Subunit Structure of AMP-deaminase from Chicken and Rabbit Skeletal Muscle*

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The AMP-deaminases from chicken and rabbit muscle have been investigated by techniques which include sedimentation equilibrium, sodium dodecyl sulfate gel electrophoresis, amino acid analysis, NH₂- and COOH-terminal analyses, and tryptic peptide mapping. The molecular weights of the native chicken (276,000) and rabbit (271,000) enzymes obtained by sedimentation equilibrium studies are in good agreement with values of 276,000 (chicken) and 275,000 (rabbit) calculated from amino acid analyses.

The enzymes were reduced, carboxymethylated, and treated with either maleic or succinic anhydride in the presence of 6 M guanidine hydrochloride. Sodium dodecyl sulfate gel electrophoresis of the chemically modified enzymes resulted in a single electrophoretic species having an apparent molecular weight of 85,000. This observation is consistent with previous studies on the nonacylated enzymes and suggests that the muscle AMP-deaminases from chicken and rabbit do not contain noncovalent linkages which are readily disrupted by a large increase in negative charge.

NH₂-terminal analyses by the method of Stark and Smyth, as well as the dansyl technique, indicate that the NH₂-terminal positions of these enzymes are blocked. The enzymes are also resistant to digestion with carboxypeptidases A or B (or both) in the presence of sodium dodecyl sulfate.

The most distinctive feature of the amino acid compositions of both the chicken and rabbit AMP-deaminases is the presence of eight half-cystine residues per 69,000 g of protein. Tryptic digests of the S-¹⁴C-carboxymethylated proteins were fractionated by ion exchange chromatography and high voltage electrophoresis. Six and five radioactively labeled peptides were detected in the electrophoreograms of the chicken and rabbit enzymes, respectively. This observation and the number of ninhydrin-position spots, together with the physical data on the molecular weights of the native enzymes and their subunits, suggest that the AMP-deaminase from chicken and rabbit muscle consist of four identical or very similar polypeptide chains.

In an earlier study (1) we found that AMP-deaminases from chicken and rabbit muscle are tetramers. The results of a recent preliminary investigation of the quaternary structure of AMP-deaminase from rat skeletal muscle are also consistent with a tetrameric structure having polypeptide chains of identical size (2).

The purposes of the investigations described in this communication were to test the effects of maleic and succinic anhydrides on the apparent molecular weights of the subunits of reduced and carboxymethylated chicken and rabbit muscle AMP-deaminase and further characterize their subunits by analysis of tryptic peptide maps of the proteins following alkylation with iodo[¹³C]acetic acid. As will be shown, these enzymes did not yield low molecular weight polypeptides following either maleylation or succinylation. The number of ¹³C-labeled tryptic peptides obtained from chicken and rabbit AMP-deaminases is consistent with a tetrameric structure in which the subunits are very similar if not identical.

EXPERIMENTAL PROCEDURE

Sodium dodecyl sulfate, trifluoroacetic acid, dansyl chloride, ninhydrin, thioglycol, N-ethylmorpholine, and phenylisothiocyanate (all Sequanal grade) were purchased from Pierce Chemical Co. Iodo[¹³C]acetic acid was obtained from New England Nuclear. Guanidine hydrochloride ("Ultrapure") was a product of Schwarz/Mann. Dowex 50W-X8 (type AA-15) was obtained from Bio-Rad. Acrylamide, N,N'-methylene bisacrylamide, and N,N,N',N'-tetramethylethylene diamine were obtained from Eastman and Bio-Rad; 2-mercaptoethanol was from Eastman. Adenosine 5'-monophosphate (free acid) was from Miles Laboratories, Inc. Phos...
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RESULTS AND DISCUSSION

The molecular weights of native AMP-deaminases from chicken and rabbit muscle determined by sedimentation equilibrium were 276,000 ± 2,000 and 271,000 ± 3,000, respectively. The value we determined for rabbit deaminase is in good agreement with those reported by Woldfend et al. (270,000; Ref. 20) and Zielke and Suelter (278,000; Ref. 21). Similar studies of the avian enzyme have not been reported previously. There was no significant dependence of molecular weight on protein concentration within the ranges studied. Furthermore, graphs of In C versus X² were linear. These observations indicate that association and dissociation are not significant under the experimental conditions employed. Molecular weights based on assumed integer values for amino acid compositions (see below) are in good agreement with those determined by sedimentation equilibrium.

