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Crystallins in water soluble-high molecular weight protein fractions and water insoluble protein fractions in aging and cataractous human lenses

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Purpose: The aim of the study was to comparatively analyze crystallin fragments in the water soluble high molecular weight (WS-HMW) and in the water insoluble (WI) protein fractions of human cataractous (with nuclear opacity) and age matched normal lenses to determine the identity of crystallin species that show cataract specific changes such as truncation and post-translational modifications. Because these changes were cataract specific and not aging specific, the results were expected to provide information regarding potential mechanisms of age related cataract development.

Methods: The WS-α-crystallin, WS-HMW protein, and WI protein fractions were isolated from normal lenses of different ages and from cataractous lenses. The three fractions were subjected to two dimensional (2D) gel electrophoresis (IEF in the first dimension and SDS-PAGE in the second dimension). Individual spots from 2D gels were trypsin digested and the tryptic fragments were analyzed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

Results: The 2D protein profiles of WS-α-crystallin fractions of normal human lenses showed an age related increase in the number of crystallin fragments. In young normal lenses, the WS-α-crystallin fragments were mostly C-terminally truncated, but in older lenses these were both N- and C-terminally truncated. The WS-HMW protein fraction from normal lenses contained mainly fragments of αA- and αB-crystallin, whereas additional fragments of βB1- and βA3-crystallin were present in this fraction from cataractous lenses. Similarly, the WI proteins in normal lenses contained fragments of αA- and αB-crystallin, but cataractous lenses contained additional fragments of βA3- and βB1-crystallin. The modifications identified in the WS-HMW and WI crystallin species of cataractous lenses were truncation, oxidation of Trp residues, and deamidation of Asn to Asp residues.

Conclusions: The results show that the components of WS-HMW and WI protein fractions of cataractous lenses differed from normal lenses. Selective insolubilization of fragments of βA3/A1- and βB1-crystallin occurred during cataract development compared to normal lenses. Further, the crystallin species of cataractous lenses showed increased truncation, deamidation of Asn to Asp residues, and oxidation of Trp residue.

The mammalian lens contains three major structural proteins known as α-, β-, and γ-crystallin. These structural proteins, by virtue of their special structural interactions and high concentration, contribute to the transparency of the lens and provide the refractive index needed for the focus of light onto the retina. With aging, crystallins undergo several modifications such as aggregation and cross linking which leads to protein insolubilization. These changes are assumed to lead to cataract development. Based on recent studies of water insoluble (WI) proteins from normal human lenses [1,2], the major in vivo modifications were observed in αA- and αB-crystallin, which included disulfide bonding, deamidation, oxidation, and backbone cleavage.

The present literature suggests a multifactorial mechanism for the development of cataract specific cross linked species. The cross linking mechanism might be driven by post-translational modifications which include disulfide bonding [3], glycation [4], oxidation of Trp and His residues [5,6], deamidation [7], and transglutaminase mediated cross linking [8]. However, despite identification of these modifications in crystallins, the mechanism of crystallin aggregation and cross linking (initiated by the above modifications) remains poorly understood.

The present literature also suggests that crystallin fragments might undergo modifications, and therefore could cause aggregation and cross linking of crystallins during cataractogenesis in human lenses. Although the majority of the evidence is indirect [9-15], these reports together implicate a potential role of modified crystallin fragments in the generation of cross linked species during cataract development. In support of this role, we recently showed that the covalent multimers (M_r>90 kDa) in the aging human lenses were composed of crystallin fragments [16].

The proportion of WI proteins increases in human lenses with aging and more so during cataract development [17]. It is believed that the age related water insolubilization of lens proteins might in turn be mediated via a precursor complex known as water soluble-high molecular weight (HMW) pro-

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teins [10,11]. Therefore, changes in the WS-HMW proteins leading to WI proteins of cataractous lenses might play a critical role in producing cataract specific aggregates and cross linked species. An understanding of these comparative changes might provide information about potential mechanisms of aggregation and cross linking during cataract development. Because these changes are presently poorly characterized, this was one of the objectives of the present study.

Although several previous studies have described extensive truncation of human lens α-, β-, and γ-crystallin during aging and cataract development [13,14,18-21], the crystallin fragments that are found in WS-HMW and WI proteins of cataractous, but not normal lenses remain yet unidentified. Additionally, because a previous report [22] identified crystallin fragments with M_r ranging between 19,951 to 27,935 Da in human lenses, the truncated species with M_r<10 kDa in the WS-α-crystallin, WS-HMW, and WI protein fractions of the two types of lenses have also not been identified. These cataract specific distinctions are important because the information might identify specific crystallin species involved in aggregation and cross linking during cataract development. To bridge these gaps, the focus of the present comparative study was to distinguish cataract specific changes from age related changes in: (1) the truncated species of αA- and αB-crystallin in the WS-α-crystallin fraction, (2) the crystallin fragments that are found in the WS-HMW proteins and WI proteins, and (3) modifications such as N-, C-, or both N- and C-terminal truncations, oxidation of Trp, and deamidation of Asn to Asp residues in crystallin species. The results show that both N- and C-terminal truncations in αA- and αB-crystallin occur during aging and cataractogenesis. The C-terminal truncation was relatively more abundant in both normal and cataractous lenses. Additionally, the WS-HMW and WI proteins of cataractous human lenses showed the presence of fragments of βB1- and βA3/A1-crystallin that were absent in these fractions of normal lenses, suggesting their cataract specific aggregation.

METHODS

Materials: Normal human lenses with no apparent opacity were obtained from Dr. Robert Church of Emory University or from the Shared Ocular Tissue Module at the University of Alabama at Birmingham. The lenses were retrieved within 48-72 h post-mortem and stored in medium-199 without phenol red at -20 °C until used. The cataractous lenses (removed intracapsularly) with nuclear opacity were obtained within 4-5 h following surgery from a local surgeon, and stored as above. The prestained and unstained molecular weight markers were from Life Science and Amersham Biosciences, respectively. All chemicals for 2D gel electrophoresis were from either Amersham Biosciences or BioRad. Unless otherwise indicated, all other chemicals used in this study were purchased from Sigma or Fisher.

Isolation of WS-α-crystallin, WS-HMW and WI protein fractions from normal and cataractous lenses: All procedures were performed at 5 °C unless otherwise indicated. Identical procedures as described below were used to isolate WS-α-crystallin, WS-HMW, and WI protein fractions from normal lenses of different ages and from cataractous lenses by a procedure previously described [9]. Lenses were thawed on ice, decapsulated, suspended (2 ml/lens) in buffer A (10 mM Tris-HCl, pH 7.9 containing 1 mM dithioerythritol, 1 mM iodoacetamide [a cysteine proteinase inhibitor], 1 mM phenylmethylsulfonyl fluoride [a serine proteinase inhibitor]), and homogenized using a tissue grinder. Following centrifugation at 25,000x g for 15 min of the lens homogenate, the supernatant was recovered, and the above process was repeated twice on the pellet material. The three supernatants after each centrifugation were pooled and designated as the water soluble (WS) protein fraction and the pellet as the water insoluble protein fraction. The WS protein fraction was filtered through a 0.45 μm filter and fractionated by a size exclusion TSK G-4000 PW_{XL} HPLC column. During the HPLC, 50 mM Tris-HCl buffer (pH 7.9) was used for both column equilibration and sample elution, and eluted fractions were maintained at 5 °C during HPLC. The α-crystallin containing fractions were identified among the column fractions by SDS-PAGE [23] using a 15% polyacrylamide gel. These fractions were pooled separately, concentrated by lyophilization, dialyzed against water at 5 °C for 24 h, and kept frozen at -20 °C until used. The WS-HMW protein from the WS protein fraction was recovered following an alternate chromatographic procedure. A size exclusion Agarose A 5 m column (2.5x75 cm) was used and the void volume peak of WS-HMW protein was collected, concentrated by lyophilization, dialyzed as above, and kept frozen at -20 °C until used. The three fractions from normal or cataractous lenses (i.e., WS-α-crystallin, WS-HMW, and WI protein fractions) were individually dissolved in a resolubilization buffer (5 M urea, 2 M thiourea, 2% 3-[C3-cholamidoproyl] dimethyl-ammonio-1-propansulfonat [CHAPS], 2% caprylyl sulfobetaine 3-10, 2 mM tri-butyl phosphine, 40 mM Tris, pH 8.0) [23], and subjected to 2D gel electrophoresis (IEF in the first dimension and SDS-PAGE in the second dimension using the Laemmli [24] method with 15% polyacrylamide gels). The IEF separation was carried out using Immobilized Dry Strips (pH range of 3-10), and by following the manufacturer's suggested method (Amersham Biosciences, Piscataway, NJ). Prior to the first IEF analysis, the desired WI protein fraction was incubated overnight with dry strips at room temperature. Following the IEF separation, the strips were consecutively treated for 15 min each, first with 100 mM dithiothreitol (in equilibration buffer: 0.1 M Tris, pH 6.8, containing 6 M urea, 30% glycerol, and 1% SDS), and next with 300 mM iodoacetamide (also dissolved in equilibration buffer). During SDS-PAGE, a 15% polyacrylamide gel of 16x14 cm (width x height) was used. For mass spectrometric analyses, the protein spots were individually excised from a polyacrylamide gel using pipette microtips. The polyacrylamide pieces containing a spot was washed with doubly distilled water, and destained after treating with ammonium bicarbonate and acetonitrile. Trypsin (12 ng/μl) was added, and the preparation was resuspended in 25 mM ammonium bicarbonate, pH 7.8. The samples were digested by trypsin (Sequencing grade from Roche) at 37 °C overnight and the

