Short Communications

C-Terminal Amino Acid Sequence of Bovine γ -Crystallin

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Proteins may be characterized by the determination of their terminal amino acid sequences, and this information also provides evidence for the homogeneity of the protein and an estimate for its molecular weight. The proteins of the vertebrate lens have been divided into three families, namely α -, β - and γ -crystallins (Waley, 1969). The lens proteins α - and γ -crystallins have been studied with respect to their *N*-terminal amino acid sequences (Corran & Waley, 1969; Björk, 1964). At present no structural information is available on the *C*-terminal amino acid sequences of these proteins. Bovine γ -crystallin has been shown to be heterogeneous and separable into six components by ion-exchange chromatography (Björk, 1961, 1964, 1970). Björk (1964) suggested that the four main components, fractions II, IIIa, IIIb and IVb, were probably homogeneous proteins with molecular weights of about 20000. The proteins had very similar amino acid compositions and the same *N*-terminal amino acid sequences (Björk, 1964):

Gly-Glu-Leu(or Ile)

This communication reports the determination of the *C*-terminal amino acid sequence of γ -crystallin (fraction II, referred to below as fraction F₂), and provides evidence for the homogeneity of this protein fraction and suggests its molecular weight to be 19200.

Materials. Bovine γ -crystallin (fraction F₂) was prepared from an aqueous lens extract by gel filtration on Sephadex G-75, followed by ion-exchange chromatography on sulphoethyl-Sephadex (Björk, 1961, 1964). Performic acid-oxidized protein was prepared as described by Croft & Waley (1971). Trypsin (salt-free, twice recrystallized) and carboxypeptidases A and B (treated with diisopropyl phosphorofluoridate) were obtained from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Reagents were of A.R. grade and those used in the Edman degradation cycle were distilled before use and stored under N₂. Anhydrous hydrazine was prepared by twice distilling commercial 99% hydrazine hydrate *in vacuo* from KOH pellets. Polyamide sheets were obtained from the

Cheng Chin Trading Co. Ltd., Taipei, Taiwan.

Methods. (a) Digestion with carboxypeptidase A. (i) Native γ -crystallin (fraction F₂) (approx. 5mg) was dissolved in 1ml of 0.5% NH₄HCO₃, containing a known amount of norleucine, which was used as an internal standard. The accurate protein concentration was determined spectrophotometrically at 280nm ($E_{1\text{cm}}^{1\%}$ 20.3; Björk, 1964). The solution was equilibrated at 37°C; 0.2ml of carboxypeptidase A solution (1mg/ml) was added to initiate hydrolysis. At intervals 0.2ml of solution was removed and the reaction terminated by 10 μ l of 6M-HCl. The solution was then made up to a final volume of 1ml with sodium citrate buffer, pH 2.2, for quantitative amino acid analysis. (ii) Performic acid-oxidized fraction F₂ was rendered water-soluble by reaction with citraconic anhydride (Dixon & Perham, 1968). The product was then dialysed overnight in 0.5% NH₄HCO₃ solution. The resulting solution was made up to a known volume, and a small sample removed for acid hydrolysis and quantitative amino acid analysis, from which the accurate protein concentration was obtained. The digestion with carboxypeptidase was carried out as described for the native protein.

(b) Isolation of the *C*-terminal tryptic peptide. The oxidized protein was digested with trypsin, as described by Croft & Waley (1971). The digest was fractionated by paper electrophoresis at pH 3.6, followed by paper chromatography in solvent BAWP [butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953)] at right-angles. Peptides were detected with the ninhydrin reagent (Heilman, Barollier & Watzke, 1957) and arginine-containing peptides with the phenanthraquinone reagent (Itano & Yamada, 1966).

(c) Amino acid analysis. Quantitative amino acid analyses were made with a Locarte amino acid analyser, with a single column for the determination of acidic, neutral and basic amino acids.

Results. (a) Carboxypeptidase A reaction. Fig. 1(a) illustrates the liberation of tyrosine from fraction F₂ by carboxypeptidase A. No other amino acids were liberated. Fig. 1(b) illustrates the

Fig. 1. (a) Release of tyrosine from γ -crystallin (fraction F_2) by carboxypeptidase A. (b) Release of amino acids from citraconylated oxidized γ -crystallin (fraction F_2) by carboxypeptidase A. Curve 1, tyrosine; curve 2, phenylalanine; curve 3, aspartic acid; curve 4, valine; curve 5, methionine sulphone.

liberation of amino acids from citraconylated oxidized fraction F_2 . Tyrosine was the most rapidly released amino acid, followed by phenylalanine and aspartic acid; valine and methionine sulphone were released more slowly.

(b) Isolation of the *C*-terminal peptide. As there are two residues of lysine in the peptide chain of fraction F_2 (Björk, 1964), it was possible after staining one peptide 'map' with ninhydrin and a second with the phenanthraquinone reagent, which is specific for arginine-containing peptides, to identify three peptides that lacked arginine. On isolation from a preparative peptide 'map', two of the peptides were found to contain lysine; the third lacked both lysine and arginine, and was concluded to be the *C*-terminal tryptic peptide. This peptide had R_{Avg} 3.0 in solvent BAWP and its mobility at pH 6.5 relative to aspartic acid was (-0.3) . Amino acid analysis indicated the composition: Asp (1.2), Mes* (0.8), Val (0.8), Phe (1.0), Tyr (1.0). Hydrazinolysis (Akabori, Ohno & Narita, 1952) revealed that tyrosine was the *C*-terminal residue. This was confirmed by reaction with carboxypeptidase A, when tyrosine and phenylalanine were released in the ratio 1.0:0.8. *N*-Terminal analysis by the dansyl method (Gray & Hartley, 1963) indicated that valine was the *N*-terminal residue. Sequence analysis by the dansyl-Edman method (Gray, 1967) indicated the sequence:

Val-Mes-Asx-Phe-Tyr

The mobility of the peptide on electrophoresis at pH 6.5 suggested the presence of aspartic acid.

* Abbreviation: Mes (in amino acid sequences), methionine sulphone.

Discussion. The results obtained from digestion of oxidized fraction F_2 with carboxypeptidase A indicated that the *C*-terminal amino acid sequence was:

(Val,Mes)Asp-Phe-Tyr

The *C*-terminal tryptic peptide was isolated and this had the structure:

Val-Mes-Asp-Phe-Tyr

These results show that the *C*-terminal pentapeptide of fraction F_2 is:

Val-Met-Asp-Phe-Tyr

The results obtained from the carboxypeptidase A reaction with both fraction F_2 and oxidized fraction F_2 suggest a molecular weight of 19200 ($\pm 3\%$) for the protein, which is in good agreement with the value of 19100 obtained from ultracentrifuge studies (Björk, 1964). These results also support the suggestion that fraction F_2 is a homogeneous protein (Björk, 1964).

Bovine and rabbit γ -crystallins both have *N*-terminal glycine (Björk, 1964; Mason & Hines, 1966); it has also been shown that one of the polypeptide chains of rabbit γ -crystallin has *C*-terminal tyrosine (Manalaysay & Hines, 1968). It therefore seems that at least one of the polypeptide chains of rabbit γ -crystallin has the same terminal residues as those of calf fraction F_2 . This gives support to the immunochemical finding that the lens proteins are organ-specific rather than species-specific.

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