Structural Studies on Bovine γ -Crystallin

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The amino acid sequences around the cysteine residues in the lens protein, γ -crystallin, were studied. Fraction II of the γ -crystallin from calf lens (Björk, 1964) was used. The protein was oxidized with performic acid and then hydrolysed with trypsin. Six peptides containing cysteic acid were isolated. One of the peptides contained three residues of cysteic acid and the others contained one residue of cysteic acid. We conclude that there are eight unique residues of cysteic acid in the oxidized protein. Amino acid analysis suggests that there are also eight residues of cysteic acid in the molecule, which thus contains only one polypeptide chain.

The structural proteins of the lens have been divided into three families α -, β - and γ -crystallin (Waley, 1969). Although they have been studied for many years no structural comparison of them has been made. The amino acid sequence around the single cysteine residue of α -crystallin is -Ser-Cys-Ser-Leu- (Mok & Waley, 1967). β -Crystallin and γ -crystallin were found to differ considerably from α -crystallin in the number and distribution of cysteine residues. The aim of the present investigation was to determine the amino acid sequences around the cysteine residues of γ -crystallin. Our results support Björk's (1970) suggestion that this protein contains only one polypeptide chain.

The term γ -crystallin was originally used to describe the protein that remained in solution after Mörner's β -complex (Mörner, 1894) had been adjusted to pH 6 (Burky & Woods, 1928). The name has since been used to describe the slowest-moving protein during electrophoresis at pH 7. γ -Crystallin constitutes about 20% of the total protein of the lens, and is more concentrated in the central part. It has been called the embryonic lens protein, as its relative concentration decreases with age (François & Rabaey, 1957).

Most of the present knowledge of γ -crystallin comes from the work of Björk (1961, 1964, 1970). Björk (1961) isolated γ -crystallin by gel filtration on Sephadex G-75 and showed the preparation to be heterogeneous. By further chromatography on sulphoethyl-Sephadex and phospho-cellulose he isolated and crystallized four fractions, II, IIIa, IIIb and IVb. Each had a molecular weight of about 20000 and the same *N*-terminal amino acid sequence, Gly-Glx-Leu(or Ile-) (Björk, 1964). The

amino acid analysis of the fractions showed them to be unusual, there being little lysine and alanine, together with relatively large amounts of arginine and tyrosine. There were distinct differences in amino acid composition between the fractions, which seemed to indicate they were each unique proteins. This finding was supported by peptide 'mapping', which indicated the fractions to be different (Björk, 1970), fraction II being more different than the others.

In this work we have isolated fraction II, using Björk's (1964) method. We decided to work with performic acid-oxidized material. The cysteine residues were converted into cysteic acid residues, which facilitated the purification of peptides from the tryptic digest.

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 1 mM with respect to mercaptoethanol and the final purification on phosphocellulose was omitted.

Reagents were generally of analytical-reagent grade. Pyridine used in the preparation of buffers for the ion-exchange chromatography of tryptic peptides was distilled twice from ninhydrin before use. The reagents used in the Edman degradation cycle were also distilled before use and stored under N_2 . Anhydrous hydrazine was prepared by twice distilling commercial 99% hydrazine hydrate *in vacuo*, from KOH pellets.

Trypsin (salt-free, twice recrystallized), chymotrypsin (three-times recrystallized), pepsin and carboxypeptidases A and B (treated with di-isopropyl phosphorofluoridate) were all obtained from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Thermolysin was obtained from Chugai Boyaki Co. Ltd. (P.O. Box Higashi, no. 106, Osaka, Japan). Polyamide sheets were obtained from the Cheng Chin Trading Co. Ltd., Taipei, Taiwan.

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METHODS

Paper chromatography. The solvents used were solvent BAWP* or butan-1-ol-acetic acid-water (40:6:15, by vol.) (Matsubara & Smith, 1963).