next day, they were analyzed by the matrix assisted laser desorption ionization-time of flight (MALDI-TOF) method (Model Voyager-DE2 PRO, Perspective Biosciences, Forest City, CA). The MALDI analysis was performed at the Comprehensive Cancer Center Mass Spectrometric Shared Facility of the University of Alabama at Birmingham. The MALDI-TOF identity of proteins was established by using the NCBInr database of Matrix Science. The mass accuracy of the MALDI instrument was ±0.1 Dalton.

RESULTS

Isolation of α-crystallin and HMW protein fractions from human lenses: The α-, β-, and γ-crystallin fractions from the WS proteins of normal aging and cataractous lenses were isolated following size exclusion HPLC using a TSK G-4000 PW_{XL} HPLC column. Figure 1A shows a typical HPLC profile with absorbance at 280 nm, and the separation of α-crystallin from β- and γ-crystallin. This was further confirmed by SDS-PAGE analysis, which showed that α-crystallin was recovered in column fractions 14-16 (Figure 1B). The WS-HMW protein fractions from normal and cataractous lenses were isolated as a void volume peak (fractions 15-28) by size exclusion Agarose A 5 m chromatography (Figure 2A). The WS-HMW protein fraction eluted prior to the elution of the α-crystallin fraction (fractions 29-38) from the WS protein fraction as was evident from their SDS-PAGE analyses (Figure 2B). Both α-crystallin (Figure 1) and WS-HMW protein (Figure 2) fractions were pooled, processed, and subjected to 2D gel electrophoresis as described in the Methods section.

Identification of species present in WS-α-crystallin fractions of normal and cataractous human lenses: The results presented in Figure 3 show 2D protein profiles of WS-α-crystallin fractions of normal human lenses from donors of four ages: (A) 13 year old, (B) 21-26 year old, (C) 40 year old, and (D) 60 year old.

The purpose of this study was to compare the protein profiles of normal and cataractous human lenses. Since the majority of cataractous lenses examined (as shown later) were from approximately 50-60 year old donors, the 2D profile from 60 year old donors was used as a reference (Figure 3D). The spots were identified by numbers starting with the spot with the lowest molecular weight. The similar spots in the profiles of 13 year old, 21-26 year old, 40 year old, and 60 year old (reference profile) were identified by black arrows whereas the spots that differed in profiles were identified by white arrows. A total of eight spots were seen in the α-crystallin fraction from 13 year old lenses (Figure 3A) whereas fifty spots were present in 60 year old lenses (Figure 3D). The increase in the number of spots with aging suggested increased truncation and heterogeneity, which might be due to post-translational modifications.

The identification of spots present in the 2D gels was carried out following tryptic digestion of individual spots and MALDI-TOF analysis, as described in the Methods section. The loss of N- or C-terminal region or both the N- and C-terminal regions was determined based on the identity of tryptic fragments from a spot with expected mass. If the N-terminal fragment was not observed, the crystallin species was considered to be truncated at the N-terminal region. A similar approach was taken to identify truncation at either the C-terminal region or both N- and C-terminal regions in a crystallin species.

In the 13 year old lenses, four spots were identified as C-terminally truncated αA-crystallin species and four spots were identified as αB-crystallin species, with one of them being truncated at the C-terminal region (Table 1). Three spots (spots 44, 67, and 68) represented untruncated αB species but no untruncated αA crystallin was seen. Among the fifty spots from the 60 year old lenses, the identity of only forty-five spots could be determined by tryptic peptide fingerprinting mass spectrometry (Table 2). Among them, twenty-five spots were derived from αA-crystallin and twenty spots were from the αB-crystallin species. The remaining five could not be identified. Among the twenty-five spots of the αA- crystallin species, two were N-terminally cleaved, nine were C-terminally cleaved, eleven were both and N- and C-terminally cleaved, and the cleavage in two αA-crystallin species was undetermined. Similarly, among the 20 spots derived from αB-crystallin, six were N-terminally cleaved, five were C-terminally cleaved, seven were both N- and C-terminally cleaved, and cleavage in two αB-crystallin species was undetermined.

The above results show that in the young 13 year old lenses, the majority of truncation in αA- and αB-crystallin occurred in the C-terminal region, whereas in the lenses of 60 year old donors, the crystallins underwent additional cleavages in either N- or both N- and C-terminal regions. The data suggest that these later cleavages were age related. The α-crystallin fraction was isolated as above from four pooled cataractous lenses (with nuclear opacity) from 60 year old donors and analyzed by 2D gel electrophoresis. In contrast to 50 spots in the WS-α-crystallin fraction of normal human lenses, the cataractous lenses showed only twenty-three spots in this fraction (Figure 4). MALDI-TOF analysis indicated that 13 spots were from αA-crystallin and the remaining 8 were from αB-crystallin. The identity of two spots was undetermined (Table 3). Among these, twelve αA-crystallin species had both N- and C-terminal cleavages and one had only N-terminal cleavage, showing that in cataractous lenses, the majority of αA-crystallin fragments were derived via both N- and C-terminal truncations. Similarly, among eight fragments of αB-crystallin, four were derived via both N- and C-terminal cleavages and the remaining four were via only N-terminal cleavage. Surprisingly, no fragments with only C-terminal cleavage of αA- or αB-crystallin was observed in this fraction of cataractous lenses.

Identification of crystallin species present in the WS-HMW protein fractions of normal and cataractous human lenses: Because previous reports have identified mostly αA- and αB-crystallin in the WI protein fraction of normal human lenses [1,2], and this fraction is apparently an intermediate between WS and WI proteins, we examined whether these fragments became part of the WS-HMW protein fraction during aging and cataractogenesis. On 2D gel electrophoresis, the WS-HMW protein fraction from normal lenses of 60 year old do-

nors showed twenty-seven spots (Figure 5A), and only eight spots in this fraction from cataractous lenses (Figure 5B). On MALDI-TOF analysis of spots from normal lenses, twelve of the twenty-seven spots were from αA-crystallin (ten spots were N- and C-terminally cleaved, and two N-terminally cleaved) and the remaining sixteen spots were from αB-crystallin (nine spots were both N- and C-terminally cleaved, four spots N-terminally cleaved, and three spots C-terminally cleaved, Table 4). In contrast to the twenty-seven spots in the WS-HMW protein from normal lenses, cataractous lenses showed only eight spots on a 2D gel (Figure 5B). Among these, four spots were from αB-crystallin (one spot was both N- and C-terminally cleaved, and three spots were N-terminally cleaved), two were a mixture of βB1-crystallin (N- and C-terminally cleaved) and βA3-crystallin (C-terminally cleaved), and two spots were from βB1-crystallin (both N- and C-terminally cleaved, Table 5). Together, the results show that both the number of spots and the composition of HMW proteins changed in the cataractous lenses compared to normal lenses. Specifically, the majority of the fragments present in the WS-HMW proteins of normal lenses were derived from αA- and αB-crystallin having both N- and C-terminal truncations, whereas in the cataractous lenses, these were derived mostly from αB-, βB1-, and βA3-crystallin.