With one exception (22), values for the molecular weights of the subunits of AMP-deaminase from skeletal muscle ranging from 65,000 to 79,000 have been reported (1, 2, 23, 24). We have assumed a subunit size of 69,000 throughout the work reported here, because it is most consistent with our sedimentation equilibrium studies of the native enzymes, as well as with our amino acid analyses.

Electrophoresis of S-carboxylated AMP-deaminases, succinylated S-carboxylated AMP-deaminases, and maleylated S-carboxylated AMP-deaminases on polyacrylamide gels containing sodium dodecyl sulfate always gave single bands. Furthermore, the carboxymethylated-succinylated and carboxymethylated-maleylated enzymes have the same electrophoretic mobilities; these mobilities are less than the mobilities of carboxymethylated AMP-deaminase and untreated enzyme, as described above. The COOH-terminal amino acids of rabbit and chicken muscle AMP-deaminases were assayed by digestion with carboxypeptidase A, carboxypeptidase B, and a mixture of the two, over a wide range of enzyme/substrate ratios and digestion times. Samples containing up to 10 mg of deaminase were digested with as much as 2.5 nmol of carboxypeptidase at 37°C for as long as 24 hours. Denaturation of substrate was accomplished by treatment with 56 mM sodium dodecyl sulfate at 100°C. Both untreated AMP-deaminases, as well as enzymes which had been modified by reduction and carboxymethylation, were examined. Sodium dodecyl sulfate (56 mM) was present during all digestions. Related amino acids were identified and quantitated by amino acid analysis after precipitation of protein with trichloroacetic acid. Carboxypeptidases A and B were assayed in the presence of 56 mM sodium dodecyl sulfate by the methods of Folk et al. (17, 18). Bovine ribonuclease was examined under conditions identical with those of a control.
The A280/A200 ratios were high (>1.9), there was no visible spectrum, and the amino acid composition accounted for >95% of the weight of the lyophilized samples. Furthermore, under conditions where the carbohydrate moiety of γ-globulin was easily detected in acrylamide gels, samples of the deaminases that had been subjected to electrophoresis were negative when similarly stained with periodic acid-Schiff.

No NH₂-terminal amino acids could be identified for either AMP-deaminase by the Stark and Smyth procedure (14) or by the dansyl technique. The significance of our NH₂-terminal studies is that they provide evidence which indicates that skeletal muscle AMP-deaminases, like many other cytoplasmic proteins from muscle (28), have blocked NH₂ termini. However, the data provide no new information regarding either the number or degree of homology among the polypeptide chains of AMP-deaminase. Inasmuch as ω-dansyl lysine and O-dansyltyrosine were the only derivatives identified, the results provide additional evidence for homogeneity of the proteins used in our studies. Any significant protein contaminants having free NH₂ termini should have contributed to the dansyl derivatives detected.

Digestion with carboxypeptidases A or B (or both) failed to liberate stoichiometric amounts of any amino acid. Resistance to digestion by these enzymes can be due to several factors. The COOH-terminal groups may be amidated, or they may be among those residues which are inherently resistant to the exopeptidases. A third possibility may be related to the conformation of the deaminase subunits in sodium dodecyl sulfate; this conformation may be a non-substrate even if the terminal residues would normally be susceptible to carboxypeptidase digestion.

By comparison with the mobilities of polypeptides of known size, the molecular weights of the acylated polypeptides can be estimated to be 85,000. Lower molecular weight species were not observed. Thus, the AMP-deaminases from chicken and rabbit muscle do not appear to be stabilized by noncovalent linkages which are readily disrupted by a large increase in negative charge. Unlike the results reported for the rat enzyme (22), treatment of carboxymethylated deaminase from chicken and rabbit muscle with either maleic or succinic anhydride causes an apparent increase in molecular weight. The magnitude of the apparent increase in the molecular weights of the subunits following acylation is 4 times the calculated amount based on lysine contents and the estimated degree of substitution. This observation is consistent with the results obtained by Tung and Knight (25) in their investigation of the effect of maleic anhydride on the apparent molecular weights of several low molecular weight proteins.

