Biochemistry and Biophysics Reports 10 (2017) 94–131

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Post-translationally modified human lens crystallin fragments show aggregation *in vitro*

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ARTICLE INFO

Keywords:
Lens
Crystallins
Cataract
Two-dimensional gel electrophoresis
Post-translational modifications
Amyloid

ABSTRACT

Background: Crystallin fragments are known to aggregate and cross-link that lead to cataract development. This study has been focused on determination of post-translational modifications (PTMs) of human lens crystallin fragments, and their aggregation properties.

Methods: Four crystallin fragments-containing fractions (Fraction I [~3.5 kDa species], Fraction II [~3.5–7 kDa species], Fraction III [~7–10 kDa species] and Fraction IV [> 10–18 kDa species]), and water soluble high molecular weight (WS-HMW) protein fraction were isolated from water soluble (WS) protein fraction of human lenses of 50–70 year old-donors. The crystallin fragments of the Fractions I–IV were separated by two-dimensional (2D)-gel electrophoresis followed by analysis of their gel-spots by mass spectrometry. The Fractions I–IV were examined for their molecular mass, particle-diameters, amyloid fibril formation, and for their aggregation by themselves and with WS-HMW proteins.

Results: Crystallin fragments in Fractions I–IV were derived from α-, β- and γ-crystallins, and their 2D-gel separated spots contained multiple crystallins with PTMs such as oxidation, deamidation, methylation and acetylation. Crystallin fragments from all the four fractions exhibited self-aggregated complexes ranging in M_r from 5.5×10^5 to 1.0×10^8 Da, with diameters of 10–28 nm, and amyloid fibril-like formation, and aggregation with WS-HMW proteins.

Conclusion: The crystallin fragments exhibited several PTMs, and were capable of forming aggregated species by themselves and with WS-HMW proteins, suggesting their potential role in aggregation process during cataract development.

General significance: Crystallin fragments play a major role in human cataract development.

1. Introduction

Vertebrate lens contains long lived crystallins (classified α-, β- and γ-crystallins) as the major structural proteins. While α- and β-crystallins exist as oligomers, only the γ-crystallin exists as a monomer. Alpha-crystallin comprises of two related subunits (αA and αB), which are derived by gene duplication and divergence, and both have chaperone function. Beta- and γ-crystallins are also derived by gene duplication and are referred as a superfamily. They share common core protein structures, with two similar domains, each composed of two characteristic-modified Greek key motifs. Beta-crystallins are subdivided into acidic and basic subunits, and while the acidic β-crystallins have N-terminal extension whereas the basic β-crystallins have both N- and C-terminal extension besides the core structure. Although the lens crystallins have been shown to be long-lived with very little turnover [1], several reports have shown extensive truncations of lens α-, β-, and γ-crystallins in aging and cataractous human lenses [2–8]. Also, it is well established that specific regions of crystallins are more susceptible to *in vivo* truncations, *e.g.* the C-terminal region of both αA- and αB-crystallins showed a greater susceptibility to truncation *in vivo* than did the N-terminal region [9,10]. Our previous reports and those of others have shown that the crystallin fragments not only showed insolubilization but also formed aggregates *in vitro* [11,12], and are part of *in vivo*-existing covalent complexes of human lenses [13–15]. Additionally, the truncation or mutation in the C-terminal extension of α-crystallin has been shown to result in myopathies [16,17]. It has been shown that *in vivo* generated crystallin peptides also interact with crystallins to enhance their aggregation and cross-linking [18,19].

Certain crystallin fragments also affected α-crystallin chaperone activity, and suppressed aggregation of proteins [20]. Further, the mini-chaperones derived from α-crystallin chaperone region suppressed the aggregation of proteins, block*ed* amyloid fibril formation, stabilized mutant proteins, sequester metal ions, and exhibit anti-apoptotic properties [20]. Together, the above reports suggest a

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http://dx.doi.org/10.1016/j.bbrep.2017.01.011
Received 21 September 2016; Received in revised form 23 December 2016; Accepted 26 January 2017
Available online 20 February 2017

potential role of crystallin fragments in aggregation and cross-linking *in vivo, and* also as therapeutic chaperones.

Crystallin fragments increase with aging in human lenses in both water soluble-high molecular weight (WS-HMW) proteins (~5% of total protein in 16- to 19-year-old lenses, and 27% in 60- to 80-year-old lenses), and also in water insoluble (WI)-proteins (up to 20% of total protein) [4]. Selective aggregation of fragments of βA3- and βB1-crystallins in the WS-HMW proteins and WI- proteins of cataractous lenses relative to normal lenses has been reported [21,22]. Furthermore, the crystallin fragments of cataractous lenses also exhibited relatively increased post-translational modifications (PTMs) such as truncation, deamidation of Asn residues to Asp, and oxidation of Trp residues. On a comparative analyses of proteins of water insoluble-urea soluble and water insoluble-urea insoluble fractions from normal and cataractous lenses, only the cataractous lenses showed an absence of αA- (but not of αB-crystallin), and preferential insolubilization of β-crystallins and their fragments [15]. This finding suggested a greater role for αB-crystallin in the process of aggregation and insolubilization relative to αA-crystallin. On a similar comparison of HMW-proteins from normal aging and cataractous human lenses, multi-protein complexes were observed that were composed of intact α-, β-, and γ-crystallins and their fragments, beaded filament proteins (filensin and/or phakinin), and aldehyde dehydrogenase [15]. Further, the age-related increasing aggregation was also supported by the sizes of polydispersed spherical protein particles, *i.e.* their sizes in the WS-HMW proteins were relatively bigger in 60- to 70-year-old normal human lenses compared to younger 20-year-old normal lenses, and their sizes were further increased in the 60- to 70-year-cataractous lenses.

Truncation of specific regions of crystallins also affect their structural stability and solubility. For example, the homomer aggregates of αA-crystallin with C-terminal extension (residue no. 140-173)-deletion became water insoluble, whereas similar aggregates of αA with deletion of the N-terminal domain (residue no. 1-63) remained water soluble [11]. A similar altered solubility property was also observed in our report on deletion of either N-terminal domain or C-terminal extension of αB-crystallin [23,24]. The crystallin fragments complexes have also been observed. For example, the WI-proteins of 25- and 41-year-old normal human lenses contained two types of covalent multimers (M_r > 90 kDa) of crystallins [25]. The first type was composed of fragments of eight different crystallins (*i.e.*, αA, αB, βA3, βA4, βB1, βB2, γS, and γD), and the second type contained α-, β-, and γ-crystallins (possibly fragments) and two beaded filament proteins (phakinin and filensin). The study further showed that αA-crystallin fragments with three post-translational modifications (*i.e.*, oxidation of M and W residues, conversion of S residue to dehydroalanine, and formylation of H residue) that are known to lead to cross-linking of proteins. An *in vivo*-generated 9 kDa γD-crystallin polypeptide (residue no. 87-173) showed covalent cross-linking by themselves and also with individual α-, β-, and γ-crystallins [26]. In summary, the above studies have provided evidence of aggregation and cross-linking of crystallin fragments *by* themselves and with intact crystallins, their insolubilization and *in vivo* existence as complex of crystallin fragments.

Although the molecular mechanism of aggregation in crystallin fragments is presently unclear, it is suspected that the aggregation could be due to either abnormal conformation of the truncated species compared to their parent crystallins, and/or induced by their post-translational modifications. In this regard, the PTMs of intact crystallins have been extensively characterized in the literature; however, the PTMs of crystallin fragments remain largely uncharacterized. We hypothesize potential roles of truncated crystallin fragments with additional PTMs might cause cumulative effects by making these species to be more prone to aggregation. Additionally, the interactions of crystallin fragments with WS-HMW proteins (believed to a precursor of aggregated and cross-linked crystallins [27]), have not been investigated. The present study was undertaken with the aim to identify crystallin fragments of four different M_r ranges and their PTMs, and characterize their properties of *in vitro* self-aggregation and aggregation with WS-HMW proteins. The study shows that the majority of crystallin fragments with M_r between 3 and 18 kDa from 50 to 70-year old normal human lenses contained a variety of PTMs, and possibly exist as covalent multi-crystallin fragments complexes. These fragments show self-aggregation and also aggregation with WS-HMW proteins to form amyloid fibril-type aggregated products.

2. Methods

2.1. Materials

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involved human lenses. Normal human lenses with no apparent opacity were obtained from Dr. Robert Church (Emory University, Atlanta, GA). The retrieved lenses were stored at −20 °C until used. The pre-stained and unstained protein molecular weight markers were from GE Biosciences (Piscataway, NJ). All chemicals used in the 2D-gel electrophoresis were from either GE Biosciences or BioRad (Hercules, CA). Unless indicated otherwise, all other chemicals used in this study were purchased from Sigma (St. Louis, MO) or Fisher (Atlanta, GA) companies.

2.2. Isolation of crystallin fragments-containing fractions from water soluble (WS) protein fraction of human lenses

The WS-protein fraction from 20-pooled human lenses of 50–70-year-old donors was prepared as previously described [21,22]. All procedures were performed at 5 °C unless described otherwise. Briefly, lenses after their retrieval were immediately frozen at −20 °C in medium 199 without phenol red and were stored frozen −20 °C. The lenses were kept frozen until utilized. Lenses were thawed on ice, decapsulated, suspended (2 ml/lens) in buffer A (50 mM Tris-HCl, pH 7.9, containing 1 mM dithiothreitol [DTT], 1 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride), and homogenized using a tissue grinder (Polytron, model PT-1200C). The lens homogenate was centrifuged at 25,000×*g* for 15 min. The supernatant was recovered and the pellet was homogenized in buffer A and centrifuged twice as above. The supernatants recovered after each centrifugation were pooled and designated as the WS-protein fraction, and the pellet as the water insoluble (WI)-protein fraction. The WS-protein fraction was subjected to a preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE, 15% acrylamide gel) by the Laemmli's [28] method using the BioRad Prep Cell (Model 491, Hercules, CA). The eluted individual crystallin fragments-containing fractions were collected on their exit from gel using a fraction collector, and analyzed by SDS–PAGE. Next, based on the M_r, four individual fractions (designated Fractions I–IV) were collected with increasing molecular weights between 3 and 18 kDa (Fig. 1). The four crystallin fragments-containing fractions were: Fraction I [~3.5 kDa species], Fraction II [~3.5–7 kDa species], Fraction III [~7–10 kDa species] and Fraction IV [> 10–18 kDa species]. Each fraction was dialyzed against 50 mM phosphate buffer, pH 7.5 using 1000-Da molecular cut-off dialysis tubing with change of the buffer every 8 h for up to 48 h. Next, the fractions were dialyzed against deionized water with changes every 4 h during 24 h of dialysis, which was followed by their lyophilization. The amount of SDS present in each fraction was quantified using the stain-all solution as described [29]. Briefly, 5 µl of sample were dissolved in 1 ml buffer containing 90 µM stains-all, 2.5% (v/v) isopropanol, and 5% (v/v) formamide and their absorbance at 438 nm was compared to a standard curve prepared with known concentrations of SDS. If any fraction contained more than 0.05% SDS, it was passed through a Detergent-OUT™ SDS-300 spin micro column using the manufac-

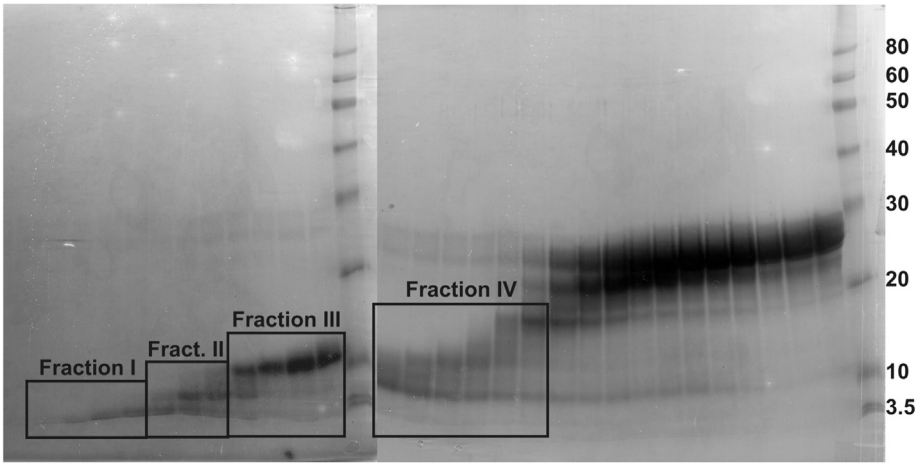

Fig. 1. SDS-PAGE analysis of crystallin fragments eluted in different fractions during a preparative SDS-PAGE separation of the WS-protein fraction from human lenses of 50–70 year-old donors. The WS-protein fraction was prepared and fractionated by the preparative SDS-PAGE method as described in Section 2. The following four fractions of crystallin fragments (identified in the Figure) with increasing M_r's were collected: Fraction I (~3.5 kDa species), Fraction II (~3.5–7 kDa species), Fraction III (~7–10 kDa species) and Fraction IV (> 10–18 kDa species).

turer's protocol (G-Biosciences, St. Louis, MO). Briefly, after the columns were equilibrated, 300 µl of sample were applied to each column, then incubated at room temperature for 5 min, and finally centrifuged at 1000×*g* for 30 s to collect the preparation that was passed through the resin. The amount of SDS was again quantified as described above to confirm the removal of SDS. Next, the samples were lyophilized and stored at −20 °C until used.

2.3. Isolation of water soluble-high molecular weight (WS-HMW)-protein fraction from human lenses

The WS-protein fraction was fractionated by HPLC using a size-exclusion TSK G-4000 PW_{XL} column (TosoHaas, Montgomeryville, PA, capable of fractionation of proteins with M_r ranging between 2×10^4 and 7×10^6 D) to collect fractions of the void volume peak, which were pooled and designated as the WS-HMW protein fraction. The HMW-protein peak was distinguished from the α-crystallin peak following SDS–PAGE analysis of the column fractions [28]. During HPLC, the column equilibration and sample elution were performed with 50 mM phosphate buffer, pH 7.5.

2.4. Amyloid fibril formation by crystallin fragments-containing fractions

Aliquots from Fractions I–IV (Fig. 1) were dissolved at 1–2 mg/ml in 10% (v/v) trifluoroethanol (TFE), adjusted to pH 2.0 with HCl, and incubated at 60 °C for 5 h. Next, the amyloid formation was determined by assay with either Congo Red (CR) or Thioflavin (ThT). For the Congo Red Assay [30], CR was dissolved in 5 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl, and was filtered through a 0.2 µm filter. The CR solution was then added to 100 µg/ml protein solutions to a final dye concentration of 0.5 µM. The absorption spectrum of each sample was recorded between 400 and 700 nm on a Shimadzu UV–vis scanning spectrophotometer (Model UV2101 PC), corrected for contributions from buffer and protein. The spectrum of CR alone was compared with that of CR solutions in the presence of a protein preparation. Amyloid fibril formation was determined by an increase in absorption and a red shift of the absorption band toward 540 nm. During thioflavin (ThT) assay [30], the spectrum of ThT alone was compared with that of protein solutions (100 µg/ml) containing ThT at a final dye concentration of 50 µM in 10 mM phosphate buffer, pH 7.5, containing 150 mM NaCl, pH 7.0, filtered through a 0.2 µm filter. The fluorescence spectra were recorded using a Shimadzu RF-5301PC spectrofluorometer using 1-cm path length cuvettes with an excitation at 442 nm and emission between 460 and 550 nm. An increase in the fluorescence emission intensity at 490 nm suggested the amyloid fibril formation.

2.5. Miscellaneous methods

2.5.1. Molecular mass determination by dynamic light scattering method

To determine the molecular mass of the aggregates of crystallin fragments in the Fractions I–IV, the aggregated peak observed during a size-exclusion chromatography were analyzed by multi-angle dynamic light scattering method as described previously [11]. A multi-angle laser light scattering instrument (Wyatt Technology, Santa Barbara, CA), coupled to a HPLC column (TSK G-4000 PW_{XL}) was used to determine the absolute molar mass of homomers of aggregated peak of fractions I–IV. Briefly, protein preparations in 50 mM sodium phosphate buffer, pH 7.5, were filtered through a 0.22 µm filter prior to their analysis. Results used 18 different angles, and the angles were normalized with the 90° detector.

2.5.2. 2D-gel electrophoresis and mass spectrometric analysis:

During 2D-gel electrophoresis, the first dimensional isoelectric focusing (IEF) was performed by active rehydration of a 17 cm immobilized pH gradient gel (IPG) strip with pH 5–8 range (BioRad, Hercules, CA), using a PROTEAN IEF System (BioRad, Hercules, CA). An aliquot from each of the Fractions I–IV containing crystallin fragments (Fig. 1) were processed to remove SDS as previously described, *i.e.*, dialysis against water at 5 °C for 48 h using 1000-Da molecular cut off dialysis tubing, and lyophilization. Next, each fraction reconstituted with IEF rehydration buffer (7 M urea, 2 M thiourea, 0.5% CHAPS, and 2% (v/v)) pharmalyte (pH 3–10) and subjected to IEF electrophoresis. During the electrophoresis, the desalting was carried out at 300 V for 4 h, focusing at 3500 V for 17.5 h, then 500 V for 1–3 h and finally 3500 V for 1.5 h. Prior to running the second dimension, focused strips were equilibrated for 15 min with an equilibration buffer (6 M urea, 50 mM Tris-HCl, pH 8.8, 2% SDS, 30% glycerol 0.01% bromophenol blue) containing 2% DTT and 15 min with equilibration buffer containing 2.5% iodoacetamide. In the second dimension, proteins were separated by SDS-PAGE using 23×20 cm-15% polyacrylamide gels. IPG strips were immobilized at the top of second dimensional slab gels using 1% low-melt Agarose in 3X running buffer containing bromophenol blue (24 mM Tris-HCl, 192 mM glycine, 1% SDS, 0.01% bromophenol blue), which was added as a tracking dye. The second dimensional gels were run for 1 h at 1 W/

Table 1
Crystallin fragments and their PTMs in Fraction I.

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
1	αA: ([13-21]/173) [17.3] αB: ([83-90]/175) [5.2]		γD: ([154-163]/174) [5.7] γS: ([8-155]/178) [10.7]	
3	αA: ([55-65]/173) [6.4]	βA3: ([33-44]/215) [6.1] βA4: ([14-25]/196) [6.1] {Oxidation (M14)} βB2: ([161-168]/205) [3.9]	γD: ([154-163]/174) [5.7] {Deamidation (N161)} γS: ([149-155]/178) [5.8]	
4	αA: ([13-21]/173) [17.3]	βA3: ([33-44]/215) [6.1] βB2: ([161-168]/205) [3.9]	γD: ([143-163]/174) [11.5] γS: ([8-155]/178) [10.7]	
5	αA: ([146-157]/173) [19.1] {Oxidation (F17, Y18)} ([108-116]/175) [23.4] {Oxidation (M68, R69)}	βA3: ([33-137]/215) [26.3] {Deamidation (Q38, N103); Oxidation (M46, F48, R45, M126)} βA4: ([14-192]/196) [13.8] {Oxidation (M14)} βB1: ([51-214]/252) [19.4] {Deamidation (N58)} βB2: ([69-198]/205) [23.4] {Dioxidation (W85)}	γD: ([4-163]/174) [9.8]; γS: ([149-155]/178) [5.8]	
6	αA: ([146-157]/173) [17.9] αB: ([57-69]/175) [18.7] {Oxidation(D62, M68, R69)}	βA3: ([33-211]/215) [14.1] βA4: ([14-25]/196) [6.1] {Oxidation (M14)} βB1: [51-214]/252) [27.0] {Oxidation (C80, M137); Methylation (S81)} βB2: ([82-160]/205) [11.2];	γB: ([4-164]/175) [9.7] {Dioxidation (F155, K164) γD: ([4-163]/174) [16.1] {Oxidation (Y144, M147, P148, D150, Y151, Y154, D156, W157, N161); Dioxidation (M147); Trp > Kynurenin (W158)} γS: ([8-174]/178) [29.8] {Trp > Hydroxykynurenin (W163); Oxidation (W73, M74); Dioxidation (M74)}	
7	ND (Not determine)			
8	ND			
9			γS: ([8-174]/178) [15.7]	
9B	αB: ([164-174]/175) [6.3]		γS: ([149-155]/178) [5.8]	
10	αA: ([79-88]/173) [17.9] {Oxidation (F17)}; αB: ([83-90]/175) [20.6] {Oxidation (D62)}	βA3: ([33-211]/215) [14.1]; βB1: ([51-86]/252) [13.9] {Methylation (C80, S81); Oxidation(C80)} βB2: ([146-168]/205) [11.2]	γC: ([4-10]/174) [4.0] {Oxidation (F6)} γD: ([4-152]/174) [9.8] {Oxidation (D150)}; γS: ([8-52]/178) [10.1] {Dimethylation (K14)}	Phakinin: ([212-220]/415) [2.2]
11	αA: ([89-99]/173) [25.4] {Dioxidation (F17)} αB: ([164-174]/175) [17.1]	βA3: ([33-44]/215) [6.1] βB1: ([51-214]/252) [19.4] βB2: ([90-168]/205) [9.8]	γC: ([4-10]/174) [4.0] γD: ([4-163]/174) [9.8] γS: ([8-174]/178) [12.9] {Dioxidation (K159) Dimethylation (K14)}	Phakinin: ([77-103]/415) [6.5] Filensin: ([482-491]/665) [1.9]
12	αA: ([12-157]/173) [25.4] {Oxidation (F17, Y18)} αB: ([12-174]/175) [23.4]	βA3: ([197-211]/215) [7.6] βB2: ([69-188]/205) [22.9] βB1: ([51-202]/252) [15.1] {Oxidation (N82)}	γB: ([4-164]/175) [9.7] γD: ([4-163]/174) [15.5] {Dioxidation (W157); Oxidation (P148, D150)} γS: ([8-174]/178) [15.7] {Dimethylation (K14); Trp- > Oxolactone (W163)}	Phakinin: ([212-220]/415) [2.2]
13	αA: ([1-157]/173) [37.6] {Deamidation (Q6); Methylation (L57)} αB: ([12-174]/175) [49.1] {Deamidation (Q108)}	βA3: ([33-211]/215) [28.3] {Deamidation (N40, Q42); Oxidation (Y36, D37, C170); Methylation (C170)} βA4: ([14-192]/196) [33.7] {Deamidation (Q23, Q189); Oxidation(M14); Methylation (C166)} βB1: ([51-214]/252) [33.7] {Deamidation (N58, N68, Q70, N158, Q205); Oxidation (F64, N68, M113, F114)} βB2: ([69-188]/205) [28.8] {Deamidation (Q147, Q155, Q185)} βB3: ([26-83]/211) [32.7]	γB: ([4-164]/175) [9.7] γC: ([4-122]/174) [8.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150, Y154, D156, W157, N161); Deamidation (N161, Q143)} γS: ([1-116]/178) [25.0] {Dioxidation (K101); Oxidation (K101)} γS: ([8-174]/178) [15.7] {Dioxidation (K159); Oxidation (K159, D162); Dimethylation (K14)}	Phakinin: ([77-239]/415) [11.6] {Oxidation (M221, D222); Deamidation (Q86)} Filensin: ([482-491]/665) [1.9]

Table 2
Crystallin fragments and their PTMs in Fraction II.

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
1	αA: ([12-65]/173) [12.1] {Methylation (I61, S62); Oxidation (Y18, F17)} αB: ([83-90]/175) [5.2]	βB2: ([69-76]/205) [3.9]	γS: ([8-155]/178) [16.3] {Dioxidation (W137, F139)}; ([74-97]/120) [18.3] {Oxidation (M74)}	
1B	αA: ([13-99]/173) [11.6] αB: ([83-90]/175) [5.2]	βB2: ([161-168]/205) [3.9]	γS: ([132-146]/178) [12.5] {Dioxidation (W137, F139)} ([74-88]/120) [12.5] {Oxidation (M74, R84)}	
2	αA: ([12-99]/173) [23.1] {Oxidation (F17, Y18); Dimethylation (R21); Dioxidation (P16, F17)} αB: ([12-90]/175) [12.3]; ([12-90]/175) [10.9]	βB1: ([203-214]/252) [4.8]	γD: ([4-10]/174) [4.0] γS: ([132-146]/178) [12.5] {Dioxidation (W137, F139)}; ([74-88]/120) [12.5] {Oxidation (M74)}	
3	αA: ([13-99]/173) [28.3] αB: ([12-116]/175) [22.3]	βA3 ([197-211]/215) [7.6] {Deamidation(Q203, Q206)}	γS: ([8-174]/178) [30.3] {Dioxidation (W137, F139)}	
4	αA: ([79-99]/173) [15.4] αB: ([12-90]/175) [12.3]; ([12-90]/175) [10.9]		γD: ([118-152]/174) [19.0] {Oxidation (M147, D150); Deamidation (N119, N125)}; ([132-174]/178) [25.8] {Dioxidation (W137)}; ([74-116]/174) [25.8] {Oxidation (M74)}	
5	αB:([83-90]/175) [5.2]	βA4: ([178-192]/196) [7.7]	γD: ([118-140]/174) [13.2] {Dioxidation (F118, W131); Deamidation (N125); Oxidation (H122, W131, N119, N125, W131, Y144 W157, R163)} γS: ([132-174]/178) [25.8] } ([74-116]/120) [25.8] {Dioxidation (K101, M108); Oxidation (M774, K101, F104)}	
5B	αA: ([13-21]/173) [5.2] αB: ([12-22]/175) [7.1]		γD: ([118-140]/174) [13.2] γS: ([132-174]/178) [25.8] {Dioxidation (W137, F139)}; ([74-116]/120) [25.8] {Oxidation (P75)}	
5C	αA: ([13-21]/173) [5.2] αB: ([12-90]/175) [12.3]; ([12-149]/175) [25.7]	βA4: ([159-192]/196) [15.8] {Dioxidation (W179); Deamidation (Q187); Oxidation (W179, H182)}	γS: ([132-155]/178) [18.3] {Dioxidation (W137, F139)} ([74-97]/178) [18.3] {Oxidation (M74)}	
6	αA: ([13-157]/173) [35.8] {Oxidation (F17, Y18, D58); Deamidation (Q90); Methylation (L57)} αB: ([12-116]/175) [22.3]	βA3: ([33-109]/215) [20.2] {Oxidation (M46, F48); Deamidation (N40, Q42, N103); Trp > Kynurenin (W99)} βA4: ([14-90]/196) [15.8] {Oxidation (M14) βB1: ([51-214]/252) [22.6] {Oxidation (C80); Methylation (C80, R86, S81); Methylation + Deamidation (N82)} βB2: ([69-168]/205) [17.6] {Deamidation (Q71)}	γC: ([4-59]/174) [29.3] {Methylation + Deamidation (N25); Oxidation (R10, F12, C23, W43); Dioxidation (M44)} γ-D ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (Q155, N161); Methylation (H16, H23)} γS: ([8-174]/178) [32.0] {Acetylation (K159); Deamidation (N15, Q17, N144, Q149, Q171); Oxidation (M74, Y150, P160, D162, W163); Trp > Kynurenin (W163); Dioxidation (M74, W73, Y150, K159, W163)}	
7	αA: ([13-157]/173) [43.4] {Deamidation(Q90); Oxidation (H79, F80); Acetylation (K70); Dioxidation(P16)} αB: ([75-149]/175) [24.0] {Deamidation (N146)}	βA3: ([33-211]/215) [39.9] {Deamidation (N40, Q42, N103); Oxidation (H78, F81); Methylation (C170, S80, C82, H78); Methylation + Deamidation (Q84); Trp- > Kynurenin (W99)} βA4: ([14-118]/196) [34.2] {Methylation (R26, H27, S35, C33); Deamidation (Q63, Q65, N114, Q155); Oxidation (R26, H27, P34, C33)} βB1: ([51-214]/252) [36.5] {Deamidation (N58, N68, Q70, N108); Methylation (C80, S81, R86); Methylation + Deamidation(N82); Oxidation (C80)} βB2: ([69-89]/205) [7.8]	γC: ([4-77]/174) [14.4] {Deamidation (Q68); Oxidation (M70, D74)} γD: ([118-163]/174) [24.7] {Oxidation (M147, P148, D150); Deamidation (N119, N125, N138, N161, Q155); Trp > Kynurenin (W137)} γS: ([8-174]/178) [60.1] {Deamidation (Q121, N144, Q149, Q171); Acetylation (K159); Oxidation (F55, Y58, M59, Y60, W73, M74, Y109, D114, C115, P116, M119, M124, R125, W137, F139, Y140, Y150, K159, P160, D162, W163, F173, R174); Methylation + Deamidation (Q121); Dioxidation (K159, W137, F139, Y150, C115, M74, P116, W163); Trp > Kynurenin (W163); Trp > Oxolactone (W73); Dimethylation (K14); Methylation (S117, C115)} ([15-116]/178) [66.7] {Deamidation (Q64, Q93, Q121); Acetylation (K101); Methylation + Deamidation (Q63); Dioxidation (C37, Y58, M74, Y58, K101); Oxidation (R20, C37,Y51, D56, M59, K101, F104,Y109), Trp > Kynurenin (W105); Trp > Hydroxykynurenin (W163); Trp > Oxolactone (W73)}	

