The Amino Acid Sequence of γ -Crystallin (Fraction II) from Calf Lens

By L. R. CROFT

Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford OX2 6AW, U.K.

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The amino acid sequence of γ -crystallin (fraction II) from calf lens has been determined; this indicates it to be a single-chain polypeptide of 165 amino acid residues.

The γ -crystallins are a group of low-molecular-weight proteins present in the vertebrate lens. They are unusual in that they behave as cryoproteins. Four homogeneous proteins (fractions II, IIIa,b, and IVb) were isolated and crystallized from calf lens (Björk, 1964). Recently interest has been centred on these proteins, as it has been suggested that they may be involved in cataract formation (Charlton & van Heyningen, 1968; Zigman, 1971).

In the present paper I give evidence for the amino acid sequence of γ -crystallin (fraction II) from calf lens. A preliminary report of this work has been published (Croft, 1972).

Experimental

Materials

γ -Crystallin (fraction II) was prepared from calf lenses by the method of Björk (1961, 1964), the only modifications being that the buffers used were 10 mM with respect to β -mercaptoethanol and the final chromatography on phosphocellulose was omitted. Other materials were as described by Croft & Waley (1971).

Methods

Preparation of derivatives. (a) Performic acid-oxidized γ -crystallin. The protein was oxidized with performic acid as described by Croft & Waley (1971). (b) *S*-Carboxymethyl γ -crystallin. The protein was reduced and carboxymethylated by the method of Björk (1970).

Enzymic digestion. (a) Trypsin. The protein was digested with trypsin as described by Croft & Waley (1971). (b) Chymotrypsin. The performic acid-oxidized protein (200 mg) was digested with chymotrypsin (3 mg) for 3 h in 1% (w/v) NH_4HCO_3 solution at 37°C. (c) Thermolysin. The performic acid-oxidized protein (100 mg) was digested with thermolysin (1.5 mg) for 3 h in 0.2 M ammonium acetate (pH 8.5), at 37°C.

Cyanogen bromide cleavage. Cyanogen bromide (250 mg) was added to a solution of γ -crystallin (100 mg) in 2 ml of 70% (v/v) formic acid, and the

solution was left at room temperature overnight. The reaction mixture was diluted to 10 ml with water and freeze-dried. The residue was dissolved in 1 ml of 50% (v/v) acetic acid and loaded on a column (2.5 cm $\times$ 26 cm) of Sephadex G-10, equilibrated with 50% acetic acid. The column was operated at room temperature at a flow rate of 40 ml/h and 4 ml fractions were collected. Peptide material was detected by measuring the E_{280} of each fraction and was eluted in the void volume of the column. All peptide material was combined and freeze-dried. The residue was subjected to performic acid oxidation (Hirs, 1967) and was then diluted with water and freeze-dried.

Sequential Edman degradation of γ -crystallin. Approximately 0.5 μmol of performic acid-oxidized γ -crystallin was used in the Edman degradation procedure. Reaction of the protein with phenyl isothiocyanate, removal of excess of reagents, cleavage with trifluoroacetic acid and extraction of the 2-anilino-5-thiazolinone derivatives were performed as described by Matsubara *et al.* (1968). After extraction of the 2-anilino-5-thiazolinone derivative, the protein was returned to the coupling step of the procedure. The thiazolinones were converted into the substituted phenylthiohydantoin in acetic acid-conc. HCl (5:1, v/v) at 40°C for 1 h (DeLange *et al.*, 1968). The amino acid phenylthiohydantoin derivatives were identified by chromatography on silica-gel thin-layer plates, by the method of Jeppsson & Sjöquist (1967). The procedure was slightly modified so that after each degradation step, the newly released *N*-terminal amino acid was identified after dansylation. The Dns-amino acids were identified by the method of Woods & Wang (1967).

Electrophoresis and chromatography. High-voltage electrophoresis of the digests was done at 50-100 V/cm in a cooled-plate apparatus at pH 1.9, pH 3.6 or pH 6.5, with the buffers described by Sargent (1965). Mobilities (*m*) at pH 6.5 refer to the distance from the leading edge of the peptide to the centre of the neutral zone as a fraction of the distance moved by aspartic acid (*m* = -1.0). Mobilities (*M*) at pH 1.9, refer to the distance from the leading edge of the peptide to the origin as a fraction of the distance moved

by arginine ($M = 1.0$). Descending paper chromatography was done in BAWP solvent [butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953)] on Whatman no. 1 paper. Peptides were detected with ninhydrin-cadmium reagent (Heilmann *et al.*, 1957); arginine peptides were detected with the phenanthraquinone reagent (Itano & Yamada, 1966). Tryptophan peptides were detected with the Ehrlich reagent (Smith, 1960). The positions of peptides were calculated relative to arginine (R_{Arg}).