Identification of water insoluble crystallin fragments in normal and cataractous human lenses: Upon comparison of

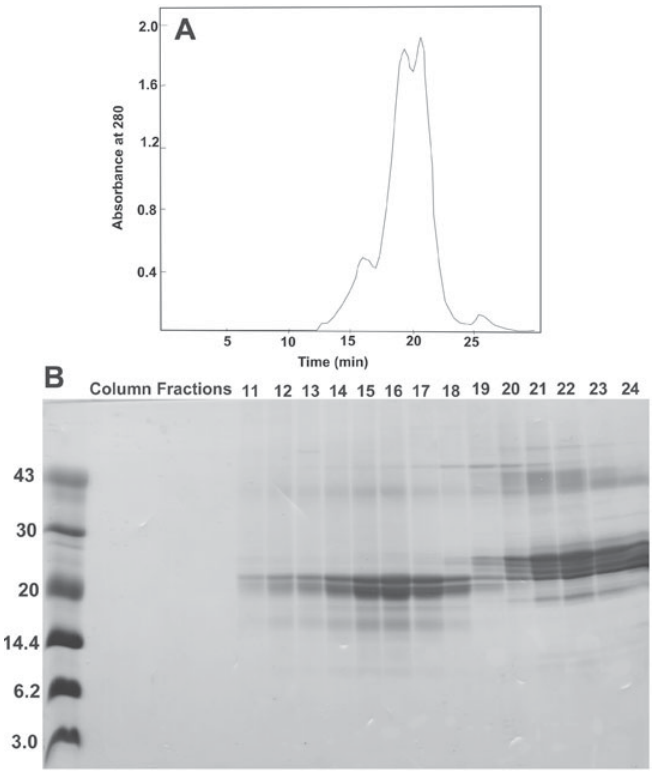

Figure 1. Isolation and identification of WS-α-crystallin protein fractions. This figure shows the separation of α-crystallin fractions from the WS proteins of human lenses following size-exclusion chromatography. Size exclusion HPLC using a TSK G-4000 PW_{XL} column of WS proteins from lenses of 21-26 year old donors. During HPLC, 50 mM Tris-HCl buffer (pH 7.9) was used for both column equilibration and sample elution, and eluted fractions were maintained at 5 °C during HPLC. **A**: Protein elution profile at 280 nm of WS proteins from 21-26 year old lenses during size exclusion HPLC using a TSK G-4000 PWXL column. **B**: Identification of α-crystallin containing fractions by SDS-PAGE using a 15% polyacrylamide gel among column fractions. Note that α-crystallin eluted at 3-16 min as shown in **A** and are represented by fractions 13-16 in **B**.

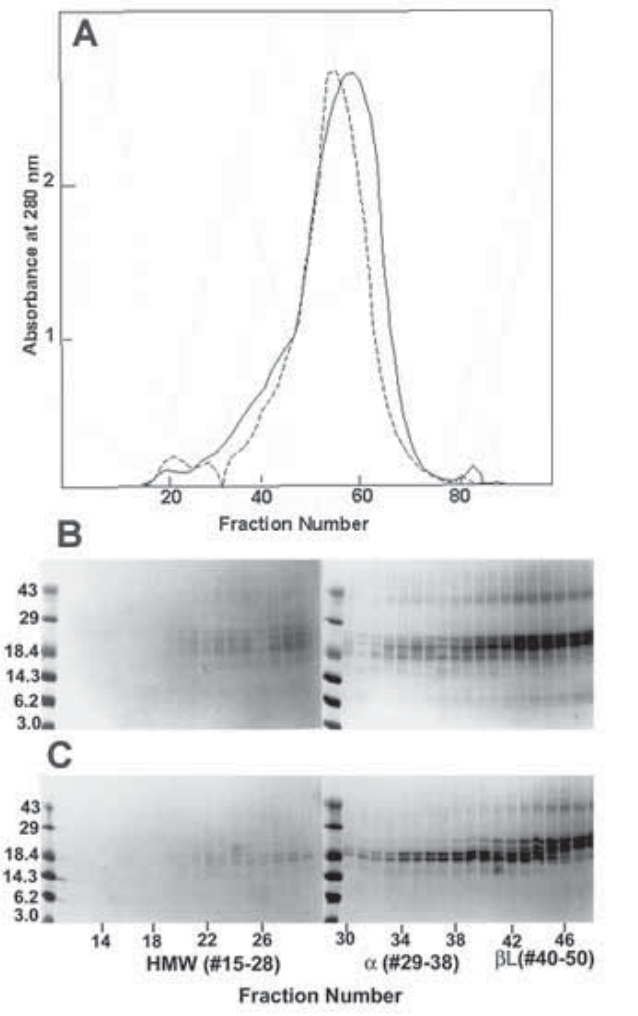

Figure 2. Isolation and identification of WS-HMW protein fractions from normal and cataractous human lenses. This figure shows separation of the HMW-protein and α-crystallin fractions from the WS proteins of human lenses following size-exclusion chromatography. Isolation of WS-HMW protein fraction following size exclusion Agarose A 5 m chromatography from WS protein fraction of normal and cataractous lenses (both from 50-60 year old donors). The HMW protein eluted as a void volume peak (fractions 15-28). **A**: Protein elution profile at 280 nm of WS-HMW proteins from 50-60 year old normal (solid line) and cataractous (dashed line) lenses during size exclusion Agarose A 5m chromatography. **B**: Identification of WS-HMW protein fractions (fractions 15-28) and WS-α-crystallin protein fractions (fractions 29-38) by SDS-PAGE using a 15% polyacrylamide gel.

2D protein profiles of the WI protein fractions from normal lenses from 16, 27, 40, and 50 year old donors, it was discovered that the abundance of crystallin fragments increased with aging (Figure 6A, Figure 6B, and Figure 6C). The common spots present in the WI protein fractions of 27 and 50 year old lenses are shown in Figure 6. Because the numbers of spots observed were highest (38) in the WI protein profile of 27 year old lenses, and the WI protein profiles of both 27 and 35 year old lenses were identical (results not shown), the spots present in the 27 year old were used for MALDI-TOF analysis (Table 6). Of the thirty-eight spots, thirty-six were identified. Among these, twenty spots were from αA-crystallin (fifteen spots both N- and C-terminally cleaved and four spots C-terminally cleaved), and fourteen were from αB-crystallin (two spots both N- and C-terminally cleaved, five spots N-terminally cleaved, one spot C-terminally cleaved, and cleavages in five αB-crystallin spots were undetermined). A single spot of βB2-crystallin was observed that was both N- and C-terminally cleaved. Together, the results show that the majority of the fragments present in the WI protein fraction from 27 year old normal lenses were derived from αA- and αB-crystallin.

The WI protein fractions from lenses with nuclear cataracts from donors of three ages (40-50 years, 50-60 years, and 70 years) were separated by 2D gel electrophoresis and analyzed as above by the MALDI-TOF method. The profiles showed that no crystallin fragment with M_r<16.9 kDa was present in 40-50 year old cataractous lenses but these appeared in 50-60 and 70 year old cataractous lenses (results not shown). A total of thirty-four spots of truncated species of four major crystallins (αA-, αB-, βA3/A1-, and βB1-crystallin) were present in the WI protein fraction of cataractous lenses of 50-60 year old donors (Figure 7). Among these, eighteen spots were from αB-crystallin (one spot was both N- and C-terminally cleaved, eight spots N-terminally cleaved, and cleav-

Table 1. Parent crystallins of species present in WS-α-crystallin from 13 year old lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)
36	αA	C	16.5
37	αA	C	16.5
38	αA	C	16.5
41	αB	UD	19
42	αB	C	20
44	αB	UD	20
67	αB	UD	20
68		UD	
69	αA	C	16.5

Identification of parent crystallins of spots present in the 2D gel of α-crystallin fraction isolated from WS protein fraction of lenses from normal 13 year old donors (Figure 3A) by tryptic peptide fingerprinting mass spectrometry. The spot numbers correspond to numbers shown in reference gel (Figure 3D). Molecular weights were determined based on migration of protein markers. In the cleavage column, "C" indicates C-terminus cleavage and "UD" indicates undetermined.