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Table 2 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
7A	αA: ([12-157]/173) [46.2] {Deamidation (Q90, N101, Q104); Acetylation (K70); Methylation (L85)} αB: ([57-149]/175) [32.0]	βA3: ([33-109]/215) [31.3] {Oxidation (M46, F48, C52, Y76, C82); Methylation (S51, C52, R58); Deamidation (Q38, N40, Q42, N103); Methylation + Deamidation (N54, Q84)} βA4: ([14-118]/196) [43.9] {Deamidation (Q23, Q112, N114); Methylation (H27, C33, S35,); Oxidation (M14, R26, H27, F29, C33, P34, W54, F57, H59); Dioxidation (C33, P34, W54,); Trp > Kynurenin (W17, W54, W77); Trp- > Hydroxykynurenin (W80)} βB1: ([51-214]/252) [42.1] {Deamidation (N58, N68, Q70, N125, Q197, Q205); Methylation (C80, S81, R86); Methylation + Deamidation (N82); Dioxidation (C80); Oxidation (C80)} βB2: ([19-168]/205) [46.3] {Methylation (C67, K68); Oxidation (C38, C67, N66, P37); Deamidation (Q71, N114, N116, Q147, Q163); Methylation + Deamidation (N66)} βB3: ([56-72]/211) [15.0]	γA: ([4-15]/174) [6.9] {Methylation (R10, Q13)} γC: ([4-77]/174) [27.0] {Deamidation (Q52, Q67, Q68); Oxidation (C42, W43, M44, Y46, R48, M70); Dioxidation (C42, W43); Methylation (C42, R48); Trp- > Oxolactone (W43); Trp- > Kynurenin (W43, W69)} γD: ([143-163]/174) [12.1] {Deamidation (Q143, Q155); Oxidation (M147, D150, Y151)} γS: ([8-174]/178) [60.7] {Dimethylation (K14, K159); Oxidation (M59, M74, F104, M108, Y109, D114, C115, P116, F136); Dioxidation ((C115, P116, P160, W163); Acetylation (K159); Deamidation (Q64, N77 Q171)} ([1-116]/178) [65.8] {Dimethylation (K101); Oxidation (M1, M59, Y50, D56, C37, Y58, Y150); Dioxidation (C37, Y58, F104); Acetylation(K101); Deamidation (Q13, N17, Q64)}	Filensin: ([482-491]/526) [1.9]; Phakinin: ([174-239]/415) [13.7] {Oxidation (M175, M221, D222); Deamidation (Q196, N207)}
8	αA: ([71-112]/173) [30.9] {Deamidation (Q90, N101, Q104)} αB: ([83-149]/175) [25.7] {Deamidation (N146)}	βA3: ([33-109]/215) [13.1] {Deamidation (N103)} βA4: ([26-118]/196) [16.3] {Deamidation (N114)} βB1: ([51-214]/252) [32.9] {Methylation + Deamidation (N82); Methylation (C80, S81,); Deamidation (N68, Q70, Q10, N108, N125); Oxidation (C80); Dioxidation (C80); Trp > Kynurenin (W101)} βB2: ([49-76]/205) [13.7] {Methylation (C67, K68); Oxidation (N66)}	γD: ([118-163]/174) [25.3] {Trp > Kynurenin (W131); Oxidation (M147, D150, P148); Deamidation (Y60, N119, N125, N138, Q155, N161, N146)}, γS: ([8-174]/178) [66.9] Oxidation (C37, F55, M59, M74, Y109, D114, C115, P116, F136, W137, F139, Y140, Y150, K159, P160, D162, W163, P168; Acetylation (K154, K159); Trp > Oxolactone (W163); Methylation (K159, I161,), Dioxidation (P116, F139, K158, W163)} ([15-116]/178 Deamidation (Q13, Q17, K41, Q121, N144, Q149, Q171); Oxidation (C37, F55, M59, Y60, M74, F104, Y109, D114, C115, P116, R125, F136, W137, F139, Y140, P143, Y150, K159, P160, D162, W163, P168); Acetylation (K154, K159); Trp > Oxolactone (W163); Methylation (K159, I161); Dioxidation (K159, F139, W137, Y150, P116, W163); Trp > Hydroxykynurenin (W163); Methylation + Deamidation (Q121); Trp- > Kynurenin (W137, W163)}	
9	αA: ([13-157]/173) [38.2] {Deamidation(N101, Q104)} αB: ([57-116]/175) [28.0] {Oxidation (D62, M68, R69); Deamidation (N78; Q108)}	βA3: ([33-109]/215) [34.8] {Oxidation (Y36, D37, R45, M46, F48, C52, Y76, H78, C82, W96, D97, N103, Y105); Deamidation (Q84, N103, Q175); Methylation + Deamidation (Q84, N103, Q175); Methylation (C52, H78, S80, C82, R90, N103, H106); Dioxidation (W99); Trp- > Kynurenin (W73); Trp- > Oxolactone (W99)} βA4: ([27-192]/196) [35.2] {Trp- > Kynurenin (W54); Deamidation (Q63, Q65, N114)} βB1: ([51-230]/252) [27.4] {Deamidation (N58, Q70, N158, Q205, Q223, Q227); Oxidation (M225)} βB2: ([69-101]/205) [9.8]	γC: ([4-77]/174) [39.7] {Oxidation (D9, R10, C42, W43, M44, Y46, R48, M70, D74); Dioxidation (C42, W43, M44); Methylation (S40, C42, R48); Trp > Oxolactone (W43); Deamidation (N50, Q68, Q143); Trp > Kynurenin (W43); Methylation + Deamidation (Q68)} γD: ([16-163]/174) [34.5] {Oxidation (C19, M147, P148, D150, Y154, D156, W157); Methylation (C19, H23); Deamidation (N25, N119, N125, N161); Trp- > Kynurenin (W137); Dioxidation (W157); Methylation + Deamidation (Q155)} γS: ([8-174]/178) [49.4] {Acetylation (K159); Deamidation (N15, N77, Q149, Q171), Oxidation (F55, Y58, M59, W73, M74, Y150, P160, D162,W163); Dioxidation (W73, M74, F136, W137); Dimethylation (K14); Trp > Oxolactone (W163)}	
10	αA:([13-112]/173) [28.9] {Deamidation (Q104)} αB: ([57-90]/175) [13.5] {Oxidation (M68, R69)}	βA3: ([68-90]/215) [11.6] {Oxidation (Y76, H78, C82); Methylation (H78, Q84); Methylation + Deamidation (Q84)}		

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Table 2 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
		βA4: ([49-192]/196) [25.5] {Deamidation (Q65, N114)}; βB1: ([51-118]/252) [24.6] {Trp- > Kynurenin (W101); Methylation (C80, S81, R86); Methylation + Deamidation (N82); Deamidation (N68, Q70, Q106); Oxidation (C80)} βB2: ([49-101]/205) [23.4] {Oxidation (W59, N66, C67); Methylation + Deamidation(N66)}		
11	αA: ([55-157]/173) [35.3]	βB1: ([51-132]/252) [28.2] {Dioxidation (C80); Methylation (C80, S81, R86); Methylation + Deamidation (N82); Deamidation (N58, N68, Q70, N82, N108, N125); Trp- > Kynurenin (W101); Oxidation (C80, M113,)}		
12	αA: ([13-157]/173) [35.8] {Deamidation (Q90)} αB: ([57-116]/175) [21.7] {Oxidation (D62, M68, R69)}	βA3: ([96-211]/215) [28.3] {Deamidation (N103, N133, Q180, Q203, Q208)} βA4: ([49-118]/196) [17.9] {Deamidation (Q65, N114)}; βB1: ([51-214]/252) [40.1] {Deamidation (N58, N68, Q106, N125); Oxidation (C80, M113); Methylation (C80, S81, R86); Methylation + Deamidation (N82)} βB2: ([49-120]/205) [23.4]	γC: ([4-122]/174) [8.0]; γD: ([118-163]/174) [24.7] {Oxidation (M147, P148, D150, Y154, D156, W157); Methylation + Deamidation (Q155)} γS: ([8-174]/178) [62.9] {Deamidation (Q17, Q64, Q107, Q121, Q163, Q171); Acetylation (K159); Oxidation (M74, Y109, D114, P116, F136, W137, F139, Y150, K159, P160, D162, W163, C1105, R3110, M108, C115, R125, Y140, M4102, M119, M124); Trp- > Kynurenin (W163); Dioxidation (C115, M119, M124, W137, F139, Y150, K159, W163); Methylation + Deamidation (Q121, Q150); Trp- > Hydroxykynurenin (W163); Dimethylation (K14)} ([15-116]/178) [57.5] {Deamidation (Q13, Q64); Acetylation(K101); Trp- > Kynurenin (W137); Oxidation (M1, C37, D56, Y58, M59, Y60, M74,Y82, K101, D114, R174); Methylation + Deamidation (Q63, Q93)}	Phakinin ([344-353]/415) [2.4]
13	αA: ([13-157]/173) [30.6] αB: ([12-82]/175) [20.6]; ([12-82]/175) [18.3]	βA3: ([96-211]/215) [20.7] {Deamidation (N103); Oxidation (M126)} βA4: ([107-192]/196) [13.8] {Deamidation (N114)} βB1: ([51-252]/252) [36.1] {Oxidation (M137)} βB2: ([49-168]/205) [21.5] {Methylation (C67, K68); Methylation + Deamidation (N66); Oxidation (N66, C67)}	γC ([4-152]/174) [16.7] {Deamidation (Q143); Oxidation (R10)} γD: ([143-163]/174) [12.1] {Oxidation (M147, P148, D150, Y154, D156, W157); Dioxidation (W157); Methylation + Deamidation(Q155)}; γS: ([8-174]/178) [62.9] {Deamidation (N15, Q107); Dioxidation (F55, M59, W73, M74, W137, F139, K159, W163, M119); Oxidation (F10, Y11, F55, Y58, M59, M74, M108, Y150, K159, D162) Acetylation (K159)}; ([15-116]/120) [57.5] { Dioxidation (M1, K101, M61); Oxidation (M16, P75, Y92, K101, D104, W15); Acetylation (K101)}	
14	αA: ([13-157]/173) [31.8] {Deamidation (Q104)} αB: ([12-174]/175) [24.6] {Oxidation (D62, M68, R69)}	βA3: ([68-211]/215) [26.3] {Oxidation (Y76, H78, C82); Deamidation (N103); Methylation + Deamidation (Q84)} βA4: ([178-192]/196) [7.7] βB1: ([61-214]/252) [13.5] {Deamidation (Q70)}	γB: ([4-164]/175) [22.9] {Oxidation (D9, R10, M70, D74)}; γD: ([118-169]/174) [28.7] {Oxidation (M147, P148, D150, Y154, D156, W157, N161); Dioxidation (W157); Deamidation (N119, N138, N161, Q155, Q143); Methylation + Deamidation (Q155); Methylation (Q155); Trp > Kynurenin (W137)} γS: ([8-174]/178) [64.6] {Dimethylation (R174); Deamidation (N15; Q17, N54, Q71, N77, Q171); Acetylation (K159); Dioxidation (M59, W73, M74, C115, P116, Y150); Oxidation (Y21, D26, F55, Y58, M59, W73, M74, Y109, D114, P116, K159, P160, W163); Methylation (S172); Trp > Kynurenin (W163)}	
15	αA: ([89-99]/173) [8.1] {Deamidation (Q90)} αB: ([12-90]/175) [25.8]; ([12-90]/175) [22.9]	βA3: ([126-211]/215) [13.6] βB1: ([61-214]/252) [9.5] βB2: ([82-101]/205) [9.8]	γB: ([4-164]/175) [12.6] {Oxidation (D9, R10)} γD: ([118-163]/174) [25.3] {Deamidation (N119, N138, N161); Oxidation (M147)}	

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Table 2 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
			γS: ([8-174]/178) [64.0] Methylation + Deamidation (Q121); Oxidation (M59, M74, M108, Y109, D114, C115, P116, M119, F122, F136, W137, Y150, K159, P160, D162, W163, M4102, F139, Y58); Deamidation (N15, Q17, Q121, Q107, N144, Q171); Trp- > Oxolactone (W163); Methylation (C115, S117, H123, K159, I161, S167, Q171); Dioxidation ((M74, C115 P116, Y150, K159, W163); ([15-116]/178) [59.2] {Acetylation (K101); Methylation + Deamidation (Q63); Oxidation (M1, D56, Y58, M59, Y82, K101); Deamidation (Q64); Methylation (C37, K101,); Dioxidation (M1, C37, K101, Y58, Y82)}	
16	αA: ([79-157]/173) [24.3] {Deamidation (Q90)} αB: ([57-103]/155) [20.6] {Oxidation (M68, R69)}	βA3: ([33-211]/215) [19.7] {Oxidation (M126, F129)} βA4: ([107-118]/196) [6.1] {Deamidation (N114)} βB1: ([61-214]/252) [12.7] {Oxidation (M113)}	γB: ([4-164]/175) [9.7] γD: ([4-163]/174) [28.7] {Dioxidation (W137, W157); Oxidation (Y7, M119, H122, N125, W137, M147, P148, D150, W157)} γS: ([8-174]/178) [35.4] {Oxidation (M74); Dimethylation (K14)}; ([15-116]/120) [37.5] {Oxidation (M1, M74)}	
17	αA: ([79-157]/173) [24.3]; αB: ([12-90]/175) [25.8] {Oxidation (D62, M68, R69)} ([12-90]/175) [22.9] {Oxidation (D62, M68, R69)}	βA3: ([33-137]/215) [19.2] {Deamidation (N103)} βA4: ([107-118]/196) [6.1] βB1: ([61-230]/252) [22.2] {Oxidation (M226, R230); Deamidation (Q197)}	γC: ([4-10]/174) [4.0] γD: ([4-169]/174) [51.7] {Dioxidation (F118, M147, P148, Y154, W157); Oxidation (Y7, M70, N119, H122, N125, W131, M147, P148, D150, Y154, D156, W157); Deamidation (Q143, Q155, N161); Trp > Oxolactone (W157); Methylation + Deamidation (Q101, Q113, Q155)} γS: ([8-174]/178) [23.6] {Deamidation (N15, Q17, Q171); Dimethylation (K14); Oxidation (M74)}	
18	αA: ([55-99]/173) [12.7] αB: ([12-116]/175) [34.3] {Oxidation (D62, M68, R69); Deamidation (Q108)}	βA3: ([33-211]/215) [27.3] {Oxidation (M126); Deamidation (N103, Q38)} βA4: ([107-119]/196) [6.6] βB2: ([161-168]/205) [3.9]	γC: ([4-10]/174) [4.0] γD: ([4-169]/174) [53.4] {Deamidation (Q155, N161); Oxidation (Y7, Y17, M70, N119, H122, N125, W131, M147, P148, D150, Y151, Y154, D156, W157, N161); Dioxidation (W131, M147, P148, W157); Methylation + Deamidation (Q155); Methylation (H16, C19, Q155); Trp > Oxolactone (W157)} γS: ([8-174]/178) [29.8] {Oxidation (M74); Dimethylation (K14)}	
19	αA: ([79-99]/173) [15.4] {Deamidation (Q90)} αB: ([57-103]/175) [25.8] {Oxidation (M68, R69)}	βA3: ([33-211]/215) [13.6] {Deamidation (Q42, Q206)} βA4: ([107-192]/196) [13.8] βB1: ([51-72]/252) [8.7]	γB: ([4-164]/175) [9.7] γD: ([4-169]/174) [37.4] {Dioxidation (F118, W131, M147, P148, W157); Dimethylation (N161, R163); Oxidation (N119, H122, N125, W131, Y144, M147, P148, D150, Y151, R152, Y154, D156, W157); Deamidation (N125, Q143 Q155, N161); Methylation + Deamidation (Q155, N161); Trp > Oxolactone (W157)} γS: ([8-174]/178) [37.1] {Oxidation (F55, Y58, M59, M74); Deamidation (Q171); Dimethylation (K14); Dioxidation (M74)}	
20A	αB: ([57-90]/175) [18.7] {Oxidation (D62, M68, R69)}	βA3:([68-211]/215) [26.3] {Oxidation(C82)} βA4: ([107-192]/196) [13.8] βB1: ([61-252]/252) [34.1] {Methylation (C80, S81, R86); Methylation + Deamidation (N82); Deamidation (Q106, N108); Oxidation (C80)} βB2: ([82-89]/205) [3.9]	γB: ([4-164]/175) [20.0] {Deamidation (Q68)} γD: ([4-169]/174) [55.7] {Oxidation (Y7, D39, C42, W43, F118, H122, W131, Y134, Y144, M147, P148, D150, Y151, Y154, D156, W157); Deamidation (Q67, Q68, N119, N125, N138); Methylation (C42); Dioxidation (C42, M44, F118, W131, M147, P148, Y154, W157); Trp > Oxolactone (W43, W157); Dimethylation (N161, R163); Methylation + Deamidation (Q155)}	

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Table 2 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
			γS: ([8-174]/178) [43.8] {Deamidation (Q171); Oxidation (F10, Y11, M74, D114, F122, Y150, K159, P160, D162, W163); Methylation + Deamidation (Q107); Dioxidation (K159, M74, W73); Trp- > Oxolactone (W163); Methylation (K159, I161); Dimethylation (K14); Acetylation (K159)} ([15-116]/178) [59.2] Oxidation (D56, Y94, K101); Methylation (K101); Acetylation (K101)}	
20C	αA: ([55-65]/173) [6.4] αB: ([12-90]/155) [17.4]; ([12-90]/175) [15.4]	βA3: ([33-44]/215) [6.1] βB2: ([109-120]/205) [5.9]	γD: ([4-77]/174) [24.0] {Oxidation (M70, D74, R77); Deamidation (N25)} ([4-163]/174) [35.6] {Oxidation (Y7, H23, M70, D74, R77, M147, P148, D150); Deamidation (N25)} γS: ([149-155]/178) [5.8]	
21	αA: ([12-157]/173) [43.9] {Oxidation (H79, F80, P82); Dioxidation (P16); Acetylation (K70); Deamidation (Q90, Q104)} αB: ([83-90]/175) [5.2]	βA4: ([14-71]/196) [17.9] {Oxidation (M14)} βB1: ([51-143]/252) [24.2] {Deamidation (N58, Q70); Methylation (S77, C80, S81, R86); Methylation + Deamidation (N82); Oxidation (M137, C80)} βB2: ([69-89]/205) [7.8]	γC: ([4-10]/174) [4.0] γD: ([154-163]/174) [5.7] γS: ([8-174]/178) [33.1] {Deamidation (Q93, Q149, Q171); Oxidation (M74, Y150); Dimethylation (K14); Dioxidation (Y150)}	
22	αA: ([13-157]/173) [42.8] {Deamidation (N101, Q104)} αB: ([83-90]/175) [5.2]	βA3: ([96-109]/215) [7.1] {Deamidation (N103)} βB1: ([51-230]/252) [35.3] {Deamidation (Q70, Q225); Methylation (C80, S81, R86); Oxidation (C80, M113, M137); Methylation + Deamidation(N82)} βB2: ([49-76]/205) [13.7] {Methylation (C67, K68); Deamidation (Q71)}	γC:([4-10]/174) [4.0] γD: ([143-163]/174) [11.5] {Deamidation (N161)} γS: ([15-116]/120) [59.2] {Acetylation (K101); Deamidation (Q93); Oxidation (M1, D56, P58, P75, F78, K101, D104); Dioxidation (K101, Y82); Methylation (K101)} ([8-174]/178) [50.0] {Acetylation (K159); Deamidation (Q171, Q149); Oxidation (W73, M74, Y109, D114, C115, P116, R125, F136, Y150, K159, D162, W163); Trp > Oxolactone (W163); Dioxidation (M74, C115, W137, F139, Y150, K159); Dimethylation (R174); Methylation (K159)}	
23	αA: ([13-99]/173) [17.9] {Deamidation (Q90)}	βB1: ([51-214]/252) [8.7] βB2: ([49-89]/205) [20.0] {Methylation (C67, K68); Trp > Oxolactone (W82); Methylation + Deamidation (N66); Oxidation (C67)}	γD: ([154-163]/174) [5.7] γS: ([8-174]/178) [34.3] {Dioxidation (W137, F139, K159); Oxidation (K159, D162)}	
24	αA: ([13-99]/173) [17.9] αB: ([83-90]/175) [5.2]	βA3: ([33-44]/215) [6.1] βB1: ([51-214]/252) [25.8] {Deamidation (N82)} βB2: ([69-89]/205) [7.8]	γB: ([4-164]/175) [9.7] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [37.1] {Deamidation (N15, Q171); Dimethylation (K14, R174); Oxidation (M74, K159, P160, D162, W163, Y11, Y150, P168); Dioxidation (K159, W163); Acetylation (K159); Methylation + Deamidation (Q149)}	
25	αA: ([13-157]/173) [30.6] αB: ([83-90]/175) [5.2]	βA3: ([33-211]/215) [26.8] {Oxidation (M126); Deamidation (N103)} βB1: ([51-214]/252) [22.6] βB2: ([90-168]/205) [15.6]	γC: ([4-122]/174) [8.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [44.4] {Deamidation (Q17, Q93, Q149, Q171); Trp > Oxolactone (W163); Oxidation (M74, Y109, P116, K159, P160, D162, W163); Dioxidation (K159, W163); Acetylation (K159); Dimethylation (R174); Methylation (K159, S167); Trp > Hydroxykynurenin (W163); Methylation + Deamidation (Q171)}	
26	αA: ([13-157]/173) [24.9] αB: ([12-22]/175) [7.1]; ([12-22]/175) [6.3]	βA3: ([126-211]/215) [13.6] {Oxidation (M126); Deamidation (Q208)}	γB: ([4-164]/175) [12.6] {Dioxidation (F155, K164); Oxidation (R10)}	

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Table 2 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
		βB1: ([51-214]/252) [25.4] {Oxidation (M137)} βB2: ([69-101]/205) [13.7] {Deamidation (Q70)}	γD: ([154-163]/174) [5.7] {Deamidation (Q155, N161)} γS: ([8-174]/178) [46.1] {Deamidation (N15, Q17, Q93, Q149, Q171); Oxidation (W73, M74, M108, Y150, , K159, P160, W163, D162); Dimethylation (K14, R174); Dioxidation (M74, M119, Y150, K159); Methylation (S167, S172); Trp > Kynurenin (W163); Methylation + Deamidation (Q149); Phosphorylation (Y157)}	
28	αA: ([146-157]/173) [17.4] αB: ([75-92]/175) [11.6]	βA3: ([96-137]/215) [13.1] {Oxidation (M126)} βA4: ([107-118]/196) [6.1] βB1: ([51-214]/252) [24.6] {Oxidation(M113)} βB2: ([146-168]/205) [11.2] {Trp > Oxolactone (W151)}	γC: ([116-152]/174) [9.8] γD: ([143-163]/174) [12.1] {Oxidation (M147, P148, D150, D156, W157)} γS: ([8-174]/178) [27.0] {Oxidation (K159, P160, D162, W163); Dimethylation (R174); Dioxidation (K159, W163)}	
29	αB: ([57-174]/175) [24.6] {Oxidation (D62, M68, R69)}	βA3: ([96-137]/215) [13.1] {Oxidation (W96, H106)} βA4: ([107-118]/196) [6.1] βB1: ([51-160]/252) [27.8] {Methylation + Deamidation (N82); Deamidation (N68); Oxidation (C80); Methylation (R86, C80, S81); Trp- > Oxolactone(W174)}	γB: ([155-164]/175) [5.7] γD: ([143-152]/174) [5.7]; {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [43.8] {Dimethylation (K159); Deamidation (Q121, Q171); Trp > Kynurenin (W163); Oxidation (M108, P160, W163); Methylation (C23, C25, C37)}	
30	αA:([146-173]/173) [31.9]	βB1: ([51-132]/252) [7.5] {Methylation (N125)} βB2: ([109-168]/205) [9.8] {Deamidation (Q163)}	γD: ([143-152]/174) [5.7] {Oxidation (M147, D150)} γS: ([8-155]/178) [16.9]	
31	αA: ([13-157]/173) [40.5] {Methylation (S62, R65); Deamidation (Q90)}	βB1: ([51-118]/252) [7.1] {Oxidation (M113)} βB2: ([161-168]/205) [3.9]	γS: ([73-174]/178) [34.2] {Oxidation (M74)}	Phakinin ([344-353]/415) [2.4]
32	αA: ([13-157]/173) [24.9] αB: ([83-90]/175) [5.2]	βB1: ([51-143]/252) [24.2] {Methylation (C80, S81, R86, N125); Methylation + Deamidation (N82); Oxidation (C80, M137)}	γB: ([155-164]/175) [5.7] γD: ([143-152]/174) [5.7] γS: ([149-174]/178) [19.2] {Trp > Kynurenin (W163); Oxidation (Y150, P160, D162, W163)}	
33	αA: ([13-157]/173) [23.7]	βB1: ([51-118]/252) [7.1] βB2: ([69-101]/205) [13.2]	γC: ([4-10]/174) [4.0] γD: ([143-152]/174) [5.7] {Oxidation (M147, P148, D150)} γS: ([147-174]/178) [20.8] {Oxidation (Y150)}	
34	αA: ([79-88]/173) [7.4] αB: ([83-90]/175) [5.2]	βB2: ([109-168]/205) [9.8]	γD: ([143-152]/174) [5.7] {Oxidation (M147, D150)} γS: ([85-174]/178) [30.0] {Dioxidation (K159); Oxidation (K159, P160, D162, W163); Methylation (I161)}	
35	αB: ([83-103]/175) [12.3]	βB2: ([109-198]/205) [17.1] {Oxidation (D192, M193)}	γD: ([154-163]/174) [5.7] γS: ([85-155]/178) [15.0]	
36	αA: ([55-65]/173) [6.4] αB: ([83-90]/155) [5.2]	βB1: ([51-118]/252) [7.1] {Oxidation (M113)} βB2: ([109-198]/205) [14.1] {Oxidation (D192, M193); Deamidation (N116, Q163)}	γC: ([78-152]/174) [12.6] γD: ([143-152]/174) [5.7] {Oxidation (M147, P148, D150)}	

Table 3
Crystallin fragments and their PTMs in Fraction III.

