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Human α-Crystallin: Characterization of the Protein Isolated from the Periphery of Cataractous Lenses†

Debdutta Roy and Abraham Spector*

ABSTRACT: α-Crystallin has been isolated from the peripheral region of old cataractous lenses. It was found to be closely related to bovine α-crystallin and to human newly synthesized α-crystallin in terms of its amino acid composition, the size of its polypeptide chains and the lack of free NH_2-terminal groups. However, in contrast to the simple urea gel electrophoretic polypeptide patterns obtained with the reference proteins, 11 polypeptides were detected in the preparation. Ten of the polypeptides were isolated and shown to be either A or B chains on the basis of their amino acid compositions and comparison of the peptide maps of their tryptic hydrolysates. The four B chains as well as the six A chains were closely related, with most of the tryptic peptides being common to all members of their respective group. A nomenclature based upon the urea gel electrophoretic mobilities of the polypeptides has been proposed to define each chain. It was found that this α-crystallin preparation is composed of at least two populations of macromolecules, one of which contains macromolecules greater than 5×10^6 daltons on the basis of gel filtration with Bio-Gel A-5m. The compositions of the two fractions were found to be essentially identical.

Bovine α-crystallin has been more thoroughly investigated than any other lens protein. There are a number of reasons for such attention. The protein is easily isolated, has an unusual macromolecular structure, and displays an age-dependent increase in molecular weight which may be related to cataract formation (Spector, 1972). Only the newly synthesized protein is physically homogeneous with a weight average molecular weight (M_w[1]) of approximately 7×10^5 (Spector et al., 1968). Within a short period of time, the macromolecular population becomes heterogeneous with an increase in M_w to approximately 1×10^6 (Spector and Katz, 1965) and with further aging, primarily in the interior region of the tissue, macromolecular aggregates greater than 50×10^6 daltons have been observed (Spector et al., 1971; Jedziniak et al., 1972; Spector, 1972).

A change in the composition of the polypeptides accompanies the development of size heterogeneity. While bovine NSα contains essentially two chemically different polypeptides designated A_2 and B_2 (Stauffer et al., 1974), post-translational changes quickly produce two additional polypeptides, A_1 arising from A_2 (Palmer and Papaconstantinou, 1969; Bloemendal et al., 1972; Delcour and Papaconstantinou, 1972) and B_1 from B_2 (Stauffer et al., 1974). With further aging, the complexity of the polypeptide population increases further, particularly in the nuclear region of the lens (Stauffer et al., 1974). A number of modified A and B chains having different charge densities, lower molecular weights, and elimination of some of their carboxy-terminal amino acids have been observed (van Kleef et al., 1974).

Investigation of human lens α-crystallin has progressed slowly because normal lens material has been difficult to obtain and the relationship of the human protein to the well characterized bovine α-crystallin has not been defined. However, recently a human NSα has been isolated and characterized (Spector et al., 1976). Sedimentation equilibrium studies indicated a relatively homogeneous preparation with an M_w of 7.5×10^5. Gel electrophoresis in alkaline urea buffers revealed essentially three polypeptide chains, one corresponding to bovine B_2 and two polypeptides with mobilities in the A chain region. The molecular weights of these polypeptides were similar to the bovine A and B chains. With a few exceptions, the amino acid composition of the newly synthesized human protein corresponded to its bovine counterpart.

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[1] M_w, weight average molecular weight; NSα, newly synthesized α-crystallin; DTE, dithioerythritol; TPCK, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.

On the basis of the studies of human NSα, it appeared possible to examine the structure of this protein in older normal and cataractous lenses. Because of the considerable problems involved in obtaining good fractionation of the polypeptides and proteins of the nuclear region, i.e., the inner 50% or so of the lens, the protein of the outer region, the periphery, was first investigated. Another problem complicating the investigation of human lens proteins was the marked increase in the diversity of the polypeptide chain population probably caused primarily by post-translational alterations. The appearance of such components made it difficult to ascertain whether a particular polypeptide was in fact related to α-crystallin or was a contaminant contributed by another protein. Only by the characterization of a particular polypeptide could it be unequivocally demonstrated that the material was a modified α-crystallin polypeptide.

This paper reports the isolation and the characterization of the α-crystallin polypeptide population isolated from the peripheries of older cataractous lenses. Preliminary experiments with α-crystallin polypeptides from older normal lens periphery suggest that most of the polypeptides reported in this communication are present in such tissue as well.

Methods and Materials

Preparation of Lens Proteins. Human cataractous lenses from patients 60 years and older were usually obtained within a few hours after surgery and were stored at −84 °C. In most cases the opacities were primarily located in the central region of the lens. Calf eyes were received from a local slaughter house and the lenses were immediately removed and stored at −84 °C. The periphery which for these experiments is defined as the outer 40–45% of the lens was isolated from the whole lens by removal of the inner section of the tissue with a No. 3 cork borer. Usually 20 to 30 human lenses were used for a single experiment. Whole lenses or peripheries were homogenized at a concentration of 1 g (wet weight) per 7 to 10 ml of 0.01 M Tris, pH 7.6, 0.1 M KCl. The homogenate was centrifuged at 27 000*g* for 15 min at 4 °C. The supernatant which contained the soluble proteins was immediately applied to a Bio-Gel A-1.5m column and the precipitate was stored at −84 °C.

Gel Filtration. A Bio-Gel A-1.5m column (2.6 × 100 cm) was used for the separation of soluble proteins. The column was equilibrated and run at 4 °C with 0.01 M Tris, pH 7.6, 0.1 M KCl. Aliquots of 5 ml were collected. Pooled fractions were dialyzed against water, lyophilized, and in certain cases added to a Bio-Gel A-5m column (1.6 × 100 cm) equilibrated with the same Tris-KCl buffer as described above. The dissolved lyophilized material was first centrifuged at 10 800*g* for 5 min to remove small amounts of insoluble material.

