which suggest a minimum of four binding sites for ATP (6) and the observation of multiple binding sites for zinc ion (7).

Tissue specific enzymes are often more closely related to isodynamic enzymes found in functionally-related tissues in different organisms than to proteins exhibiting the same catalytic activities in different tissues of the same

organism. In certain cases these relationships are also reflected in patterns of appearance of various enzymes during development (18). Adenosine 5'-phosphate deaminase appears to fit this general pattern. Vertebrate skeletal muscle is the richest known source in adult animals and there is a correlation between the appearance of deaminase activity, as well as other enzymes of sarcoplasmic origin, and functional development of skeletal muscle from various organisms (19).

The strong similarities in biological activities, catalytic as well as immunogenic, of muscle adenylate deaminases from mammals and birds suggest common physiological roles. Although the nature of the biological importance of this enzyme is somewhat uncertain, a rather attractive hypothesis has been proposed by Lowenstein and Tornheim (20) on the basis of their studies of purine nucleotide interconversions in extracts of rat muscle. It remains to be seen whether the suggested pathway is operative in other organisms.

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