Paper electrophoresis. This was carried out at 10 V/cm at pH 3.5 or 6.5 (Sargent, 1965) and at 100 V/cm in a Locarte high-voltage apparatus at pH 1.9 (Sargent, 1965).

Peptides were detected by dipping the paper in ninhydrin-cadmium reagent (Heilman, Barollier & Watzke, 1957); arginine peptides were detected with the phenanthraquinone reagent (Itano & Yamada, 1966). Histidine and peptides containing histidine were detected with the Pauly reagent (the diazotized sulphanilic acid was prepared from equimolar amounts of sulphanilic acid and NaNO_2).

Amino acid composition. Quantitative amino acid analyses were made on fraction II, and cysteic acid peptides by using a Locarte amino acid analyser, with a single column for the determination of acidic, neutral and basic amino acids. Protein (1 mg) or peptide (0.05 μmol) was hydrolysed at 110°C in evacuated Pyrex tubes with redistilled 6M-HCl for 24 h. For proteins and peptides containing tyrosine, 5% (v/v) of 0.1M-thioglycolic acid was added with the HCl (Blackburn, 1968). Tryptophan was determined by reaction of the protein with ninhydrin (Gaitonde & Dovey, 1970).

Performic acid oxidation of γ -crystallin (fraction II). The protein (10 μmol) was oxidized by performic acid (prepared as described by Hirs, 1967a). After 2 h at -5°C the protein was precipitated with 20 ml of 25% (w/v) trichloroacetic acid, and the precipitate was washed with 10% (w/v) trichloroacetic acid until the washings gave a negative starch-iodine reaction. Finally the oxidized protein was washed with ethanol followed by ether and air-dried overnight.

Digestion of the oxidized protein with trypsin. Performic acid-oxidized γ -crystallin (fraction II) (200 mg) was suspended in 20 ml of 1% NH_4HCO_3 . The digestion was performed at 37°C by the addition of 3 mg of trypsin dissolved in 300 μl of mM-HCl at zero time and a further 1 mg of trypsin in 100 μl of mM-HCl after 2 h, by which time the oxidized protein had dissolved. The reaction was terminated 1 h later by the addition of 1% acetic acid, to pH 4.5. The digest was then freeze-dried.

Fractionation of peptides by ion-exchange chromatography. The freeze-dried digest was dissolved in 2 ml of water and the pH adjusted to 2 with M-HCl, then added to a column (90 cm $\times$ 0.9 cm diam.) of Bio-Rad Aminex 50 W (X2) (Bio-Rad Laboratories, Richmond, Calif., U.S.A.), prepared as described by Schroeder, Jones, Cormick & McCalla (1962), equilibrated with 0.2M-pyridine-acetate buffer, pH 3.1. The column was eluted with a linear gradient from 0.2M-pyridine-acetate buffer, pH 3.1, to 2M-pyridine-acetate buffer, pH 5, at 40°C; 600 ml of each buffer was used. Fractions (2 ml) were collected every 20 min. Peptides were detected by reaction with ninhydrin (Rosen, 1957) after alkaline hydrolysis (Hirs, 1967b). Each ninhydrin-positive fraction was concentrated

on a rotary evaporator at 40°C and repeatedly dried down after the addition of water; finally the fractions were freeze-dried.

Detection of cysteic acid peptides. Peptides were tested for the presence of cysteic acid by hydrolysing a small portion and examining the hydrolysate by electrophoresis at pH 1.9.

Identification of N-terminal residues. The dansyl procedure was used to identify N-terminal amino acids (Gray & Hartley, 1963). The DNS-amino acids were identified by two-dimensional t.l.c. on polyamide layers (Woods & Wang, 1967) or on layers of silica gel G (Gros & Labouesse, 1969).