Amino acid analysis. Peptides were hydrolysed, under vacuum, with redistilled 6M-HCl, containing 5% (v/v) of 0.1M-thioglycolic acid, for 24h at 110°C and the hydrolysates were analysed on a Locarte amino acid analyser, with a single column for the determination of acidic, neutral and basic amino acids.

Identification of N- and C-terminal amino acid residues. The methods of Croft & Waley (1971) were used. The only modification of these methods was the substitution of toluene for benzene in the solvent system of Woods & Wang (1967) for the resolution of Dns-amino acids on polyamide thin layers.

Amino acid sequence determination. This was done by the dansyl-Edman procedure of Gray (1967). Arrows above the peptides show the amino acids that were identified. [Arrows under peptides show the amino acids identified after treatment of the peptide with carboxypeptidases A and B (Croft & Waley, 1971).] The dansyl-Edman procedure was sometimes checked by analysing the peptide at each stage of the degradation.

Amide residues. Amide residues were assigned on the basis of electrophoretic mobilities of the peptides at pH 6.5 (Offord, 1966).

Partial acid hydrolysis of γ -crystallin. The protein (25mg) was dissolved in 20ml of 2% acetic acid and hydrolysed at 110°C in an evacuated Carius tube for 86h. The hydrolysate was then freeze-dried.

Results

Amino acid analysis of γ -crystallin

The amino acid composition of calf γ -crystallin is shown in Table 1. Tryptophan was determined by two independent methods, which agreed with each other within 7%. The amino acids subject to either destruction during acid hydrolysis, or incomplete liberation have been corrected for. The distinguishing features of this composition are the relatively high tyrosine and arginine content and the low lysine and alanine content.

N-Terminal sequence determination

The oxidized protein was subject to six steps of the Edman degradation, and the N-terminal sequence

Table 1. *Amino acid analysis of γ -crystallin*

Values are molar proportions, calculated relative to valine (=6). Half-cystine was determined separately as cysteic acid and S-carboxymethylcysteine; an average of these values was taken. Tryptophan was determined by the method of Gaitonde & Dovey (1970) and by the method of Liu & Chang (1971); an average value was taken. The analyses for serine, threonine and tyrosine were extrapolated to zero time of hydrolysis, and analyses for valine and isoleucine were corrected for incomplete hydrolysis after hydrolysis for 20h, 44h and 72h.

Amino acid	Amino acid composition	
	Molar proportions	Nearest integer
Lys	1.87	2
His	4.95	5
Arg	19.40	19
Asp	18.40	18
Thr	4.70	5
Ser	12.40	12
Glu	18.30	18
Pro	8.30	8
Gly	11.70	12
Ala	1.87	2
Val	6.00	6
Met	6.80	7
Ile	6.19	6
Leu	12.50	12-13
Tyr	15.10	15
Phe	8.40	8
Cys	6.30	6
Trp	3.30	3

was concluded to be Gly-Lys-Ile-Thr-Phe-Tyr-Glx. Residues 1, 3 and 4 were identified by both their Dns- and phenylthiohydantoin derivatives; residue 2 was identified only as its phenylthiohydantoin derivative; residues 5, 6 and 7 were identified only as their dansyl derivatives.

Characterization and amino acid sequence of the tryptic peptides

The composition of the tryptic peptides that were obtained from γ -crystallin is given in Table 2, together with the methods used to isolate and purify the peptides. The sequences and partial sequences of these peptides are shown in Fig. 1, and were determined by the dansyl-Edman procedure.

Peptides T₃, T₄, T₈ and T₁₃ correspond to peptides T₂, T₃, T₅ and T₁ (Croft & Waley, 1971) and evidence for their sequences has already been pres-

Table 2. Amino acid compositions and properties of tryptic peptides from γ -crystallin

Purification procedures (for details see the text) were as follows: electrophoresis at pH 6.5 (A); paper chromatography in solvent BAWP (B); electrophoresis at pH 1.9 (C); electrophoresis at pH 3.6 (D); ion exchange on Dowex 50 (E) (Croft & Waley, 1971); gel filtration on Sephadex G-25 [S(G-25)].