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/ Phakinin**
1	αA ([71-78]/173) [5.9] αB ([75-82]/175) [5.2]	βB2 ([109-168]/205) [9.8] {Deamidation (Q163)}		
2	αA: ([13-157]/173) [40.5] {Deamidation (Q104)} αB: ([83-90]/175) [5.2]	βA3: ([96-211]/211) [14.6] βA4: ([72-90]/196) [9.7] βB1: ([51-214]/252) [29.4] {Methylation + Deamidation (N82); Methylation (C80, S81, R86, N125); Deamidation (N125); Oxidation (C80, M113); Trp > Oxolactone (W124)} βB2: ([69-89]/205) [7.8]	γC: ([4-10]/174) [4.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150, D156, W157); Deamidation (Q155, N161)} γS: ([8-174]/178) [43.8] {Deamidation (Q149, Q171); Acetylation (K154, K159,); Oxidation (W73, M74, M108, Y109, D114, C115, P116, M119, Y150, K159, P160, D162, W163,R174); Dioxidation (M74, C115, P116, K159,W163); Dimethylation (R174); Methylation (K159, S167); Trp > Oxolactone (W163)}	
3	αA: ([13-88]/173) [17.3] αB: ([83-90]/175) [5.2]	βB1: ([51-60]/252) [4.0] βB2: ([69-89]/205) [7.8] {Oxidation (W85); Trp > Oxolactone (W82)}	γD: ([143-163]/174) [11.5] {Oxidation (M147, P148)} γS: ([8-174]/178) [20.8] {Oxidation (W73, M74, Y150); Dioxidation (M74)}	
4	αA: ([89-99]/173) [8.1] αB: ([83-90]/175) [5.2]	βB2: ([69-76]/205) [3.9]	γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [27.0] {Oxidation (M74, Y150)}	
5	αA: ([89-157]/173) [16.9] αB: ([83-90]/175) [5.2]	βA3: ([197-211]/215) [7.6] βB1: ([51-214]/252) [25.4] {Deamidation (N58, N125); Trp- > Oxolactone (W124); Methylation (N125)} βB2: ([82-168]/205) [7.8] {Deamidation (Q163)}	γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150); Deamidation (Q155, N161)} γS: ([73-174]/178) [45.0] {Deamidation (Q171); Acetylation (K159); Dioxidation (M119, M124, Y150, K159, W163,); Oxidation (M74, M108, Y150, K159, D162)}	
6	αA: ([13-99]/173) [17.9]	βB1: ([51-132]/252) [10.7] {Oxidation (M113)} βB2: ([161-168]/205) [3.9]	γD: ([143-163]/174) [11.5] {Deamidation (N161); Oxidation (M147, D150)} γS: ([73-174]/178) [25.0] {Oxidation (M74, Y150)}	
7	αA: ([13-99]/173) [16.2] {Oxidation (D76, K78); Dioxidation (K78)} αB: ([57-90]/175) [13.5] {Oxidation (D62, M68, R69)}	βA3: ([33-44]/215) [6.1]	γB: ([4-164]/175) [9.7] γD: ([143-163]/174) [12.1] {Oxidation (M147, P148, D150, D156, W157, N161); Dioxidation (W157); Trp > Kynurenin (W157); Deamidation (Q155, N161)} γS: ([8-174]/178) [37.1] {Oxidation (W73, M74, Y150, K159, D162); Dioxidation (W73, M74, K159, W163); Dimethylation (K14)}	
8	αA: ([79-99]/173) [15.4] {Deamidation (Q90)} αB: ([57-90]/175) [13.5] {Oxidation (M68, R69)}	βA3: ([33-211]/215) [19.7]	γB: ([60-164]/175) [16.0] {Deamidation (Q67)} γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150, Y154, D156, W157, N161); Dioxidation (W157); Methylation + Deamidation (Q155)} γS: ([8-174]/178) [46.6] {Oxidation (W73, M108, Y109, D114, C115, P116, M119, F136, W137, F139, Y150, K159, P160, D162, W163, P168); Acetylation(K159); Deamidation (N15, Q121, Q171); Trp > Kynurenin (W163); Dioxidation (M74, Y109, M119, R125, K159, Y150, K154, W163); Methylation + Deamidation (Q121); Dimethylation (K154, R174); Trp > Oxolactone (W163); Methylation (C115, S117, H123, K159, I161, S167)}	
9	αA: ([55-99]/173) [18.5]		γB: ([4-164]/175) [9.7] γD: ([143-163]/174) [12.1] {Dioxidation (M147, P148, W157); Oxidation (D156, W157, M147); Deamidation (Q155)} γS: ([8-155]/178) [7.9]	
10	αA: ([13-99]/173) [23.7] αB: ([83-90]/175) [5.2]	βA3: ([33-44]/215) [6.1]	γA: ([4-152]/174) [9.8] {Dimethylation(R147)} γD: ([4-163]/174) [16.1] {Oxidation (M147, P148, D150, D156, W157, N161); Dioxidation (M147, P148, W157); Deamidation (Q143, Q155, N161)} γS: ([8-79]/178) [10.7] {Oxidation (M74); Dimethylation(K14)}	

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Table 3 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/ Phakinin**
10B	αA: ([89-99]/173) [8.1]	βB2:([161-168]/205) [3.9]	γA: ([4-152]/174) [9.8] {Dimethylation (R147); Methylation (L145, L146)} i γD: ([4-163]/174) [16.1] {Oxidation (Y7, M147, P148, D150, D156, W157, N161); Dioxidation (M147, W157); Deamidation (Q155, N161)} γS: ([8-155]/178) [18.0] {Oxidation (M74); Dimethylation (K14)}	
11	αA: ([13-99]/173) [17.9] αB: ([83-90]/175) [5.2]	βB1: ([203-214]/252) [4.8] βB2: ([161-168]/205) [3.9]	γB: ([4-164]/175) [9.7] γD: ([4-163]/174) [17.2] {Oxidation (M147, D150, D156, W157); Deamidation (N161); Dioxidation (W157)}; γS: ([8-155]/178) [20.8] {Dimethylation (K14); Oxidation (M74)}	
12	αA: ([13-157]/173) [40.5] {Deamidation (Q90)}	βB1: ([51-214]/252) [21.8] {Deamidation (N58, N82, N125)} βB2: ([69-89]/205) [7.8]	γD: ([143-152]/174) [5.7] {Oxidation (M147, P148, D150)} γS: ([42-174]/178) [23.0] {Deamidation (Q149, Q171); Dimethylation (R174); Oxidation (W73, M74,Y150); Trp > Kynurenin (W163); Dioxidation (M74)}	
13	αA: ([12-157]/173) [41.0] {Dimethylation (R21, R65); Methylation (I61, S62, R65)}	βB1: ([51-160]/252) [28.2] {Oxidation (M113); Methylation (C80, S81); Methylation + Deamidation (N82)} βA3: ([96-109]/215) [7.1] βA4: ([91-103]/196) [6.6] {Methylation + Deamidation (N101)}	γC: ([4-122]/174) [8.0] γS: ([8-174]/178) [23.0] {Oxidation (Y150); Dioxidation (Y150)}	Phakinin: ([344-353]/415) [2.4]
14	αA: ([13-99]/173) [23.7] αB: ([83-90]/175) [5.2]	βB1: ([51-118]/252) [7.1]	γB: ([4-164]/175) [9.7] γC: ([4-152]/174) [9.8] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [35.4] {Deamidation (Q171); Oxidation (M74, M108, Y109, P116, Y150, P160, D162, W163); Trp > Hydroxykynurenin (W163); Dioxidation (M119, Y150); Dimethylation (K14)}	
16	αA: ([12-157]/173) [41.0] {Dimethylation (R21); Methylation (S62, R65); Deamidation (Q90); Oxidation (F93)} αB: ([83-90]/175) [5.2]	βA3: ([33-44]/215) [6.1] βA4: ([14-118]/196) [28.6] {Oxidation (C99); Deamidation (N114); Methylation + Deamidation (N101); Methylation (C99)} βB1: ([51-214]/252) [32.9] {Oxidation (M113, F114); Deamidation (N125, N158, Q205); Dioxidation (C80, M113); Trp > Oxolactone (W124)} βB2: ([82-168]/205) [13.7]	γC: ([4-10]/174) [4.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, D150); Deamidation(Q155)} γS: ([8-174]/178) [36.0] {Oxidation (M74, Y150, P160, D162, W163); Dimethylation (K14); Deamidation (Q93); Trp > Kynurenin (W163)}	Phakinin: ([344-353]/415) [2.4]
17	αA: ([13-157]/173) [32.4] αB: ([83-90]/175) [5.2]	βA3: ([197-211]/215) [7.6] βB1: ([51-160]/252) [24.6] {Methylation + Deamidation (N82); Deamidation (N158, N125); Oxidation (C80, M113, F114); Methylation (C80, S81, R86)} βB2: ([82-188]/205) [15.6] {Deamidation (Q163)}	γC: ([4-10]/174) [4.0] γD: ([143-152]/174) [5.7] {Oxidation (M147, D150)} γS: ([8-174]/178) [29.8] {Oxidation (M74)}	
18	αA: ([55-157]/173) [30.6] {Deamidation (Q104)} αB: ([75-90]/175) [10.3]	βB1: ([51-214]/252) [22.6] {Methylation (C80, S81, R86); Methylation + Deamidation (N82); Oxidation (C80)} βB2: ([69-188]/205) [31.2] {Oxidation (W82)}	γC: ([4-122]/174) [8.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [29.8] {Dioxidation (K159, W163); Oxidation (M74, K159, D162); Dimethylation (K14)}	
19	αA: ([13-173]/173) [46.2] {Deamidation (Q147); Methylation (I61, S62); Oxidation (Y109, H107)} αB: ([57-174]/175) [34.3] {Oxidation (D62, M68, R69); Deamidation (N78)}	βA3: ([126-211]/215) [13.6] {Oxidation (M126); Deamidation (Q208)} βB1: ([51-214]/252) [25.4] {Oxidation (M113); Acetylation (S189); Deamidation (Q197); Trp- > Oxolactone (W124)} βB2:([69-168]/205) [30.7] {Deamidation (N116, Q147, Q155, Q163); Trp > Oxolactone (W82)}	γC: ([116-122]/174) [4.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150); Deamidation(N161)}; γS: ([8-174]/178) [29.8] {Oxidation (M74, Y150, P160, D162, W163); Deamidation (N15, Q17)}	
20	αA: ([13-112]/173) [23.1]	βA3: ([33-44]/215) [6.1]	γC: ([4-163]/174) [22.4] {Oxidation (M160, D161); Deamidation	

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Table 3 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/ Phakinin**
	αB: ([12-174]/175) [40.6] {Oxidation (D62, M68, R69)}	βB1: ([51-214]/252) [21.8] {Oxidation (M113)} βB2:([109-198]/205) [21.5] {Deamidation (N116); Oxidation (M193)}	(Q84, Q143, Q149)} γD: ([143-163]/174) [11.5] γS: ([8-174]/178) [23.0] {Dimethylation (R174); Trp > Oxolactone (W163); Oxidation (Y150, P160, D162, W163); Methylation (K159, I161); Deamidation (Q149, Q171); Acetylation (K155); Dioxidation (K159)}	
21	αA: ([13-157]/173) [35.8] αB: ([75-103]/175) [17.4]	βA3: ([197-211]/215) [7.6] βB1: ([51-160]/252) [23.4] {Methylation + Deamidation (N82); Dioxidation (C80, M113); Oxidation (C80, M113, F114, M137); Methylation (C80, S81, R86)} βB2:([109-198]/205) [22.0] {Oxidation (M193)}	γC:([78-152]/174) [16.7] {Methylation (C79, C80)}; γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150)} γS: ([8-155]/178) [18.0] {Oxidation (M74)}	
22	αA: ([55-157]/173) [30.6] αB: ([12-103]/175) [34.2] {Oxidation (D62, M68, R69)}	βB1: ([51-72]/252) [8.7] βB2: ([109-188]/205) [36.6] {Oxidation (K121, M122); Deamidation (N116)}	γD: ([143-163]/174) [11.5] γS: ([85-174]/178) [28.3] {Deamidation (Q171); Trp > Hydroxykynurenin (W163)}	
23	αA: ([13-157]/173) [24.3] αB: ([83-174]/175) [30.9] {Deamidation (N146)}	βA3: ([126-211]/215) [13.6] {Dioxidation (W198); Oxidation (M126, W198, H201)} βB1: ([51-202]/252) [20.6] {Oxidation (M113) Deamidation (N58); Trp- > Oxolactone (W124); Acetylation (S189)} βB2: ([91-198]/205) [35.1] {Oxidation (W151, Y154, D192, M193); Deamidation (N114, Q147, Q155, Q163, Q183, Q194, Q197); Dioxidation (M193); Methylation + Deamidation (Q147)}	γC: ([78-163]/174) [22.4] {Oxidation (C80, M160, D161) Methylation (H117) Deamidation (Q143)} γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161); Dioxidation (W157)} γS: ([8-174]/178) [29.8] {Oxidation (W73); Dioxidation (M74)}	
24	αA: ([13-157]/173) [42.8] {Deamidation (Q90, Q147)} αB: ([75-103]/175) [12.3]	βA3: ([33-137]/215) [12.1] {Oxidation (M126)} βA4: ([14-25]/196) [6.1] {Oxidation (M14) βB1: ([51-160]/252) [24.6] {Deamidation (N58, N82, N158); Dioxidation (M113); Oxidation (M113)} βB2: ([109-168]/205) [9.8]	γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [32.0] {Trp > Kynurenin (W163)}	
25	αA: ([13-157]/173) [35.8] {Deamidation (Q90)} αB: ([57-174]/175) [24.6] {Oxidation (M68, R69)}	βB1: ([51-160]/252) [19.4] {Deamidation (N58, N158); Oxidation (M113)} βB2:([146-168]/205) [11.2] {Deamidation (Q155, Q163)}	γD: ([154-163]/174) [5.7] γS: ([85-174]/178) [28.3] {Oxidation (W163); Trp > Oxolactone (W163)}	
26	αA: ([13-157]/173) [40.5] {Oxidation (H79, F80, P82); Methylation (S62, R65); Deamidation (Q90, Q147)} αB: ([12-174]/175) [36.0] {Oxidation (D62, M68, R69)}	βA3: ([33-211]/215) [13.6] {Deamidation (Q38)} βA4: ([107-118]/196) [6.1] {Deamidation (Q112)} βB1: ([51-214]/252) [33.3] {Deamidation (N68, N158, Q197); Oxidation (M113, F114, M137,); Acetylation (S189)} βB2:([82-168]/205) [21.0] {Deamidation (N114, N116, Q147, Q163)}	γC: ([116-122]/174) [4.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [25.8] {Oxidation (K159, P160, D162, W163); Dioxidation (K159, W163)}	Filensin ([78-90]/ 665) [2.0]
27	αA: ([50-157]/173) [38.2] {Deamidation (Q147); Dioxidation (F53)} αB: ([57-174]/175) [29.1] {Oxidation (D62, M68, R69); Dimethylation (K166)}	βB1: ([51-160]/252) [7.9] {Deamidation (N58)}; βB2:([109-188]/205) [24.9] {Deamidation (N116, Q155, Q163)}	γD: ([4-163]/174) [35.1] {Oxidation (M70, D74, M147, P148, D150)} γS: ([85-174]/178) [28.3] {Dimethylation (R174); Oxidation (K159, W163, D162); Dioxidation (K159, W163); Trp > Oxolactone (W163); Deamidation (Q93, Q149,)}	
28	αA: ([13-157]/173) [35.8] {Methylation (S81); Deamidation (Q147)} αB: ([108-174]/175) [11.4]	βB1 ([51-214]/252) [26.6] {Oxidation (M113)}; βB2([90-188]/205) [26.8] {Deamidation (N116, Q155)}	γD: ([4-10]/174) [4.0]	
29	αA: ([13-157]/173) [35.8] αB: ([83-174]/175) [26.9] {Dimethylation (K166); Acetylation (K150)}	βB1: ([51-160]/252) [7.9] {Deamidation (N158)} βB2:([109-188]/205) [24.9] {Deamidation (N114, N116, Q155, Q163)}	γD: ([154-163]/174) [5.7] γS: ([8-95]/178) [10.1]	
30	αA: ([55-157]/173) [18.5] αB: ([93-116]/175) [11.4]	βB1: ([151-202]/252) [9.9] {Acetylation (S189)} βB2: ([109-188]/205) [24.9] {Deamidation (N114, N116, Q147, Q163)}		
31	αA: ([13-157]/173) [45.7]	βB1: ([51-160]/252) [19.4] {Deamidation (N158); Oxidation (M113)}	γS: ([85-174]/178) [22.5] {Deamidation (Q93); Oxidation (W163)}	

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Table 3 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/ Phakinin**
		βB2:([109-120]/205) [5.9] {Deamidation (N116)}		
32	αA: ([13-157]/173) [35.8] {Methylation (S62)} αB: ([12-174]/175) [48.6] {Oxidation (D62, M68, R69); Acetylation (K92); Deamidation (Q151)}	βA3: ([126-137]/215) [6.1] {Oxidation(M126)} βA4: ([107-118]/196) [6.1] βB1: ([51-160]/252) [24.6] {Deamidation (N158); Methylation (C80, S81, R86); Oxidation (C80, M113); Methylation + Deamidation (N82)} βB2:([90-188]/205) [23.9] {Deamidation (N116, Q163)};	γC: ([4-152]/174) [9.8] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [23.6] {Trp > Kynurenin (W163); Oxidation (M74)}	Filensin ([78-90]/ 665) [2.0]
33	αA: ([13-157]/173) [35.8] {Oxidation (D106, H107); Deamidation (Q90)} αB: ([75-116]/175) [20.6]	βB1: ([51-160]/252) [7.9] βB2:([109-168]/205) [9.8]	γD: ([4-163]/174) [9.8]	
34	αA: ([13-88]/173) [11.0]			
35	αA: ([13-157]/173) [29.5] αB: ([12-174]/175) [24.6] {Oxidation (D62, M68, R69)}	βB1: ([61-214]/252) [13.5]	γD: ([143-152]/174) [5.7] {Oxidation (M147, P148, D150)} γS: ([85-174]/178) [22.5] {Dioxidation (K159, W163); Oxidation (K159, D162)}	
36	αA: ([55-157]/173) [35.3] αB: ([75-103]/175) [12.3]	βB2: ([109-120]/205) [5.9]	γD: ([4-10]/174) [4.0]	
37	αA: ([13-157]/173) [35.8] {Dimethylation (R21); Methylation(I61, S62)}	βB2:([109-168]/205) [9.8]		
38	αA: ([1-157]/173) [54.3] {Oxidation (M1, D2); Methylation (I61, S62, R65, H79, S81, I96, K99, I110, S111); Dimethylation (R21); Deamidation (Q90, Q147); Acetylation (K99); Dioxidation (Y109)} αB: ([57-69]/175) [8.4] {Oxidation (M68, R69)}	βA3: ([33-109]/215) [13.1] {Deamidation (Q38, N40, Q42, Q50)} βA4: ([107-118]/196) [6.1] βB1: ([151-160]/252) [4.0] {Deamidation (N158)}	γS: ([73-174]/178) [19.2] {Oxidation (M74)}	
39A	αA: ([12-157]/173) [37.6] {Deamidation (Q90, Q104); Methylation (S62, R65); Dimethylation (R21)} αB: ([93-174]/175) [12.6]	βB2: ([109-120]/205) [5.9]		
40	αA: ([12-157]/173) [38.7] {Methylation (S62, R65, S81); Deamidation (Q90); Dimethylation (R12)} αB: ([83-90]/175) [5.2]	βB1: ([124-160]/252) [7.5] {Deamidation i(N158)}		
41	αA: ([1-157]/173) [50.3] {Dimethylation (R12, R21, R65); Methylation (S59, S62, R65, I61); Deamidation (Q90); Trp > Kynurenin (W9); Phosphorylation (T13)} αB: ([83-90]/175) [5.2]	βA3: ([126-137]/215) [6.1] {Oxidation (M126)} βB2:([109-168]/205) [9.8] {Deamidation (N114)}	γD:([4-10]/174) [4.0] γS: ([149-174]/178) [19.2]	
42	αA: ([12-157]/173) [36.4] {Methylation (I96) Oxidation (D84,Y109); Deamidation (Q90, Q104)} αB: ([83-90]/175) [5.2]			
43	αA: ([13-157]/173) [40.5] αB: ([83-103]/175) [12.3]	βA3: ([33-44]/215) [6.1]		
44	αA: ([13-157]/173) [35.8] {Oxidation (D105, H107)} αB: ([12-174]/175) [42.9] {Oxidation (P58, W60, D62, M68, R69); Dioxidation (M68, R69); Deamidation (Q108)}	βA3: ([33-137]/215) [12.1] {Methylation (I128)} βA4: ([107-192]/196) [14.3] βB1: ([51-160]/252) [15.9] {Oxidation (M113, F114); Deamidation (N58)} βB2:([109-168]/205) [9.8]	γC: ([4-10]/174) [4.0] γD: ([4-152]/174) [9.8] {Oxidation (M147, P148, D150)} γS: ([149-174]/178) [19.2]	
45	αA: ([13-157]/173) [23.7] αB: ([12-174]/175) [40.6] {Oxidation (D62, M68, R69); Deamidation (Q108)}	βA3: ([126-137]/215) [6.1] {Oxidation (M126)}; βB2:([109-168]/205) [9.8]	γD: ([154-163]/174) [5.7]; γS: ([149-155]/178) [5.8];	
46	αA: ([13-157]/173) [17.9]; αB: ([12-174]/175) [40.0] {Oxidation (P58, W60, D62, M68, R69, K72); Dioxidation (M68, R69); Dimethylation (R12, K166)}	βA3: ([96-137]/215) [13.1] {Oxidation (M126)} βB2: ([109-168]/205) [9.8]	γC:([4-10]/174) [4.0]	

Table 4
Crystallin fragments and their PTMs in Fraction IV.