Identification of C-terminal residues. (a) Hydrazinolysis by the method of Akabori, Ohno & Narita (1952). The peptide (0.05–0.1 μmol) was dried *in vacuo* over P_2O_5 in a small hydrolysis tube. Anhydrous hydrazine (100 μl) was added, and the tube sealed and placed in a heated oven at 110°C for 5 h. The tube was then opened and the hydrazine removed *in vacuo* over conc. H_2SO_4 . The residue was dissolved in 200 μl of water and extracted three times with 500 μl of benzaldehyde followed by two extractions with ethyl acetate. The aqueous residue was then examined for amino acids by either electrophoresis or chromatography. (b) Digestion with carboxypeptidase A and B. The peptides were digested with 0.2 mg of carboxypeptidase A and/or B/ μmol of peptide in 0.5% NH_4HCO_3 , pH 8.5, at 37°C. The amino acids were detected by either electrophoresis or chromatography.

Amino acid sequence determination. This was done by the dansyl-Edman procedure of Gray (1967). This procedure was sometimes checked by analysing the peptide at each stage of the degradation.

Thermolysin digestion of tryptic peptides. The peptides in 0.2M-ammonium acetate, pH 8.5, were digested with thermolysin (0.02–0.5 mg/ μmol of peptide) as described by Ambler & Meadway (1968).

Chymotrypsin digestion of tryptic peptides. The peptides in 1% NH_4HCO_3 were digested with chymotrypsin (0.05 mg/0.2 μmol of peptide) at 37°C.

Pepsin digestion of tryptic peptides. The peptides in 5% formic acid were digested with pepsin (0.1 mg/mol of peptide) at 37°C.

Partial acid hydrolysis of peptides. Peptides were hydrolysed in 1M-acetic acid (300 μl / μmol of peptide) at 110°C for 6–20 h in a sealed tube.

Determination of amides. The presence of amides was determined by measuring the electrophoretic mobility of the peptide at pH 6.5 (Offord, 1966).

RESULTS

Amino acid analysis of γ -crystallin (fraction II)

γ -Crystallin (fraction II) was hydrolysed for 24 h and the hydrolysate analysed on a Locarte amino acid analyser. The results obtained are in reasonable agreement with those of Björk (1964) (Table 1).

Isolation of cysteic acid peptides

The performic acid-oxidized protein was readily digested by trypsin, and the resulting peptides were completely soluble. The digest was fractionated on an ion-exchange column and eluted with a gradient

* Abbreviations: solvent BAWP, butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.) (Waley & Watson, 1953); Mes, methionine sulphone; Cya, cysteic acid.

of pyridine-acetate buffers. The results (Fig. 1) showed that good resolution was achieved. Cysteic acid peptides were detected mainly in the first peaks

Table 1. *Amino acid analysis of γ -crystallin (fraction II)*

The amino acid analysis of γ -crystallin (fraction II) (an average obtained from two separate preparations) is shown below; the values are calculated on the basis of a molecular weight of 20000. Half-cystine was determined separately as cysteic acid after performic acid oxidation, and corrected for 10% loss. No other corrections for destruction or incomplete liberation of amino acids during hydrolysis have been made. Tryptophan was determined by the method of Gaitonde & Dovey (1970). For comparison the values obtained by Björk (1964) are also shown.

Amino acid	Amino acid composition (mol/mol of protein)	
	Present work	Björk (1964)
Lys	1.9 ± 0.3	2.0
His	4.8 ± 0.5	5.0
Arg	19.8 ± 1.0	19.4
Cys	7.5 ± 0.4	6.7
Asp	18.4 ± 1.4	17.4
Thr	4.8 ± 0.3	4.4
Ser	11.7 ± 0.2	10.8
Glu	19.5 ± 0.1	17.8
Pro	8.3 ± 0.7	9.0
Gly	13.0 ± 0.5	13.4
Ala	2.5 ± 0.3	2.0
Val	5.2 ± 0.2	5.7
Met	6.4 ± 0.1	6.8
Ile	5.4 ± 0.3	5.7
Leu	11.8 ± 0.9	12.2
Tyr	15.1 ± 0.3	14.2
Phe	8.9 ± 0.1	8.4
Trp	3.0 ± 0.2	Not determined

to emerge from the column. Some were essentially pure and needed no further purification. Peaks 1 and 2 corresponded to peptides T1 and T2 and required no further purification. Peak 3 contained nearly pure peptide T3, which was further purified by paper electrophoresis. Peaks 4 and 5 contained several peptides; the cysteic acid peptides were isolated after paper chromatography and electrophoresis. None of the other peaks were found to contain cysteic acid peptides.