Peptide Amino acid	Composition (mol of amino acid/mol of peptide)																			
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇ *	T ₈	T ₉	T ₁₀	T ₁₁ †	T ₁₂	T ₁₃	T ₁₄ *	T ₁₅	T ₁₆	T ₁₇	T ₁₈ *	T ₁₉	T ₂₀
Lys	1.0																	1.0		
His			1.0		1.0	0.8			0.9					1.0						
Arg		1.0	1.0	0.8	1.0	1.0	1.1	1.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0		1.0	
Cys(O ₃ H)		2.8	2.2	1.1				1.0					1.0							
Asp		1.2	2.2	1.2	1.1	1.1	3.4			1.0		2.0	3.3	1.3	0.8			2.2		1.0
Met(O ₂)					0.6				1.8				0.8	0.7						0.7
Thr		0.9											1.1	1.1						
Ser			3.0	1.0	1.0		0.9	1.0					2.2	2.0					1.0	
Glu		1.0	3.1		1.2	1.2	1.8		1.0		1.0		3.3	2.8		2.0				
Pro			1.6			1.0	1.0	1.0					1.0	1.0		1.0				
Gly	1.0		2.0		0.2	0.9	1.8		1.0				1.2	1.2	1.0	1.0		1.1	1.0	
Ala																		2.0		
Val					1.6									1.8					1.1	0.9
Met							0.8											1.0		
Ile		1.0		0.6				0.8	0.8		0.9		1.2	2.2						
Leu		1.0	1.0		2.6				1.0				1.2			2.0		1.0	1.1	
Tyr		1.0	2.2		1.2	1.6	1.7				0.5			1.8		2.0		0.6		0.9
Phe		0.9	2.0				0.8		1.0			1.0		1.0						0.9
Trp							†							†				†		
m	+0.70	-0.25	-0.35	0.0	+0.14	+0.18	-0.16	0.0	+0.40	+0.56	0.0	-0.38	-0.60	Streak	+0.84	0.0	+0.90	0.0	+0.50	-0.26
M	1.12	0.46		0.32	0.30		0.32		0.37		0.41				1.02	0.33	1.0	0.35	0.65	
R(Arg)	0.69	1.84	1.10	0.90	1.80	1.04	1.64	0.70	2.52	1.12	2.70	1.18	0.50		0.77	2.58	1.0	1.80	2.45	2.95
N-Terminal			Gly	Cys(O ₃ H)	Val	Pro	Gly	Ser	Leu	Met(O ₂)	Ile	Asp	Gly	Phe	Gly	Glu-	Arg	Tyr	Val	Val
amino acid			I	I, A	I, A, B	I, A, B	B, C	I, A, B	I, B	I, A, B	I, A, B	I, C	I	S(G-25)	I, S	I, A, B	I, C	B, C	I, A	I, A
Purification	I, B, C	I, B, C	I	I, A	I, A, B	I, A, B	B, C	I, A, B	I, B	I, A, B	I, A, B	I, C	I	S(G-25)	I, S	I, A, B	I, C	B, C	I, A	I, A

* Isolated from a digest of S-carboxymethyl protein.

† Hydrolysis for 90 h.

‡ Measured with Ehrlich reagent.

Fig. 1. Tryptic peptides from γ -crystallin

Residues identified by the dansyl-Edman method are shown by $\rightarrow$. Amino acids liberated on treatment with carboxypeptidases A and B are indicated by $\leftarrow$. Amide groups were assigned from electrophoretic mobility. Evidence for the sequence of the larger peptides is discussed in the text or in the references indicated, *Croft & Waley (1971); †Croft (1971).

ented. Peptides T₇, T₁₄ and T₁₈ were isolated from a tryptic digest of *S*-carboxymethyl- γ -crystallin and contained tryptophan. Peptide T₁₄ was digested with chymotrypsin and the resulting tryptophan peptide, T_{14a}, was purified by electrophoresis at pH6.5. Peptide T_{14a} (*m*, -0.28) had the composition Val(0.9), Gly(1.0), Glu(1.0), Ser(1.0), Leu(0.7), Trp(+); the dansyl-Edman procedure indicated the sequence Val-Leu-Glu-Gly-Ser-Trp.