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
1	αA: ([13-157]/173) [40.5] {Deamidation (Q90); Methylation (S62, R65); Oxidation (D106)} αB: ([73-150]/175) [38.3]	βA3: ([33-177]/215) [32.3] {Trp > Oxolactone (W73, W168); Deamidation (N103); Methylation (H78, S165, C170, Q164, S80, C82); Oxidation (C170, W168); Methylation + Deamidation (Q172, Q164)} βA4: ([14-118]/196) [24.0] βB1: ([51-143]/252) [24.2] {Methylation + Deamidation (N82); Methylation (C80, S81, R86); Deamidation (N58, N125); Oxidation (M137); Dioxidation (C80)} βB2: ([82-168]/205) [13.7]	γC: ([4-10]/174) [4.0] γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150); Deamidation (Q143, Q155, N161)} γS: ([8-174]/178) [29.8] {Deamidation (N77, Q149); Oxidation (M74, Y150); Methylation (L152); Dioxidation (Y150); Dimethylation (K14)}	
2	αA: ([13-112]/173) [33.5] αB: ([73-90]/175) [11.6]	βA3: ([33-109]/215) [13.1]; βA4: ([14-71]/196) [17.9] {Oxidation (M14)}; βB1: ([51-202]/252) [25.4] {Methylation (C80); Oxidation (C80, N82); Methylation + Deamidation (N82); Dioxidation (C80)} βB2: ([146-160]/205) [7.3]	γB: ([4-164]/175) [9.7] γD: ([143-163]/174) [11.5] {Oxidation (M147, D150, D156, W157)} γS: ([8-175]/178) [47.8] {Trp > Kynurenin (W163); Dimethylation (K14, R174,); Deamidation (Q171); Trp > Oxolactone (W163); Oxidation (Y109, P116, Y150, K159, P160, D162, W163, F173); Methylation (K159, I161, Q171, S172, R175); Dioxidation (K159, W163); Acetylation (K159); Trp > Hydroxykynurenin (W163); Methylation + Deamidation (Q171)}	
3	αA: ([13-157]/173) [40.5] {Deamidation (Q90); Methylation (S62)}; αB: ([12-149]/175) [39.4] {Oxidation (D62, M68, R69); Deamidation (N146)}	βA3: ([68-211]/215) [19.2] {Methylation (H78, C82); Oxidation (Y76, C82); Methylation + Deamidation (Q84)} βA4: ([14-192]/196) [42.3] {Trp- > Kynurenin (W54); Dioxidation (W54); Oxidation (M14, W54, F57, H59, F62, Y67); Deamidation (Q63, Q112, N114, Q155, Q189)} βB1: ([51-214]/252) [34.9] {Methylation (C80, S81, R86); Dioxidation (C80); Deamidation (N82); Methylation + Deamidation (N82); Oxidation (C80, M113, M137); Trp > Oxolactone (W124)}	γD: ([143-163]/174) [11.5] {Dioxidation (W157); Oxidation (M147, P148, D150, D156, W157); Deamidation (N161)} γS: ([8-174]/178) [29.8] {Deamidation (N77, Q149, Q171); Acetylation (K159); Trp > Kynurenin (W163); Trp > Oxolactone (W163); Oxidation (W73, M74, K159, P160, D162, W163); Methylation (K159); Dioxidation (K159, W163); Dimethylation (K14)}	
4	αA: ([13-157]/173) [41.6] {Acetylation (K70); Dimethylation (R21); Deamidation (Q90, Q104)} αB: ([83-90]/175) [5.2]	βA3: ([33-109]/215) [13.1] {Deamidation (N103)} βA4: ([14-118]/196) [34.2] βB1: ([51-118]/252) [11.9] {Deamidation (N58, N68, Q70); Oxidation (M113)} βB2: ([69-168]/205) [13.7]; {Deamidation (Q64, N114, Q155); Trp > Kynurenin (W59); Methylation (S51)}	γC: ([4-10]/174) [4.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150, D156, W157) γS: ([8-155]/178) [23.6] {Oxidation (Y150); Deamidation (Q149); Dimethylation (K14); Methylation (C37, N38)}	Filensin: ([19-328]/526) [5.1] {Phosphorylation (T321, T327)}
5	αA: ([13-112]/173) [33.5] {Oxidation (D105, D106)}; αB: ([75-90]/175) [10.3] {Oxidation (F75)}	βA4: ([49-192]/196) [19.4] {Trp > Kynurenin (W54)} βB1: ([51-132]/252) [15.5] {Oxidation (M113); Deamidation (N58)} βB2: ([69-76]/205) [3.9]	γC: ([4-122]/174) [8.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150, D156, W157); Dioxidation (W157); Deamidation (Q155, N161)} γS: ([8-174]/178) [36.0] {Oxidation (M74, Y150); Deamidation (Q93, Q149,); Dioxidation (Y150)}	
6	αA: ([13-112]/173) [33.5] {Dimethylation (R65); Methylation (I61, S62); Deamidation (Q90); Oxidation (D105, D106)} αB: ([57-149]/175) [37.7] {Deamidation (N78, N146)}	βA3: ([33-211]/215) [13.6] {Deamidation (Q208)} βA4: ([49-192]/196) [25.5] {Deamidation (Q189)}; βB1: ([51-143]/252) [26.2] {Dioxidation (C80); Methylation (C80, S81, R123, N125); Trp > Oxolactone (W124); Oxidation (C80, N82); Methylation + Deamidation (N82, N125); Deamidation (N58, Q70, N82, N125)} βB2: ([82-89]/205) [3.9]	γC: ([4-10]/174) [4.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([8-174]/178) [56.7] {Dimethylation (K159, R174); Methylation (C25, L151, L152, K159, I161, S167, S172); Trp- > Kynurenin (W163); Acetylation (K159); Oxidation (Y21, Y109, P116, W137, F139, Y150, K159, P160, D162, W163, F173); Deamidation (Q149, Q171); Trp >	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
			Oxolactone (W163); Dioxidation (C115, Y150, W163); Methylation + Deamidation (Q171)}	
7	αA: ([13-157]/173) [35.8] {Methylation (S62)} αB: ([57-90]/175) [18.7]	βA3: ([33-211]/215) [13.6] βB1: ([51-214]/252) [24.2] {Oxidation (C80, M113, M137); Methylation (C80, S81); Deamidation (N82, N125); Methylation + Deamidation (N82)} βB2: ([82-160]/205) [11.2] {Acetylation (S148)}	γC: ([4-163]/174) [16.7] {Methylation (C154); Oxidation (R10); Methylation + Deamidation (Q155)} γD: ([4-163]/174) [16.7] {Oxidation (M147, P148, D150, Y154, D156, W157); Deamidation (Q143, N161); Methylation + Deamidation (Q143)} γS: ([8-174]/178) [29.8] {Deamidation (N77, Q149, Q171); Trp > Kynurenin (W163); Trp > Oxolactone (W163); Methylation (K159, I161); Dioxidation (Y150, W163); Oxidation (M74, Y150, K159, P160, D162, W163); Dimethylation (K14)}	
8	αA:([13-157]/173) [55.5] {Methylation (C131, C142)}; αB: ([12-90]/175) [20.6] {Oxidation (D62, M68, R69)}	βA3: ([33-211]/215) [38.4] {Deamidation (Q42, N103, N133); Methylation (H78)} βA4: ([14-25]/196) [6.1] βB1: ([51-214]/252) [27.0] βB2: ([69-89]/205) [7.8]	γA: ([4-59]/174) [13.2] {Methylation (R10, Q13)} γB: ([4-164]/175) [29.1] {Oxidation (D9, R10, M70, D74)} γC: ([4-163]/174) [32.8] {Oxidation (D9, R10, M70, D74); Methylation (C154)} γD: ([141-163]/174) [13.2] {Dioxidation (R142, W157); Oxidation (R142, Y144, M147, P148, D150, Y154, D156, W157); Trp > Oxolactone (W157); Deamidation (Q155, N161); Methylation (Q155)} γS: ([8-174]/178) [53.4] {Acetylation (K159); Oxidation (M59, Y60, M74, Y150, K159, D162); Methylation (R20, C23, C25, C27, R52) Deamidation (Q17, N77, Q171); Dioxidation (K159, P160, W163)};	
9	αA: ([13-112]/173) [27.2] αB: ([57-149]/175) [38.9]	βA3: ([33-211]/215) [28.3] {Trp > Oxolactone (W168); Methylation (C170); Oxidation (C170); Dehydration (C170); Deamidation (Q172, Q208)} βB1: ([61-214]/252) [18.7] {Deamidation (Q70)} βB2: ([69-89]/205) [7.8] {Deamidation (Q71)}	γB: ([4-164]/175) [20.0] γD: ([4-163]/174) [16.1] {Dioxidation (W157); Trp > Oxolactone (W157); Oxidation (M147, P148, D150, Y154 D156, W157); Methylation (Q155); Deamidation (Q155, N161)} γS: ([8-174]/178) [72.5] {Dimethylation (K14, R20, K154, K159, R174); Oxidation (F55, M59, M74, M108, Y109, D114, C115, P116, F136, W137, F139, Y150, K159, P160, D162, W163); Acetylation (K159); Trp- > Oxolactone (W163); Methylation (K159, I161, S167, S172); Dioxidation (C115, K159); Deamidation (N77, Q149, Q171); Methylation + Deamidation (Q107, Q121)}	
10	αA: ([13-112]/173) [28.9] αB: ([57-116]/175) [21.7] {Oxidation (D62, M68, R69)}	βA3: ([33-211]/215) [20.7] βA4 ([49-192]/196) [25.5] βB1: ([51-202]/252) [21.4] {Oxidation (M113)} βB2: ([69-76]/205) [3.9]	γB: ([4-164]/175) [12.6] {Deamidation (N162); Oxidation (R10)} γC: ([4-163]/174) [22.4] {Deamidation (Q155); Methylation (C154); Oxidation (R10, C154, M160, D161); Methylation + Deamidation (Q155)} γD: ([143-163]/174) [11.5] {Oxidation (M147, P148, D150); Deamidation (, Q155, N161); Dioxidation (W157)}; γS: ([8-174]/178) [44.4] {Trp > Kynurenin (W163); Dimethylation (K159, R174); Trp- > Oxolactone (W163); Oxidation (Y109, P116, Y150, K159, P160, D162, W163, R3110, P168, R174, M74); Methylation (I161, K159); Dioxidation (K159, W163, F173); Deamidation (Q171, Q149, N77); Acetylation (K154)}	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
11	αA: ([13-145]/173) [37.6]; αB: ([12-149]/175) [30.3]	βA3: ([33-211]/215) [26.8] {Deamidation (Q38, N133)} βA4: ([14-192]/196) [20.4]; βB1: ([51-202]/252) [23.4] {Methylation (L61, C80, S81); Oxidation (C80); Deamidation (N58); Methylation + Deamidation (N82)} βB2: ([82-160]/205) [11.2] {Deamidation (Q155)}	γB: ([4-164]/175) [12.6] {Oxidation (R10, F12)} γC: ([4-163]/174) [19.0] {Deamidation (Q155); Dimethylation (R147); Oxidation (P148, D156, W157); Dioxidation (W157); Deamidation (Q143, Q155); Methylation (Q143); Methylation + Deamidation (Q143)} γD: ([4-163]/174) [16.1] γS:([8-174]/178) [53.9] {Dimethylation (R20, D26, M108, K159, R174); Oxidation (Y21, C25, Y109, D114, C115, P116, F136, Y150, K159, P160, D162, W163); Dioxidation (Y109, C115, P116, R125, K159, W163); Methylation (R20, I161, S167, S172); Methylation + Deamidation (Q107); Deamidation (Q17, Q171); Acetylation (C23, C27)}	
12A	αA: ([13-157]/173) [35.8] αB: ([57-149]/175) [37.7] {Oxidation (D109)}	βA3: ([96-211]/215) [14.6] {Dioxidation (W198); Oxidation (W198, H201)} βA4: ([14-192]/196) [50.0] {Methylation (H27, C33, S35, H59, C166); Oxidation (H27, M14, C33, P34); Trp > Kynurenin (W54); Deamidation (Q23, Q112, N114); Trp > Oxolactone (W54)} βB1: ([51-202]/252) [25.0] {Deamidation (N125); Oxidation (C80, M113, M137); Methylation (C80, S81, R86); Methylation + Deamidation (N82)} βB2: ([109-168]/205) [9.8]	γB: ([4-164]/175) [12.6] {Oxidation (R10)} γC: ([4-163]/174) [16.7] {Deamidation (Q155); Methylation (C154); Oxidation (R10, D161); Methylation + Deamidation (Q155)} γD:([4-163]/174) [15.5] {Oxidation (M147, P148, D150, D156, W157); Dioxidation (W157); Deamidation (N161, Q155)} γS: ([1-174]/178) [42.1] {Deamidation (Q17, N144, Q171); Trp- > Kynurenin (W163); Methylation (S2); Oxidation (W73, M74); Dioxidation (M74); Dimethylation (K14)}	
12B	αA: ([55-99]/173) [18.5] αB: ([57-174]/175) [49.7] {Deamidation (N78); Oxidation (D62, M68, R69); Acetylation (K150)}	βA3: ([33-137]/215) [20.7] {Dioxidation (M126)} βB1: ([51-214]/252) [36.9] {Deamidation (N58, N68, Q70, N82, N158); Methylation (C80, S81, R86); Oxidation (C80, M113); Methylation + Deamidation (N82); Acetylation (S189)} βB2: ([91-198]/205) [46.8] {Deamidation (N114, N116, Q147, Q155, Q163, Q183, Q185, Q194, Q197); Oxidation (M122, W151, Y154, Y156, D192, M193); Methylation (I109, I110, N114, Q147, S148, L166, S174, S175, Q183); Dioxidation (W151); Dimethylation (K172); Trp- > Oxolactone (W151); Methylation + Deamidation (N114); Trp- > Kynurenin (W151)}	γC: ([78-152]/174) [16.7] {Methylation (S78, C79, C80, L81); Deamidation (Q143)} γD: ([4-163]/174) [25.9] {Dioxidation (W157); Oxidation (H23, M147, P148, D150, D156, W157, N161); Deamidation (N161, Q155, Q143); Methylation (C19, S21, H23)} γS: ([8-174]/178) [29.8] {Deamidation (Q171); Oxidation (M74)}	
13	αA: ([12-157]/173) [43.9] {Dimethylation (R12, R21, R65); Dioxidation (R12, F80, P82,); Methylation (I61, S62, R65, S81); Deamidation (Q90); Oxidation (D105, D106)} αB: ([12-150]/175) [39.4] {Deamidation (N146)}	βA3: ([68-177]/215) [26.3] {Deamidation (N103); Oxidation (W168, C170, Y171, H78); Trp > Oxolactone (W73, W168); Methylation + Deamidation (Q172); Methylation (S80, C170)} βA4: ([14-25]/196) [6.1] {Oxidation (M14)} βB1: ([51-132]/252) [16.3] {Deamidation (N58, N125); Methylation (C80, S81, R86); Methylation + Deamidation (N82); Oxidation (C80)} βB2: ([161-168]/205) [3.9]	γC: ([4-10]/174) [4.0] γD: ([4-163]/174) [15.5] {Oxidation (M147)} γS: ([8-174]/178) [29.2] {Deamidation (Q149); Trp > Kynurenin (W163); Dimethylation (K14)}	
14	αA: ([1-157]/173) [45.7] {Dimethylation (R12, R21); Oxidation (M1, D2); Deamidation (Q90); Methylation (I61, S62, R65); Phosphorylation (S51)}	βA2: ([160-167]/197) [4.1] βA3: ([33-109]/215) [13.1] βB1: ([51-160]/252) [21.8] {Deamidation (N58, N82); Methylation (C80, S81, R86); Methylation + Deamidation	γC: ([116-122]/174) [4.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([42-174]/178) [29.2]	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
	αB: ([57-116]/175) [17.1]	(N82)} βB2: ([109-168]/205) [9.8]	{Deamidation (N77, Q93, Q149); Oxidation (Y150)}	
15A	αA: ([13-157]/173) [38.7] {Oxidation (D105, H107, Y109); Methylation + Deamidation (Q104)} αB: ([57-103]/175) [25.8] {Oxidation (M68, R69)}	βA3: ([33-109]/215) [23.2] {Deamidation (Q38, N40, Q42, N103); Trp- > Kynurenin (W99)} βA4: ([14-25]/196) [6.1] {Oxidation (M14)} βB1: ([51-252]/252) [37.3] {Acetylation (S189); Deamidation (N125, Q197); Methylation (C80, S81, R86); Oxidation (C80); Methylation + Deamidation (N82)} βB2: ([49-188]/205) [48.3] {Methylation (C67, K68); Deamidation (Q71, N114, N116, Q147, Q185); Oxidation (W85); Methylation + Deamidation (N66)}	γC: ([4-163]/174) [26.4] {Methylation (C154)} γD:([143-163]/174) [11.5] {Oxidation (M147, P148, D150, Y154, D156, W157); Deamidation (Q155, N161)} γS: ([8-174]/178) [43.8] {Deamidation (N77, Q93, Q171); Trp > Hydroxykynurenin (W163); Dioxidation (Y150); Dimethylation (K14)}	
15B	αA: ([50-78]/173) [13.9] αB: ([57-174]/175) [52.6] {Oxidation (D62, M68, R69, H83, F84); Deamidation (N146, Q108); Dimethylation (K166)}	βA3: ([33-44]/215) [6.1] βB1: ([51-252]/252) [48.8] {Methylation + Deamidation (N82); Methylation (C80, S81, N82, R86); Oxidation (C80, M113); Deamidation (N58, N68, N82, N158, Q197, Q205)} βB2: ([90-198]/205) [46.8] {Oxidation (M122, W151, Y154, Y156, M193); Methylation (I109, I110, L111, N114); Acetylation (, K120, K121, K168); Dioxidation (W151); Deamidation (N116, Q147, Q155, Q183, Q185); Methylation + Deamidation (N114)}	γC: ([4-89]/174) [10.9] γD: ([143-163]/174) [12.1] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([73-174]/178) [34.2] {Oxidation (M74, W163)}	
16	αA: ([12-157]/173) [43.9] {Deamidation (Q90, Q104, Q147); Oxidation (D105, D106, H107, Y109) Methylation (I61, S62, R65, S81)} αB: ([57-174]/175) [39.4]	βA3: ([33-109]/215) [13.6] {Deamidation (N103)} βA4: ([14-118]/196) [18.9] {Oxidation (M14, C99); Deamidation (Q23); Methylation + Deamidation (N101)} βB1: ([51-214]/252) [28.6] {Deamidation (N58, N125); Oxidation (C80); Methylation (C80, S81); Methylation + Deamidation (N82)} βB2: ([69-168]/205) [17.6]	γC: ([78-89]/174) [6.9]; γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([42-174]/178) [29.2] {Deamidation (N77, Q93); Oxidation (M74)}	
16A	αA: ([12-157]/173) [46.2] {Deamidation (Q90, N101, Q104, Q147); Acetylation (S66, K70, K88); Oxidation (D105, D106, H107, Y109); Methylation (I61, S62); Methylation + Deamidation (N101, Q104); Dioxidation (Y109)} αB: ([83-149]/175) [20.6] {Deamidation (N146)}	βA3: ([33-137]/215) [25.8] {Oxidation (M46, F48); Deamidation (N40, Q42, N103, N133)} βA4: ([14-118]/196) [40.8] {Deamidation (Q23, Q112, N114); Oxidation (M14, R26, H27, F29, C33, P34, C99); Methylation (H27, C33, S35, L50, S51); Dioxidation (C33)} βB1: ([51-214]/252) [39.7] {Deamidation (N58, N68, Q70, N82, N125, N158, Q205); Methylation (C80, S81); Oxidation (C80); Methylation + Deamidation (N82)}	γD: ([143-152]/174) [5.7] {Oxidation (M147, P148, D150); Deamidation (Q143)} γS: ([42-174]/178) [51.1] {Deamidation (Q64, Q71, Q93, N144, Q171); Acetylation (K159); Oxidation (C115, P116)}	Filensin: ([78-303]/665) [7.4] {Deamidation (Q251)} Phakinin: ([77-89]/415) [3.1] {Deamidation (Q86)}
17	αA: ([12-157]/173) [46.2] {Deamidation (Q90, Q104, Q147); Dimethylation (R65); Oxidation (D105, D106) Acetylation (K70, S66); Methylation (I61, S62, S81)} αB: ([57-103]/175) [20.6]	βA3: ([33-109]/215) [13.6] {Deamidation (Q42, N103)} βA4: ([14-118]/196) [34.2] {Oxidation (M14) Deamidation (Q23, N114)} βB1: ([51-202]/252) [27.0] {Methylation (C80, S81, R86); Oxidation (C80, M113); Deamidation (N58, N68, N125); Methylation + Deamidation (N82)} βB2: ([82-160]/205) [17.1] {Deamidation (N116)}	γC: ([4-77]/174) [14.4] {Trp > Kynurenin (W69)} γD: ([4-163]/174) [15.5] γS: ([73-174]/178) [25.0] {Oxidation (M74)}	Filensin: ([78-90]/665) [2.0] Phakinin: ([77-89]/415) [3.1]
18	αA: ([13-157]/173) [63.6] {Deamidation (Q90, Q147); Oxidation (F53, R54, D105, D106, H107, Y109, H154); Dimethylation (R21, R65); Methylation	βA3: ([33-211]/215) [38.9] {Deamidation (Q203, Q206, Q208); Methylation (S80, C82)} βA4: ([14-192]/196) [13.8]	γC: ([4-152]/174) [12.6] {Oxidation (R10)} γD: ([4-163]/174) [35.6] {Oxidation (M70, M147, P148, D150);	Filensin: ([78-90]/665) [2.0]

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
	(R54, L57, S59, I61, S62, R65, S81); Acetylation (S66, K70)}; αB: ([12-116]/175) [35.4] {Oxidation (D62, M68, R69); Acetylation (K92)}	{Oxidation (M14)} βB1: ([51-214]/252) [38.9] {Methylation (S77, C80, S81); Methylation + Deamidation (N82); Deamidation (N58, N82, N125); Oxidation (C80, N82, M113, F114,) Acetylation (S189)} βB2: ([82-188]/205) [38.0] {Deamidation (N116, Q147)}	Deamidation(N161)} γS: ([8-174]/178) [33.1] {Deamidation (N77, Q93)}	
19	αA: ([12-157]/173) [38.7] {Deamidation (Q147); Dimethylation (R65); Methylation (I61, S62, S81)} αB: ([12-174]/175) [40.6] {Oxidation (D62, M68, R69); Deamidation (N78, Q108)}	βA3: ([96-211]/215) [28.8] {Oxidation (M126); Trp > Kynurenin (W96, W99)} βA4: ([14-118]/196) [24.0] {Trp > Kynurenin (W54)} βB1: ([51-214]/252) [35.7] {Oxidation (C80); Methylation (C80, S81); Methylation + Deamidation (N82); Deamidation (Q70, N158)} βB2: ([69-121]/205) [20.0] {Deamidation (N114)}	γC: ([4-152]/174) [13.8]; γD: ([4-163]/174) [35.1] {Oxidation (C19, H23, M147, P148, D150); Methylation (C19); Deamidation (N161, Q155)} γS: ([73-175]/178) [35.0] {Deamidation (N77)}	
20	αA: ([12-157]/173) [38.7] {Deamidation (Q90, N101, Q147); Methylation + Deamidation (N101, Q104); Oxidation (H107)} αB: ([12-116]/175) [33.1] {Oxidation (D62, M68, R69); Dimethylation (R12); Deamidation (Q108); Acetylation (K92)}	βA3: ([33-211]/215) [34.8] {Oxidation (M126); Deamidation (N103, Q180, Q208)} βA4: ([14-118]/196) [24.0] {Deamidation (Q112)} βB1: ([51-202]/252) [31.0] {Methylation (C80, S81, R86); Methylation + Deamidation (N82); Deamidation (N82, N158); Oxidation (C80, N82, M113, F114,); Acetylation (S189)} βB2: ([109-198]/205) [21.0] {Deamidation (N116, Q147)}	γC: ([4-15]/174) [6.9] {Oxidation (R10)} γD: ([16-152]/174) [31.0] {Oxidation (H23, M70, M147, P148, D150); Deamidation (N25, Q67, Q68, Q143); Methylation (C19, L81, I82); Trp > Kynurenin (W69)} γS: ([8-174]/178) [29.8] {Deamidation (N77, Q171); Dimethylation (K14); Oxidation (M74)}	Filensin ([78-90]/665) [2.0]
21	αA: ([12-157]/173) [61.3] {Methylation (I61, S62, C142, K145); Oxidation (D105, H107, Y109, M138); Deamidation (Q90, N101, Q104, Q147); Acetylation (S66, K70); Dimethylation (R65)} αB: ([57-174]/175) [42.9] {Oxidation (D62, M68, R69)}	βA3: ([33-177]/215) [32.3] {Deamidation (Q38, N40, Q50, N103); Trp > Kynurenin (W73); Trp > Oxolactone (W168); Oxidation (H78, C82, C170); Methylation + Deamidation (Q84, Q172); Methylation (H78, C82, C170)} βA4: ([49-118]/196) [17.9] {Trp > Kynurenin (W54); Deamidation (Q112, N114)} βB1: ([51-214]/252) [36.1] {Deamidation (N58, N82, N125, N158); Methylation (C80, S81, R86, R123, N125); Methylation + Deamidation (N82, N125); Trp > Oxolactone (W124); Oxidation (C80, F114, N125, M137); Acetylation (S189, S190)} βB2: ([49-160]/205) [32.2] {Methylation (C67, K68); Deamidation (N116, Q147, Q155); Oxidation (N66, C67); Methylation + Deamidation (N66)}	γD: ([4-163]/174) [25.3] {Oxidation (M147, P148, D150); Methylation (C19)} γS: ([42-174]/178) [19.1] {Deamidation (Q171)}	
22	αA: ([13-157]/173) [63.6] {Methylation (I96, H97, K99, K145); Deamidation (Q90, Q147); Oxidation (Y109, H107); Acetylation (K70, S66); Dioxidation (Y109); Dimethylation (R54)}; αB: ([57-174]/175) [42.9] {Oxidation (P58, W60, D62, M68, R69); Deamidation (N78, N146); Dioxidation (F113)}	βA3: ([33-211]/215) [32.3] {Oxidation (Y76, H78); Methylation (H78); Deamidation (Q42); Methylation + Deamidation (Q84)} βA4: ([49-118]/196) [17.9] {Deamidation (N114)} βB1: ([51-160]/252) [23.8] {Deamidation (N58, N82, N158); Methylation + Deamidation (N82); Methylation (C80, S81, R86); Oxidation (C80)} βB2: ([109-120]/205) [5.9] {Deamidation (N114)}	γC: ([123-140]/174) [10.3] γD: ([4-152]/174) [9.8] {Oxidation (M147, P148, D150)} γS: ([42-174]/178) [21.3]	Phakinin: ([77-89]/415) [3.1]
23	αA: ([12-157]/173) [51.4] {Dimethylation (R12); Deamidation (Q90, Q104); Methylation (H107)} αB: ([57-174]/175) [18.9] {Oxidation (D62, M68, R69); Dimethylation	βA3: ([33-109]/215) [13.1] βA4: ([49-192]/196) [25.5] {Deamidation (N114)} βB1: ([51-160]/252) [21.0] {Deamidation (N58, Q70, N82,	γC: ([123-140]/174) [10.3] γD: ([4-152]/174) [9.8] {Oxidation (M147, P148, D150)} γS: ([85-174]/178) [22.5] {Oxidation (P160, W163)}	Phakinin: ([77-89]/415) [3.1]

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
	(K166); Phosphorylation (S59)}	N158)} βB2:([109-188]/205) [17.6] {Deamidation (N116, Q163)}		
24	αA: ([12-173]/173) [54.9] {Dimethylation (R21); Deamidation (Q90, Q147); Acetylation (K88, K99, K166); Methylation (S59, I61, S62, R65, S81, H107, I110, L150, H154); Dioxidation (Y109); Oxidation (F80, H100, D106, H107, Y109)} αB: ([57-174]/175) [50.9] {Deamidation (N78, N146); Oxidation (D62, M68, R69, K72, F84, P86, F113); Phosphorylation(S59); Trp- > Kynurenin (W60)}	βA3: ([96-177]/215) [14.6] {Trp > Oxolactone (W168); Methylation (S165, C170); Oxidation (C170); Methylation + Deamidation (Q172)} βA4: ([14-192]/196) [25.5]; βB1 ([61-202]/252) [27.0] {Deamidation (Q70, N158); Acetylation (S189)} βB2: ([82-188]/205) [28.8] {Deamidation (N116, Q147, Q155, Q163)}	γC: ([60-77]/174) [10.3]; γD: ([16-163]/174) [31.0] {Oxidation (C19, M147, P148, D150); Deamidation (Q143, Q155); Methylation (C19)} γS: ([59-174]/178) [30.9] {Deamidation (Q93, Q171); Oxidation (M74)} γS: ([1-116]/120) [45.8]	Phakinin: ([77-89]/415) [3.1]
25	αA: ([12-157]/173) [43.4] {Methylation (S62, R65, I96, H97, K99, I146); Oxidation (D105, D106, H107, Y109); Deamidation (Q90, Q147); Acetylation (K70)} αB: ([12-174]/175) [39.4] {Oxidation (D62, M68, R69, H83, F84); Acetylation (K92)}	βA3: ([96-109]/215) [7.1] βA4: ([49-192]/196) [25.5] {Deamidation (Q63, Q189); Trp > Kynurenin (W54)} βB1: ([51-202]/252) [22.2] {Deamidation (N58, N158); Oxidation (M113, F114); Acetylation (S189)} βB2: ([90-168]/205) [22.9] {Deamidation (N116, Q163)}	γC: ([4-152]/174) [16.7] {Deamidation (Q84, Q149)} γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([85-155]/178) [15.0]	
26	αA: ([13-157]/173) [44.5] {Deamidation (Q90, Q147); Oxidation (Y109); Dimethylation (R65); Methylation (I61, S62); Dioxidation (Y109)} αB: ([12-174]/175) [50.9] {Dimethylation (K166); Oxidation (D62, M68, R69); Phosphorylation (S59); Deamidation (Q108)}	βA3: ([197-211]/215) [7.6] βA4: ([14-192]/196) [19.9] βB1: ([51-252]/252) [36.9] {Acetylation (S189); Deamidation (N58, N68, N158)} βB2: ([109-188]/205) [21.0] {Deamidation (N116, Q147, Q155)}	γC: ([143-152]/174) [5.7] γD: ([4-163]/174) [25.9] {Oxidation (M147, P148, D150); Deamidation (Q143, Q155)} γS: ([42-174]/178) [25.3] {Deamidation (Q171)}	Phakinin: ([77-89]/415) [3.1] {Deamidation (Q86)}
27	αA: ([13-157]/173) [42.8] {Deamidation (Q90, Q147)} αB: ([12-174]/175) [57.7] {Oxidation (D62, M68, R69, H83, F84); Acetylation (K92); Deamidation (Q108)}	βA3: ([96-137]/215) [13.1] βA4: ([49-192]/196) [25.5] βB2: ([69-198]/205) [40.5] {Deamidation (N114, Q147)} βB1: ([51-202]/252) [23.8] {Acetylation (S189)}	γB: ([155-164]/175) [5.7] γD: ([60-163]/174) [21.8] {Oxidation (M147, P148, D150); Dioxidation (W157); Deamidation (Q155)} γS: ([73-174]/178) [34.2] {Deamidation (Q93); Oxidation (M74, Y150)}	
28A	αA: ([13-157]/173) [45.7] {Methylation (I61, S62, R65, S81, Q90, R157); Methylation + Deamidation (Q90, Q104); Deamidation (Q90, Q104); Oxidation (D105); Dimethylation (R65)} αB: ([12-174]/175) [39.4] {Oxidation (D62, M68, R69, H83, F84, P86); Dioxidation (P86, F84); Acetylation (K92)}	βA3: ([96-211]/215) [14.6] βB1: ([51-202]/252) [21.4] {Acetylation (S189)} βB2: ([82-188]/205) [29.3] {Deamidation (N116, Q147, Q163)}	γC: ([4-122]/174) [8.0] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (Q155)}	
28B	αA: ([12-157]/173) [31.2] {Deamidation (Q90)} αB: ([12-174]/175) [46.9] {Oxidation (P58, W60, F61, D62, M68, R69, K72, K166); Trp > Oxolactone (W60); Deamidation (Q108, N78); Dioxidation (F84, P86); Methylation (S59, K166); Acetylation (S66, K90); Trp- > Kynurenin (W60); Trp- > Hydroxykynurenin (W60)}	βA3: ([96-109]/215) [7.1] βB1: ([51-214]/252) [38.9] {Deamidation (N58, Q70, N125, N158, Q205); Methylation (C80, S81); Oxidation (C80, M113, F114, M137); Methylation + Deamidation (N82); Trp- > Oxolactone (W124)} βB2: ([69-198]/205) [54.6] {Oxidation (M122); Deamidation (N116, Q147, Q155, N114); Phosphorylation (S93)}	γC: ([49-152]/174) [22.4] {Deamidation (Q143); Oxidation (D74)} γD: ([60-163]/174) [22.4] {Oxidation (D74, M147, P148, D150); Deamidation (Q155, N161); Trp- > Oxolactone (W157); Methylation + Deamidation (Q143, Q155); Methylation (Q143, Q155)} γS: ([159-174]/178) [13.3]	Filensin: ([78-90]/665) [2.0]
29	αA: ([12-157]/173) [61.3] {Acetylation (S66, K70, K78, K99); Dioxidation (K88, R103, Y109, M138); Deamidation (Q90, N101, Q104, Q147); Oxidation (D105, D106, H107, Y109); Methylation + Deamidation (N101, Q104); Methylation (H79, S81)} αB: ([57-150]/175) [32.6] {Deamidation (Q108); Acetylation (K150); Oxidation (N146)}	βA3: ([33-177]/215) [61.1] {Methylation + Deamidation (N54, Q84, N103, Q164, Q172); Deamidation (Q38, N40, Q42, N54, N62, N103, N133, Q164, Q172); Acetylation (K131, S147); Oxidation (R45, M46, F48, C52, Y76, H78, C82, Y105, H106, M151, F154, N155, W168, C170, Y171); Dioxidation (W153, F154); Methylation (S51, C52, R58, H78, S80, N103, H106, I107, Q164, S165, C170); Trp > Oxolactone (W99,	γD: ([143-152]/174) [5.7] {Deamidation (Q143) γS: ([132-174]/178) [25.8] {Deamidation (N144); Dioxidation (K159)}	Phakinin: ([319-339]/415) [5.1] {Oxidation (H322, N323, C326); Methylation (H322, S325); Methylation + Deamidation (Q327)}