Characterization and amino acid sequence of the cysteic acid peptides

The amino acid compositions of the cysteic acid peptides are shown in Table 2. The yield and total number of residues of each peptide are also shown, together with a summary of the isolation procedure. The determination of the sequences of these peptides is discussed below.

Peptide T1. This peptide was isolated pure from the ion-exchange column. It gave a yellow colour with the ninhydrin reagent, consistent with the *N*-terminal residue being glycine, found by dansylation. The dansyl-Edman method gave the sequence Gly-Glx-Mes-Ser-Glx. Digestion with carboxypeptidases A and B gave arginine, aspartic acid, glutamine and leucine. Thermolysin digestion gave three products, which were isolated by pH 6.5 electrophoresis (Table 3). Peptide T1a was the *N*-terminal fragment; the mobility of the peptide suggested the presence of one amide. This was placed on the first glutamic acid residue by determining the mobilities of the Edman degradation products. The sequence as determined by the dansyl-Edman method was Gly-Gln-Mes-Ser-Glu. Peptide T1c was the only fragment to contain arginine and was therefore the *C*-terminal fragment.

Fig. 1. Ion-exchange chromatography of peptides from digestion of performic acid-oxidized γ -crystallin with trypsin. Chromatography was carried out on a column (90 cm $\times$ 0.9 cm diam.) of Bio-Rad Aminex 50W (X2) at 40°C; elution was carried out with a gradient from 0.2M-pyridine-acetate buffer, pH 3.1, to 2M-pyridine-acetate buffer, pH 5, with a flow rate of 6 ml/h. Peptides were detected by ninhydrin after alkaline hydrolysis.

Table 2. *Cysteic acid peptides obtained by trypsin digestion of performic acid-oxidized γ -crystallin*

The amino acid compositions of cysteic acid peptides are presented below; the values in parentheses are the compositions deduced from the sequence study. Also shown is the purification procedure, which, after column chromatography, usually involved electrophoresis at the indicated pH, paper chromatography in solvent BAWP, or both. The mobility on electrophoresis at pH 6.5 relative to aspartic acid is also shown (the sign indicating the charge), together with the R_F on paper chromatography (solvent BAWP) relative to arginine. Amino acids present in amounts less than 0.1 mol/mol of peptide have been omitted from this and subsequent tables.

Peptide	Purification	N-Terminus	R_F	Mobility at pH 6.5	Yield (%)	Amino acid composition (mol/mol of peptide)
T1		Gly	0.5	-0.6	50	Arg 1.0 (1), Cya 1.0 (1), Asp 3.3 (3), Thr 1.1 (1), Ser 2.2 (2), Glu 3.3 (3), Pro 1.0 (1), Gly 1.2 (1), Mes 0.8 (1), Ile 1.2 (1), Leu 1.2 (1)
T2		Gly	1.1	-0.4	50	Arg 1.0 (1), His 1.0 (1), Cya 2.8 (3), Asp 2.2 (2), Ser 3.0 (3), Glu 3.1 (3), Pro 1.6 (2), Gly 2.0 (2), Leu 1.0 (1), Tyr 2.2 (2), Phe 2.0 (2)
T3	6.5	Cya	0.9	0	80	Arg 0.8 (1), Cya 1.1 (1), Asp 1.2 (1), Ser 1.0 (1), Ile 0.6 (1)
T4	6.5; BAWP	Leu	1.1	0	8	Arg 1.3 (1), Cya 1.0 (1), Ser 1.6 (2), Leu 0.8 (1)
T5	6.5; BAWP	Ser	0.7	0	6	Arg 1.2 (1), Cya 1.0 (1), Ser 1.0 (1)
T6	6.5; BAWP	Glu	0.5	0	4	Lys 1.1 (1), His 0.8 (1), Cya 1.1 (1), Asp 0.2 (0), Ser 1.1 (1), Glu 1.2 (1), Val 1.0 (1), Leu 0.2 (0), Tyr 0.2 (0), Gly 0.2 (0)