DEAE Chromatography. DE52 (Whatman Laboratories, England) was suspended in 6 M urea, 0.006 M Tris, pH 8.0, 0.02% DTE, pH 8.0, and, after equilibration, packed in a column 1.6 × 14 cm, equipped with flow adaptors. A flow rate of 30 ml/h was maintained with a peristaltic pump. The sample was dissolved in the initial buffer, pumped into the column and a Tris-HCl gradient from 0.006 to 0.1 M, pH 8.0, in 6 M urea; 0.02% DTE was then applied. The gradient was made with an LKB ultragrad and the eluant was monitored for 280 and 230 nm absorption with an LKB UVicord III connected to a recorder. Three-milliliter fractions were collected and pooled fractions were dialyzed against water and lyophilized. The column was run at 4 °C.

Gel Electrophoresis. Sodium dodecyl sulfate gel electrophoresis of the polypeptides were performed with 10% gels according to the method of Weber and Osborn (1969). Urea gel electrophoresis was conducted according to Ornstein (1964) and Davis (1964) with 10% gels. The urea gels were run for 3 h at 3 mA/gel at room temperature.

Fingerprinting. Proteins at a concentration of 1 mg/ml were dissolved in 0.1 M ammonium bicarbonate, pH 8.5, and digested with TPCK-trypsin at 37 °C. The initial enzyme to substrate ratio was 1:100 but was increased to 1:50 after 1 h. After 2 h the reaction was stopped by lyophilization. The material was redissolved and lyophilized a second time to completely eliminate ammonium bicarbonate. The tryptic digest was then dissolved in pyridine acetate buffer, pH 6.5 (pyridine–acetic acid–water–ethyl alcohol, 25:1:255:25), and 50–100 μg was applied to thin-layer cellulose plates with glass backing (E. Merck). The plates have 0.1-mm thick cellulose layers. The plates were chromatographed in 1-butanol–pyridine–acetic acid–water (15:10:3:12) until the solvent front moved to within 1 cm of the top. The plates were dried at 80 °C for 5 h and then electrophoresed for 1 h in the second dimension on a cooled flat plate electrophoresis apparatus at a voltage of 800 V. Dried plates were sprayed with cadmium acetate ninhydrin reagent (Spector et al., 1975) and the color was developed at 70 °C.

Amino Acid Analysis. In most cases proteins were hydrolyzed in evacuated sealed tubes at 110 °C for 24, 48, and 72 h with three-times distilled, constant-boiling HCl. Amino acid analyses were performed on a Beckman amino acid analyzer Model 121 using Durrum DC6A resin and the Durrum Pico buffer system II.

Reduction, Alkylation, and Determination of Cysteine. Proteins were reduced with DTE in 6 M guanidine-HCl at 60 °C according to the method of Konigsberg (1972). Alkylation with iodoacetamide was performed in the same solvent at room temperature. A tenfold excess of iodoacetamide over protein sulfhydryl was used. Reduced and alkylated proteins were hydrolyzed with constant boiling HCl at 110 °C for 24 h and carboxymethylcysteine was determined by amino acid analysis.

Sedimentation Velocity. A Beckman analytical Model E ultracentrifuge was used for determination of the $s_{20,w}$ values of the lens proteins. The proteins were dissolved in 0.01 M Tris, pH 8.5, 0.1 M KCl. Centrifugation was conducted at 60 000 rpm at 20 °C.

End-Terminal Determinations. Amino-terminal determinations were carried out utilizing dansylation techniques (Gray, 1972). The dansyl amino acids were identified by chromatography on polyamide plates using the solvent systems of Woods and Wang (1967). The carboxy terminals were determined with carboxypeptidase A and B as described previously (Spector et al., 1975).

Results

α-Crystallin has the highest molecular weight of all soluble proteins in both bovine and young human lenses, and this characteristic previously has been utilized for its isolation (Spector et al., 1971; Spector et al., 1975). Therefore, the soluble lens proteins from the peripheral region of human cataractous lenses were first fractionated on the basis of size upon Bio-Gel A-1.5m. The results obtained from a typical experiment with the peripheries of 30 lenses are shown in Figure 1. An excellent separation of the first peak which represents 26% of the initial 280-nm absorption

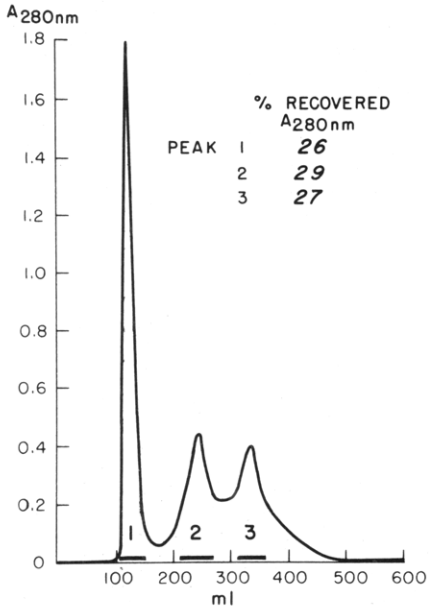

FIGURE 1: Fractionation of soluble lens proteins from the peripheral region of human cataractous lenses on Bio-Gel A-1.5m (2.5 × 100 cm). The eluting buffer is 0.01 M Tris, pH 7.6, 0.1 M KCl. The fractions pooled are shown by solid bars.