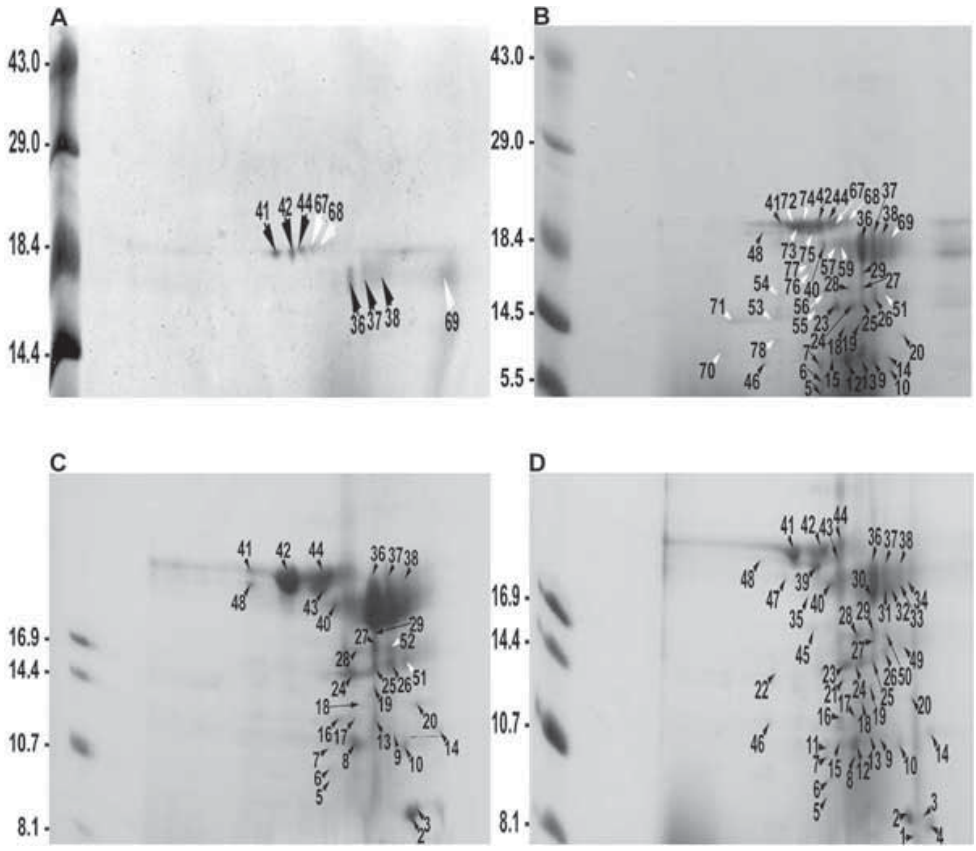

Figure 3. Two dimensional gel electrophoretic separation of WS-α-crystallin fractions isolated from aging normal lenses. Two dimensional (2D) gel electrophoretic profiles of WS-α-crystallin fractions isolated from aging normal lenses. **A**: 13 year old. **B**: 21-26 year old. **C**: 41 year old. **D**: 60 year old. The WS-α-crystallin fractions were isolated from lenses of different ages by size-exclusion HPLC as described in Figure 1. 2D gel electrophoresis was performed as described in Methods. The spots-profile of 60 year old lenses was used as a reference gel. Similar spots in all the gels are shown with black arrows and the spots that differed by white arrows.

ages in six were undetermined), two spots were of αA-crystallin (one spot was both N- and C-terminally cleaved, and one spot C-terminally cleaved), eleven spots were of βA3/A1-crystallin (five spots were both N- and C-terminally cleaved, four spots N-terminally, and one spot C-terminally cleaved), and one spot's cleavage site(s) was unidentified, and two spots were of βB1-crystallin (both were N- and C-terminally cleaved, Table 7). Together, the results show that the WI protein fraction of cataractous lenses differed in its composition from that of normal lenses; the cataractous lenses contained several truncated species of βA3/A1- and βB1-crystallin that were absent in the normal lenses. Further, the WI proteins of normal lenses contained mostly N- and C-terminally

Table 2. Parent crystallins in species present in the WS-α-crystallin fraction from 60 year old normal lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)	Percent sequence detected
1	αA	N	7.0	35
2	αA	C	8.1	23
3	αA	C	8.2	23
4	αA	N and C	8.1	21
5	αB	UD	8.2	UD
6	αB	N and C	9.0	45
7	αB	N and C	9.5	25
8	αB	C	9.5	26
9	αA	UD	9.9	UD
10	αA	N and C	9.9	46
11	αB	N	10.2	21
12	αA	C	9.9	27
13	αA	C	10.5	50
14	αA	N and C	11.0	34
15	αA	N	11.0	51
16	αB	C	11.4	41
17	αA	UD	11.4	UD
18	αB	N and C	11.9	63
19	αA	C	12.5	50
20	αA	C	12.1	39
21	αB	N	13.0	63
22	αB	C	13.5	31
23	αB	C	13.5	57
24	αA	C	13.5	42
25	αA	N and C	13.5	43
26	αA	C	14.0	55
27	αB	N and C	14.4	43
28	αA	C	14.4	34
29	αA	N and C	15.4	31
30	αA	N and C	17.7	37
31	αA	N and C	17.7	31
32	αA	N and C	17.7	37
33	αA	N and C	17.7	40
34	αB	N	17.7	67
35	αA	N and C	17.2	46
36	αA	N and C	18.5	40
37	αA	N and C	18.5	49
38	αB	N	18.5	49
39	αB	N and C	19.5	64
40	αB	N	15.4	54
41	αB	UD	20.0	UD
42	αB	N and C	20.1	59
43	αB	N	19.8	71
44	αB	C	20.1	51
45	αB	N and C	15.0	57

Identification of parent crystallins of spots present in the 2D gel of the α-crystallin fraction of lenses from normal 60 year old donors (Figure 3D) by tryptic peptide fingerprinting mass spectrometry. The spot numbers correspond to numbers shown in reference gel (Figure 3D). In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage. Molecular weights were determined from migration of protein markers. "UD" indicates undetermined. The percent sequence detected was during MALDI-TOF.

Table 3. Parent crystallins of spots in a 2D gel of WS-α-crystallin fraction from 60 year old cataractous lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)
1	αA	N and C	3.1
2	αA	N and C	3.7
3	αB	N and C	6.1
4	αA	N and C	6.1
5	αA	N and C	5.0
6	αB	N and C	9.0
7	αA	N and C	9.5
8	αA	N	9.0
9	αA	N and C	15.2
10	αA	N and C	15.2
11	αA	N and C	15.3
12	αA	N and C	15.3
13	Mixture of αA and αB	N and C	15.3
14	αB	N and C	19.7
15	αA	N and C	19.5
16	αB	N	19.2
17	αB	N and C	19.2
18	αB	Intact	20.7
19	αB	Intact	20.7
20	αA	Intact	20.7
21	αB	Intact	20.7

Identification of parent crystallins of spots present in 2D gels of the WS crystallin fraction of cataractous lenses with nuclear opacity Spot number corresponds to numbers shown in Figure 4. In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage. Molecular weights were determined based on migration of protein markers.

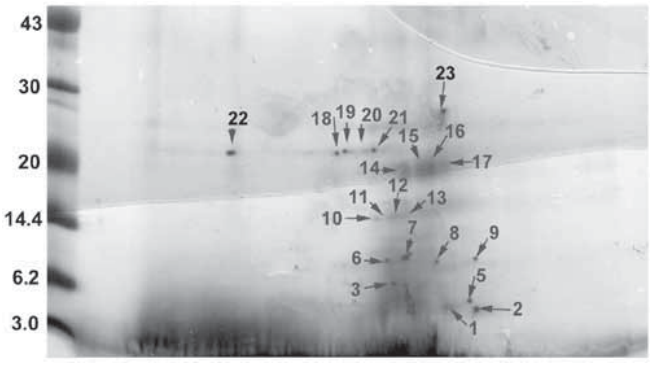

Figure 4. Two dimensional gel electrophoretic proteins profile of the WS-α-crystallin fraction from 60 year old cataractous lenses. Two dimensional (2D) gel electrophoretic profiles of WS-α-crystallin fractions isolated from cataractous lenses with nuclear opacity from 60 year old donors.

cleaved αA-crystallin and N-terminally cleaved αB-crystallin, whereas the cataractous lenses contained mostly N-terminally cleaved αB-crystallin fragments.