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
		W168); Trp- > Kynurenin (W73, W139, W168); Trp- > Hydroxykynurenin (W153)} βA4: ([14-118]/196) [12.2] {Oxidation (M14) Deamidation (Q23, Q112)} βB1: ([51-202]/252) [24.2] {Deamidation (N58, Q70, N82, N158); Methylation (C80, S81); Acetylation (S189); βB2: ([49-160]/205) [22.9] {Deamidation (N116, Q147, Q155); Oxidation (N66, C67); Methylation + Deamidation (N66)}		
30	αA:([12-157]/173) [61.3] {Acetylation (S66, K70); Deamidation (Q90, N101, Q104, Q147); Oxidation (H107, F80, Y109, H79, D106); Methylation (I61, S62, R65, H79, H107, I110, S111, K145); Dimethylation (R65)} αB: ([57-149]/175) [33.1] {Deamidation (N146); Oxidation (M68, R69)}	βA3: ([33-177]/215) [58.1] {Oxidation (R45, M46, F48, C52, Y76, H78, F81, C82, D143, D144, Y145, P146, F154, N155, N156, K162, W168, C170, Y171); Deamidation (Q38, N40, Q42, N54, Q84, N103, Q138, Q149, Q164, Q172, Q180, N214); Methylation + Deamidation (N54, Q84, Q172); Methylation (S51, C52, R58, H78, S80, C170); Trp > Oxolactone (W168); Trp > Kynurenin (W73); Trp > Hydroxykynurenin (W168); Dioxidation (F81); Acetylation (K131)} βA4: ([14-192]/196) [19.9] {Oxidation (M14); Deamidation (Q23); Dimethylation (K118)} βB2: ([109-120]/205) [5.9] {Deamidation (N114)}	γD: ([143-152]/174) [5.7] {Deamidation (Q143)} γS: ([159-174]/178) [13.3] {Deamidation (Q171)}	
31	αA:([12-157]/173) [41.0] αB: ([12-174]/175) [61.7] {Oxidation (P58, W60, F61, D62, M68, R69, K72, H83, F84, P86, H111, F113); Acetylation (K90, K92, K166); Deamidation (N78, Q108, N146); Dimethylation (R12, K103, K166); Dioxidation (W60, F61, F84, P86,); Methylation (K166); Trp > Kynurenin (W60)}	βA3: ([33-211]/215) [36.4] {Oxidation (M126); Deamidation (N103, Q203, Q206, Q208)} βA4: ([49-118]/196) [24.5] {Oxidation (C99); Methylation + Deamidation (N101)} βB1: ([51-214]/252) [30.2] {Methylation (L61); Deamidation (N158); Oxidation (F64, M113)} βB2: ([82-168]/205) [26.8] {Deamidation (N116, Q163)} βB3: ([76-195]/211) [20.4]	γB: ([60-164]/175) [16.0] {Oxidation (D74); Trp > Hydroxykynurenin (W69)} γC: ([60-163]/174) [28.7] {Oxidation (D74); Methylation (C154); Deamidation (Q84, Q143); Methylation + Deamidation (Q155); Trp > Oxolactone (W158); Trp > Hydroxykynurenin (W69)} γD: ([60-163]/174) [21.8] {Deamidation (Q67, Q68, Q143, N161); Oxidation (M70, M147, D150, P148,)} γS: ([1-116]/120) [45.8] {Deamidation (N15)}	Filensin: ([78-90]/665) [2.0]
32	αA: ([12-157]/173) [60.7] {Oxidation (H79, F80, F93, H97, D106, H107, Y109, C131, D136, M138, P144); Methylation (I61, S62, H79, Q126, S127, S130, L129, C131, C142, K145); Deamidation (Q90, Q147); Dioxidation (Y109, M138); Acetylation (K78); Methylation + Deamidation (Q104)} αB: ([57-69]/175) [8.4] {Oxidation (D62, M68, R69)}	βA3: ([33-177]/215) [58.1] {Methylation + Deamidation (N103, Q172); Trp > Oxolactone (W168); Deamidation (Q38, Q42, N103); Methylation (Q42, N103, H106, C170); Trp > Kynurenin (W73); Oxidation (N155, C170, Y171, W168)} βA4: ([14-192]/196) [41.8] {Methylation (H27, C33, S35); Oxidation (R26, H27, C33); Deamidation (Q23, Q112, N114)} βB2: ([90-160]/205) [19.0] {Deamidation (N114, N116, Q155)}	γD: ([118-140]/174) [13.2] {Trp > Kynurenin (W131)} γS: ([159-174]/178) [13.3] {Deamidation (Q171)}	
33	αA: ([1-157]/173) [65.3] {Dimethylation (R12); Oxidation (M1, D2, C131, D136, M138, P144,); Deamidation (Q90, N123, Q126, Q147); Dioxidation (F80, P82, F93); Methylation (S62, R65, H97, K145); Methylation + Deamidation (Q126); Acetylation (S66, K70, C131, S172)} αB: ([12-149]/175) [49.1] {Deamidation (N146); Oxidation (F84); Phosphorylation(S59); Trp- > Kynurenin	βA3: ([33-193]/215) [53.5] {Acetylation (K131); Oxidation (M46, F48, Y76, H78, C82, H106); Deamidation (Q38, N40, Q42, N103, N133, Q172); Methylation (C52, R58, S80, N103, C170, C185, H78, S51, H187, H106, S50, I107); Methylation + Deamidation (N54, Q84, N103); Trp > Oxolactone (W99)} βA4: ([107-118]/196) [6.1] βB1:	γS: ([159-174]/178) [13.3]	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
	(W60)}	([51-72]/252) [8.7]		
34	αA: ([12-157]/173) [46.8] {Deamidation (Q90); Methylation (S59, I61, S62, R65, H79, S81, H107, I110, S111); Dioxidation (Y109); Oxidation (D105, D106, H107, Y109); Dimethylation (R65, R112)} αB: ([57-174]/175) [23.4] {Oxidation (M68, R69, F113); Phosphorylation (S59)}	βA3: ([33-211]/215) [42.9] {Oxidation (M46, F48, C170, Y171); Trp > Oxolactone (W168); Methylation (S165, C170); Deamidation (Q42); Methylation + Deamidation (Q172)} βA4: ([8-71]/196) [20.9] {Acetylation (K13)} βB1: ([51-202]/252) [25.8] {Acetylation (S189); Deamidation (N158)} βB2: ([109-168]/205) [17.1] {Deamidation (N116, Q147, Q155, Q163)}	γC: ([60-122]/174) [14.4] γD: ([143-163]/174) [11.5] {Deamidation (N161)} γS: ([73-174]/178) [37.5] {Oxidation (M74)}	Phakinin ([77-89]/415) [3.1]
35A	αA: ([12-157]/173) [63.6] {Acetylation (S66, K70); Oxidation (F93, C131, D136, M138, P144); Dioxidation (M138); Deamidation (Q90, N123, Q126); Methylation (H79, C131, C142, K145,)} αB: ([12-174]/175) [54.3] {Oxidation (D62, M68, R69, H83, F84); Dioxidation (M68, R69,); Deamidation (Q108, N146,) Phosphorylation (S59)} ([12-174]/175) [54.3] {Oxidation (D62, M68, R69, H83, F84); Dioxidation (R69, M68); Deamidation (Q108, N146); Phosphorylation (S59)}	βA3: ([33-177]/215) [45.5] {Oxidation (R45, M46, F48, Y76, H78, C82, M126, W168, C170, Y171); Methylation (R45, H78, S100, I128); Methylation + Deamidation (Q172); Trp- > Kynurenin (W73); Trp- > Oxolactone (W96); Deamidation (Q42, Q164, Q180)} βA4: ([107-118]/196) [6.1] βB2: ([90-168]/205) [22.9] {Deamidation (N114, N116, Q155, Q163)}	γC: ([60-122]/174) [14.4] {Oxidation (D74)} γD: ([4-152]/174) [9.8] {Oxidation (M147, P148, D150)} γS: ([132-174]/178) [25.8] {Deamidation (N144)}	
35B	αA: ([12-157]/173) [44.5] {Deamidation (Q90, Q104, Q147); Methylation (S62, R65, H79); Oxidation (R12)} αB: ([12-116]/175) [38.9] {Oxidation (P58, W60, F61, D62, M68, R69); Acetylation (K90, K92,); Dioxidation (W60, F61); Phosphorylation(S59); Deamidation (N78)}	βA3: ([33-211]/215) [53.0] {Oxidation (H78, C82, W168, C170, Y171); Deamidation (N103, Q164, Q172); Methylation (S51, C52, R58, S100, H201); Methylation + Deamidation (N54, Q84, Q172); Trp > Oxolactone (W168, W198); Dioxidation (W198)} βA4: ([14-192]/196) [40.3] {Oxidation (C99); Deamidation (Q23, Q189)} βB1: ([111-202]/252) [16.7] {Acetylation (S189)} βB2: ([90-160]/205) [19.0] {Deamidation (N116, Q147); Trp > Kynurenin (W151)}	γC: ([4-122]/174) [8.0]) γD: ([143-163]/174) [12.1] {Oxidation (M147, D150); Deamidation (Q143)} γS: ([42-174]/178) [23.0] {Methylation + Deamidation (Q171); Oxidation (F173)}	
36	αA: ([13-157]/173) [40.5] {Deamidation (Q90, Q104)} αB: ([12-174]/175) [62.9] {Deamidation (N78, Q108, N146); Oxidation (F61, D62, M68, R69); Dioxidation (W60, F61, M68, R69, F84, P86); Dimethylation (K166); Acetylation (K92); Phosphorylation (S59)} ([12-174]/175) [62.9] {Deamidation (N78, Q108, N146); Oxidation (M68, R69, F61, D62); Dioxidation (W60, F61, F84, P86, M68, R69); Dimethylation (K166); Acetylation (K92); Phosphorylation (S59)}	βA3: ([33-211]/215) [49.5] {Deamidation (Q42, N103, Q164, Q172, Q180, Q203, Q206, Q218); Trp > Oxolactone (W168); Methylation (S51, C52, R58, C170); Methylation + Deamidation (N54, Q172); Oxidation (C52, C170, Y171); Acetylation (K131)} βA4: ([49-118]/196) [17.9] {Deamidation (Q63, Q155)} βB1: ([188-214]/252) [10.7] {Acetylation (S189)} βB2: ([82-188]/205) [38.0] {Deamidation (N116, Q138, Q147)}	γC: ([78-89]/174) [6.9] {Deamidation (Q84); Methylation (C80)}; γD: ([16-163]/174) [31.6] {Oxidation (Y17, C19, M147, P148, D150); Methylation (C19); Deamidation (N25, Q68, N161,)} γS: ([42-174]/178) [15.2] {Deamidation (Q171)}	
37	αA: ([12-157]/173) [38.7] {Deamidation (N101, Q104)} αB: ([12-174]/175) [63.4] {Oxidation (W60, F61, D62, M68, R69, H83, F84); Deamidation (Q108, N146); Dimethylation (R12, R69, R116); Dioxidation (W60, F61, F84, P86, F113); Methylation (L65, S66, I114, S115); Trp > Kynurenin (W60); Phosphorylation (S59, S76); Acetylation (K92)}	βA3: ([33-177]/215) [39.9] {Methylation (H78, S80, C82); Deamidation (N103, N133, Q172); Oxidation (D97); Dioxidation (M126)} βA4: ([49-192]/196) [40.3] {Oxidation (C99, W179, H182); Methylation (C99, C166); Deamidation (N114); Trp- > Kynurenin (W179); Methylation + Deamidation (N101)} βB1: ([51-132]/252) [20.6] {Deamidation (N68, N125)} βB2: ([82-121]/205) [15.6] {Deamidation (N114, N116)}	γC: ([4-152]/174) [24.1] γD: ([4-152]/174) [9.8] {Deamidation (Q143)}; γS: ([8-174]/178) [43.3] {Oxidation (M74); Dimethylation (K14)}	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
38	αA: ([12-157]/173) [50.9] {Deamidation (N123, Q126); Acetylation (K70); Oxidation (C131)} αB: ([12-174]/175) [61.7] {Oxidation (P58, W60, F61, D62, M68, R69, H83, F84); Dioxidation (M68, R69, F84, P86); Acetylation (K90, K92); Deamidation (N78, Q108)}	βA3: ([33-211]/215) [46.0] {Dioxidation (W198); Deamidation (Q42, N103, Q172, Q203, Q206, Q208); Oxidation (D97, C170, W198, H201); Trp > Oxolactone (W96, W168); Trp > Kynurenin (W73); Methylation (S80, S100, C170); Methylation + Deamidation (Q172)} βA4: ([107-118]/196) [6.1] βB1: ([61-202]/252) [18.3] {Acetylation (S189); Deamidation (N125); Trp > Oxolactone (W124)} βB2: ([109-160]/205) [13.2] {Deamidation (Q147, Q155)}	γC: ([4-152]/174) [9.8] γD: ([4-163]/174) [15.5] γS: ([42-174]/178) [15.2]	
39	αA: ([12-157]/173) [35.3] {Oxidation (D105)} αB: ([1-174]/175) [53.1] {Oxidation (D62, M68, R69, K72); Acetylation (K90, K92); Deamidation (Q108); Dioxidation (F84, P86)}	βA3: ([33-211]/215) [34.8] {Dioxidation (W198); Oxidation (M126, W198, H201); Methylation (C185); Deamidation (Q38, Q206)} βA4: ([107-192]/196) [13.8] {Deamidation (Q112)} βB1: ([73-214]/252) [20.2] {Deamidation (Q197, Q205)}; βB2: ([69-160]/205) [21.0] {Deamidation (N114, N116)}	γC: ([4-152]/174) [16.7] {Deamidation (Q84)} γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N161)} γS: ([8-174]/178) [27.0] {Deamidation (N77); Dimethylation (K14); Oxidation (M74)}	Filensin: ([78-90]/665) [2.0]
40	αA: ([12-157]/173) [46.2] {Deamidation (Q90)} αB: ([1-174]/175) [69.1] {Oxidation (D2, P58, W60, F61, D62, M68, R69, H83, F84, P86); Deamidation (Q108, N146); Methylation (S19, S21, L65, S66, I114, S115); Dimethylation (R12, R22, R69, R116, K166); Dioxidation (F84, F113); Acetylation (S66, K90)}	βA3: ([33-211]/215) [42.4] {Dioxidation (W198); Oxidation (W168, C170, Y171, W198, H201); Methylation (C185); Deamidation (Q42, N103, Q203, Q206, Q208); Methylation + Deamidation (Q172); Dimethylation (R211); Trp] > Oxolactone (W168); Acetylation (K131)} βA4: ([49-192]/196) [25.5] {Deamidation (Q189)} βB1: ([61-214]/252) [19.4] {Acetylation (S189)} βB2: ([109-198]/205) [26.3] {Deamidation (N114, N116)}	γC: ([4-152]/174) [31.0] {Oxidation (M70, D74)} γD: ([4-152]/174) [20.1] {Oxidation (M147, P148, D150)} γS: ([8-174]/178) [19.7] {Deamidation (Q171); Oxidation (M74)}	
41A	αA: ([13-157]/173) [45.7] {Oxidation (Y109); Deamidation (Q90); Acetylation (K70)} αB: ([12-174]/175) [46.9] {Oxidation (P58, W60, F61, D62, M68, R69, K72, F84); Dioxidation (R69, M68, F61, W60); Acetylation (K92); Deamidation (N78, Q108)}	βA3: ([33-211]/215) [27.3] {Dioxidation (W198); Oxidation (M126, W198, H201); Deamidation (N103); Methylation (H201); Methylation + Deamidation (Q203)} βA4: ([14-71]/196) [17.9] βB1: ([51-214]/252) [30.2] {Deamidation (N58, Q197); Acetylation (S189)} βB2: ([69-188]/205) [21.5] {Deamidation (N116)}	γB: ([4-164]/175) [9.7] γC: ([4-152]/174) [9.8] γD: ([4-163]/174) [15.5] {Oxidation (M147, P148, D150); Deamidation (N77); Dimethylation (K14)} γS: ([8-175]/178) [33.7]	Filensin: ([78-90]/665) [2.0]
41B	αA: ([12-157]/173) [46.8] {Acetylation (K70)}; αB: ([12-174]/175) [46.9] {Oxidation (P58, W60, F61, D62, M68, R69, H83, F84); Dioxidation (W60, F61, M68, R69, K72,) Deamidation (N78, Q108); Methylation (I98, H101, K166); Acetylation (K92)}	βA3: ([33-44]/215) [6.1] βA4: ([49-71]/196) [11.7] βB1: ([51-214]/252) [38.5] {Deamidation (N58, Q70, N158, Q197) Oxidation (M113, F114)} Acetylation (S189)} βB2: ([82-198]/205) [50.7] {Oxidation (K121, M122, D126, W151, Y154, M193); Deamidation (N114, N116, Q147, Q163)}	γC: ([143-152]/174) [5.7] {Deamidation (Q149)} γD: ([4-163]/174) [15.5]	Phakinin: ([77-89]/415) [3.1]
42	αA: ([12-157]/173) [46.8] {Dimethylation (R12); Deamidation (Q90); Methylation (S62, R65, S81, R157,); Acetylation (S66, K70,); Dioxidation (Y109); Oxidation {D58)} αB: ([57-90]/175) [13.5] {Oxidation (M68, R69)}	βA3: ([33-211]/215) [55.6] {Deamidation (N103); Oxidation (C52, H78, C82, H106); Methylation (S51, C52, R58, H78, S80, C82, C170); Trp > Oxolactone (W99, W168); Methylation + Deamidation (N40, Q42, N54, Q172)} βA4: ([14-192]/196) [60.2] {Methylation (H27, C33, S35, L50, S51, L107, I109); Oxidation (M14, R26, H27, F29, C33, P34, W179, H182); Deamidation (Q23, Q112,	γC: ([4-122]/174) [25.3] γD: ([4-152]/174) [9.8] γS: ([42-174]/178) [15.2]	Phakinin: ([77-89]/415) [3.1]

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
		N114, Q189); Dioxidation (C33, P34, W179); Trp > Kynurenin (W77); Trp > Hydroxykynurenin (W80)} βB1: ([51-214]/252) [32.1] {Deamidation (N68, Q205)} βB2: ([82-168]/205) [13.7] {Deamidation (N116, Q163)}		
43	αA: ([12-157]/173) [46.2] {Acetylation (K70, K99); Deamidation (Q90, Q147); Oxidation (H107, Y109); Methylation (H100, L150, H154); Dimethylation (R163)} αB: ([57-174]/175) [22.9] {Oxidation (D62, M68, R69)}	βA3: ([33-211]/215) [39.9] {Deamidation (Q42, N103, Q203, Q206, Q208); Trp- > Oxolactone (W168); Oxidation (C170); Methylation (C170)} βA4: ([8-192]/196) [69.9] {Methylation (H27, C33, S35, L50, S51, H59, L107, I109, Q112, C166, S181, H182); Deamidation (Q23, Q63, N101, Q112, N114, Q187, Q189); Oxidation (M14, W17, R26, H27, F29, C33, P34, Y67, F94, C99, F110, W179, H182); Trp > Oxolactone (W54, W179); Phosphorylation (T30, S42); Trp > Kynurenin (W54, W77, W80, W179); Dioxidation (M14, C33, P34, C99); Acetylation (K118); Trp > Hydroxykynurenin (W12, W80); Methylation + Deamidation (Q112)} βB1: ([51-214]/252) [38.1] {Oxidation (M137); Methylation (C80); Deamidation (N125)} βB2: ([82-120]/205) [9.8]	γC: ([38-89]/174) [19.5] γD: ([143-152]/174) [5.7] γS: ([8-174]/178) [35.4] {Deamidation (Q171)}	
44	αA: ([12-157]/173) [61.3] {Acetylation (S66, K70, K88, K99); Methylation (S130, C131, K145); Oxidation (C131, M138, P144); Deamidation (Q90, Q147); Methylation + Deamidation (N101, Q104)} αB: ([83-174]/175) [25.7] {Deamidation (N146); Dimethylation (K166)}	βA3: ([33-177]/215) [38.9] {Methylation + Deamidation (Q172); Oxidation (M46, F48, H78, W168, C170); Deamidation (Q42, N103, Q172); Trp > Oxolactone (W168); Methylation (H78, C170)} βA4: ([14-192]/196) [58.7] {Methylation (H27, C33, S35, L50, S51, H59, L107, I109, Q112, C166, H168, K174, S181, H182); Deamidation (Q23, N101, Q112, N114, Q189); Oxidation (M14, R26, H27, F29, C33, P34, W54, F57, F94, C99, C166); Dimethylation (R26, K118); Trp > Kynurenin (W54); Trp > Oxolactone (W54, W179); Trp- > Hydroxykynurenin (W54, W179); Methylation + Deamidation (Q112)} βB1: ([61-202]/252) [17.9] {Deamidation (N68)} βB2: ([109-120]/205) [5.9] {Deamidation (N116)}	γC: ([4-140]/174) [14.4] {Trp- > Hydroxykynurenin (W131)} γD: ([118-163]/174) [19.0] {Deamidation(N161)} γS: ([8-174]/178) [31.5] {Acetylation (K159); Deamidation (N144); Oxidation (M74)}	
45	αA: ([12-157]/173) [46.2] {Deamidation (Q90, Q147); Methylation + Deamidation (Q50, Q90, N101, Q104); Phosphorylation (T148); Acetylation (K99); Oxidation (F93, H107, Y109); Dimethylation (R12); Dioxidation (Y109)} αB: ([57-174]/175) [38.3] {Deamidation (Q108); Dioxidation (P58)}	βA3: ([33-211]/215) [46.0] {Oxidation (W168, C170, Y171); Trp > Oxolactone (W168); Methylation + Deamidation (Q172); Deamidation (Q42, Q206, Q208); Methylation (C170)} βA4: ([8-192]/196) [71.4] {Methylation (H27, C33, C99, Q112, L164, C166, H168, S170); Deamidation (Q23, Q63, N83, N101, Q112, N114Q187, Q189); Oxidation (M14, R26, H27, F29, C33, P34, W54, F57, C99, C166, H182, P184); Trp > Kynurenin (W17, W54, W179); Trp > Hydroxykynurenin (W12); Dioxidation (M14, W179); Methylation + Deamidation (N101)} βB2: ([146-160]/205) [7.3] {Deamidation (Q147)}	γC: ([123-152]/174) [16.1] {Trp > Oxolactone (W131); Methylation (L127, C130, H125); Deamidation (Q143)} γD: ([118-163]/174) [19.0] {Trp > Kynurenin (W131)} γS: ([21-174]/178) [64.0] {Deamidation (Q64, Q71, Q93, N144, Q171); Oxidation (M74); Acetylation (C23, C25, C27)}	

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	**β-crystallin**	**γ-crystallin**	**Filensin/Phakinin**
46	αA: ([12-157]/173) [61.3] {Methylation (L57, S59, I61, S62, R65, H79, S130, K145, I146, L150, H154, R157); Oxidation (D106, H107, Y109, M138, D151, H154); Dimethylation (R21, R65); Methylation + Deamidation (Q147); Deamidation (Q90, Q147); Dioxidation (Y109, C131)} αB: ([57-174]/175) [44.6] {Oxidation (M68, R69); Dimethylation (K166)}	βA3: ([33-177]/215) [21.2] {Deamidation (N40, Q42); Trp > Oxolactone (W168); Methylation (C170); Methylation + Deamidation (Q172); Oxidation (C170)} βA4 ([14-192]/196) [65.8] {Methylation (C33, S35, L107, I109); Oxidation (M14, H27, P34, C99,); Deamidation (Q23, N114, Q189); Methylation + Deamidation (N101)} βB1: ([111-202]/252) [9.1] βB2: ([109-188]/205) [17.6]	γD: ([143-163]/174) [11.5] γC: ([4-89]/174) [21.3] γS: ([85-174]/178) [22.5]	
47	αA: ([12-157]/173) [58.4] {Oxidation (H79, F80, P82, Y109, C131, M138); Methylation (I146, Q147); Methylation + Deamidation (Q147); Deamidation (Q90, Q104, Q147)} αB: ([12-174]/175) [60.0] {Oxidation (M68, R69); Deamidation (N146)};	βA3: ([33-211]/215) [28.3] {Deamidation Q42, Q172); Oxidation (W198)} βA4: ([8-192]/196) [69.9] {Deamidation (Q23, Q63, N83, N101, Q112, N114, Q153} βB1: ([73-214]/252) [27.0] {Oxidation (C80); Deamidation (Q197, Q205); Methylation (C80, S81); Methylation + Deamidation (N82)} βB2: ([91-188]/205) [26.3] {Deamidation (N114, N116, Q147, Q155, Q185); Trp > Kynurenin (W151); Methylation (C33, I109, N114); Oxidation (M1, F27, N114, H181); Methylation + Deamidation (N114)}	γC: ([60-152]/174) [33.3] {Methylation (H125, L127, C130); Oxidation (M70, D74, C130, W131); Trp > Kynurenin (W131); Trp- > Oxolactone (W131); Deamidation (Q143); Trp > Hydroxykynurenin (W69)} γD: ([4-163]/174) [28.7] {Dioxidation (W157); Oxidation (Y17, Y144, M147, P148, D150, Y151); Methylation (H16, C19); Deamidation (Q143, Q155, N161)} γS: ([8-174]/178) [59.0] {Oxidation (Y109, P116, R125, W137, F139); Deamidation (Q71, N77, Q171)}	Phakinin: ([77-89]/415) [3.1]
48	αA: ([12-157]/173) [46.2] {Deamidation (Q90, Q104, Q147); Methylation (S62, R65, H79, H100) Dimethylation (R103)}; αB: ([12-174]/175) [45.1] {Oxidation (P58, W60, F61, D62, M68, R69); Dimethylation (K166); Acetylation (K92); Methylation (K166); Phosphorylation (S59); Deamidation (N78); Trp- > Kynurenin (W60)}	βA3: ([33-211]/215) [42.4] {Trp > Oxolactone (W168); Deamidation (N40, Q42, Q172); Methylation + Deamidation (Q172); Methylation (C170); Oxidation (C170)} βA4: ([14-192]/196) [68.4] {Oxidation (M14, W54, F57, C99); Trp > Kynurenin (W77, W179); Methylation (L107, I109, C166, H168); Deamidation (Q23, Q112, N114, Q189i); Dimethylation (R26); Trp > Hydroxykynurenin (W80); Methylation + Deamidation (Q23, Q112)} βB1: ([61-230]/252) [38.1] {Acetylation (S189); Methylation + Deamidation (N82); Methylation (C80, S81, R86); Deamidation (Q197, Q205); Oxidation (C80, M226)} βB2: ([90-168]/205) [22.9] {Deamidation (N116, Q147, Q155, Q163)} βB3: ([97-115]/211) [9.0]	γB: ([4-164]/175) [22.9] {Oxidation (R10, M70, D74)} γC: ([4-163]/174) [46.6] {Methylation (S78, C79, C80, L81, C154); Oxidation (R10, M70, D74, M160, D161); Methylation + Deamidation (Q155); Deamidation (Q143)} γD: ([4-163]/174) [36.2] {Deamidation (Q143, Q155, N161); Oxidation (Y17, M147, P148, D150); Methylation (H16, C19); Dioxidation(W157)} γS: ([8-174]/178) [57.3] {Deamidation (Q71, N77, Q93, Q171); Oxidation (M74, M108, Y109, D114, P116, M124, R125, F136, D162, W163); Methylation + Deamidation (Q107)}; ([1-116]/120) [65.8] {Deamidation (Q13, N15); Oxidation (D56, Y58, F104); Methylation + Deamidation (Q149)}	
49	αA: ([13-157]/173) [59.0] {Deamidation (Q90, Q104); Methylation (S62, R65, L150, H154); Oxidation (M138)} αB: ([12-174]/175) [60.6] {Oxidation (P58, W60, F61, D62, M68, R69, K72); Dioxidation(R69, M68, K72); Dimethylation (K166); Deamidation (N78, Q108)}	βA3: ([33-211]/215) [35.4] {Deamidation (Q42, N103); Oxidation (R45, M46, F48)} βA4: ([91-192]/196) [20.9] {Deamidation (Q112, N114, Q189); Oxidation (C99) βB1: ([61-214]/252) [22.6] {Deamidation (N158, Q197, Q205)} βB3: ([97-167]/211) [16.1] {Deamidation (N155)}	γB: ([60-164]/175) [16.0] {Trp- > Kynurenin (W69)} γD: ([4-163]/174) [16.1] {Oxidation (M147, P148, D150); Deamidation (Q155, N161, N163); Phosphorylation (Y154); Methylation (R153)} γS: ([42-174]/178) [33.1] {Deamidation (Q171)}	Filensin: ([78-90]/665) [2.0]
50	αA: ([13-157]/173) [40.5] {Methylation (S62, R65); Deamidation (Q90, Q147)} αB: ([12-174]/175) [44.6] {Dioxidation (W60, F61, M68, R69); Oxidation (P58, W60, F61, D62, M68, R69); Acetylation (K90, K92); Phosphorylation (S59); Deamidation (Q108)}	βA3: ([33-211]/215) [34.3] {Dioxidation (W198); Oxidation (W198, H201); Deamidation (N103)} βA4: ([14-192]/196) [46.9] {Methylation (C99, L107, C166); Trp > Kynurenin (W179); Oxidation (M14, C99); Deamidation (Q189); Methylation + Deamidation (N101)}	γB: ([4-164]/175) [9.7] γC: ([4-152]/174) [16.7] {Deamidation (Q143)} γD: ([4-163]/174) [26.4] {Deamidation (Q143, Q155, N161)} γS: ([8-174]/178) [34.8] {Oxidation (P160, W163); Dimethylation (K14)}	Filensin: ([78-90]/665) [2.0]