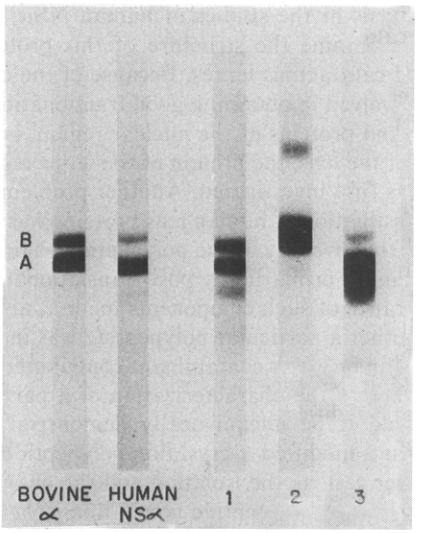

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis profiles of the three pooled peaks from Bio-Gel A-1.5m (Figure 1) designated 1, 2, and 3 respectively. Bovine α-crystallin and human NSα are shown for comparative purposes.

Table I: Amino Acid Composition of Fractions from Bio-Gel A-1.5m Filtration of Soluble Proteins of Human Cataractous Lenses.[a]

	Residues/1000 Residues				
	Peak 1	Peak 2	Peak 3	Human NSα	Calf α-Crystallin
CM-Cys	10	16	35	13	5
Asp	92	90	83	96	86
Thr	45	32	35	44	34
Ser	92	85	77	95	103
Glu	116	150	130	103	105
Pro	62	55	47	55	81
Gly	69	97	98	89	60
Ala	51	58	54	38	44
Val	61	57	43	58	58
Met	15	14	23	8	12
Ile	47	39	51	44	47
Leu	92	60	65	88	87
Tyr	34	46	73	28	31
Phe	72	50	52	80	76
Lys	44	57	43	46	48
His	40	35	30	35	39
Arg	72	60	80	76	73

[a] All values are the mean of at least three determinations. Variations of ±4% or less were usually observed.

was achieved. Two other fractions representing 29 and 27%, respectively, were also isolated. The peak regions denoted in the figure were taken for further study.

After reduction and alkylation, the amino acid compositions of the three peaks were compared with human NSα and calf α-crystallin as shown in Table I. An excellent correlation between peak 1 and human NSα was found with serious discrepancies in only methionine, alanine, and glycine. It is interesting to note that the peak 1 values for these three amino acids agree reasonably well with those found for calf α-crystallin. The major differences between the bovine protein and the peak 1 material reside in the values for cysteine, threonine, and proline. These amino acids have rather similar values in human NSα and peak 1. Peaks 2 and 3 have markedly different compositions and must represent other protein components.

Sedimentation velocity studies gave values of 20S, 9S, and 2.6S for the three peaks. Such values are in reasonable agreement with observations on isolated bovine α-, β-, and γ-crystallin, respectively. The sedimentation velocity profile of peak 1 suggested considerable physical heterogeneity, and this conclusion was confirmed by fractionation of the material on Bio-Gel A-5m. Approximately 96% of the protein added to the column was recovered with 55% appearing in the void volume, therefore suggesting a molecular weight population for this fraction of greater than 5×10^6. A second peak representing approximately 33% of the starting material was eluted in the region where low molecular weight bovine α-crystallin (1×10^6 daltons) appears. Amino acid analyses clearly indicate that these two components are essentially the same and almost identical with the peak 1 material from the Bio-Gel A-1.5m column. Alkaline urea and sodium dodecyl sulfate gel electrophoreses profiles were identical for the two fractions.

When the Bio-Gel A-1.5m peak 1 material was subjected to sodium dodecyl sulfate gel electrophoresis, a pattern similar to that for bovine α-crystallin and human NSα was obtained (Figure 2). Two major polypeptide bands with molecular weights of approximately 22 000 and 20 000 were detected. Minor, lower molecular weight components were also noted in the fraction 1 profile. The patterns for Bio-Gel A-1.5m peaks 2 and 3 were clearly different from peak 1 and α-crystallin. It is of interest to note the presence of two unusual polypeptides, an approximately 43 000 dalton component in the peak 2 material and a polypeptide of approximately 12 000 daltons in the low-molecular-weight, peak 3 fraction.

On the basis of the above observations, there is little question that peak 1 represents α-crystallin and yet when this material is examined by alkaline urea gel electrophoresis (Figure 3) little similarity to the reference α-crystallins is apparent. While human NSα contains only three polypeptides (Figure 3, panel B), one corresponding to the low mobility B_2 chain of bovine α-crystallin and two with mobilities in the A region of the bovine protein, the peak 1

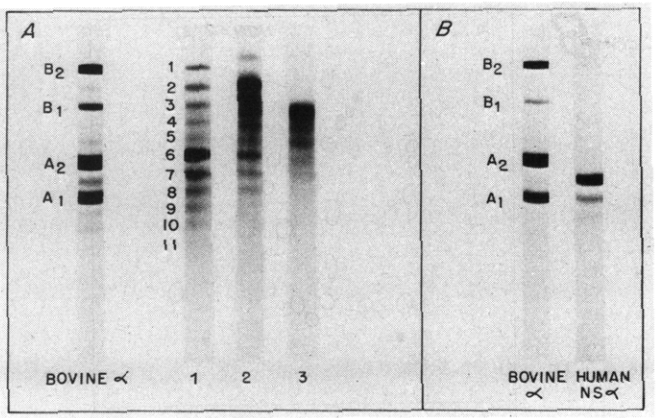

FIGURE 3: Urea–polyacrylamide gel electrophoretic profiles of (A) bovine α-crystallin and peaks 1 to 3 from Bio-Gel A-1.5m fractionation of the soluble lens proteins (Figure 1); (B) comparison of profiles of bovine α-crystallin and human NSα.