Table 3. *Peptides obtained by thermolysin digestion of peptide T1*

The digest was fractionated by paper electrophoresis at pH 6.5. The mobilities were measured relative to aspartic acid at pH 6.5, and the sign indicates the charge at this pH. The R_F values were measured in solvent BAWP, relative to arginine.

Peptide	N-Terminus	R_F	Mobility at pH 6.5	Amino acid composition (mol/mol of peptide)
T1a	Gly	0.8	-0.4	Gly (1.1), Mes (0.9), Ser (1.0), Glu (2.0)
T1b	Ile	0.8	-0.8	Cya (1.1), Asp (1.7), Thr (1.0), Ser (1.3), Pro (1.2), Ile (1.0)
T1c	Leu	1.0	Neutral	Asp (1.1), Glu (1.0), Leu (1.0), Arg (1.0)

The dansyl-Edman procedure indicated the sequence Leu-Gln-Asp-Arg; the position of the amide was determined by examining the mobility of the Edman degradation products. Peptide T1b was the fragment containing the cysteic acid residue and was acidic. The mobility at pH 6.5 suggested the presence of one amide. Hydrazinolysis indicated serine to be C-terminal. The dansyl-Edman method indicated the sequence Ile-Thr-Asx(Asx,Pro,Cya,-Ser). Partial acid hydrolysis gave aspartic acid and serine rapidly and at the same rate. This indicated the sequence Ile-Thr-Asx(Pro,Cya)Asx-Ser. Under the conditions used for the partial acid hydrolysis aspartic acid peptide bonds are preferentially cleaved (Tsung & Fraenkel-Conrat, 1965). As serine is released at the same rate as aspartic acid this indicates they are adjacent. Prolonged partial acid hydrolysis followed by purification by electrophore-

sis at pH 3.6 gave the dipeptide Pro-Cya. The structure of peptide T1b was concluded to be Ile-Thr-Asx-Pro-Cya-Asx-Ser. These results suggest that the amino acid sequence of peptide T1 is Gly-Gln-Mes-Ser-Glu-Ile-Thr-Asx-Pro-Cya-Asx-Ser-Leu-Gln-Asp-Arg.

Peptide T2. This was a very acidic peptide and stained yellow with the ninhydrin reagent. The sequence Gly-Phe-Glx-Gly-His was given by the dansyl-Edman method and was supported by the amino acid composition determined at each stage of the degradation. Reaction of peptide T2 with carboxypeptidases A and B released arginine, serine, phenylalanine and tyrosine in that order, which indicated that the C-terminal sequence was -Tyr-Phe-Ser-Arg. Digestion with thermolysin produced many fragments, of which only three were isolated and these in poor yield (Table 4).

Table 4. *Peptides obtained by thermolysin digestion of peptide T2*

The digest was fractionated by paper electrophoresis at pH 6.5 followed by electrophoresis at pH 3.6. The R_F values were measured in solvent BAWP, relative to arginine. Amino acids determined qualitatively are indicated (+) in this and subsequent tables.

Peptide	<i>N</i> -Terminus	R_F	Ionic state pH 6.5	Amino acid composition (mol/mol of peptide)
T2t1	Gly	1.0	Neutral	Glu (0.7), Gly (2.3), Phe (1.0)
T2t2	Leu	3.0	Neutral	Glu (1.3), Pro (1.9), Leu (1.0) Tyr (0.5)*
T2t3	Ser	0.8	Basic	Ser (+), Arg (+)

* Calculated as mol of amino acid/mol of leucine.