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Table 4 *(continued)*

Spot number	Protein ([Fragment]/Length), [Coverage (%)], {Modification Type (amino acid)}			
	α-crystallin	β-crystallin	γ-crystallin	Filensin/Phakinin
		βB1: ([51-202]/252) [20.2] {Deamidation (N58, Q197); Oxidation (M137)} βB2: ([109-168]/205) [9.8] {Deamidation (N114, Q163)} βB3: ([76-83]/113) [7.1]		
52	αA: ([13-157]/173) [30.1] {Deamidation (Q90)} αB: ([1-175]/175) [69.7] {Oxidation (M1, D2, D62, M68, R69, H83, F84); Deamidation (N78, N146); Acetylation (K92); Dimethylation (K166); Methylation (K166); Dioxidation (P16)}	βA3: ([33-211]/215) [20.7] {Deamidation (N103, Q208)} βA4: ([14-25]/196) [6.1] βB1: ([61-202]/252) [17.5] {Deamidation (Q197)} βB2: ([90-188]/205) [23.4] {Deamidation (Q183)} βB3: ([73-96]/113) [16.8]	γB: ([4-164]/175) [9.7] γC: ([4-152]/174) [16.7] {Deamidation (Q84, Q143)} γD: ([4-163]/174) [25.3] {Methylation (C19, S20); Oxidation (Y17, H23, M147, D150, Y154, D156, W157); Deamidation (N25)} γS: ([8-174]/178) [36.0] {Oxidation (M74); Deamidation (Q17, N77, Q171)}	
53	αA: ([1-157]/173) [37.0] {Phosphorylation (T4)} αB: ([12-175]/175) [45.7] {Oxidation (P58, W60, F61, D62, M68, R69); Dioxidation (M68, R69); Dimethylation (K103, K166); Methylation (K166); Trp > Oxolactone (W60); Deamidation (N78, Q108)}	βA3: ([96-211]/215) [14.6] βA4: ([14-192]/196) [25.5] βB1: ([51-214]/252) [25.4] {Oxidation (M113)} βB2: ([82-168]/205) [21.5] {Deamidation (Q155, Q163)} βB3: ([76-96]/113) [14.2]	γB: ([4-164]/175) [9.7] γC: ([4-152]/174) [16.7] {Methylation (C79, C80)} γD: ([4-163]/174) [25.9] {Oxidation (D74, M147, D150); Deamidation (N161); Methylation + Deamidation (Q143); Methylation (Q143)} γS: ([8-174]/178) [36.0] {Oxidation (M74)}	
54	αA: ([13-157]/173) [35.8] αB: ([12-175]/175) [47.4] {Oxidation (P58, W60, F61, D62, M68, R69, K72, K166); Dioxidation (F61, R69, M68,); Deamidation (N78, Q108); Dimethylation (K166); Methylation (K166)} ([12-175]/175) [47.4] {Oxidation (P58, W60, F61, D62, M68, R69, K72, K166); Dioxidation (F61, R69, M68,); Deamidation (N78, Q108,); Dimethylation (K166); Methylation (K166)}	βA3: ([33-109]/215) [13.1] βA4: ([14-192]/196) [19.9] {Oxidation (M14); Deamidation (Q187)} βB1: ([51-230]/252) [45.2] {Deamidation (N125, N158, Q197, Q205); Oxidation (M113, F114, W127, M137, M226, R230); Acetylation (S189); Trp- > Oxolactone (W124)} βB2: ([82-188]/205) [46.3] {Deamidation (N114, N116, Q147, Q155, Q163); Oxidation (M122)} βB3: ([73-167]/211) [21.8] {Methylation (L116, L118); Deamidation (N155, N94)}	γB: ([4-164]/175) [14.3]; γC: ([4-152]/174) [16.7]; γD: ([16-163]/174) [37.4] {Dioxidation (W157); Oxidation (M147, P148, D150, Y154, D156, W157, M70); Deamidation (N161, Q155); Methylation (C19); Trp > Oxolactone (W157)} γS: ([8-174]/178) [29.8] {Deamidation (N77, Q171); Oxidation (M74)}	Phakinin: ([77-89]/415) [3.1]

gel, and then at 15 W/gel for 6 h.

The individual spots from 2D-gels were excised, destained, and digested with trypsin before QTRAP analysis using an ABI 4000 QTRAP LC-MS/MS Mass Spectrophotometer (Applied Biosystem). During the analysis, 5 μl per sample containing tryptic peptides was injected into the spectrophotometer and eluted off of a capillary C-18 reverse-phase column using an H_2O/acetonitrile gradient, then fragmented in the QTRAP. Columns were washed between sample analyses. The spectra were analyzed using mzWiff (Seattle Proteome Center [SPC]), which requires the Analyst library (Analyst 1.4.2 software [Applied Biosystems MDS Sciex]) and the resulting peptide masses were identified using the Trans-Proteomic Pipeline (TPP). The peptide masses of each spot were submitted several times to the TPP in order to identify the different post-translational modifications (PTMs). Next, the search results were combined into a single ProteinProphet output file, which was further refined by an in-house application. Briefly, the program outputted an Excel sheet (Tables 1–4) contained the spot number their peptide compositions and lengths, the crystallins to which they belonged, 'UniRef number', probability, peptide percent coverage of a crystallin, and the peptide's sequence and sequence "number of sibling's pair" adjusted probability, the type of modification and the position of the modified amino acid. Only those peptides are reported in Tables 1–4, which had a probability of at least 0.95 and a percent coverage of at least 10% of a member of the crystallin family or part of a beaded filament proteins (filensin and phakinin). The following PTMs were searched: (1) Dioxidation (M), Oxidation (H, W and M); (2) Acetyl (K), Acetyl (C), Acetyl (S); (3) Deamidated (N and Q), phosphorylation (S, T and Y); (4) Trp (W) to hydroxykynurenin, kynurenin, and oxolactone: (4) Oxidation (C, D, F, K, N, P, R and Y), dioxidation (C, F, K, P, R, W, and Y) (5) Dimethylation (K, R, N); (6) Methylation (K, H, R, C, N, Q, I, L and S), and (6) Methylation and deamidation (N and Q).

Due to space constraints, only basic information about identified crystallin fragments and those of beaded filament protein (phakinin and filensin) fragments are reported in Tables 1–4.

2.6. *Aggregation of crystallin fragments of Fractions I–IV* per se *and with WS-HMW proteins*

The self-aggregation of crystallin fragments present in Fractions I–IV was determined by the appearance of a high molecular peak (as a void volume peak) during size-exclusion HPLC analysis using a TSK G-4000PW_{XL} column. Similarly, the complex formation between WS-HMW proteins and crystallin fragments of Fractions I–IV was also examined by the above HPLC size-exclusion method. In these experiments, crystallin fragments of individual Fractions I–IV and WS-HMW proteins were mixed at 1:1 (w/w) ratio, and then examined by the above HPLC method for the appearance of peaks representing complex formation. The aggregated complexes of either crystallin fragments of Fractions I–IV or between fragments of Fractions I–IV and WS-HMW proteins were also examined for their sizes by transmission electron microscopic (TEM) method at the High Resolution Imaging Facility of the University

of Alabama at Birmingham. The protein preparations alone or after forming complexes were negatively stained with uranyl acetate (2% v/v) for 20 s, and observed with a Tecnai T12 Spirit TWIN microscope at 80–120 kV (Field Emission Instrument [FEI], Hillsboro, OR).

3. Results

3.1. Isolation of crystallin fragments from WS-protein fraction of human lenses

During a preparative SDS-PAGE separation of the WS protein fraction from normal human lenses of 50–70 year-old donors, crystallin fragments with increasing M_r's between 3 and 18 kDa were recovered in separate fractions (Fig. 1). As stated above, based on their M_r's, the following four fractions were selectively collected: Fraction I containing ~3.5 kDa species, Fraction II ~4–7 kDa species, Fraction III ~7–10 kDa species and Fraction IV > 10–18 kDa species (Fig. 1). The four fractions were extensively dialyzed, and then passed through Detergent-OUT™ SDS-300 spin micro columns to remove SDS as described in Section 2.

3.2. 2D-gel electrophoretic separation of crystallin fragments of Fractions I–IV

On 2D-gel electrophoresis, the Fractions I, II, III and IV showed 13, 35, 46 and 54 spots, respectively (Fig. 2). To determine an potential overlap among crystallin fragment spots based on their M_r in Fractions I–IV in their 2D-gel profiles, the profiles of each of the four fractions were compared using Image J (v. 1.42q) program (http://rsb.info.nih.gov/ij). As shown in Fig. 3, those spots that did not overlap appeared with red and green fluorescence whereas those overlapped exhibited yellow fluorescence (Fig. 3; top colored panel: overlapping spots among Fractions II, III and IV). As shown in the bottom comparative panels in Fig. 3, only few overlapping spots among the Fractions I–IV were observed due to their varying M_r's.

3.3. Identification of crystallin fragments, their occurrence and PTMs of Fractions I–IV

The tryptic fragments of 2D-gel electrophoretic separated spots of Fractions I–IV were analyzed for their crystallin components and PTMs by QTRAP LC-MS/MS mass spectrometric method as described in detail in Section 2. Results in Tables 1–4 list the regions of individual crystallins that matched with the amino acid sequences of tryptic peptides of individual spots of Fractions I–IV. The identity of the parent crystallins (*i.e.* human α-, β- or γ-crystallins) of the fragments was based on their overlapping amino acid sequences of tryptic peptides. Additionally, Tables 1–4 also show the percent coverage by tryptic peptide sequences of an individual spot relative to the total amino acid sequence of its parent crystallin, and also the identity and position of the specific post-translationally modified amino acids within a tryptic peptide of a crystallin fragment. Because the majority of spots of the Fractions I–IV contained fragments of multiple crystallins, the description of the amino acid sequences of their tryptic peptides was extensive, and therefore to conserve space, only the regions that were represented in these tryptic peptides are listed in Tables 1–4. Further, because certain tryptic fragments of a crystallin in Tables 1–4 were not contiguous in the crystallin sequence, their amino acid positions in the crystallin was described with a range, and therefore, their percent coverage in certain instances does not match with the range.

Fractions I contained 13 spots with crystallin fragments of lowest M_r of ~3.5 kDa relative to the fragments observed in 2D-gel profiles of all four fractions. The major findings among these fragments of Fraction I were (Table 1): (A) Each of the spots in the Fractions I contained a mixture of fragments of αA, αB, β- and γ-crystallin species, which suggested that the spots were composed of the fragments from these crystallins. (B) Compositions: Spot 1: fragments of αA, αB, γD and γS-crystallins; spot. 3: fragments of αA, βA3, βB4, βB2, γD and γS-crystallins; spot 4: fragments of αA, βA3, βB4, βB2, γD and γS-crystallins; spot 5: αA, αB, βA3, βA4, βB1, βB2, γD and γS-crystallins; spot 6: fragments of same crystallins as spot 5 but also fragment of γB; spot 9: fragment of γS; spot no. 9B: fragments of αB- and γS; spot 10: fragments αA, αB, βA3, βB1, βB2, γC,γD and γS, and phakinin, spot 11: αA, αB, βA3, βB1, βB2, γC,γD and γS, phakinin and filensin; spot 12: αA, αB, βA3, βB1, βB2, γB, γD and γS; spot no 13: fragments of αA, αB, βA3, βA4, βB1, βB2, βB3, γB, γD, γS crystallins. (C) Based on the compositions of the spots, apparently either complexes of crystallin fragments were formed *in vivo* or the individual components could not be separated by the 2D-gel electrophoresis. However, certain major observations were that the spots showed αA alone or together with αB was always present as with βA3, βA4, βB1, βB2 and γD and γS-crystallins. Additionally, some these spots also contained fragments of βB3, γB, γC, and filensin and phakinin (the two lens-specific intermediate filament proteins). (D) The percent occurrence of different crystallin fragments in 2D-gel separated spots (see Table 5) occurred as follows: αA (20%), αB (10.6%), βA3 (10.6%), βA4 (5.3%), βB1 (8%), βB2 (10.6%), βB3 (1.3%), γB (4%), γC (2.6%), γD (20%), γS (14.4%), filensin (2.6%) and phakinin (5.3%). The results suggested that the fragments of αA, αB, βA3, βB2, γD and γS were predominately existed in the Fraction I. Several post-translationally modified amino acids in the fragments were also observed, which are described below in Table 6.

The 2D-gel profile of Fraction II showed 36 spots with M_r between ~3.5 and 7 kDa. Each spot contained fragments of multiple crystallins, which are listed in Table 2 along with PTMs of specific amino acids. The major findings of among these fragments were: (A) Almost each spot contained either αA- or αB-crystallins or both, along with major β-crystallin species (βA3-, βA4-, βB1- and βB2-crystallins), and major γ-crystallin species (γD- and γS-crystallins). (B) Compositions of crystallin fragments in spots: spot 1: αA, αB, βB2, and γS; spot 1B: αA, αB, βB2, and γS; spot 2: αA, αB, βB1, γD and γS; spot 2: αA, αB, βB1, γD and γS; spot 3: αA, αB, βA3, and γS; spot 4: αA, αB, and γD; spot 5: αB, βA4, γD and γS; spot 5B: αA, αB, γD and γS; spot 5C: αA, αB, βA4 and γS; spot 6: αA, αB, βA3, βA4, βB1, βB2, γC, γD and γS; spot 7: αA, αB, βA3, βA4, βB1, βB2, γC, γD and γS; spot 7A: αA, αB, βA3, βA4,βB1, βB2, γA, γC, γD, γS, filensin and phakinin; spot 8: αA, αB, βA3, βA4, βB1, βB2, γD, and γS; spot 9: αA, αB, βA3, βA4, βB1, βB2, γC, γD and γS; spot 10: αA, αB, βA3, βA4, βB1, and B2; spot 11: αA and βB1; spot 12: αA, αB, βA3, βA4, βB1, βB2, γC, γD and γS; spot 13: αA, αB, βA3, βA4, βB1, βB2, γC, γD, and γS; spot 14: αA, αB, βA3, βA4, βB1, γB, γD, and γS; spot 15: αA, αB, βA3, βB1, βB2, γC, γD, and γS; spot 16: αA, αB, βA3, βA4, βB1, γB, γD and γS; spot 17: αA, αB, βA3, βA4, βB1, γC, γD and γS; spot 18: αA, αB, βA3, βA4, βB2, γC, γD and γS; spot 19: αA, αB, βA3, βA4, βB1, γB, γD and γS; spot 20A: αB, βA3, βA4, βB1, βB2, γB, γD and γS; spot 20C: αA, αB, βA3, βB2, γD and γS; spot 21: αA, αB, βA4, βB1,βB2, γC, γD and γS; spot 22: αA, αB, βA3, βB1, βB2, γC, γD and γS; spot 23: αA, βB1, βB2, γD and γS; spot 24: αA, αB, βA3, βB1, βB2, γB, γD and γS; spot 25: αA, αB, βA3, βB1, βB2, γC, γD and γS; spot 26: αA, αB, βA3, βB1, βB2, γB, γD and γS; spot 27 (unidentified); spot. 28: αA, αB, βA3, βA4, βB1, βB2, γC, γD and γS; spot 29: αB, βA3, βA4, βB1, γB, γD and γS; spot 30: αA, βB1, βB2, γD and γS; spot 31: αA, βB1, βB2, γS and phakinin; spot 32: αA, αB, βB1, γB, γD and γS; spot 33: αA, βB1, βB2, γC, γD and γS; spot 34: αA, αB, βB2, γD and γS; spot 35: αB, βB2, γD and γS, and spot 36: αA, αB, βB1, βB2, γC and γD. (C) Only one spot (no. 7A) showed the presence of fragments of both filensin and phakinin along with fragments of αA-, αB-, βA3-, βA4-, βB1-, βB2-, βB3-, γA-, γC-, γD and γS-crystallins. (D) The percent occurrence of different crystallin fragments in 2D-gel separated spots of Fraction II (see Table 5) was as follows: αA (12.9), αB (14.4), βA3 (8.3), βA4 (6.1), βB1 (9.0), βB2 (9.3), βB3 (0.4), γA (0.4), γB (3.6), γC (4.3), γD (15.1), γS (14.8), filensin (0.4) and phakinin (1.08). Several post-translationally modified amino acids in the tryptic fragments of crystallins were also

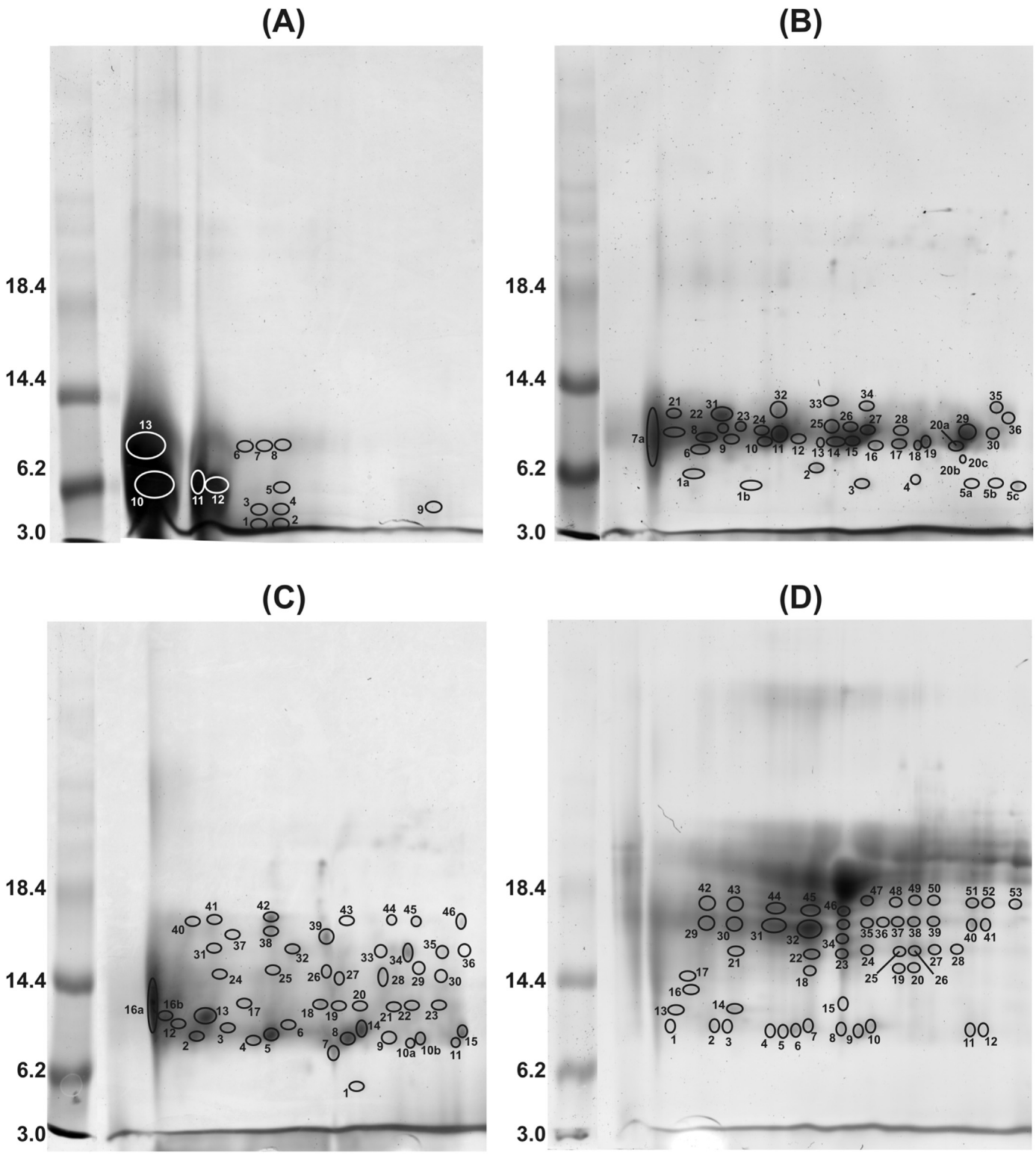

Fig. 2. Two-dimensional gel electrophoretic profiles of Fractions I–IV following their separation by IEF in the first dimension followed by SDS-PAGE in the second dimension. The spots on gels were numbered starting with lower to higher M_r's as shown in (A) to (D) of Fraction I–IV, respectively. Individual spots were trypsin-digested and the tryptic fragments were analyzed by Q-Trap mass spectrometric method as described in Section 2. The majority of spots contained multiple crystallin fragments of α-, β- and γ-crystallins.

observed, which are described in Table 2 along with their specific locations within crystallins.

The 2D-gel electrophoretic profile of Fraction III showed 46 spots with M_r of ~7–10 kDa (Table 3). Because of the large number of spots and their variable tryptic sequence compositions, these are only listed in Table 3 and not described in the text. The major findings among these spots were: (A) Individual spots showed a mixture of tryptic peptides of multiple crystallins, suggesting the presence of their complexes in each spot or their fragments were not separated during the 2D-gel electrophoresis. (B) Although each spot contained fragments of α-, β- and γ-crystallins, these spots contained either αA- and α-B crystallins or only αA but never αB alone. (C) All spots contained

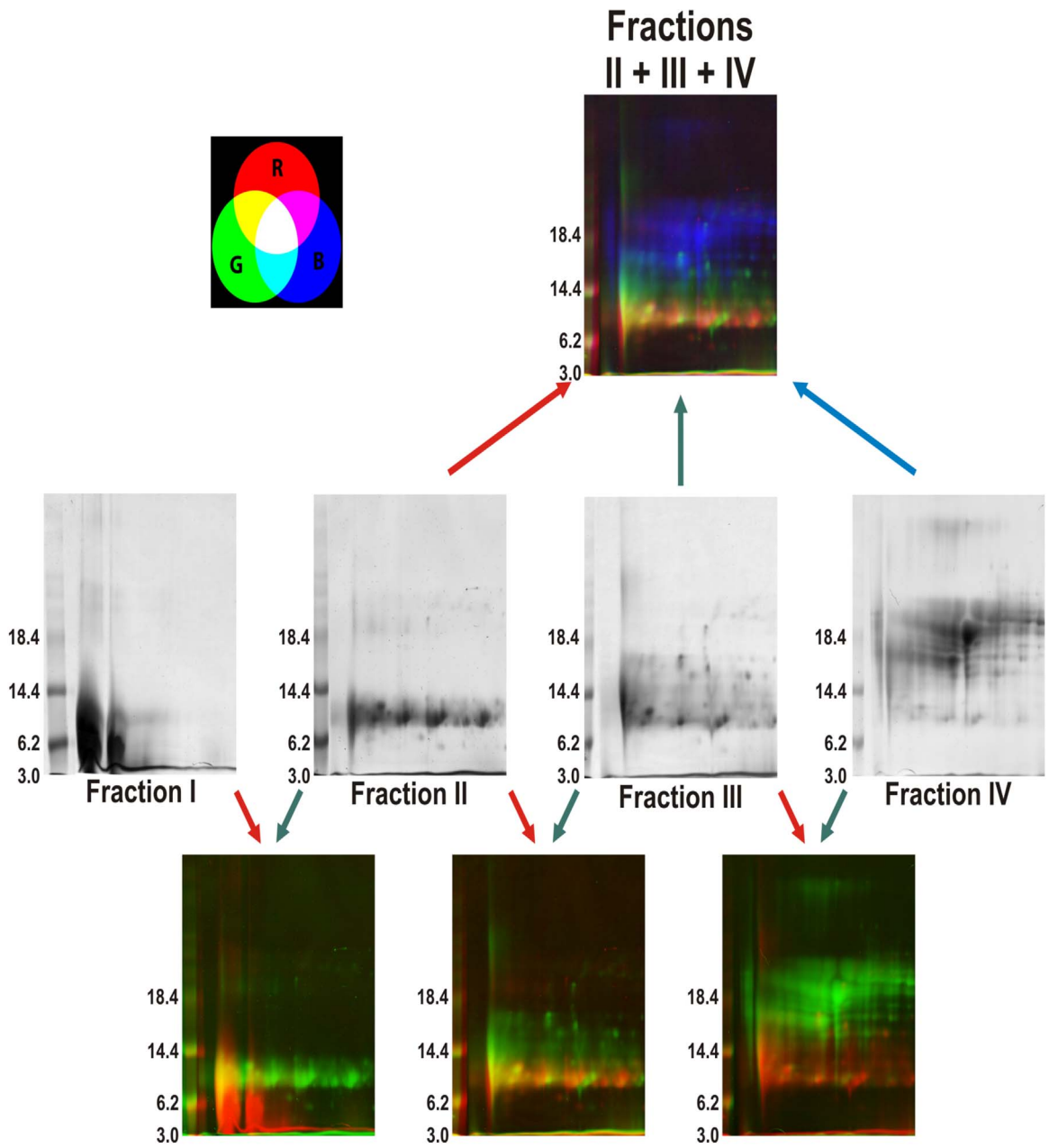

Fig. 3. Overlap among crystallin fragments of Fractions I–IV in their 2D-gel electrophoretic profiles. The protein profiles of Fractions I–IV were compared using Image J (v. 1.42q) program (http://rsb.info.nih.gov/ij). During the comparison, the non-overlapping spots showed red and green fluorescence whereas the overlapping spots the yellow fluorescence. Top panel: Overlapping spots among fractions II, III and IV. Bottom panels: Left: Overlapping spots in Fractions I and II, Middle: Overlapping spots in Fractions II and III, and right: Overlapping spots in Fractions III and IV.

fragments of α-, β- and γ-crystallins except phakinin fragments in spot nos. 13 and 16 and filensin fragments in spot nos. 26 and 32. (D) Although the fragments of β- and γ-crystallins were associated with the fragments of αA- and αB-crystallins in specific spots, certain variability in the former crystallins was observed. The β-crystallins were present in the following decreasing order: βB2 (present in 30 of 46 spots) > βB1 (present in 23 of 46 spots) > βA3 (present in 21 of 46 spots) > βA4 (present in 8 of 46 spots). (E) The occurrence of acidic- and basic β-crystallins also varied in the following decreasing order: basic βB1 plus βB2 (present in 20 of 46 spots) > basic βB1 plus βB2 with acidic βA3 and βA4 (present in 16 of 46 spots) > βB2 alone (present in 5 of 46 spots) > βB1 (present in 2 of 46 spots). (F) The percent occurrence of different crystallin fragments in 2D-gel separated spots (see Table 5) was as follows: αA (17.3), αB (14.2), βA3 (7.7), βA4 (3), βB1 (11.6), βB2 (11.6), γA (0.4), γB (1.5), γC (5.4), γD (12.7), γS (13.1), filensin (0.4) and phakinin (0.8). Together, the results suggested that the fragments of αA, αB, βA3, βB1, βB2, γD, and γS were predominately present in the Fraction III.