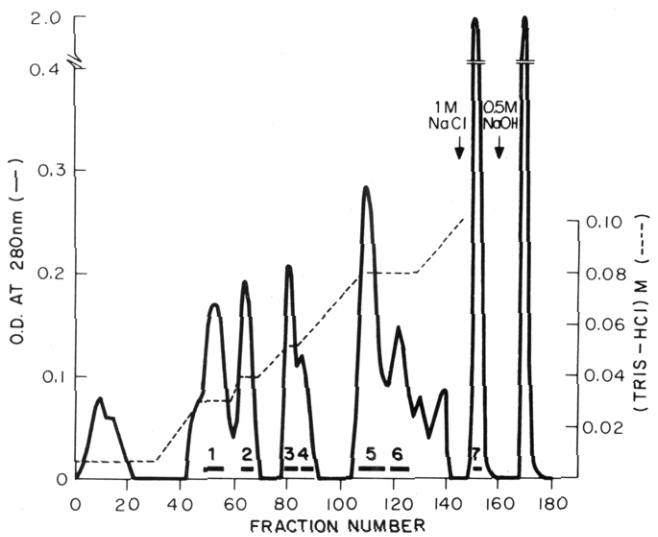

FIGURE 4: Profile of DE52 chromatography of the peak 1 material from Bio-Gel A-1.5m (Figure 1) in 6 M urea. (- - -) represents the Tris-HCl gradient. The fractions pooled are shown by solid bars.

material contains a minimum of 11 bands (Figure 3, pane A) ranging in mobility from bovine B_2 to greater than bovine A_1. Peak 2 from the Bio-Gel A-1.5m column also has a complicated multiband pattern but is different from peak 1 and the simplier peak 3. These results suggest that as a result of aging, marked changes in charge density but little change in size have developed in the polypeptide population of the human α-crystallin in the peripheral region.

In order to identify these electrophoretically delineated polypeptides as α-crystallin components, it was necessary to isolate and characterize each polypeptide. Therefore, Bio-Gel A-1.5m peak 1 protein was fractionated on a DE52 column at 4 °C in the presence of 6 M urea, utilizing a Tris gradient with 0.02% DTE (Figure 4). Approximately 87% of the material added to the column was recovered, with 27% being eluted by the gradient, 30% with 1 M NaCl in 6 M urea, 0.15 M Tris, pH 8.0, 0.02% DTE, and another 30% with 0.5 M NaOH. The latter fraction appears to represent protein that has been insolubilized during the isolation of the Bio-Gel A-1.5m peak 1 protein. If the unfractionated material was centrifuged at 20 000*g* for 10 min before addition to the DE52 column, the NaOH peak was eliminated and the relative recoveries of the other components were

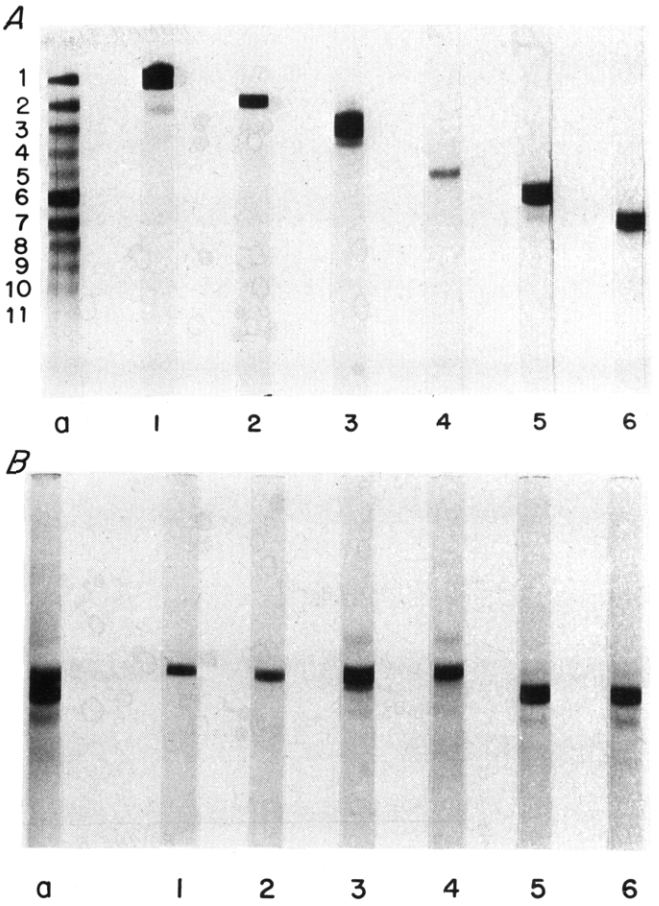

FIGURE 5: Polyacrylamide gel electrophoretic profiles. The first six peaks from the DE52 column (Figure 4) are designated 1 to 6; (a) represents the unfractionated protein; (A) alkaline urea gel patterns; (B) sodium dodecyl sulfate gel patterns.

concomitantly increased. Thus the sodium hydroxide fraction is clearly an artifact.

The first small peak eluted from the column contained no protein and appears to be an artifact frequently seen with this elution system. Six major fractions were eluted with the gradient. The peak tubes of each fraction were pooled and analyzed. Alkaline urea gel patterns indicated that all fractions were relatively pure, although some contained small amounts of contaminants which can only be detected when the gels are somewhat overloaded as shown in Figure 5A. The electrophoretic bands of the first three fractions isolated from DE52 correspond to the first three slowest moving peptides observed with the unfractionated material. Fraction four corresponds to the fifth band and fractions 5 and 6 to the sixth and seventh bands of the unfractionated material. Sodium dodecyl sulfate gel electrophoresis (Figure 5B) indicated that the first four DE52 fractions have molecular weights comparable to the bovine B chains and the human NSα first band. When a high concentration of fraction 3 was utilized for sodium dodecyl sulfate gel electrophoresis, trace amounts of additional polypeptides could be observed. The contaminant observed after urea gel electrophoresis of fraction 1 has the same molecular weight as the major component. Based on sodium dodecyl sulfate gel electrophoresis, fractions 5 and 6 have the same molecular weight as the bovine A chains and the major polypeptide present in human NSα.