Post-translational modifications in crystallin species present in HMW and WI protein fractions of cataractous lenses: As shown above, the compositions of WS-HMW proteins and WI proteins from lenses with nuclear cataracts differed from that of age matched normal lenses, which might lead to aggregation of these species following post-translational modifications. Therefore, specific modifications in the crystallin species present in the WS-HMW and WI proteins were investigated. Because previous reports have shown four major age related modifications (i.e., truncation, deamidation, disulfide bond formation, and oxidation of Trp residues) in the WS and WI crystallin species of normal human lenses, our focus was to determine the modifications in the species present in the WS-HMW and WI protein fractions of cataractous lenses. As stated above, the WS-HMW protein fraction of cataractous lenses showed only eight spots (Figure 5B) of truncated αB-, βB1-, and βA3-crystallin (Table 6). Upon examination of the deamidation among theses species, Spot 1 showed oxidation of a Trp residue and deamidation of Asn_{146} to Asp. The oxidation of the Trp residue was determined in an αB-crystallin tryptic fragment with a mass of 1,496 containing residues 57 to 69 (sequence: APSWFDTGLSEMR). Upon oxidation of Trp, another peak of 1,513 with an additional about 16 mass units was observed (results not shown). The conclusion of deamidation of αB-crystallin Asn_{146} to Asp was based on the determination of isotopic distribution of the tryptic fragment (residues 124-149; sequence: IPADVDPLTITSSDGVLTVNGF) with mass of 2,624 of αB-crystallin (Figure 8). The major isotopic species had a mass of 2,625, which was due to the gain of one mass unit following the conversion of Asn to Asp (Figure 8). Using a similar approach as described above, the modifications observed in spots 1 to 8 of WS-HMW protein (Figure 5B) and in spots 2 to 34 of WI protein (Figure 7) of cataractous lenses were determined and are summarized in Table 8.

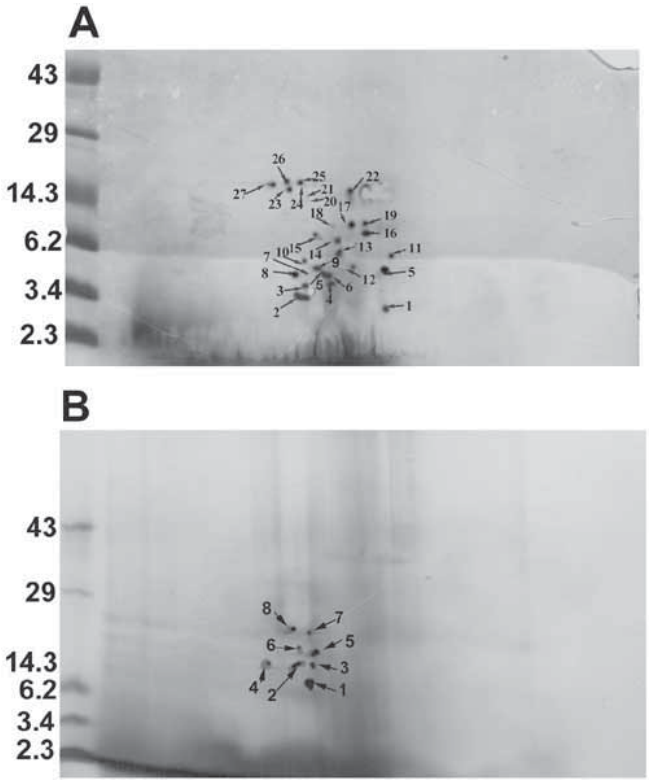

Figure 5. Two dimensional gel electrophoretic protein profiles of WS-HMW proteins of normal and cataractous lenses. Two dimensional (2D) gel electrophoretic profiles of WS-HMW protein fractions. **A**: Normal lenses of 60 year old donors. **B**: Cataractous lenses with nuclear opacities from 60 year old donors.

DISCUSSION

The aims of the present study were to comparatively identify parent crystallins of the crystallin fragments present in WS-α-crystallin, WS-HMW, and WI protein fractions of human cataractous (with nuclear opacity) and age matched normal lenses, and to identify post-translational modifications in crystallin species present in the WS-HMW and WI protein fractions of cataractous lenses. The rationale was that the results would distinguish cataract specific aggregated crystallin spe-

Table 3. Parent crystallins of spots in a 2D gel of WS-α-crystallin fraction from 60 year old cataractous lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)
1	αA	N and C	3.1
2	αA	N and C	3.7
3	αB	N and C	6.1
4	αA	N and C	6.1
5	αA	N and C	5.0
6	αB	N and C	9.0
7	αA	N and C	9.5
8	αA	N	9.0
9	αA	N and C	15.2
10	αA	N and C	15.2
11	αA	N and C	15.3
12	αA	N and C	15.3
13	Mixture of αA and αB	N and C	15.3
14	αB	N and C	19.7
15	αA	N and C	19.5
16	αB	N	19.2
17	aB	N and C	19.2
18	αB	Intact	20.7
19	αB	Intact	20.7
20	αA	Intact	20.7
21	αB	Intact	20.7

Identification of parent crystallins of spots present in 2D gels of the WS crystallin fraction of cataractous lenses with nuclear opacity Spot number corresponds to numbers shown in Figure 4. In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage. Molecular weights were determined based on migration of protein markers.

cies from those that aggregate during aging in both WS-HMW and WI protein fractions.

The major findings of this study are: (1) the majority of aggregated species in the WS-HMW and in the WI protein fractions during aging and nuclear cataract development were fragments of αA- and αB-crystallin, (2) while the younger lenses contained only the C-terminally truncated αA- and αB-crystallin, the older normal lenses showed age related truncation of both N- and C- terminal regions in the crystallins, (3) in the WS-HMW protein fraction, mostly αA- and αB-crystallin species were present in the normal lenses but additional fragments of βB1- and βA3-crystallin species were present in the cataractous lenses, (4) like the WS-HMW protein fraction, the WI proteins of the cataractous lenses contained several fragments of βA3/A1- and βB1-crystallins, which were absent in the WI proteins of age matched normal lenses, (5) among αA- and αB-crystallin, mainly αB-crystallin fragments aggregated in the WS-HMW and WI protein fractions of cataractous lenses, and (6) the crystallin species present in the WS-HMW and WI proteins showed three major modifications (truncation, oxidation of Trp residues, and deamidation of Asn to Asp residue).

Table 4. Parent crystallins of spots in a 2D gel of WS-HMW proteins from normal lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)
1	αA	N and C	2.5
2	αB	N and C	2.9
3	αB	N and C	3.6
4	αA	N and C	
	αB	N	3.9
5	αA	N and C	4.5
6	αA	N and C	4.5
7	αA	N and C	4.9
8	αB	N and C	5.1
9	αA	N	5.5
10	αA	N and C	5.9
11	αA	N and C	6.0
12	αB	N and C	6.0
13	αB	C	6.3
14	αB	N and C	7.2
15	αB	N and C	7.4
16	αA	N and C	8.85
17	αA	N and C	9.6
18	αB	N and C	9.6
19	αA	N and C	10.5
20	αB	C	12.5
21	αB	N and C	13.5
22	αA	N	10.5
23	αB	N	14.5
24	αB	N	14.5
25	αB	Mixture of both N and C terminally cleaved	18.0
26	αB	N and C	19.0
27	αB	N	18.0

Identification of spots present in 2D gel of HMW protein fraction isolated from normal lenses. Spot number corresponds to the number shown in Figure 5. In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage. Molecular weights were determined based on migration of protein markers.

The above results are unique and different from previously reported findings in the literature. Presently, the distinction between aggregates that are formed during cataract, but not during aging or vice versa, is obscure. Therefore, the identification of the cataract specific aggregates of truncated βA3/A1- and βB2-crystallin in the WS-HMW and WI protein fractions is significant, and has been reported for the first time. This finding differs from several age related changes in the WI crystallins as described in previous reports [1,2]. These reports also identified αA- and αB-crystallin as the major constituents in the WI proteins of 45 and 50-60 year old normal lenses with certain modifications relatively more prevalent than WS proteins. These included intrachain disulfide bonding among two oxidized Cys residues of αA-, partial N- and C-terminal truncations, partial oxidation of Met residues, deamidation of Gln and Asn residues, and cleavage at the deamidated sites. Additional water insoluble crystallins (in order of their decreasing abundance: γS-, βB1-, γD-, βA3/A1-, and βB2-crystallin) were also identified, and αA-, αB-, βB2-, γS-, and γD-crystallin existed in both intact and truncated forms with oxidation of Cys, Met, and Trp residues, and deamidation of Gln and Asn residues [2]. The three cataract specific modifications reported herein (truncation, oxidation, and deamidation) are similar to the age related changes except that the species involved and truncated regions differed. Another previous report has shown two cataract specific atypical αB-crystallin species, one 22.4 kDa (αB minus C-terminal Lys) and the other 16.4 kDa (αB minus 38-46 N-terminal residue) at increased levels compared to the normal lenses [13]. Similarly, an increased deamidation of N_{143} in γS-crystallin in cataractous lenses compared to normal lenses was observed [7]. A similar report showed deamidation of the Gln reside in αA-crystallin [25].