The fraction IV showed 54 spots in its 2D-gel electrophoretic profile, which showed M_r between 10 and 18 kDa (Table 4). Again, each of the spots showed the presence of tryptic peptides of multiple crystallins suggesting their presence as complexes or the fragments were not separated by 2D-gel electrophoresis. Because of the large number of spots and their variable tryptic sequence compositions, they are listed in Table 4 but not described in the text. The major findings among the spots of fractions IV were: (A) All 46 spots contained αA- and αB-crystallins along with βA3- and γS-crystallins. In addition to above crystallins, all the spots except 3 spots (spot nos. 30, 32 and 35A) contained βB1-crystallin, and similarly all but one spot (spot no. 33) also contained γD-crystallin. (B) The occurrence of fragments of αA-,

Table 5
Percent occurrence of crystallin fragments compared the total crystallin species as described in Fractions I to IV.

Crystallins	Percent occurrence of fragments of crystallins in Fractions I to IV*			
	Fraction I (~3.5 kDa)	Fraction II (~3.5 to 7 kDa)	Fraction III I(~7 -10 kDa)	Fraction IV (> 10 – 18 kDa)
αA	12.7	12.9	17.3	10.9
αB	9.8	14.4	14.2	10.8
βA3	9.8	8.3	7.7	10.8
βA4	5.6	6.1	3.0	9.5
βB1	8.6	9.0	11.6	10.0
βB2	9.8	9.3	11.6	10.2
βB3	1.4	0.4	ND**	1.1
γA	ND**	0.4	0.4	0.2
γB	4.2	3.6	1.5	2.4
γC	4.2	4.3	5.4	8.4
γD	11.3	15.1	12.7	10.8
γS	14	14.8	13.1	10.6
Filensin	4.2	0.4	0.4	1.9
Phakinin	4.2	1.08	0.8	2.2

* Percent occurrence of individual crystallin species was calculated from sum of occurrence of all the crystallin species, which was considered as 100%.

αB-, βA3-, βB1-, βB2-, γD- and γS-crystallins in the majority of the spots suggested that these crystallins are maximally truncated in the 50–70 year old human lenses. In contrast, the occurrence of βA4-, γB- and γC-crystallins with lesser frequency in theses spots suggested their relatively lower truncations compared to above described crystallins. (C) The presence of either filensin or phakinin or both with the above most frequently occurring crystallin fragments in several spots (spot nos. 4, 16A, 17, 18, 20, 22, 23, 24, 26, 28B,29, 31, 34, 39, 41A, 41B, 42, 47, 49, 50, and 54) suggested that in the lens, the two intermediate filament proteins (phakinin and filensin) also show truncation with aging, and also possibly form complexes with crystallin fragments. (D) The percent occurrence of different crystallin fragments in 2D-gel separated spots (see Table 5) was as follows: αA (10.9), αB (10.8), βA3 (10.8), βA4 (9.5), βB1 (10.0), βB2 (10.2), βB3 (1.1), γA (0.2), γB (2.4), γC (8.4), γD (10.8), γS (10.6), filensin (1.9) and phakinin (2.2). The results suggest that the fragments of αA, αB, βA3, βA4, βB1, βB2, γC, γD, and γS were predominately present in Fraction IV.

3.4. Post-translational modifications (PTMs) in crystallin fragments present in Fractions I–IV

The PTMs observed in crystallin fragments of Fractions I–IV are listed in Table 6. These PTMs included oxidation, deamidation, acetylation, methylation, methylation plus deamidation and dimethylation. αA-crystallin exhibited oxidation of M1, D2, R12 F17,Y18, F53, R54, D58, R69, D76, K78, H79, F80, P82, D84, K78, F93, H97, K99, H100, R103, Q104, D105, D106, H107, Y109, C131, D136, M138, P144,K145, D151, H154, deamidation of Q6, Q90, N101, Q104, N123, Q126, Q147, acetylation of S66, K70, K78, K88, K99, C131, K166, S172, methylation of L51, R54, L57, S59, I61, S62, R65, H79, S81, L85, Q90, I96, H97, K99, H100, H107, I110, S111, Q126, S127, L129, S130, C131, C142, K145, I146, Q147, L150, H154, and R157, methylation + deamidation of Q50, Q90, N101, Q104, Q126, and Q147, and dimethylation of R R12, R21, R54, R65, R103, R112, and R16321. Similarly, αB-crystallin showed oxidation of M1, D2, P16, P58, W60, F61, D62, M68, R69, K72, F75, H83, F84, P86, D109, H111, F113, K166, K174, N146, deamidation of N78, Q108, N146, Q151, acetylation of S66, K90, K92, K150, K166, methylation of S19, S21, S59, L65, S66, I98, H101, I114, S115, K166, and methylation + deamidation of R12, R22, R69, K103, R116, K16. Among β-crystallins, βB1-crystallin showed oxidation of F64, N68, C80, N82, M113, F114, N125, W124, W127, M137, W174, W220, M226, R230, deamidation of N58, N68, Q70, N82, Q106, N108, N125, N158, Q167, Q197,Q205, N216, Q223, Q225, Q227, acetylation of S189, S190, methylation of L61, S77, C80, S81, R86, R123, N125, and methylation and deamidation of N82, N125. Similarly, βB2-crystallin showed oxidation of M1, M14, H30, F27, P37, C38, W59, N66, C67, W82, W85, N103, F116, N114, K121, M122,D126, W151, Y154, Y156, H182, D192, M193, deamidation of Q64, Q71, Q105, N114, N116, Q138, Q147, Q155, Q163, Q183, Q185, Q194, Q197, acetylation of K120, K121, S148, K168, methylation of H27, H30, C33, S38, S51, C67, K68, I109, I110, L111, N114, Q147, S148, C166, H168, S174, S175, Q183, Q185, methylation and deamidation of N66, N114, and Q147, and dimethylation of K172. βA3-crystallin showed oxidation of Y36, D37, R45, M46, F48, C52, W73, Y76, H78, F81, C82, W96, D97, W99, N103, Y105, H106, M126, F129, W139, D143, D144, Y145, P146, M151, F154, N155, N156, K162, W168, C170, Y171, W198, H201, deamidation of Q38, N40, Q42, Q50, N54, N62, Q84, N103, N133, Q138, Q149 Q164, Q172, Q180, Q203, Q206, Q208, acetylation of K131, S147, methylation of R45, S51, C52, R58, H78, S80, C82, Q84, R90, S100, N103, H106, I107, I128, Q164, S165, C170, C185, H201, methylation + deamidation of N40, Q42, N54, Q84, N103, Q164, Q172, Q179, Q203, and dimethylation of R211. βA4 showed oxidation of M14, W17, R25, R26, H27, F29, C33, P34, S35, W54, F57, H59, F62, Y67, W77, W80, F94, C99, F110, C166, W179, H182, P184, deamidation of Q23, Q63, Q65, N83, N101, Q112, N114, Q153, Q187, Q189, acetylation of K13 and K119, methylation of R26, H27, C33, S35, L50, S51, H59, C99, L107, I109, Q112, L164, C166, H168, S170, K174, S181, and H182, methylation and deamidation of Q23, N101, Q112, and dimethylation of R26 and K118.

Among γ-crystallins, γA and γB-crystallins showed modifications of only few residues, which are shown in Table 6. However, γC, γD and γS showed several modifications, which are described. γC showed oxidation of F6, D9, R10, F12, C14, C23, S40, C42, W43, M44, Y46, R48, W69, M70, D74, C80, C130, W131, R140, Y144, C154, M160, and D161, deamidation of N50, Q52 Q67, Q68, Q84, Q143, Q149, Q155, methylation of S40, C42, R48, S78, C79, C80, L81, H117, H125, L127, C130, Q143, C154, methylation and deamidation of N2 Y7, F12, Y17, C19, H23, D39, C42, W43, M70, D74, R77, C79, F118, N119, H1225, Q68, Q143, Q155, and dimethylation of R147. Similarly, γD-crystallin exhibited oxidation of Y7, F12, Y17, C19, H23, D39, C42, W43, M70, D74, R77, C79, F118, N119, H122, N125, W131, Y134, W137, R142, Y144 M147, P148, D150, Y151, R152, Y154, D156, W157, N161, deamidation of N25, Q67, Q68, N119, N125, N138, Q143, Q155, N144, N161, methylation of H16, C19, S20, S21, H23, C42, C79, C79, L81, I82, R117, R153, Q155, methylation + deamidation Q101, Q113, Q143, Q155, N161 and dimethylation of N161, R163. γS-crystallin exhibited oxidation of M1, F10, Y21, C25, D26, C37, K41, W46, F55, D56, Y58, M59, Y60, W73, M74, P85, Y94, K101, F104, M108, Y109, D114, C115, P116, M119, M124, R125, F136, W137, F139, Y140, P143, Q149, Y150, K159, P160, D162, W163, P168, Q171, F173, R174, deamidation of Q13, N15, Q17, N54, Q64, Q71, N77, Q93, Q107, Q121, N144, Q149, Q171, acetylation of C23, C25, C27, K96, K101, K154, K155, K159, methylation of S2, R20, C23, C25, C27, C37, R52, K101, C115, S117, H123, L151, L152, K159, I161, S167, Q171, S172, deamidation + methylation of Q63, Q93, Q107, Q121, Q149, Q171, and dimethylation of K14, R20, K95, K101, K154, K159, R174. Taken together, while both fragments of αA and αB exhibited several modifications, and among fragments of β-crystallins, most PTMs were observed βA3 followed by βB2, and among γ-crystallins most PTMs were in γS followed by γD-crystallin.

3.5. Characterization of properties of crystallin fragments present in Fractions I–IV

3.5.1. Self-aggregation of crystallin fragments and with WS-HMW proteins

The crystallin fragments of Fractions I–IV (stores at 5 °C) showed aggregation by themselves and eluted in the form a void volume peak

Table 6
Post-translational modifications of specific amino acids in crystallin fragments present in Fractions I to IV.

PTMs	Oxidation	Deamidation	Acetylation	Methylation	Methylati-on + Deamida-tion	Dimethylation
αA	M1, D2, R12 F17,Y18, F53, R54, D58, R69, D76, K78, H79, F80, P82, D84, K78, F93, H97, K99, H100, R103, Q104, D105, D106, H107, Y109, C131, D136, M138, P144,K145, D151, H154	Q6, Q90, N101, Q104, N123, Q126, Q147	S66, K70, K78, K88, K99, C131, K166, S172	L51,R54, L57, S59, I61, S62, R65, H79, S81, L85, Q90, I96, H97, K99, H100, H107, I110, S111, Q126, S127, L129, S130, C131, C142, K145, I146, Q147, L150, H154, R157	Q50, Q90, N101, Q104, Q126, Q147	R12, R21, R54, R65, R103, R112, R163
αB	M1, D2, P16, P58, W60, F61, D62, M68, R69, K72, F75, H83, F84, P86, D109, H111,F113, K166,K174, N146	N78, Q108, N146, Q151	S66, K90, K92, K150, K166	S19, S21, S59, L65, S66, I98, H101, I114, S115, K166		R12, R22, R69, K103, R116, K166
βB1	F64, N68, C80, N82, M113, F114, N125, W124, W127, M137,W174, W220, M226, R230	N58, N68, Q70, N82, Q106, N108, N125, N158, Q167, Q197,Q205, N216, Q223, Q225, Q227	S189,S190	L61, S77, C80, S81, R86, R123, N125	N82, N125	
βB2	M1, M14, H30, F27, P37, C38, W59, , N66, C67, W82, W85, N103, F116, N114, K121, M122,D126, W151, Y154, Y156, H182, D192, M193	Q64, Q71, Q105, N114, N116, Q138, Q147, Q155, Q163, Q183, Q185, Q194, Q197	K120, K121, S148, K168	H27, H30, C33, S38, S51, C67, K68, I109, I110, L111, N114, Q147, S148, C166, H168, S174, S175, Q183, Q185	N66, N114, Q147	K172
βA3	Y36, D37, R45, M46, F48, C52, W73, Y76, H78, F81, C82, W96, D97, W99, N103, Y105, H106, M126, F129, W139, D143, D144, Y145, P146, M151, F154, N155, N156, K162, W168, C170, Y171, W198, H201	Q38, N40, Q42, Q50, N54, N62, Q84, N103, N133, Q138, Q149 Q164, Q172, Q180, Q203, Q206, Q208	K131,S147	R45, S51, C52, R58, H78, S80, C82, Q84, R90, S100, N103, H106, I107, I128, Q164, S165, C170, C185, H201	N40, Q42, N54, Q84, N103, Q164, Q172, Q179, Q203	R211
βA4	M14, W17, R25, R26, H27, F29, C33, P34, S35, W54, F57, H59, F62, Y67, W77, W80, F94, C99, F110, C166, W179, H182, P184	Q23, Q63, Q65, N83, N101, Q112, N114, Q153, Q187, Q189	K13, K119	R26, H27, C33, S35, L50, S51, H59, C99, L107, I109, Q112, L164, C166, H168, S170, K174, S181, H182	Q23, N101, Q112	R26, K118
γA				R10, Q13, L145, L146		R147
γB	D9, R10, F12, M70, D74, R77, W69, W158	N25, Q67, Q68, N162				
γC	F6, D9, R10, F12, C14, C23, S40, C42, W43, M44, Y46, R48, W69, M70, D74, C80, C130, W131, R140, Y144, C154, M160, D161	N50, Q52 Q67, Q68, Q84, Q143, Q149, Q155		S40, C42, R48, S78, C79, C80, L81, H117, H125, L127, C130, Q143, C154	N25, Q68, Q143, Q155,	R147
γD	Y7, F12, Y17, C19, H23, D39, C42, W43, M70, D74, R77, C79, F118, N119, H122, N125, W131, Y134, W137, R142, Y144 M147, P148, D150, Y151, R152, Y154, D156, W157, N161	N25, Q67, Q68, N119, N125, N138, Q143, Q155, N144, N161		H16, C19, S20, S21, H23, C42, C79, C79, L81, I82, R117, R153, Q155	Q101, Q113, Q143, Q155, N161	N161, R163
γS	M1, F10, Y21, C25, D26, C37, K41, W46, F55, D56, Y58, M59, Y60, W73, M74, P85, Y94, K101, F104, M108, Y109, D114, C115, P116, M119, M124, R125, F136, W137, F139, Y140, P143, Q149, Y150, K159, P160, D162, W163, P168, Q171, F173, R174,	Q13, N15, Q17, N54, Q64, Q71, N77, Q93, Q107, Q121, N144, Q149, Q171	C23, C25, C27, K96, K101, K154, K155, K159	S2, R20, C23, C25, C27, C37, R52, K101, C115, S117, H123, L151, L152, K159, I161, S167, Q171, S172, R175	Q63, Q93, Q107, Q121, Q149, Q171	K14, R20, K95, K101, K154, K159, R174
Filensin		Q251				
Phakinin	M221, D222, H322, N323, C326	Q86		H322, S325	Q327	

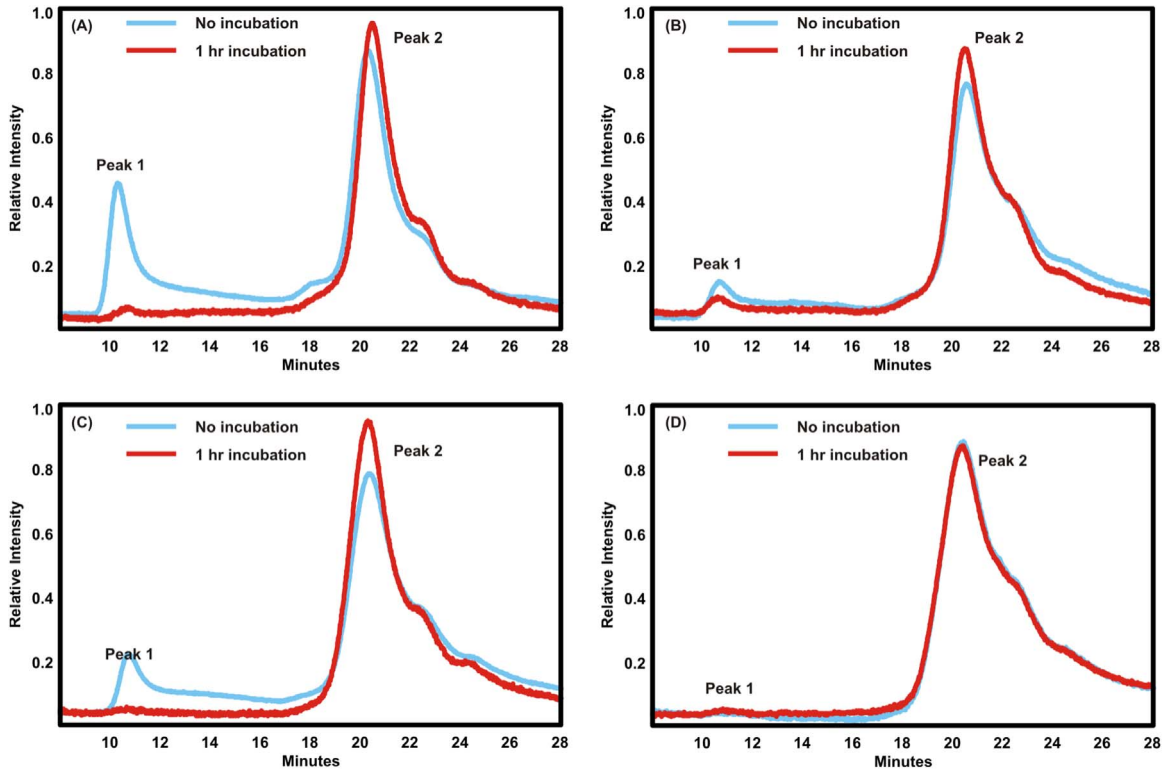

Fig. 4. HPLC examination of self-aggregation of crystallin fragments of Fractions I–IV in (A)–(D), respectively, using a TSK-G4000PW$_{XL}$ column. On storage of Fractions I–IV at 5 °C and subsequent HPLC analysis at room temperature two peaks, Peak 1 (——) in void volume and a low M_r but major peak 2 (——) were observed in the Fractions I–IV. The aggregated void volume protein peak I disappeared on incubation for 1 h at 37 °C in Fractions I–IV.

(Peak 1, Fig. 4) along with non-aggregated major low molecular peak (Peak 2, Fig. 4) during size-exclusion HPLC analysis using a TSK G-4000$_{XL}$ column. However, the void volume peak 1 disappeared on incubation of Fractions 1–4 at 37 °C for 1 h during size-exclusion HPLC, suggesting that the aggregation process was non-covalent and possibly hydrophobic in nature. The determination of the molecular mass of the peak 1 of Fractions I–IV by multi-angle light scattering (MALS), the average M_r were: $1.03 \pm 0.04 \times 10^8$, $5.5 \pm 0.27 \times 10^5$, $7.6 \pm 0.13 \times 10^5$ and $8.5 \pm 0.08 \times 10^5$, respectively (Table 7). Therefore, the crystallin fragments of fraction I showed highest M_r compared to the crystallin fragments of Fractions II, III and IV. Additionally, the hydrodynamic radii (R_H) of complexes of peak I of Fractions I–IV are also shown in Table 7. The highest R_H was of peak I of Fraction II and III (Fraction III: 217.5 ± 14.03 nm and Fraction II: 244.3 ± 20.5 nm), which was 4× and 2× greater than that of peak 1 of Fraction I (129.5 ± 4.9 nm) and of Fraction IV (52.4 ± 3.4 nm), respectively. The hydrodynamic radius (R_H) does represent the protein spheres that include hydration and shape effects. R_G value represents the radius of gyration (root mean square RMS radius- average distance of each point in a molecule from the molecule's center of gravity). The R_G value was the same for the peak I from fractions I and III, a slightly higher for peak 1 of Faction II and lowest for peak I of Fraction IV (Table 7).

The aggregates (Peak 1, Fig. 4) of Fractions I–IV were also examined by TEM as described in Section 2. The Fig. 5(A) shows TEM images of Fractions I, II, III and IV at 11,000×, 30,000× and 67,000×. For comparison purpose, the reference TEM images of recombinant human αA- and αB-crystallins with particle sizes of ~10 nm in diameter are included. The particle sizes of crystallin fragments of Fractions I, II, III and IV were 13.12 ± 1.29 nm, 20.27 ± 1.29 nm, 26.68 ± 2.46 nm and 25.4 ± 2.83 nm, respectively. The difference between the aggregated species of peak 1 following HPLC and those of particle sizes of Fractions I–IV was due to the latter containing both HPLC separated peaks I and II during particle size analysis. As shown in Fig. 5B, the diameters of aggregates of crystallin fragments of Fractions I–IV ranged between 12 and 28 nm (calculated by Image J Program using an average of 20 particles), To examine the aggregation of crystallin fragments of Fractions I–IV with WS-HMW proteins, each of the fractions were incubated at 1:1 ratio (crystallin fragments: WS-HMW proteins) or singularly, and their elution profiles determined by size-exclusion HPLC using TSK G4000PW$_{XL}$ column (Fig. 6). While the WS-HMW protein peak eluted at 10 min as a void volume peak (shown in black), and the crystallin fragments peak from Fractions I–IV eluted at 21 min (shown in red), the mixture of WS-HMW proteins and crystallin fragments eluted as major peak at about 14 min (shown in blue) with about 90% loss of the void volume WS-

Table 7
MALS analysis of Peak I of Fractions I–IV to determine their molecular mass, polydispersity and radii.

	Polydispersity	Molar mass		Radii (nm)	
		Range (kDa)	Average (Da)	R_H	R_G
Fraction I	1.017 ± 0.049	68,421–126,417	(1.029 ± 0.036)e+8	129.49 ± 4.89	83.72 ± 1.83
Fraction II	1.003 ± 0.068	499–613	(5.502 ± 0.265)e+5	244.37 ± 20.48	105.51 ± 5.77
Fraction III	1.424 ± 0.034	290.4–822	(7.594 ± 0.125)e+5	217.5 ± 14.03	85.17 ± 5.00
Fraction IV	1.038 ± 0.015	596–1201	(8.495 ± 0.083)e+5	52.45 ± 3.42	49.52 ± 2.78

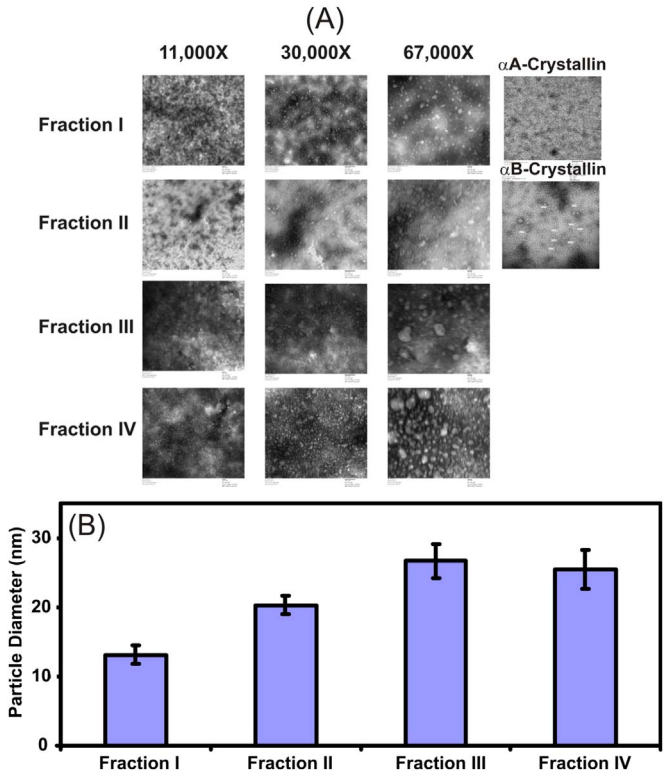

Fig. 5. Transmission electron microscopic (TEM) examination and size determination of the peak I-containing species recovered during self-aggregation of Fractions I–IV as shown in (A) to (D) in Fig. 4. (A) TEM-images of Fractions I–IV with reference images of recombinant αA- and αB-crystallins that showed particle sizes of ~10 nm in diameter. (B) Diameters of crystallin fragments of Fractions I–IV ranged between 10 and 28 nm (calculated by Image J Program).

HMW peak (Fig. 6, A–D). The results clearly suggested the aggregation of crystallin fragments with WS-HMW proteins, and the sizes of aggregates were greater than that of αA- and αB-crystallins.

Because compared to crystallin fragments of Fraction II–IV, the fragments of Fraction I exhibited aggregates with highest M_r (Table 6), it was selectively used to determine its aggregation with WS-HMW proteins. The yield of the crystallin fragments with M_r ~3.5 kDa in Fraction I was very low, and therefore we utilized an alternative method of TCA solubilization to isolate these species as described by us previously [31]. As shown in Fig. 8, the TCA solubilization method isolated crystallin fragments with M_r of ~3.5 kDa from both water soluble (Fig. 7A) and water insoluble protein fractions (Fig. 7B). On TEM examination of the TCA solubilized crystallin fragments from WS-proteins and WI-proteins, the particle sizes were 12.2 ± 1.2 nm and 23.5 ± 1.8 nm, respectively, compared to 26.0 ± 6.1 nm sizes of WS-HMW proteins (Fig. 7, top right panel). As shown in the HPLC profile in Fig. 7(bottom panel), the incubation of the 3.5 kDa species (isolated from WS- and WI-proteins, shown in red) with WS-HMW proteins (shown in black), the WS-HMW protein exhibited slight increase in its peak height suggesting their potential aggregation.