To further identify these six polypeptide chains, amino acid analyses were performed and the results compared

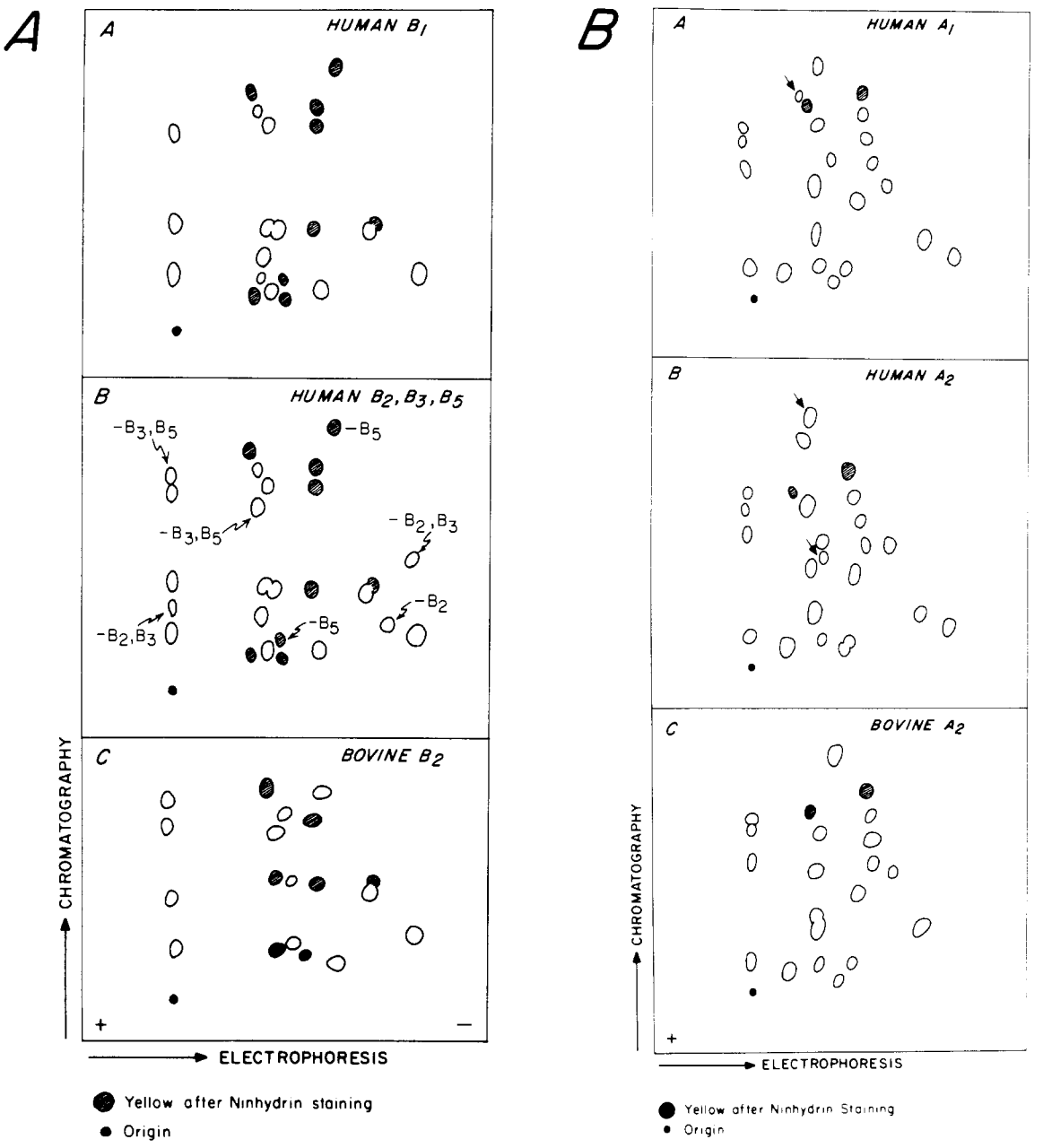

FIGURE 6: Fingerprints of the tryptic digests of α-crystallin polypeptides. Identical conditions were used in all experiments. (A) B polypeptides: human B_1 (panel A); human B_2, B_3, and B_5 (panel B); and bovine B_2 (panel C). (B) A polypeptides: human A_1 (panel A), human A_2 (panel B), and bovine A_2 (panel C). Arrows denote peptides not found in the other A polypeptide.

with bovine B_2 and A_2 polypeptides (Table II). The first four fractions appear very similar and correspond to bovine B_2. No cysteine and low tyrosine content, two major characteristics of B chains, are found for all four polypeptides. Fractions 5 and 6 have amino acid compositions similar to each other and to bovine A_2 and are distinctly different from fractions 1–4. They contain one cysteine and six tyrosine residues per molecule, characteristic of the A_2 bovine polypeptide, but differ significantly from the bovine A chains in serine and threonine content. Because of the relationships demonstrated in these experiments, fraction 1 through 3 will be designated B_1–B_3, respectively, and fraction 4, B_5 to indicate that they are clearly B chains. Fractions 5 and 6 will be designated A_1 and A_2 to reflect their relationship to the bovine A polypeptides.

In order to determine if the polypeptide sequences reflect the relationships revealed by the above experiments, tryptic digestion followed by two-dimensional fractionation of the digests were performed. The results are shown in Figure 6A for the B chains and Figure 6B for the A chains. Utilizing similar conditions, 22 peptides were identified in each of fractions B_1–B_3 and B_5 but only 19 with bovine B_2. The fingerprint obtained for human B_1 which corresponds in mobility on alkaline urea gels to bovine B_2 is shown in Figure 6A, panel A, and that for bovine B_2 in Figure 6A, panel C. A very considerable similarity between these fingerprints is apparent. The relationship between human B_2, B_3, and B_5 is shown in Figure 6A, panel B. Of the 27 different peptide spots observed in the B fingerprints, 19 are common to all four B fractions. One peptide was found to be unique to B_1, two to B_2 and two to B_5. Two peptides were found in all B chains except B_5. Thus B_5 appears to have a somewhat different pattern, although its relationship to the B chains is clearly apparent.