Characterization and partial amino acid sequence of cyanogen bromide peptides

Eight peptides were obtained after reaction of the protein with cyanogen bromide. The amino acid

compositions, properties and methods used to isolate these peptides are given in Table 3.

Peptide CB₈ was the only peptide not having C-terminal homoserine and was thus derived from the C-terminus of the protein (Croft, 1971). Dansyl-Edman degradation indicated the sequence Asp-Phe-Tyr. Peptide CB₂, was subjected to the dansyl-Edman degradation, which indicated the *N*-terminal sequence Leu-Glx-Arg-Pro-Asx-Tyr. The partial sequence of peptide CB₂ is therefore Leu-Glx-Arg-Pro-Asx-Tyr (Gly₂, His, Arg, Asx, Glx₃, Pro, Tyr₃)Hse*.

*Abbreviations: Met(O₂), methionine sulphone; Cys(O₃H), cysteic acid; Hse, homoserine; $\overrightarrow{\text{Glu}}$, pyrrolid-2-one-5-carboxylic acid.

Table 3. *Amino acid composition of cyanogen bromide peptides from γ -crystallin*

The peptides from the cyanogen bromide reaction were separated into three fractions on the basis of solubility in (i) pyridine-acetate buffer, pH 6.5; (ii) 1% acetic acid; (iii) 0.5% NH_3 . Purification procedures (for details see the text) were as follows. The peptides in fraction (i), CB_3 , CB_4 , CB_7 and CB_8 , were purified by electrophoresis at pH 6.5 (A), followed by paper chromatography in BAWP (B). Fraction (ii), contained peptides CB_2 and CB_6 , which were fractionated on Sephadex G-25 in 1% acetic acid [S(G-25)], followed by electrophoresis at pH 1.9 (C). Peptides CB_1 and CB_5 , which were in fraction (iii), were purified by chromatography on Sephadex G-50 in 0.5% NH_3 [S(G-50)].

Composition (mol of amino acid/mol of peptide)

Peptide ... Amino acid	CB_1	CB_2	CB_3	CB_4	CB_5	CB_6	CB_7	CB_8
Hse	1.0	1.0	1.0	1.0	0.9	1.0	1.0	
Lys	1.0						1.0	
His	1.6	0.7	1.0		1.3			
Arg	3.3	2.3	3.2	2.9	1.2	4.5	2.0	
Cys(O_3H)	3.4		0.8		1.0			
Asp	4.9	2.0	2.4	2.0	3.8	1.2	1.0	1.0
Met(O_2)								
Thr	1.2	0.2	1.6		1.8			
Ser	4.5		2.0		2.9	1.2	0.9	
Glu	4.2	4.4	1.0	2.0	4.6	2.2		
Pro	1.7	2.2	1.0		1.3	1.7		
Gly	2.7	2.0	2.0	1.0	1.4	2.6	1.0	
Ala						0.6	0.9	
Val	2.1				1.8		2.0	
Met								
Ile	1.8		1.7	1.0	1.0			
Leu	3.3	1.3	1.3		3.0	2.7	1.0	
Tyr	3.6	3.6		1.0	1.2	3.3		1.0
Phe	2.8	0.3	2.0	1.0	1.2			1.0
<i>m</i> ...	Streaks		+0.42	+0.27	Streaks		+0.80	-0.44
<i>M</i> ...	Streaks	0.60			Streaks	0.64		
$R_{(\text{Arg})}$...	Streaks		1.54	1.18	Streaks		1.28	2.80
<i>N</i> -Terminal amino acid...	Gly	Leu	Gly	Arg	Ser	Pro	Asx	Asp
Purification...	S(G-50)	S(G-25), C	A, B	A, B	S(G-50)	S(G-25), C	A, B	A, B

The remaining cyanogen bromide peptides were digested with trypsin and the resulting peptides (some of which are listed in Table 4) were purified by suitable combinations of paper electrophoresis and chromatography. In many cases the resulting tryptic peptides could be identified as the same peptide isolated from tryptic digests of the protein. For this identification the minimum criteria were identical quantitative amino acid analysis and the same *N*-terminus. Peptide CB_1 was digested with trypsin and peptides T_1 , T_2 , T_3 and T_4 were isolated, but the *C*-terminal fragment was not isolated. Reaction of peptide CB_1 with carboxypeptidase A gave homoserine followed by tyrosine.