Rabbit and chicken muscle AMP-deaminases have very similar amino acid compositions (Table I). The composition reported here for the rabbit enzyme is consistent with an earlier study (20), whereas the amino acid composition of the chicken AMP-deaminase has not been previously reported. Several observations indicate that the enzyme preparations used in these studies contain negligible amounts of nonprotein constituents. The A₂₅₀/A₂₈₀ ratios were high (>1.9), there was no visible spectrum, and the amino acid composition accounted for >95% of the weight of the lyophilized samples. Further-
unique tryptic peptides if the chicken and rabbit AMP-deaminases are composed of four unrelated, nonidentical subunits, whereas 75 should be formed if the four polypeptide chains are identical. Thus, it was anticipated that the pattern of ninhydrin-positive spots which constitute the tryptic peptide map would be quite complex. However, on the basis of their half-cystine contents, we expected a maximum of 32 radioactively labeled peptides if the subunits are nonidentical and only 8 if the subunits are either identical or very similar. The tryptic peptide maps of $S^{14}\text{C}$-carboxymethylated chicken and rabbit muscle AMP-deaminases are shown in Figs. 2 and 3, respectively.

There was a total of 91 ninhydrin-positive spots on the peptide map of rabbit muscle AMP-deaminase. Assuming maximum identity of adjacent spots, the minimum number of soluble tryptic peptides detected was 44. Five radioactively labeled peptides were detected on the map of rabbit muscle AMP-deaminase. These may represent five unique $S^{14}\text{C}$-carboxymethylcysteine-containing tryptic peptides. On the other hand, it is likely that some spots represent single peptides containing more than 1 $S^{14}\text{C}$carboxymethylcysteine residue or 2 radioactively labeled peptides having the same electrophoretic mobilities. This hypothesis is consistent with the relative heights of the peaks in the radioactivity profile.

A similar assessment of the number and position of the ninhydrin-positive spots on the electrophoretogram for chicken muscle AMP-deaminase shows a maximum of 112 and a minimum of 63. The elution profile shows at least six radioactively labeled peptide pools. The radioactivity found in the breakthrough peak (Pool I) in both figures was attributed to carboxymethylated 2-mercaptoethanol.

There was no insoluble core in the tryptic digest of chicken muscle deaminase. The insoluble tryptic core of the rabbit enzyme contained an amount of radioactivity equal to only one-half that found under Peak XV or XXII (Fig. 3). This might have represented a single radioactive peptide which was obtained in low yield. The core peptides were chromatographed on a column of Sephadex G-75 (fine) which was equilibrated

![Fig. 2. Tryptic peptide map of AMP-deaminase from chicken muscle. A tryptic digest of $S^{14}\text{C}$-carboxymethylated protein (0.9 $\mu$mol) was chromatographed on a column (2.0 x 25 cm) of Dowex 50W-X8 at 55°. The column was developed at a flow rate of 80 ml/hour with a double linear gradient of pyridine acetate buffers as described by Bradshaw et al. (19). Fractions of 6 ml were collected. Peptides were monitored automatically by ninhydrin analysis (-) after alkaline hydrolysis, and radioactivity (-----) was determined on 0.2 ml aliquots of each fraction. Fractions were pooled as indicated by the solid bars and Roman numerals above the ninhydrin and radioactivity profiles; the pooled fractions were subjected to electrophoresis at pH 3.75 at 2000 volts for 90 min. The ninhydrin-positive pattern of each pool is shown immediately above the corresponding bar. The most intensely stained spots are shown in solid black, those of intermediate intensity are shown in solid outline, and the least intensely stained spots are shown in broken outline. Each radioactive peptide is indicated by an X immediately to the left of the spot. STD, standard mixture of amino acids.]

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Fig. 3. Tryptic peptide map of AMP-deaminase from rabbit muscle. Experimental conditions were the same as given in the legend for Fig. 2, as are the symbols.

with 6 M guanidine hydrochloride plus 0.1 M acetic acid. The elution profile showed that only a very small amount of radioactivity was associated with a peak. This trace of radioactive label was regarded as insignificant.

The results which have been presented indicate that both chicken and rabbit muscle AMP-deaminase consist of four identical or very similar polypeptide chains. The most significant data supporting this hypothesis are the patterns obtained when tryptic digests were subjected to ion exchange chromatography and electrophoresis, particularly the low number of radioactively labeled peptides.

The AMP-deaminases from chicken and rabbit muscle share many structural and catalytic properties. Furthermore, as indicated in an earlier communication (1), they exhibit similarities in tissue distribution, and they probably serve similar physiological roles. At the time we reported that rabbits produce an autoantibody to AMP-deaminase when immunized with enzyme from appropriate sources (4), virtually no structural information was available. The data presented in this report, together with our previous investigations of the subunit structure of these AMP-deaminases (1), support the hypothesis that their similarities as antigens can most likely be attributed to similarities in primary sequence or conformational determinants (or both), rather than common or related polysaccharide constituents.

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REFERENCES

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