Table II: Amino Acid Composition of Fractions from DE52 Column.

	Residues/Mole of Proteins[a]							
	1	2	3	4	5	6	Bovine B_2	Bovine A_2
CM-Cys	0	0	0	0	1	1	0	1
Asp	14	14	13	14	18	15	13	16
Thr	8	9	8	8	9	9	7	5
Ser	16	16	15	16	19	18	17	23
Glu	17	18	17	19	18	16	17	17
Pro	15	14	14	14	11	12	17	12
Gly	9	10	10	12	12	11	8	10
Ala	6	6	6	8	7	7	9	6
Val	11	11	11	11	11	11	10	10
Met	2	2	2	2	2	2	2	2
Ile	9	9	9	9	8	8	10	9
Leu	15	15	15	15	16	14	15	14
Tyr	2	3	3	3	6	6	2	6
Phe	14	14	13	13	14	14	13	14
Lys	10	10	10	10	7	8	10	7
His	9	8	8	7	7	8	9	7
Arg	14	14	14	14	13	13	14	13

[a] Results for bovine B_2 and A_1 are based upon the molecular weights of these polypeptides as determined from the primary sequences. The reported composition is in agreement with that determined from the sequence of the polypeptides. The amino acid composition of fractions 1–4 is based on the molecular weight of the bovine B chains (20 070), and fractions 5 and 6 are based on the molecular weight of the bovine A chain (19 832). The values for fractions 1 to 6 are based on an average of six determinations upon three preparations.

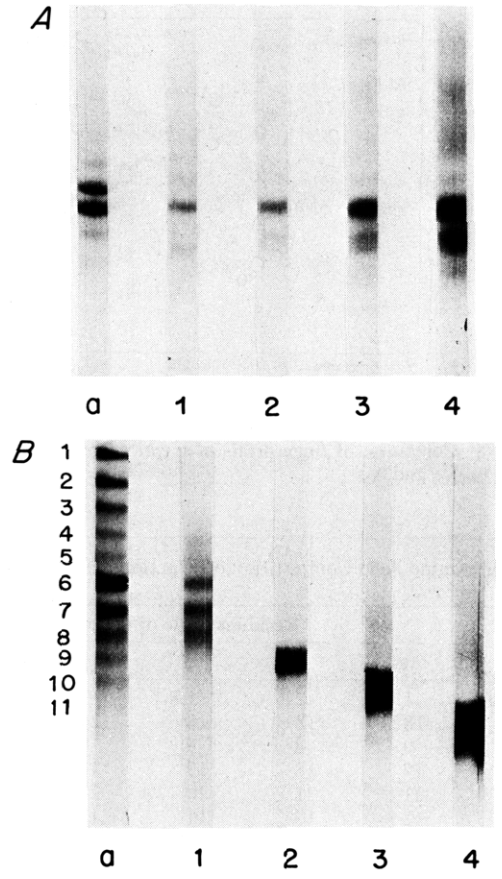

FIGURE 7: Gel electrophoretic profiles of the four peaks from DE52 chromatography of reduced and alkylated protein from 1 M NaCl peak from first DE52 chromatography (Figure 4); (a) represents unfractionated material; (A) sodium dodecyl sulfate gel patterns; (B) alkaline urea gel patterns.

The bovine A_2 chain (Figure 6B, panel C) gave 21 spots after tryptic digestion and fingerprinting, while the human fractions designated A_1 (Figure 6B, panel A) gave 23 spots and A_2 (Figure 6B, panel B) 24 spots. The profile of the bovine A_2 polypeptide appears to be similar to the human A polypeptides, although differences in certain regions of the fingerprints are apparent. The human A_1 chain has one peptide not found in human A_2, and human A_2 has two peptides not found in A_1. These peptides are denoted in the figures by arrows.

Carboxypeptidase A and B release serine in equimolar quantities from all four human B chains within 10 min. Under similar conditions, an equimolar amount of lysine was released from bovine B_2. In comparable experiments A_1 and A_2 gave 0.35 and 0.5 mol of serine per mole of polypeptide and, with bovine A_2, 0.8 mol of serine was released. In all of these experiments only trace amounts of other amino acids were detected. These results suggest that the carboxy-terminal residues of all four human B chains differ from their bovine counterpart, while the human A chains appear to have the same carboxy terminals as their bovine counterpart. No free amino-terminal residues could be detected in any of the polypeptides by dansylation techniques.

While these experiments clearly define the six fractions obtained from the DE52 urea fractionation as either B or A chains, a number of the bands appearing on the alkaline urea gel pattern of the unfractionated material remain unidentified. It is probable that these polypeptide chains were eluted with the NaCl wash. Further study of this fraction was therefore undertaken. The material was reduced and alkylated and then fractionated again on a DE52 urea column utilizing the same gradient as previously described. A relatively poor separation was obtained with four fractions being isolated with the Tris gradient. These components represent approximately half of the material with most of the remaining protein again being eluted from the column with 1 M NaCl. The peak regions of each fraction were concentrated and characterized. On sodium dodecyl sulfate gel electrophoresis (Figure 7A), all four components gave a major band corresponding to the bovine A chain and only a very faint band in the B region. A number of lower molecular weight components were also observed in all four fractions, particularly in the 14 000 to 16 000 dalton range. With alkaline urea gel electrophoresis (Figure 7B), fraction 1 was found to contain a high concentration of a component corresponding to band 8 of the unfractionated material and two other major components with somewhat lower mobilities corresponding to the human A_1 and A_2. Fractions 2 through 4 each appear to have polypeptide bands which are clearly different from each other and from fraction 1 and have mobilities comparable to bands 9–11, respectively, of the unfractionated protein. However, these fractions also appear to contain components not initially found in the starting material. This is particularly true of fraction 4 which contains polypeptides with mobilities greater than those which were observed initially.