Peptide CB_3 , on reaction with carboxypeptidases A and B gave homoserine, arginine and phenylalanine, in that order. Peptide CB_3 was subjected to the dansyl-Edman degradation; this indicated the *N*-terminal sequence Gly-Phe-Asx-Asx-Ser. Digestion of peptide CB_3 with trypsin gave peptides T_8 and T_9 , together with peptide CB_3Ta . The partial sequence of peptide CB_3 is therefore Gly-Phe-Asp-Asp-Ser-Ile-Arg-Ser-Cys(O_3H)-Arg-Leu-Ile-Pro-Gln-His-Thr-Gly-Thr-Phe-Arg-Hse.

Peptide CB_4 was digested with trypsin; this gave the free amino acid, arginine, peptides T_{11} and T_{12} and peptide CB_4Ta . Peptide CB_4Ta had the sequence Gly-Glx-Hse. These results suggest the sequence of

Table 4. *Amino acid compositions of some tryptic peptides isolated from cyanogen bromide peptides*

For abbreviations of purification procedures see Table 2.

		Composition (mol of amino acid/mol of peptide)						
Peptide	...	CB ₃ Ta	CB ₄ Ta	CB ₅ Ta	CB ₅ Tb	CB ₆ Ta	CB ₆ Tb	CB ₇ Ta
Amino acid								
Lys								1.1
His					1.0			
Arg		1.0		1.0		1.0		
Cys(O ₃ H)				1.0				
Asp		1.8		3.0	1.0		1.0	1.0
Thr				0.7	0.6			
Ser		1.0		1.9	1.2	1.0		
Glu			1.0	1.8	2.8			
Hse			0.5		0.6		0.4	
Pro				0.8		1.0		
Gly		1.0	1.0		0.8		1.1	
Ala							1.2	1.0
Val					1.7			
Ile		0.7		0.9				
Leu				1.0	2.1		1.0	
Tyr					0.8	0.6	0.8	
Phe		0.9			0.9			
<i>m</i>	...	-0.40		-0.80	Streaks	+0.42	0.0	
<i>M</i>	...	0.50	0.46			0.70	0.23	0.89
<i>N</i> -Terminal								
amino acid	...	Gly	Gly	Ser	Phe	Pro	Tyr	Asx
Purification	...	C	C	A	S(G-25)	A, C	A, C	C

peptide CB₄ to be Arg(Ile-Tyr-Glu-Arg;Asp-Asp-Phe-Arg)Gly-Glx-Hse. (The symbol ; indicates that the order of the peptides it separates was not yet defined).

Peptide CB₅, on digestion with trypsin gave only two peptides, CB₅Ta and CB₅Tb. Peptide CB₅Tb was subjected to the dansyl-Edman degradation which indicated the *N*-terminal sequence to be Phe-His-Leu-Thr-Glx-Val-Asx-Tyr-Val-Leu. The partial sequence of peptide CB₅ was thus Ser(Cys(O₃H), Asx₃, Thr, Ser, Glx₂, Pro, Ile, Leu)Arg-Phe - His - Leu - Thr - Glx - Val - Asx - Tyr - Val - Leu(Glx₂, Gly, Ser)Hse.

Peptide CB₆, on reaction with carboxypeptidase A gave homoserine and alanine in the molar ratio 1:0.6. Dansyl-Edman degradation of peptide CB₆ indicated the *N*-terminal sequence Pro-Ser-Tyr. Digestion of peptide CB₆ with trypsin gave arginine, peptides T₁₅, T₁₆ and peptides CB₆Ta and CB₆Tb. The partial sequence of peptide CB₆ is therefore Pro-Ser-Tyr-Arg(Gly - Arg; Arg; [Glx₂, Tyr₂, Leu₂, Pro, Gly, Arg₂]) Tyr(Leu, Asx, Gly)Ala-Hse.

Peptide CB₇, on reaction with carboxypeptidase A gave homoserine and valine. Digestion of peptide

CB₇ with trypsin gave arginine, peptide T₁₉, and peptide CB₇Ta. These results suggest the partial sequence of peptide CB₇ to be Asx-Ala-Lys(Arg;Val-Gly-Ser-Leu-Arg)Val-Hse.