Table 5. Parent crystallins of spots present in a 2D gel of WS-HMW proteins from cataractous lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)
1	αB	N and C	8.9
2	αB	N	18.0
3	αB	N	18.0
4	αB	N	18.0
5	βB1	N and C	20.5
	βA3	C	
6	βB1	N and C	21.5
	βA3	C	
7	βB1	N and C	22.0
8	βB1	N and C	22.5

Identification of parent crystallins of spots present in a 2D gel of HMW protein fraction from cataractous lenses with nuclear opacity. Spot number corresponds to the number shown in Figure 5B. Molecular weights were determined based on migration of protein molecular weight markers on the gel. In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage.

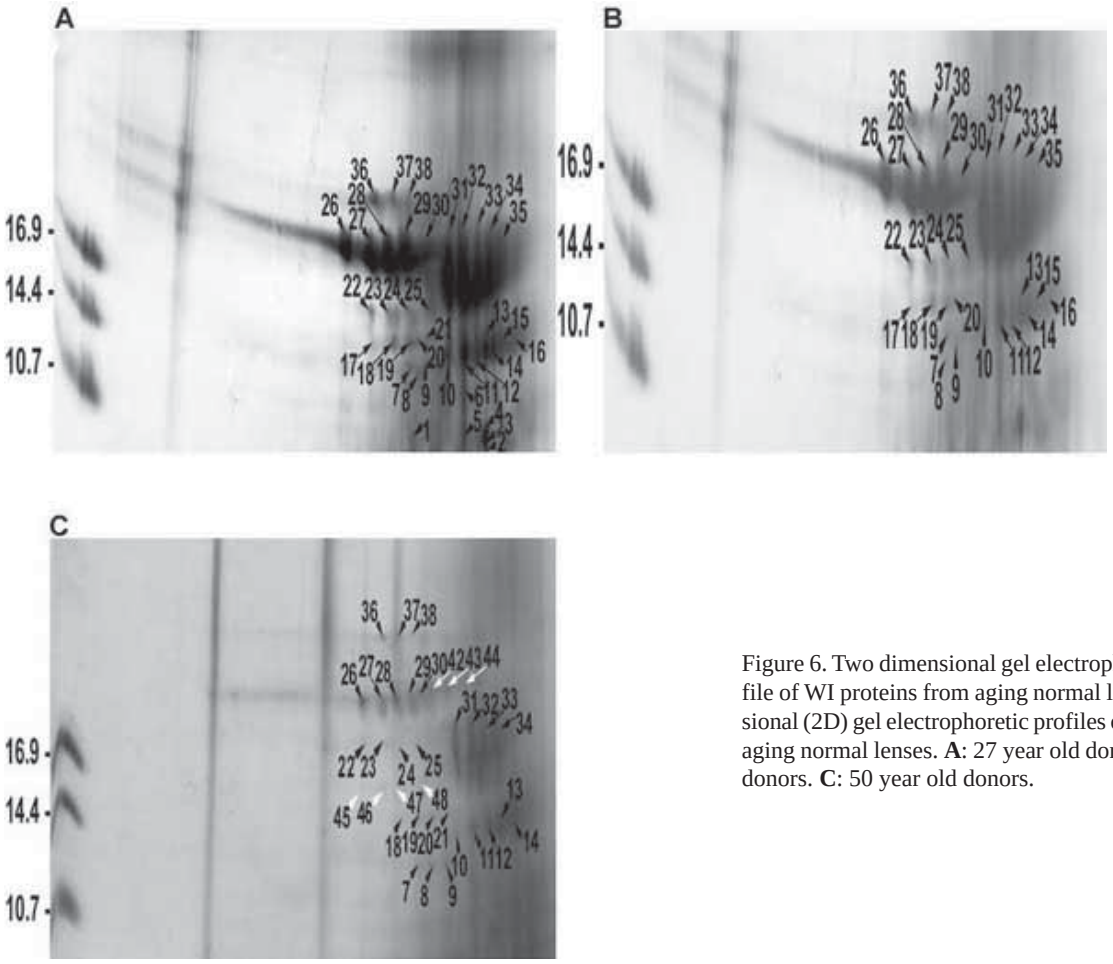

Figure 6. Two dimensional gel electrophoretic protein profile of WI proteins from aging normal lenses. Two dimensional (2D) gel electrophoretic profiles of WI proteins from aging normal lenses. **A**: 27 year old donors. **B**: 40 year old donors. **C**: 50 year old donors.

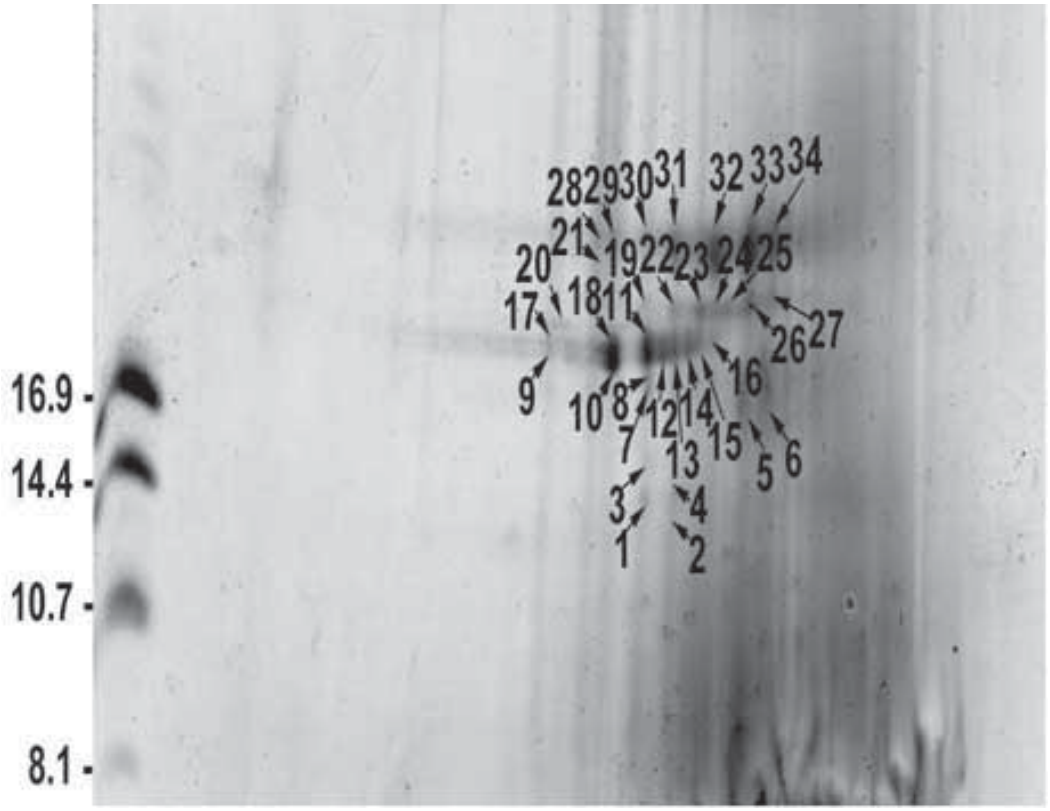

Figure 7. Two dimensional gel electrophoretic protein profile of WI proteins from 50-60 year old cataractous lenses. Two dimensional (2D) gel electrophoretic profiles of WI proteins isolated from cataractous lenses with nuclear opacity from 50-60 year old donors.

The results of this report show that only the C-terminal truncation in αA- and αB-crystallin occurs in normal young lenses. With aging, additional N- and both N- and C-terminal truncations occurred, which have been previously reported [26]. In contrast to the normal lenses, the α-crystallin fraction of cataractous lenses contained mainly both N- and C-terminally truncated αA- and αB-crystallins. The fact that most of the N- and C-terminally truncated αA- and αB-crystallin showed aggregation into the WS-HMW proteins of normal and cataractous lenses suggest that the N- and C-terminally truncated species are more prone to form aggregates than those having only N- or C-terminal truncation. These truncations have a significant effect on the chaperone activity of the α-crystallin, as described below.