In Table III, the amino acid compositions of these fractions are compared with that of human A_1. With a few exceptions, a good correlation was found. Of considerable interest is the indication that these polypeptides contain two cysteine residues in contrast to the one cysteine usually associated with the A chains. A higher level of glutamic acid was also observed and, in the case of fractions 1 and 2, gly-

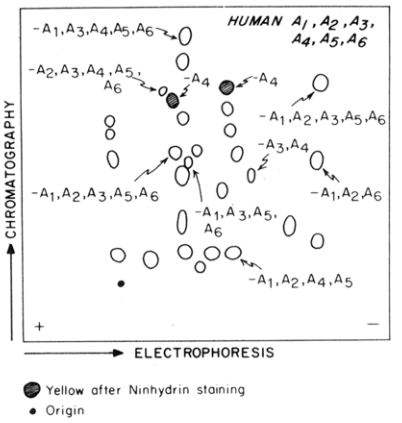

FIGURE 8: Composite of fingerprints of tryptic digest of human A_1, A_2, A_3, A_4, A_5, and A_6.

Table III: Amino Acid Composition of Fractions from NaCl Wash.

	Residues/Mole of Protein[a]				
	1	2	3	4	Human A_1
CM-Cys	1.7	2.1	1.8	1.5	1
Asp	18	18	18	20	18
Thr	9	8	9	9	9
Ser	17	18	19	18	19
Glu	21	21	20	20	18
Pro	12	12	11	11	11
Gly	14	13	12	12	12
Ala	9	8	7	7	7
Val	11	11	11	11	11
Meth	2	2	2	2	2
Ileu	9	9	9	9	8
Leu	16	17	16	16	16
Tyr	6	6	6	6	6
Phe	13	14	14	14	14
Lys	8	8	8	8	7
His	6	7	7	7	7
Arg	13	13	13	13	13

[a] Based on the bovine A_2 molecular weight of 19 832. The values are based on an average of six determinations on three preparations.

cine levels may also be somewhat elevated. No amino terminal groups could be detected by dansylation techniques.

It is clear from these results that the components contained in the NaCl wash of the original fractionation represent modified A chains. The appearance of the new higher mobility bands on urea gel electrophoresis may represent material degraded during the isolation procedure. To ascertain the degree of difference in the sequences of the polypeptides in the NaCl wash, they were subjected to tryptic digestion, fingerprinted, and then compared with the already defined A chains. A composite diagram of the patterns of all the A chains is shown in Figure 8. A total of 29 different peptides were detected. Of this number, 19 peptides were found in all six isolated fractions. There are two polypeptides which are found in all chains except A_4 and one polypeptide found in all chains except A_3 and A_4. A_2, A_3, and A_4 have one unique polypeptide not common to any of the other polypeptides.

Discussion

The relationship of the isolated protein to α-crystallin was elucidated by comparing it with young bovine α-crystallin and to NSα. A good correlation was found among these proteins with regard to amino acid composition and

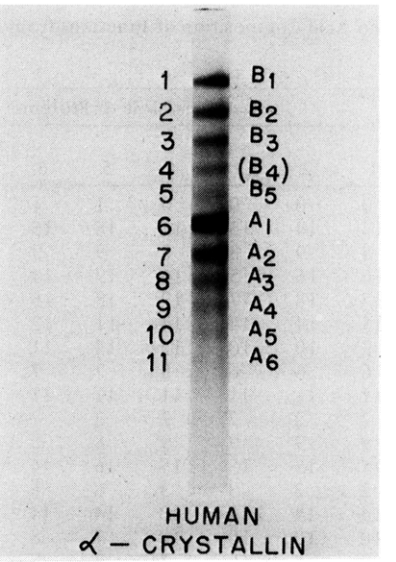

FIGURE 9: Human α-crystallin identification of electrophoretic bands.

the size of both the aggregated macromolecules and their polypeptides. However, in contrast to the simple polypeptide composition of the reference proteins, the presumptive α-crystallin isolated from cataractous lens peripheries contained at least 11 polypeptide chains. It was thus necessary to isolate and characterize each of these chains. The unidentified polypeptides were designated 1 through 11 based upon increasing electrophoretic mobility on alkaline urea gels (Figure 9). Ten of the eleven polypeptides were isolated and defined as α-crystallin polypeptides. Only polypeptide 4 did not correspond to an isolated fraction. No explanation for the failure to isolate this component is apparent. With the exception of this fraction, polypeptides 1 through 5 were unequivocally defined as B chains and fractions 6 through 11 as A chains. The polypeptides have therefore been noted as B_1 through B_5 and A_1 through A_6 as indicated in Figure 9. Polypeptide 4 has tentatively been assumed to be a B chain on the basis of its electrophoretic mobility and designated B_4. The brackets denote the tentative nature of the assignment. It is proposed that this nomenclature be used in the future in delineating the α-crystallin polypeptides.

The B_1 polypeptide corresponds in mobility on alkaline urea gel electrophoresis to the presumptive B chain of human NSα and to the B_2 polypeptide of calf α-crystallin (Figure 3). B_3 corresponds to bovine B_1. On the basis of this criterion A_1 corresponds to bovine A_2 but appears to move more slowly than the major human NSα presumptive A chains, suggesting that it is more positively charged. Since no material was available to make a direct comparison between the cataractous and NSα, this conclusion is based on comparisons with bovine α-crystallin and must be considered tentative. However, the results clearly suggest that most of the α-crystallin polypeptides found in older lenses have markedly different charge characteristics than are found in human NSα and are a much more complex group of components.