The results obtained from the cyanogen bromide experiment enabled the overall structure of the protein to be constructed. Clearly peptides CB₁ and CB₈ were from the *N*- and *C*-terminus of the protein respectively. The tryptic peptides T₂₀, T₁₈, T₁₃ and T₁₀ suggest that the cyanogen bromide peptides CB₇, CB₆, CB₄, CB₃ are adjacent to peptides CB₈, CB₇, CB₅ and CB₄ respectively. The *C*-terminus of peptide T₁₄ is Pro-Ser-Tyr-Arg, which suggests that peptide CB₅ is adjacent to CB₆. The remaining cyanogen bromide peptide, CB₂ thus links peptides CB₁ and CB₃.

Overlap of tryptic peptides

Digestion of the protein with thermolysin, chymotrypsin and partial acid hydrolysis were used to obtain peptides that would overlap the tryptic peptides. The amino acid compositions of selected

Table 5. Amino acid composition of selected peptides from γ -crystallin, after digestion with chymotrypsin (C) thermolysin (L), and partial acid hydrolysis (A)

For abbreviations of purification procedures see Table 2.

Peptide Amino acid	Composition (mol of amino acid/mol of peptide)																							
	C ₁	C ₂	C ₃	C ₄ (†)	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	L ₁	L ₂	L ₃	L ₄	L ₅	L ₆	L ₇ (†)	L ₈	A ₁	
Lys	1.0											1.1												
His			0.8																					
Arg		1.2	1.1	1.0		1.0	1.0	1.6	1.0	2.0	0.9	2.0		2.0	1.0	0.8	1.9	2.0	2.8					1.1
Cys(O ₃ H)								1.0									1.0							
Asp		1.1	1.0	1.3	1.0		2.0	2.8	0.7					1.0	2.2	1.3	1.0				1.0			
Met(O ₂)								0.9																
Thr	1.0							0.9	0.7										1.0	0.9				
Ser								1.7					0.9		2.0	2.2	1.0		1.0			1.0	1.0	
Glu		1.0	1.3	2.8			1.0	2.6	1.0	1.1	1.0				1.7				1.3	1.0				
Pro		1.0	0.6					1.0		1.0	1.0								1.0	1.0				
Gly	1.0	1.0	0.9	0.9	1.0			0.9		1.0	1.2		1.0		1.3				1.0	1.0	1.0			
Ala													0.9											
Val								0.6					0.8	1.0									1.0	
Met																								
Ile	0.9					0.8		0.9									0.8							0.8
Leu								1.0	1.0		2.0	1.0	1.0	1.2	1.0				0.9	1.0	1.0			
Tyr						0.9		0.7		1.0	1.0			1.0		1.0			1.0					
Phe	1.0	1.0	1.6	2.2	1.0		1.0	0.9							1.0	1.0		1.0						
Trp																								
m ...	+0.34	-0.35	+0.14	+0.20	0.0	+0.36	-0.60	-0.40	0.0	+0.48	0.0	+0.55	+0.50	+0.20	-0.40	0.0	+0.30	+0.55	-0.48			+	-0.40	
M ...	0.61	0.53	0.54	0.43		0.72	0.56			0.80	0.47	0.94		0.60	-0.08			0.87	0.70			-0.43	0.40	
R(Arg) ...	2.40	1.42	0.95	1.32	2.35	3.00	1.10	0.50	1.80	1.29	2.85	1.31	2.2	2.39	0.48	0.85		1.70	1.06					2.56
N-Terminal amino acid...	Gly	Glu	Gln	Glx	Met(O ₂)	Arg	Glu	Arg	His	Arg	Leu	Arg	Ala	Arg	Phe	Phe	Ile	Phe	Glu	Leu	Leu	Leu	Ser	
Purifica- tion...	A, B, C	A, B, C	A, B, C	S(G-25), D	A, B	A, B, C	A, B, C	A, B, D	A, B	A, B, C	A, B, C	A, B, C	A, B	A, B, C	A, B, C	A, B, D	A, B, D	A, B, D	A, B, C	A, B, C	S(G- 25), C	A, B, C	A, C	

* Measured with Ehrlich reagent.

† Isolated from a digest of unoxidized protein.