Although the WS-HMW proteins of cataractous lenses showed a relatively lower number of truncated species than normal lenses, it contained new species of βB1- and βA3/A1-crystallin. This reduced number could be due to either proteolysis of αA- and αB-crystallin species or their cross linking in species (M_r>100 kDa) that did not enter the 15% polyacrylamide gel. The selective aggregation of truncated βB1- and βA3/A1-crystallin in the cataractous lenses could be due to cataract specific modifications. The two modifications, truncation and deamidation in Asn_{54}, Asn_{62}, and Asn_{108} in βA3/A1-crystallin, and Asn_{106}, Asn_{162}, and Asn_{218} in βB1-crystallin (Table 8), might be responsible for this aggregation. These deamidation sites in the two crystallins have been identified

Table 6. Parent crystallins of spots present in a 2D gel of WI proteins from normal lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)	Percent sequence detected
2	αA	N and C	8.1	38
3	αA	N and C	8.3	28
4	αA	N and C	8.5	41
5	αA	N and C	8.5	38
6	αA	C	9.8	45
7	αB	C	10.5	49
8	αA	N and C	10.3	43
9	αA	N and C	10.5	60
	αB	N and C		43
10	αA	N and C	10.7	59
11	αA	C	10.7	50
12	αA	N and C	10.7	59
13	αA	N and C	10.7	38
14	αA	N and C	10.7	62
15	αA	N and C	10.7	42
16	αA	N and C	11.0	36
17	αB	N	11.6	61
18	αB	N and C	11.6	50
19	αB	N and C	11.6	57
24	αA	C	13.5	42
25	αA	N and C	13.5	43
26	αA	C	14.0	55
27	αB	N and C	14.4	43
28	αA	C	14.4	34
29	αA	N and C	15.4	31
30	αA	N and C	17.7	37
31	αA	N and C	17.7	31
32	αA	N and C	17.7	37
33	αA	N and C	17.7	40
34	αB	N	17.7	67
35	αA	N and C	17.2	46
36	αA	N and C	18.5	40
37	αA	N and C	18.5	49
38	αB	N	18.5	49
39	αB	N and C	19.5	64
40	αB	N	15.4	54
41	αB	UD	20.0	UD
42	αB	N and C	20.1	59
43	αB	N	19.8	71
44	αB	C	20.1	51
45	αB	N and C	15.0	57

Identification of parent crystallins of spots present in 2D gel of WI protein fraction of lenses of a normal 27 year old donor (Figure 6A) by tryptic peptide fingerprinting mass spectrometry. The spot numbers correspond to numbers shown in reference gel (Figure 5). In the cleavage column, "C" indicates C-terminus cleavage, "N" indicates N-terminus cleavage, and "UD" indicates undetermined cleavage. Molecular weights were determined based on the molecular weight of protein markers. Percent sequence was detected during MALDI-TOF.

Table 7. Parent crystallins of spots present in a 2D gel of WI proteins from cataractous lenses

Spot number	Parent crystallin	Cleavage	Molecular weight (kDa)	Percent sequence detected
1	UD	UD	14.7	UD
2	αB	UD	15.0	UD
3	αB	N	16.1	58
4	αB	N and C	16.3	55
5	αA	C	18.9	40
6	αA	N and C	19.5	24
7	αB	N	18.9	37
8	αB	N	19.5	62
9	αB	Intact	20.3	100
10	αB	N	19.9	39
11	αB	UD	20.3	UD
12	αB	UD	20.3	UD
13	αB	UD	20.3	UD
14	αB	Intact	20.7	100
15	αB	Intact	21.2	100
16	αB	Intact	21.2	100
17	αB	N	21.2	63
18	αB	UD	20.7	UD
19	αB	UD	21.4	UD
20	αB	UD	20.7	UD
21	αB	UD	22.0	UD
22	βA3/A1	N	22.7	55
23	βA3/A1	N	22.7	32
24	βA3/A1	N	22.7	42
25	βA3/A1	UD	22.7	UD
26	βA3/A1	N	22.7	37
27	βB1	Intact	22.7	100
28	βA3/A1	C	25.4	30
29	βA3/A1	N	26.8	23
30	βA3/A1	Intact	25.4	100
31	βA3/A1	N and C	25.4	20
32	βA3/A1	C	25.4	20
33	βA3/A1	C	25.4	12
34	βA3/A1	N	26.2	UD

Identification of parent crystallins of spots present in the 2D gel of WI protein fractions of cataractous lenses with nuclear opacity from 50-60 year old donors (Figure 7) by tryptic peptide fingerprinting mass spectrometry. Molecular weights were determined based on migration of protein markers. "UD" indicates undetermined. In the cleavage column, "C" indicates C-terminus cleavage and "N" indicates N-terminus cleavage.

for the first time although deamidation of βA3/A1-crystallin in both WS [18,22] and WI proteins [1,2] of normal lenses has previously been shown.

Among different β-crystallin species, the human lens βA3/A1- and βB1-crystallins are extensively truncated at the hydrophilic N-terminal region [18,22] in the WS protein fraction of normal human lenses. We have previously reported a preferred cleavage site at the Glu_{39}-Asn_{40} bond in the human βA3/A1-crystallin [20]. Similarly, the bovine lens βA3- and

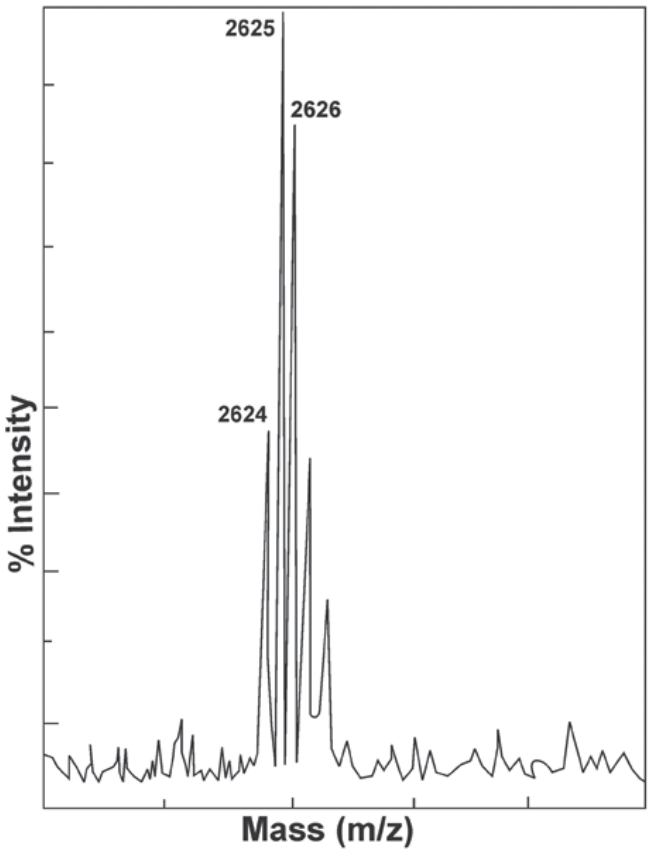

Figure 8. Determination of deamidation of Asn to Asp in tryptic fragments of αB-crystallin. Isotopic distribution of a tryptic fragment (residues 124-149; sequence: IPADVDPLTITSSDGVLTVNGF; mass of 2,624) of αB-crystallin during MALDI-TOF mass spectrometric analysis showing deamidation of αB-crystallin Asn_{146} to Asp. The major isotopic species had mass of 2,625, which was due to the gain of one mass unit following the conversion of Asn to Asp. Using a similar approach, the modifications observed in the spots 1-8 of WS-HMW proteins (Figure 5B) and in spots 2-34 of WI proteins (Figure 7) of cataractous lenses were determined and are summarized in Table 8.

Identification of post-translational modifications in HMW and WI proteins of cataractous lenses. The 2D gel electrophoretically separated protein spots of WS-HMW proteins (Figure 5B) and WI proteins (Figure 7) from cataractous lenses were examined using the MALDI-TOF mass spectrometric method. The oxidation or deamidation of specific residues in tryptic fragments was identified based on the changes in the mass of the fragments.