In the bovine lens it appears that only the B_2 and A_2 polypeptides are directly synthesized and the appearance of the additional B and A chains are due to post-translational reactions (Palmer and Papaconstantinou, 1969; Decour and Papaconstantinou 1972; Bloemendal et al., 1972; Stauffer et al., 1974). The peripheries of 2 year old bovine lenses contain essentially four α-crystallin polypeptide chains B_2,

B_1, A_2, and A_1. However, in the nuclear region, additional polypeptides are present in the α-crystallin macromolecule (Stauffer et al., 1974; van Kleef et al., 1974; van Kleef, 1975). In contrast to the human situation, the bovine B_2 and A_2 polypeptides appear to be predominant at all ages. From the urea gel electrophoresis profiles, it seems that at least as extensive a group of transformations has occurred with α-crystallin in the human lens periphery as is observed in the entire bovine lens. However, further study of old bovine lenses is required to substantiate this viewpoint.

There is little question that the human A chains as well as the B chains are closely related groups of polypeptides. Based on the uniformity in the size of the polypeptides in both the A and B groups, only a small number of amino acid residues could have been removed from the polypeptides during the modification process. However, it should be noted that some of the A polypeptides particularly A_5 and A_6 contain smaller molecular weight components. Since the amino acid compositions and fingerprints of A_5 and A_6 are very similar to those of the other A chains, it is likely that the low molecular weight components represent cleaved products of the intact polypeptides. In bovine lenses, all such reported cleavages occur at the carboxy-terminal end of the polypeptide (van Kleef et al., 1974; van Kleef, 1975). The amino-terminal group remains blocked in all cases. All the human α-crystallin polypeptides also appear to have a blocked amino-terminal residue probably similar to the acetyl group found in the bovine protein (Hoenders and Bloemendal, 1967; Mok and Waley, 1968).

Examination of the amino acid analyses of the polypeptides indicates that, while A_1 and A_2 contain one cysteine residue, A_3–A_5 and possibly A_6 appear to have two such residues. The abundance of a few other amino acids also appears to vary significantly in both A and B chains. Such observations raise the question of whether all the transformations observed in the human periphery are post-translational or whether some changes may be the result of the synthesis of modified polypeptides. It has previously been shown that, even in young bovine lenses, the nuclear region is not capable of significant synthesis of protein (Wannemaker and Spector, 1968). With aging there is further contraction of the region of the lens in which significant protein synthesis occurs. A similar situation probably exists in the human lens. Thus it is probable that most of the α-crystallin polypeptides found in older lenses were synthesized at a much earlier time and that the large number of different polypeptides is primarily a result of post-translational changes.

The presence of high concentrations of glutathione in the lens (Kinoshita and Merola, 1958) suggests the possibility that this tripeptide may be covalently linked by a disulfide bond to some of the α-crystallin chains, particularly A_3–A_6 which contain an enriched cysteine content. However, since these polypeptides were isolated after reduction and carboxymethylation, this possibility does not appear likely.

It is of interest that, although the A chains are closely related polypeptides, A_3–A_6 could only be eluted with the Tris gradient after reduction and carboxymethylation. Since no evidence of significant concentrations of polypeptides in the 40 000 daltons range were observed in the starting material, these polypeptides initially could not have been linked via disulfide bonds. However, the presence of two SH groups per chain suggests that inter- or intrachain oxidation may have occurred during the original isolation or that internal disulfide linkages were initally present. It should also be noted that, even after reduction and carboxymethylation, some material continues to bind to the column requiring high salt concentration for elution. The polypeptide composition of this fraction remains to be elucidated.

Preliminary work with α-crystallin isolated from old normal human lens peripheries indicates that essentially all the polypeptides present in the preparations from cataract lenses are also present in the normal tissue. Thus at present there is no evidence that the presence of these modified polypeptides are directly related to the development of cataract. Since senile cataract frequently involves primarily the nuclear region of the lens, it is possible that, if α-crystallin is associated with the development of opacity, further transformations or cooperative interaction with other components may be involved.

The development in the central region of bovine lens of high molecular weight species of α-crystallin with molecular weights greater than 50×10^6 may possibly be related to cataract formation (Spector, 1972). This view is supported by theoretical studies reported by Benedek (1971). It has been shown that calcium is capable of causing the transformation of low-molecular-weight α-crystallin macromolecules to such high-molecular-weight components (Jedziniak et al., 1972). Investigation of the reaggregation of the isolated polypeptides of bovine α-crystallin in the presence of calcium suggests that only A chains with sterically hindered SH groups are capable of directing the formation of these giant macromolecules (Spector and Rothschild, 1973). These A chains appear to have the same charge distribution as other A chains since they cannot be differentiated by urea gel electrophoresis.

The high-molecular-weight species found in the nuclear region of the human lens (Spector et al., 1974) has not as yet been characterized. However, it is evident from studies upon human NSα and the present investigation, that size transformations of this macromolecule occur with aging even in the peripheral region of the lens. Human NSα is a physically homogenous macromolecule with a molecular weight of 7×10^5. The preparation investigated in this study and in old normal human lens periphery appears to be physically heterogeneous with a significant proportion of the protein having aggregate sizes greater than 5×10^6 daltons. Such observations suggest that the giant aggregates of the nuclear region may contain α-crystallin components.

Recently Dilley and Harding (1975) have reported the isolation of a presumptive human α-crystallin from normal lenses of varying age. Their amino acid composition is in reasonable agreement with that observed in the present work. Although these investigators did not isolate and characterize the α-crystallin polypeptides, their work suggests that deamidation did not contribute significantly to the development of modified α-crystallin polypeptides. However, the close similarity of many of the polypeptides elucidated in this investigation suggests that deamidation may account for some of the observed differences in the charge distribution. Such deamidation reactions have previously been reported with other proteins, such as aldolase (Lai and Horecker, 1970; Midelfort and Mehler, 1972) and cytochrome *c* (Flatmark and Sletten, 1968), and the possibility that similar reactions occur with the α-crystallin has been previously proposed (van Vanrooij et al., 1974).

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