Fig. 2. Selected peptides from γ -crystallin, after digestion with chymotrypsin (C), thermolysin (L), and partial acid hydrolysis (A)

For abbreviations, see Fig. 1; in addition ($\leftarrow$) is used to indicate C-terminal amino acids identified after hydrazinolysis.

peptides are presented in Table 5. The sequences of these peptides, together with details of the methods used are presented in Fig. 2. In Fig. 3 the use of these peptides and the cyanogen bromide peptides, in overlapping the tryptic peptides is summarized. Although many more peptides were isolated during this work, only the ones necessary to establish the final structure are described. No peptides were isolated that did not support the proposed sequence.

Discussion

γ -Crystallin (fraction II) is the first protein isolated from the lens to have its amino acid sequence described. The total number of amino acid residues is 165, from which the molecular weight was calculated to be 19870. This value is consistent with the value of 19100 from sedimentation equilibrium measurements (Björk, 1964) and the value of 19200 ± 600 obtained from measurements of C-terminal tyrosine (Croft, 1971). Preliminary work (Björk, 1964) had suggested the sequence of residues 1-3 to be Gly-Glu-Leu(or Ile). In the present paper the assignment Gly-Lys-Ile was based on results obtained after application of the Edman degradation procedure. The same N-terminal amino acid sequence has been found in the other proteins of the γ -crystallin group (L. R. Croft, unpublished work).

The amino acid analysis of the protein used in this work agrees with the results from the sequence analysis, except for the number of glycine residues. Sequence analysis suggests 13 residues, whereas 12 residues is suggested from amino acid analysis; Björk (1964) reports 13 residues. γ -Crystallin contains six cysteine residues and the sequence (Fig. 3) shows these to be at positions 15, 18, 23, 32, 74 and 105. Some oxidation to cystine occurs during isolation of the protein and consequent storage. No attempt has been made in this work to determine which cysteine residues are preferentially oxidized, if any. The sequence thus deduced represents the protein as it is in the lens, cystine being absent (Testa *et al.*, 1968).

An interesting position of tryptic cleavage has been observed in this work, that of hydrolysis of the peptide bond between residues 48 and 49. Normally, trypsin does not cleave the peptide bond between arginine and proline, and the comparable peptide bond between residues 138 and 139 was not hydrolysed. It may be significant that the sequence around the susceptible bond was Gln-Arg-Pro-Asp, since it has been reported that the Arg-Pro peptide bond in the sequence Gln-Arg-Pro-Gly is also hydrolysed by trypsin (Carnegie, 1969). Peptide T₁₆, when isolated from ion-exchange columns was ninhydrin-negative and had no free amino group owing to the cyclization of the N-terminal glutamine to form a pyrrolid-2-one-5-carboxylic acid derivative. Peptide maps of tryptic digests of the protein indicated this peptide to exist in several forms of slightly differing electrophoretic mobility, because of the gradual cyclization of the N-terminal glutamine residue (Smyth *et al.*, 1963). Two tryptic peptides, Leu-Ser-Ser-Cys-Arg and Glu-Val-His-Ser-Cys-Lys previously isolated, albeit in low yield (Croft & Waley, 1971), are now thought to have been due to the presence of a small cross contaminant of fraction I, as their isolation could not be repeated.

An interesting feature of the sequence is the irregular distribution of cysteine residues. There is a

Fig. 3. Proposed amino acid sequence of γ -crystallin showing the component peptides

Chymotryptic ($\leftarrow \rightarrow$), thermolytic ($\leftarrow \cdots \rightarrow$), partial acid ($\leftarrow \text{---} \rightarrow$), and cyanogen bromide ($\leftarrow \cdots \rightarrow$) peptides were used to overlap tryptic peptides ($\leftarrow \rightarrow$).

clustering of four cysteine residues in the *N*-terminal region of the protein, between residues 15–32. This is a characteristic feature of fraction II; such a cysteine cluster is not present in the other fractions (L. R. Croft & C. Slingsby, unpublished work). It is interesting that fraction II is much more stable to denaturation than any of the other fractions. The γ -crystallins are oxidized very easily. Fraction II is oxidized without denaturation, fractions III and IV are oxidized and denatured readily. This difference in stability is of great interest, as disulphide bond formation and protein denaturation are believed to play an important role in cataract formation (Testa *et al.*, 1968).

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