3.5.2. *Amyloid fibril formation by Fractions II–IV*

Amyloid fibril formation by crystallin fragments of Fractions II–IV was determined at 0 time and after 5 h incubation by assays with Congo red (CR) and Thioflavin T (ThT) as described in Section 2. During CR assay (Fig. 8, Upper panels A to D), a red shift of the absorption band toward 540 nm was observed in the fragments of Fractions II–IV, whereas an increase in fluorescence was observed only in the Fraction III. Both red shift in and its increase were indicative of amyloid structure. During ThT assay (Fig. 8, lower panel, A–D), an increase in the fluorescence emission intensity at 490 nm was observed in Fractions II, III and IV suggested the amyloid fibril formation. Fig. 9 shows the TEM images of Fractions II and III, which showed amyloid fibril-like

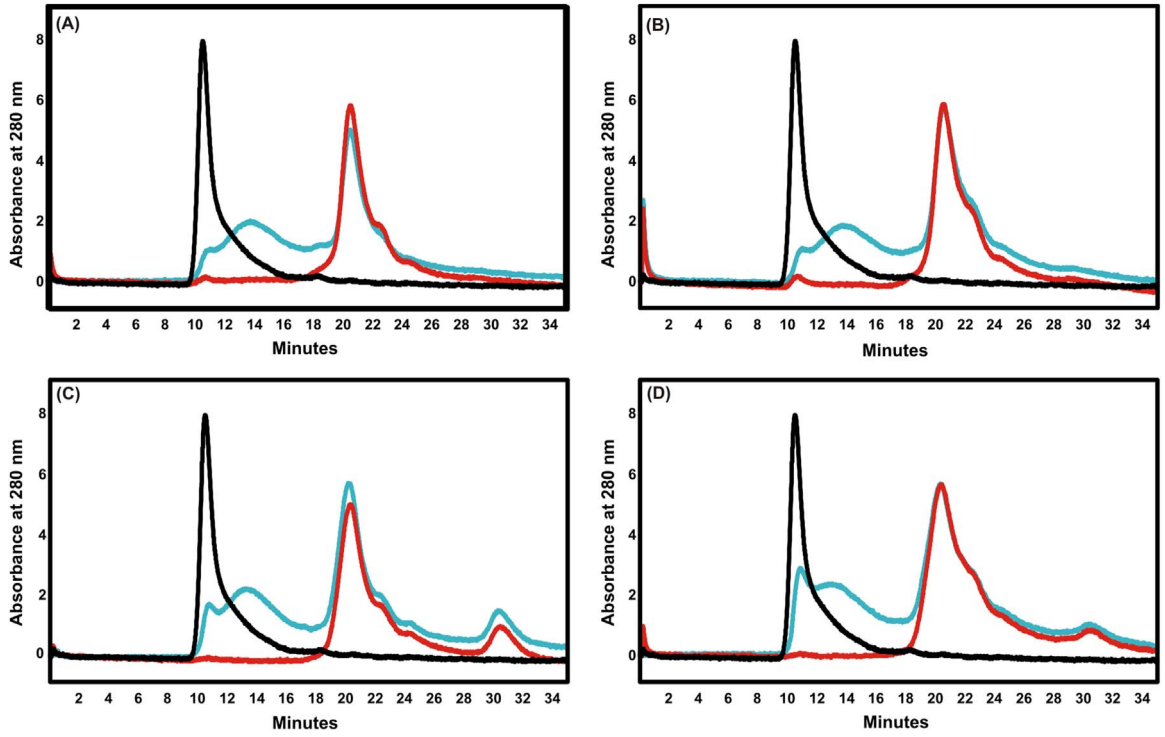

Fig. 6. HPLC analysis of complex formation between WS-HMW-proteins and crystallin fragments of Fractions I–IV (all fractions isolated from same human lenses of 50–70 year old donors). Individual fractions I–IV containing crystallin fragments (——) and WS-HMW protein fractions (——) were mixed at 1:1 ratio (w/w), and examined by HPLC using a size-exclusion TSK G-4000PW_{XL} column. Note the complex formation (——) between WS-HMW proteins and crystallin fragments of Fractions I–IV. (A) to (D) represents Fractions I–IV alone, HMW-proteins alone and together in a mixture (each of the Fractions 1–4 and WS-HMW proteins), respectively.

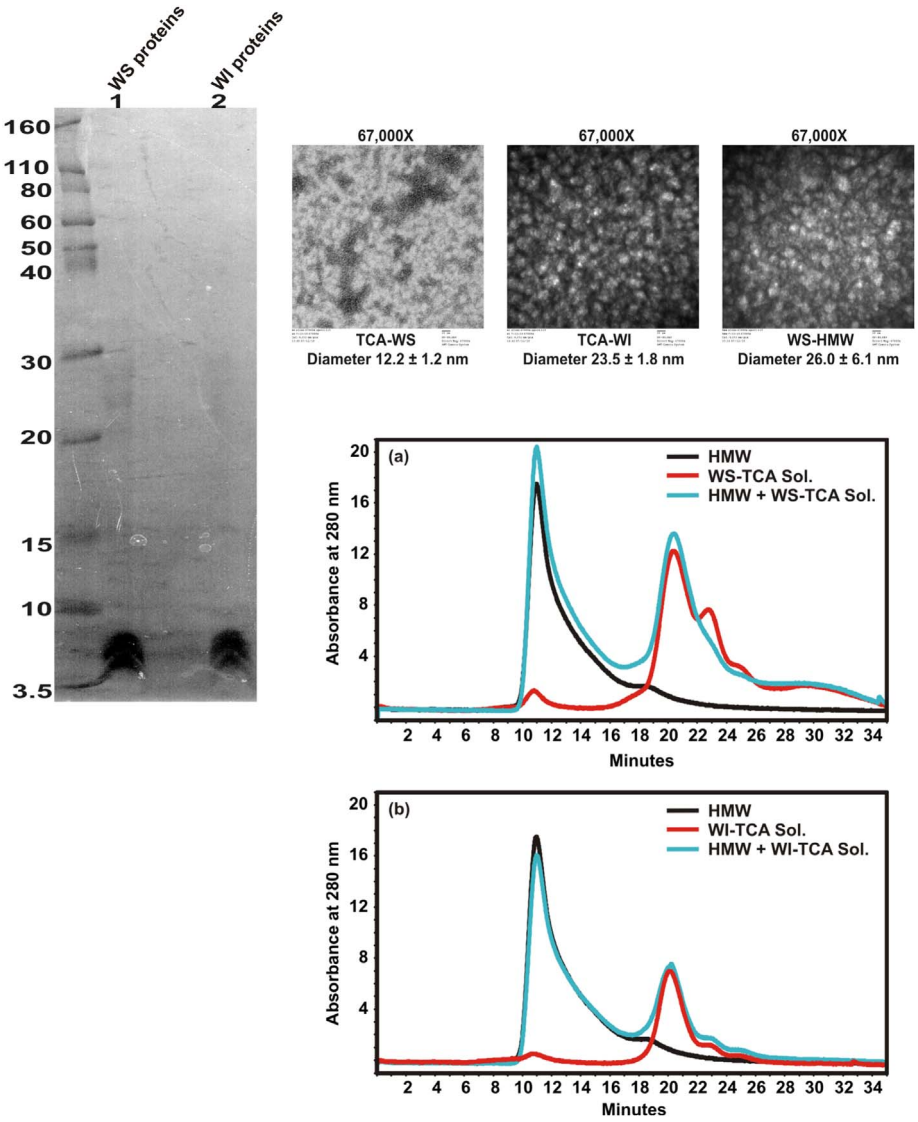

Fig. 7. Analysis of 3–4 kDa polypeptides isolated by TCA-solubilzation method from WS-proteins and WI-proteins of human lenses of 50–70 year-old donors as described by us previously (31). Upper left panel: SDS-PAGE analysis of TCA-solubilized polypeptide of 4–5 kDa from WS-proteins (lane 1) and from WI-proteins (lane 2). Upper right panel showing TEM-images of: TCA-soluble fractions from WS-(left image), TCA–soluble fraction isolated from WI-proteins (middle image), and WS-HMW proteins (right image). Bottom right panel (A) and (B): Determination of aggregation between WS-HMW proteins and TCA-solubilized polypeptides by HPLC using a TSK G-4000PW$_{XL}$ column. Aggregation complex between (A) WS-HMW proteins and TCA-solubilized polypeptides from WS-proteins. (B) WS-HMW proteins and TCA-solubilized polypeptides from WI-proteins.

structures in the form of strings at low pH (2.0) and heating at 60 °C for 5 h. The aggregated particles were of ~100 nm in sizes (Fig. 9).

4. Discussion

The three major aims of the study were to: (A) Selectively fractionate the WS crystallin fragments of 3–18 kDa into four fractions based on their M_r from lenses of 50–70 year-old human donors, (B) Identity the origin of fragments from crystallins along with PTMs of specific amino acids in fragments, and (iii). Determine aggregation of crystallin fragments *per se* and with WS-HMW proteins. The selective fractionation of 3–18 kDa crystallin fragments by a preparative SDS-PAGE in four fractions (*i.e.*, Fraction I with ~3.5 kDa species, Fraction II ~3.5–7 kDa species, Fraction III ~7–10 kDa species and Fraction IV > 10–18 kDa species) was accomplished for the first time. However, the major drawback of the methodology was that a further characterization of the fragments required SDS removal from the fragments, which was accomplished by an extensive dialysis followed by a passage through an affinity Detergent-OUT SDS-300 spin microcolumn.

Unlike only a few bands generally seen during one-dimensional SDS-PAGE, the 2D-gel electrophoretic method (IEF followed by SDS-PAGE) separated 13, 36, 46 and 54 distinct spots in the Fractions I, II, III and IV, respectively (Fig. 1). The majority of spots contained multiple crystallin fragments as determined by Q-TRAP mass spectrometric analysis. The identification as reported in Tables 1–4, was definitive because it was based on an overlap of amino acid sequences of tryptic fragments of a spot with an individual crystallin. Almost all the spots contained αA or αB or both, and also showed the presence of fragments of mostly αA-, αB-, βA3-, βB2-, γD and γS-crystallins. The results suggest that the crystallin fragments might exist *in vivo* as covalent complexes or were not dissociated by first dimensional IEF and the second dimensional SDS-PAGE during 2D-gel electrophoretic method. We have previously reported similar covalent complexes of crystallin fragments in both normal and cataractous human lenses [2,15,21,22,25]. In one of the report [2], an analysis of laser microdissected-cellular proteins from cortical and nuclear regions of a normal 69-year old human lens showed that the truncation of crystallins began in cortical region and progressively extends to the nuclear region but crystallin aggregation mainly occurs in the nuclear region.

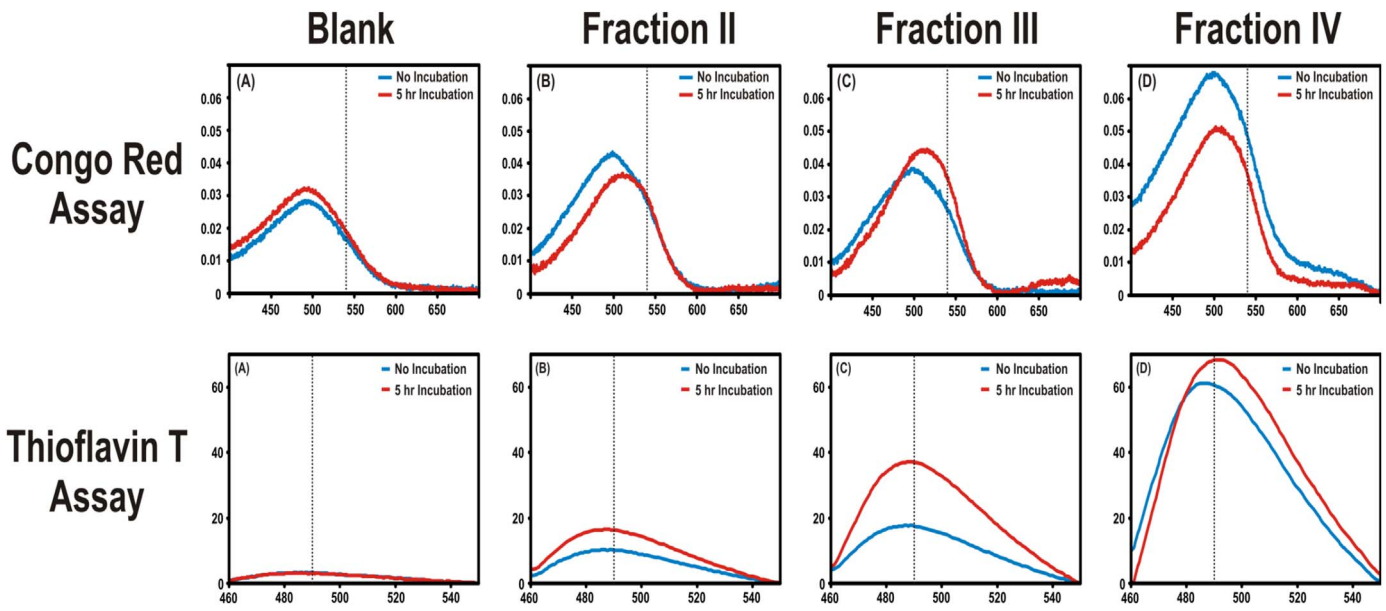

Fig. 8. Amyloid fibril formation of crystallin fragments of Fractions II–IV by assays with Congo red (CR, upper (A) to D) and thioflavin T (ThT, lower A to D). During CR assay (Upper panel, A to D), a red shift of the absorption band towards 540 nm and an increase in absorption were indicative of amyloid structure. During ThT assay (Lower panel, A to D), an increase in the fluorescence emission intensity at 490 nm suggested the amyloid fibril formation. (——) No incubation and (——) 5 h incubation of Fraction II to IV with CR or ThT.

Additionally, the 2D-gel separated spots with M_r < 20 kDa contained multiple crystallin fragments (possibly as covalent multimers), which included fragments of either αA or αB or both, along with those of β- and γ-crystallins. The results suggest a real possibility that the covalent molecular weight complexes of crystallin fragments might exist in human lenses. This finding was further supported by our previous reports showing similar complexes in the WS-HMW proteins of normal and cataractous human lenses [15], and also in the water insoluble proteins of normal lenses [25]. In this previous study [25], among the two types of covalent multimers (M_r > 90 kDa) of crystallins in the WI-proteins of 25- and 41-year-old human lenses, the first type contained fragments of eight different crystallins (*i.e.*, αA, αB, βA3, βA4, βB1, βB2, γS, and γD), and the second type fragments of α-, β-, and γ-crystallins and two beaded filament proteins (phakinin and filensin), with αA showing three PTMs (oxidation of M and W residues, conversion of S residue to dehydroalanine, and formylation of H residue) These αA-associated PTMs are known to lead to cross-linking of proteins. Together, the results suggest that likely covalent complexes of crystallin fragments exist in WS-, WS-HMW- and WI-proteins, suggesting their potential role in the aggregation and cross-linking process leading to lens opacity.

Certain specific truncations of crystallins are known to cause loss of their structural stability and sometimes water insolubilization. For example, among the human αA-crystallin deletion mutants that contained either the N-terminal hydrophobic domain (residues 1-63) alone, the core domain (residues 64-142) alone, or among similar αB mutants consisting of either the N-terminal hydrophobic domain (residues 1-66) alone, the core domain (residues 67-146) alone , or the C-terminal extension (residues 147-175) alone, the mutants with only the N-terminal domain became water insoluble while those with only the C-terminal extension remained soluble [23,32]. Similarly, human βA3-crystallin showed insolubility on deletion of either N-terminal extension plus motif I, N-terminal extension plus motifs I and II, N-terminal extension plus motifs I, II and III, or motif IV [33]. Together, the results suggest that depending of the region of crystallin fragments that is part of covalent multimers, these multimers could become water soluble or insoluble.

Our study also identified several PTMs (oxidation, deamidation, acetylation deamidation plus methylation and dimethylation [Table 7]) of specific amino acids in the fragments, but their roles in formation of covalent complexes of crystallin fragments *in vivo* have not well understood. Presently, it is not known whether these PMTs in the crystallin fragments occur prior to or after their truncation from crystallins. Results show that fragments of αA, αB, βA3, βB2, γD and γS-crystallins showed relatively greater PTMs compared to those from remaining species of β- and γ-crystallins. A previous report that analyzed eleven different PTMs in normal and cataractous human lenses showed that major PTMs were deamidation, oxidation and methylation [34]. In spite of an exhaustive list of PTMs of specific amino acids in fragments of α-, β- and γ-crystallins (Table 6), their specific effects on crystallin fragments are yet to be determined. Therefore, the description below is mainly restricted to the PTMs effects on αA- and αB-crystallins. This particular emphasis was undertaken because soluble α-crystallin has been reported to disappear from the center of normal lenses (presumably due to its modification) by age [35,36], and results of our study show that almost all the spots of Fractions I–IV contained either αA or αB or both.

Deamidation that introduces a negative charge when an amide group is replaced with a carboxylic group in Asn and Gln has been identified as the most abundant PTM of crystallins. As shown in our previous reports, the deamidation of N101 but not of N123 in αA [37], and N146 but not of N78 in αB [38] had greater effects on their structural and functional properties. A deamidated-truncated αB crystallin fragments in human lenses has also been identified [43]. Our reports have also shown that the truncation of the C-terminal extension but not the N-domain severely destabilized αa [11] – and αB [23]-crystallins, whereas the deamidation not the truncations of αA and αB resulted in relatively greater changes in their structural and functional properties [11,24]. Lampi et al. have shown that deamidation of β-crystallins leads to their structural destabilization and aggregation [39]. In human aging and cataractous lenses, an equal number of Asn and Gln residues are deamidated in crystallins, but the extent of deamidation of Asn was three times greater than that of Gln [40]. Further, although the deamidation has been identified as molecular clocks for biological events such as protein turnover, development, and aging [41], such roles of this PTM in the lens remain unknown.

Oxidation of crystallins has also been reported to be the key PTM in development of age-related nuclear cataract [42]. Our study showed the oxidation of αA fragments of M1, D2, R12 F17, Y18, F53, R54,

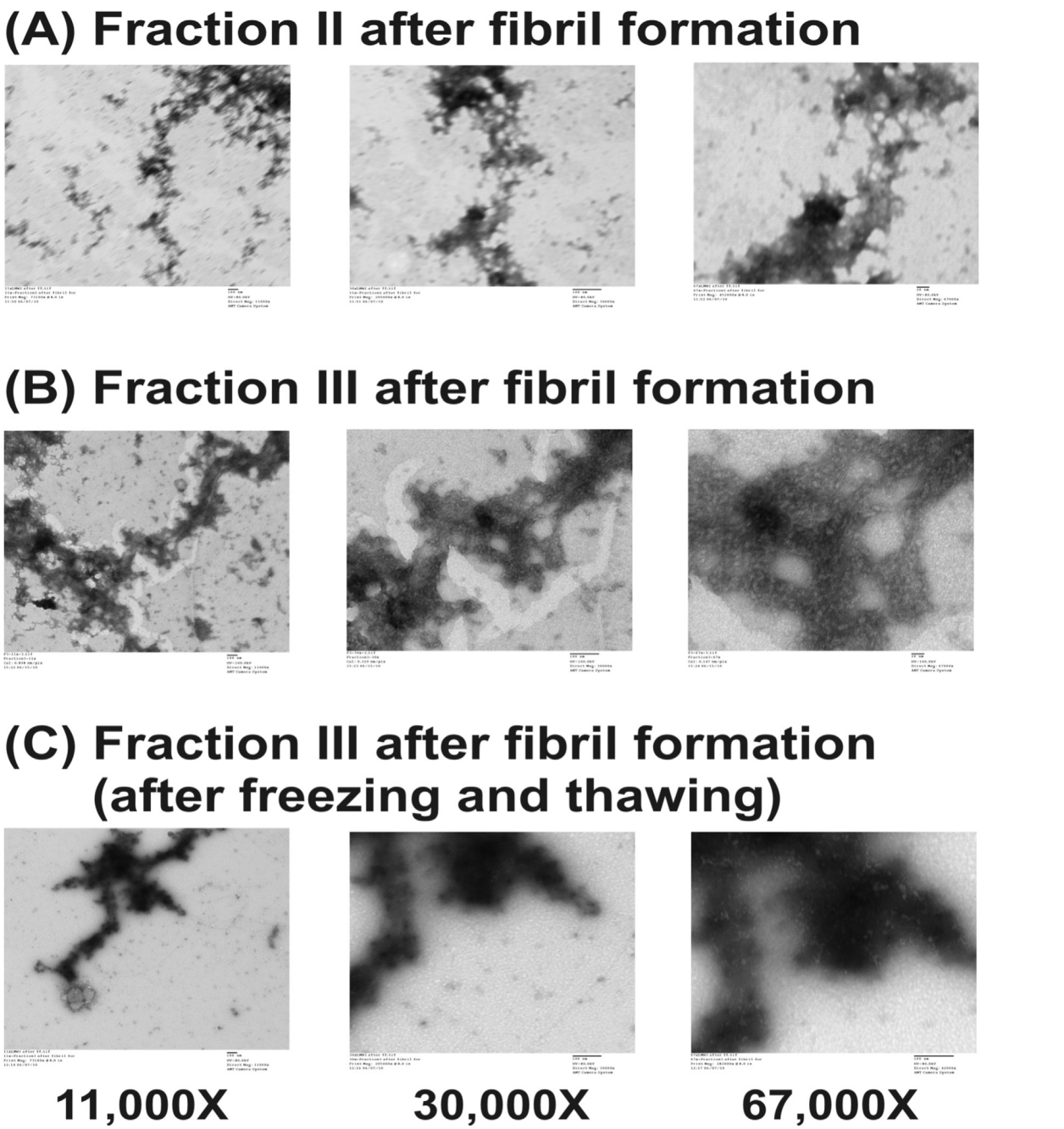

Fig. 9. TEM-images of Fractions II and III to determine amyloid fibril formation. (A) and (B): Fractions II and III showed amyloid fibril-like structure in the form of strings at low pH, 2.0, respectively. (C): Fraction III showing fibril formation after freezing and thawing of Fraction III. The aggregated particles were of ~100 nm in sizes.

D58, R69, D76, K78, H79, F80, P82, D84, K78, F93, H97, K99, H100, R103, Q104, D105, D106, H107, Y109, C131, D136, M138, P144, K145, D151, and H154 residues, and in αB fragments of M1, D2, P16, P58, W60, F61, D62, M68, R69, K72, F75, H83, F84, P86, D109, H111, F113, K166, K174, and N146 resides. Because a steep oxygen concentration gradient exists in the lens with highest at the periphery and lowest in the center [43], the oxidative modifications of specific amino acids of crystallins in nucleus might be minimal in young lenses. However, oxidation of Y18, Y34 and M138 residues of αA and Y48, W60 and M68 residues of αB and of C-142 and C-142 residues of αA [44] have been reported. Several initiating factors are proposed that could lead to oxidation of crystallins and eventually their cross-linking. For example, an exposure to UV light of covalently attached lens protein to kynurenine is believed to cause their UV-induced oxidation [45], but similar oxidation of crystallin fragments has not been investigated.

In our report, αA fragments showed methylation of L51, R54, L57, S59, I61, S62, R65, H79, S81, L85, Q90, I96, H97, K99, H100, H107, I110, S111, Q126, S127, L129, S130, C131, C142, K145, I146, Q147, L150, H154, and R157 residues, whereas αB fragments of S19, S21, S59, L65, S66, I98, H101, I114, S115, and K166 residues. A previous report showed that in αA, K70, K78, K88 and K145 residues were acetylated and R21 and K88 residues were methylated [44]. Methylation of proteins typically occurs at side chains of Lys, Arg, Glu and Asp in residues at their N and C-terminal ends [46]. Although both acetylation and methylation are believed to play a major role in signal transduction, their role in the truncated crystallin fragments might be simply to alter their structures.

The crystallin fragments of fractions I–IV exhibited aggregation on storage at 5 °C but such aggregates were dissociated on incubation at

37 °C. The Fraction I on aggregation exhibited a mass of 1.03×10^8 Da, Fraction II 5.5×10^5 Da, Fraction III 7.6×10^5 Da, and Fraction IV 8.5×10^5 Da (Table 7). The results suggested that among the four fractions, the crystallin fragments of Fraction I generated the aggregates with highest M_r. Similarly, the crystallin fragments also exhibited their aggregation with WS-HMW proteins. The aggregation of crystallin fragments *per se* or with WS-HMW proteins could be due to hydrophobic interactions as suggested previously by us [4,15]. The HMW-protein-associated crystallin fragments increased 14× and 69× between ages of 16–19 years and 60–80 years, respectively [4]. Similarly, the percent of crystallin fragments increased 4.8× with aging constituting about 27% of the HMW proteins in 60–80 year-old donors compared to 5.6% in 16–18 year old donors. Additionally, it was noted that the hydrophobic amino acid contents in crystallin fragments in WS-HMW proteins were 40% and 45% in 16–18 year and 60–80-year-old human lenses, respectively [4]. This suggested that the crystallin fragments that are rich in hydrophobic amino acids could form complexes due to hydrophobic interactions. This was supported by the fact that a αA-crystallin peptide of residue no. 66–80 (SDRDKFVIFLDVKHF) that existed in normal and cataractous human lenses showed aggregation and amyloid fibril formation due to hydrophobic interactions [18]. Based on these findings, we hypothesize that hydrophobic interactions among crystallin fragments with aberrant conformations and PTMs lead to their proximity causing them to aggregate and cross-link.

Another interesting property of the crystallin fragments present in Fractions I–IV was amyloid fibril-type structure formation. The amyloid formation was determined by assay with Congo Red (CR) or Thioflavin (ThT). Amyloid fibrils bind to CR or ThT and show a shift in fluorescence emission [47]. Proteins unrelated to any amyloid disease could also produce amyloid fibrils [48]. To avoid this pitfall that amyloid fibril formation is a generic property of polypeptides, studies have been directed to understand mechanism of protein misfolding and aggregation that might lead to amyloid fibril formation. Amyloidosis could result from the increased concentrations of the incompletely folded states of globular proteins due to mutagenesis or post-translational modifications, environment changes around proteins or “molecular crowding” (*i.e.*, a high concentration of proteins [300–400 mg/ml]) [49,50]. These conducive conditions to amyloidosis of crystallins do exist in the lens, *i.e.* high protein concentrations in the center of older lenses, molecular crowding and altered environment around proteins due to their PTMs as described in the present study. Similarly, in several hereditary cataracts, the mutations caused a reduction in solubility of the native state crystallins that in turn lead to solid state complexes and lens opacity. The mechanism of misfolding of crystallins and amyloid fibrils formation in these cataracts have not been investigated although conformational changes leading to crystallin misfolding were shown. Formation of amyloid deposits in the eye lens would potentially disturb the short range order of the crystallins and thus lead to lens opacity and cataract.

In summary, the present study shows the existence of potential covalent complexes of fragments of multi-crystallins with several PTMs *in vivo* in the WS proteins of human lenses. These complexes could aggregate with WS-HMW proteins *in vitro* and form amyloid fibril-type aggregates that might a mechanism of development of lens opacity. Because our previous studies have shown that the complexes of crystallin fragments exist in WS-HMW- and WI-proteins in aging and cataractous human lenses, it is likely that such complexes of the crystallin fragments play an important role in the development of lens opacity.

Grant information

Supported by the PHS grants EY06400 and P30 EY03039 and a grant from the EyeSight Foundation of Alabama.

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