Table 5. *Peptides obtained by pepsin digestion of peptide T2*

The digest was fractionated by paper electrophoresis at pH 6.5. The mobilities were measured relative to aspartic acid at pH 6.5, and the sign indicates the charge at this pH. The R_F values were measured in solvent BAWP, relative to arginine.

Peptide	<i>N</i> -Terminus	R_F	Mobility at pH 6.5	Amino acid composition (mol/mol of peptide)
T2p1	Tyr	1.0	-0.6	Cya (2.1), Asp (2.1), Ser (1.9), Glu (2.1), Pro (2.1), Tyr (1.3), Leu (1.0)
T2p2	Gly	0.6	-0.5	Cya (2.5), Asp (2.1), Ser (2.0), Glu (2.8), Pro (1.8), Gly (1.7), Tyr (1.4), Phe (0.9), Leu (1.0), His (1.0)
T2p3	Gly	0.8	0	His (1.0), Cya (0.9), Glu (1.0), Gly (1.8), Phe (0.8)
T2p4	Phe	1.8	+0.5	Arg (1.1), Ser (1.0), Phe (1.0)

Peptide T2t1 was the *N*-terminal peptide. The dansyl-Edman method indicated the sequence Gly-Phe-Gln-Gly. The peptide was neutral so the presence of a glutamine was indicated. Peptide T2t2 was also neutral, and this also contained a glutamine residue and the sequence as determined by the dansyl-Edman method was Leu-Gln-Pro(Pro,Tyr). Peptide T2t3 was basic and the sequence was Ser-Arg. Partial acid hydrolysis of peptide T2 gave several peptides, but only one was isolated pure by electrophoresis at pH 6.5, peptide T2a. The amino acid composition was Cya (2.0), Ser (2.0), Glu (2.2), Gly (1.9), Tyr (1.0), Phe (1.1), His (+). This peptide stained yellow with the ninhydrin reagent and had *N*-terminal glycine, suggesting that it was derived from the *N*-terminal of peptide T2. Hydrazinolysis indicated that serine was *C*-terminal. No further study was made on this peptide. Chymotrypsin digestion of peptide T2 and fractionation of the digest by electrophoresis at pH 6.5 gave a basic peptide, peptide T2c. Peptide T2c had the amino acid composition Ser (+), Phe (+), Arg (+), determined qualitatively by electrophoresis. The sequence of this peptide was Phe-Ser-Arg and this was derived from the *C*-terminal of peptide T2. Digestion of peptide T2 with pepsin produced four peptides, which were purified by electrophoresis at pH 6.5. The properties and amino acid composition of these peptides are described in Table 5. Peptide T2p1 was degraded by the Edman method and

Table 6. *Amino acid compositions of peptides determined at successive steps in the Edman degradation of peptide T2p1*

The amino acid compositions of peptides determined at each stage of the Edman degradation of peptide T2p1 are given below; each analysis is based on leucine.

Amino acid	Amino acid composition (mol/mol of peptide)			
	Peptide T2p1	Step 1	Step 2	Step 3
Cya	2.1	2.1	2.1	1.3
Asp	2.1	2.1	2.1	2.0
Ser	1.9	1.8	2.0	2.0
Glu	2.1	2.0	1.3	1.4
Pro	2.1	2.0	2.1	2.1
Leu	1.0	1.0	1.0	1.0
Tyr	1.3	0.8	0.8	0.8

analysed at each stage for three steps (Table 6) to indicate the partial sequence Tyr-Glx-Cya(Ser₂-Asx₂-Cya-Leu-Glx-Pro₂-Tyr). Peptides T2p2, T2p3 and T2p4 were not further studied. The sequence of peptide T2 was concluded to be Gly-Phe-Gln-Gly-His-Cya-Tyr-Glx-Cya-Ser-Ser-Asx(Asx,Cya)-Leu-Gln-Pro-Pro-Tyr-Phe-Ser-Arg. A summary of the enzymic and acid hydrolytic studies on peptide T2 is given in Fig. 2.

