The Amino-acid Sequence of Gamma Crystallin

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Summary The amino-acid sequence of gamma crystallin, fraction II, has been determined, which indicates it to be a single chain polypeptide of 165 amino-acid residues.

THE γ-crystallins are a group of cryoproteins present in the vertebrate lens. Four homogeneous proteins (fractions II, IIIa, b, and IVb) were isolated and crystallized from calf lens by Björk.[1-3] Recently, interest has been centred on these proteins as it has been suggested that they may be involved in cataract formation.[4] This communication describes the determination of the amino-acid sequence of fraction II, which is the first lens protein to have its amino-acid sequence described.

The protein used in this study was prepared from calf lenses by the method of Björk.[1,2] Amino-acid analysis indicated the following composition in molar ratios: Lys (2), His (5), Arg (19), Asp (18), Thr (5), Ser (12), Glu (18), Gly (12), Ala (2), Val (6), Met (7), Ile (6), Leu (12·5), Tyr (15), Phe (8), Pro (8), Cys (6), Trp (3). This agrees with the sequence analysis, except for Gly and Leu, which from the

sequence are 13 and 12 respectively. A molecular weight of 19,870·6 was calculated for the correct 165 residues, consistent with a value of 19,200 ± 600 found by determination of *C*-terminal tyrosine[5] and 19,100 by sedimentation equilibrium.[2]† The *N*-terminal residue is known to be

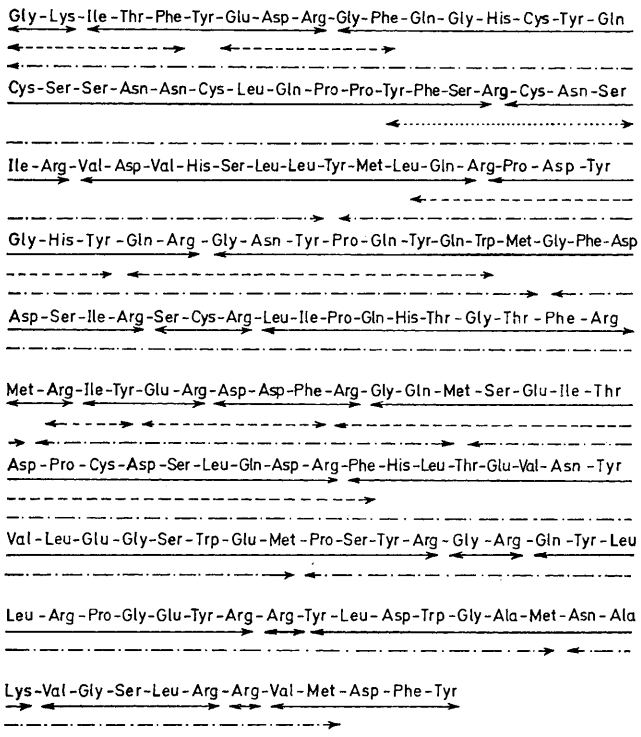

FIGURE. *Proposed amino-acid sequence of gamma crystallin, fraction II, from calf lens. Chymotryptic* (←——→), *thermolytic* (←···→), *and cyanogen bromide* (←·—·—·→) *peptides used to overlap tryptic peptides* (←————→).

glycine.[2] The *N*-terminal sequence was determined by the Edman stepwise degradation,[6] to be Gly-Lys-Ile-Thr-Phe-Tyr-Glx. This was confirmed by isolation from a tryptic digest of citraconoylated protein, the peptide *N*-citraconoyl-Gly(N^{ε}-citraconoyl)Lys-Ile-Thr-Phe-Tyr-Glu-Asp-Arg. The methods used to identify the *C*-terminal tryptic peptide have already been described.[5] Samples of performic acid oxidized and *S*-carboxymethylated protein were digested with trypsin and from these digests 19 unique peptide fragments were obtained, together with the free amino-acid arginine. (The peptide fragments Leu-Ser-Ser-Cys-Arg, Glu-Val-His-Ser-Cys-Lys, previously isolated,[7] albeit in low yield, are now thought to have been due to a cross contaminant of fraction I; their isolation could not be repeated.) All the peptide fragments were purified by suitable combinations of column chromatography, paper chromatography, and electrophoresis. The peptide fragments were completely, or partially sequenced by standard methods, much use being made of the dansyl Edman procedure.[8] Asparagine and glutamine residues were assigned from the electrophoretic mobilities of the purified peptides.[9] Reaction of the protein with cyanogen bromide in 70% formic acid,[10] gave rise to eight peptides which were purified by column chromatography and paper electrophoresis. The cyanogen bromide peptides were partially sequenced, in a similar manner to that used for the tryptic peptides. These results enabled the derivation of almost the entire amino-acid sequence. Further support for this sequence was obtained from studies of the peptides that were isolated after digestion of the protein with the enzymes, chymotrypsin, pepsin, and thermolysin. Full details on the isolation, characterization, and sequences of these peptides will be published elsewhere.

An interesting feature of the sequence is the irregular distribution of sulphur containing amino-acids, as indicated in the Table. This feature may be related to the relative

TABLE

Distribution of Cysteine and Methionine in Gamma Crystallin, Fraction II

Region	Residues of Cysteine	Residues of Methionine
1—40	4	0
41—81	1	2
82—122	1	2
123—165	0	3

stability of this protein fraction to oxidation and consequent denaturation as compared to the other gamma crystallin fractions, the amino-acid sequences of which are at present being investigated.

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† The overall shape (from viscosity measurements) was spherical with 22% β-structure and 0% helical content (from c.d. measurements). The electrophoretic mobility relative to bovine serum albumin at pH 9·0 was found to be 0·58.

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