Table 8. Post-translational modifications identified in HMW and WI proteins of cataractous lenses

Spot number	Crystallin fragment	Modification(s)
HMW Proteins		
1	αB	Oxidation (W60) Deamidation (N146)
2	αB	Oxidation (W60) Deamidation (N146)
3	αB	Oxidation (W60) Deamidation (N146)
4	αB	Deamidation (N146)
5	βB1 βA3/A1	Deamidation βA3/A1 (N54, N62)
6	βB1 βA3/A1	Deamidation βA3/A1 (N54, N62, N108)
7	βB1	Deamidation (N216)
8	βB1	Deamidation (N107, N161)
WI Proteins		
2	αB	Oxidation (W60) Deamidation (N78, N146)
3	αB	Oxidation (W60) Deamidation (N146)
4	αB	Oxidation (W60) Deamidation (N146)
5	αA	-
6	αA	-
7	αB	Oxidation (W60) Deamidation (N78)
8	αB	Oxidation (W60) Deamidation (N78, N146)
9	αB	Deamidation (N78, N146)
10	αB	Oxidation (W60) Deamidation (N78, N146)
11	αB	Oxidation (W60) Deamidation (N146)
12	αB	Deamidation (N146)
13	αB	Oxidation (W60) Deamidation (N78, N146)
14	αB	Deamidation (N146)
15	αB	Oxidation (W60) Deamidation (N78, N146)
16	αB	Deamidation (N146)
17	αB	Oxidation (W60) Deamidation (N146)
18	αB	Oxidation (W60) Deamidation (N78, N146)
19	αB	Deamidation (N78, N146)
20	αB	-
21	αB	-
22	βA3/A1	Deamidation (N133)
23	βA3/A1	-
24	βA3/A1	-
25	βA3/A1	Deamidation (N40, N133)
26	βA3/A1	Oxidation (W96) Deamidation (N40, N133)
27	βB1	-
28	βA3/A1	Deamidation (N40)
29	βA3/A1	Deamidation (N40)
30	βA3/A1	Deamidation (N40)
31	βA3/A1	Deamidation (N40)
32	βA3/A1	Deamidation (N40)
33	βA3/A1	Deamidation (N40)
34	βB1	-

βA1-crystallin (βA3- and βA1-crystallin have identical sequence except that βA3-crystallin has 13 additional N-terminal amino acid residues) showed two major truncated species with βA3-crystallin losing 11 and 22 amino acid residues at the N-terminal region [26]. Age related truncations of human lens βB1-crystallin in the WS proteins of normal lenses have been reported with the βB1-crystallin species missing N-terminal 15, 33, 34, 35, 36, 39, 40, and 41 amino acid residues [18]. It was also noted that the N-terminal truncation in the βB1-crystallin started within the first year of life [18]. In fact, the earliest post-translational modification in human lenses has been reported to be truncation of βA3/A1- and βB1-crystallin [27,28]. These studies also showed that most age related modifications have occurred by age 20 with few additional changes occurring in lenses of ages between 20 to 70 years [27,28]. Our results show that the truncation and aggregate formation of crystallins occurred beyond age 20 and the truncated βA3/A1- and βB1-crystallin aggregated in the WS-HMW proteins and WI protein of only the cataractous lenses. This finding of an absence of βA3/A1- and βB1-crystallin in the WI proteins of normal lenses differs from previous studies [1,2], which could be due to cataract specific truncation of both N- and C-terminal regions along with certain yet unknown modifications in these crystallins.

The X-ray crystallographic structure of βB2-crystallin [29] showed that unlike γ-crystallin, its two domains were separated due to a relatively longer connecting peptide, and in the β_L-crystallin (a dimer of βB2-crystallin), the opposite domains showed interaction with free terminal tails. However, NMR studies [30,31] showed that in the β_H-crystallin aggregate, the C-terminal tail of βB2-crystallin was fixed, whereas the N-terminal tail was free, which suggested that the C-terminal tail may be involved in inter-crystallin bonding during aggregation. Based on these results, we speculate that the truncation of both N- and C-terminals in βA3/A1- and βB2-crystallin might cause destabilization of β-crystallin aggregates in vivo, and would result in the formation of new aggregates.

An additional major finding was that the WS-HMW and WI protein fractions of the cataractous lenses contained only truncated αB-crystallin species in addition to βB1- and βA3/A1-crystallin fragments. The absence of αA-crystallin species could be due to either degradation or cross linking into >100 kDa species that could not enter the 15% polyacrylamide gel. The αB-crystallin species of cataractous lenses showed deamidation of Asn_{78} and Asn_{146} and oxidation of the Trp_{60} residue, which might cause their aggregation because these modifications are identified as causative factors for the formation of WI proteins [1,2].

As described in detail in the Introduction, several studies have shown that crystallin fragments per se could form aggregates and cross linked species. Because these fragments could be separated by SDS-PAGE, they must be held as aggregates through hydrophobic interactions. Indeed, our previous study has shown an age related increase in hydrophobic amino acid contents in species that form WS-HMW protein fractions [10]. Therefore, it appears that the increased hydrophobicity and the above described three post-translational modifications of crystallin fragments might be responsible for aggregation in WS-HMW and WI protein fractions.

In this report, the exact cleavage sites in αA-, αB-, βB1-, and βA3/A1-crystallin fragments that form aggregates were not determined. However, several previous studies have identified these cleavage sites [1,2,17-22,27,28]. Further, these sites have been identified in aging human lenses, but very few cataract specific cleavage sites have been identified.

One major question raised by this study is how the post-translational modifications (i.e., truncation, deamidation of Asn residues, oxidation of Trp residues, and aggregation) affect chaperone activity of α-crystallin. Both truncation and aggregation of αA and αB-crystallin have been shown to affect their chaperone activity [32]. Losses of greater chaperone activity and increased C-terminal truncation in diabetic cataracts compared to normal lenses have been reported [14]. Deamidation of the Asn residue has also been shown to reduce chaperone activity of human αB-crystallin [33].

Like other small heat shock proteins (hsp), α-crystallin also contains a highly conserved sequence of 80-100 residues (residues 62-143 in αA-crystallin and 66-147 in αB-crystallin) called the α-crystallin domain [34,35]. Based on similarities with the structure of other hsp, it is believed that the N-terminal region (residues 1-62 in αA-crystallin and 1-66 in αB-crystallin) of α-crystallin forms independently folded domains whereas the C-terminal (referred as the C-terminal extension; residues 143-173 in αA-crystallin and 147-175 in αB-crystallin) is flexible and unstructured [35]. The α-crystallin domain is engaged in subunit-subunit interactions because recombinant αB-crystallin containing only the α-domain region formed a dimer [36].

The N-terminal region of αA and αB-crystallin plays a role in oligomerization because human αA-crystallin, devoid of the first 20 amino acids, retained its high molecular weight oligomeric structure. However the removal of first 56 residues reduced the complex to a trimer or tetramer [37]. The C-terminal extensions of αA- and αB-crystallin are 34 and 32 residues, respectively. The function of the C-terminal region of α-crystallin is not well understood but it has been proposed to act as a solubilizer of crystallin because of a preponderance of charged amino acids. Deletion of the last 17 amino acids from human αB-crystallin caused precipitation with reduced chaperone activity [38], and a deletion of 25 residues from C-terminal in *Xenopus* Hsp30C reduced its solubility and showed impaired chaperone activity [39]. The C-terminal region seems to be needed to preserve the native structure of the molecule [39]. A recent study has shown that cleavage of eleven C-terminal residues including Arg_{163} in αA-crystallin showed substantial decease of oligomeric size and chaperone activity, whereas cleavage of 10 residues had either a small effect or no effect at all [40]. Together, these reports suggest that both N- and C-terminal regions are important for proper folding of α-crystallin, subunit interactions between αA- and αB-crystallin, and chaperone activity. Therefore, the age and cataract related truncation of αA- and αB-crystallin will have detrimental effects on properties of crystallins such as folding, oligomerization, and chaperone activity.

Presently, the mechanism of covalent multimer formation of crystallins in both normal and cataractous lenses is poorly understood. Past studies have provided evidence for several modifications that might be causing unfolding of native structures of crystallins and lead to aggregation of proteins. The question is whether the aggregation alone is sufficient to begin lens opacity or the formation of both aggregates and covalent multimers are needed for opacity to progress over a period of time. Therefore, it is important to characterize covalently cross linked species in the WI proteins from normal and cataractous lenses to determine the type of bonding and specific amino acids and crystallins involved in the process. This study showed selective insolubilization of the βA3/A1- and βB1-crystallin fragments in the WI protein fraction of cataractous lenses but not during normal aging. This finding warrants further investigation to determine whether selective modifications (i.e., truncation, oxidation of Trp residues and deamidation of Asn to Asp residues) in βA3/A1- and βB1-crystallin during cataractogenesis differ from those that occur during aging.

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