Peptide T3. This peptide was neutral, which

Fig. 2. Peptides obtained from peptide T2 after acid and enzymic hydrolysis. The peptides are described in the text. $\leftarrow$ denotes release of amino acids by carboxypeptidases A and B.

- T1 Gly-Gln-Mes-Ser-Glu-Ile-Thr-Asx-Pro-Cya-Asx-Ser-Leu-Gln-Asp-Arg
 T2 Gly-Phe-Gln-Gly-His-Cya-Tyr-Glx-Cya-Ser-Ser-Asx(Asx, Cya)Leu-Gln-Pro-Pro-Tyr-Phe-Ser-Arg
 T3 Cya-Asn-Ser-Ile-Arg
 T4 Leu-Ser-Ser-Cya-Arg
 T5 Ser-Cya-Arg
 T6 Glu-Val-His-Ser-Cya-Lys

Fig. 3. Amino acid sequences of cysteic acid peptides obtained from tryptic digestion of γ -crystallin (fraction II).

indicated the presence of an asparagine residue. The dansyl-Edman method gave the sequence Cya-Asn-Ser-Ile-Arg, this being supported by the amino acid composition determined at each stage of the degradation.

Peptide T4. This peptide was neutral. The sequence Leu-Ser-Ser-Cya-Arg was given by the dansyl-Edman method, and was supported by the amino acid composition determined at each stage of the degradation.

Peptide T5. This peptide was neutral and gave a yellow colour with the ninhydrin reagent. The dansyl-Edman method gave the sequence Ser-Cya-Arg.

Peptide T6. The dansyl-Edman method indicated the sequence Glx-Val-His-Ser(Cya,Lys). The peptide was neutral, during electrophoresis at pH 6.5; therefore the *N*-terminus was glutamic acid. Of the two possible structures for this peptide the structure with cysteic acid as *C*-terminal was not considered, as cysteic acid is not the *C*-terminal residue of the protein (L. R. Croft, unpublished work); therefore, from the specificity of trypsin the lysine was placed *C*-terminal and peptide T6 was Glu-Val-His-Ser-Cya-Lys.

The amino acid sequences of the cysteic acid peptides obtained from the performic acid-oxidized γ -crystallin (fraction II) are summarized in Fig. 3.

DISCUSSION

The amino acid analysis of our preparation of γ -crystallin (fraction II) (Table 1) is in reasonable

agreement with the values reported by Björk (1964). Our preparation was prepared as described by Björk (1964) with the omission of the final chromatography on phospho-cellulose. The small differences in content of some amino acids could be accounted for by the different methods of calculation. Björk (1964) calculated on the basis of two residues of lysine per molecule of protein. We have compared our preparation with a sample kindly given us by Dr Björk by peptide 'mapping', and they appeared to be identical. The physicochemical information on fraction II is consistent with it being a homogeneous protein (Björk, 1964). Our results support this suggestion. Amino acid analysis suggests eight residues of cysteic acid per molecule of oxidized protein. The isolation of six cysteic acid peptides containing eight residues of cysteic acid supports the finding from amino acid analysis. As the number of ninhydrin-positive spots on peptide 'maps' of tryptic digests corresponds approximately to the number of peptides eluted from the ion-exchange column, it seems unlikely that any cysteic acid peptides had been irreversibly retained on the resin. The variable yield of the cysteic acid peptides can be accounted for by the different procedures of purification; it is to be noted that the peptides recovered in small amount had been purified by paper chromatography and electrophoresis. These methods are known to produce considerable losses.

These studies are consistent with the suggestion that γ -crystallin (fraction II) is a homogeneous protein, consisting of a single polypeptide chain (Björk, 1964, 1970), and are a beginning towards an

understanding of the structural chemistry of the proteins of the lens.

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