Identification and Properties of Anti-chaperone-like Peptides Derived from Oxidized Bovine Lens β\textsubscript{L}-Crystallins*

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Thermal aggregation of β\textsubscript{L}-crystallin was higher in the presence of peptide fragments generated from oxidized and trypsin-digested β\textsubscript{L}-crystallin compared with thermal aggregation of the control proteins without oxidized β\textsubscript{L}-crystallin fragments. Increased aggregation of β\textsubscript{L}-crystallin was also observed despite the presence of α-crystallin (which has anti-aggregating properties) in the system. Self-aggregation of the oxidized β\textsubscript{L}-crystallin fragments per se was not observed under the experimental conditions. Reverse-phase HPLC analysis of the precipitate obtained after heating a mixture of β\textsubscript{L}-crystallin and oxidized β\textsubscript{L}-crystallin fragments revealed that more than one peptide co-precipitates with β\textsubscript{L}-crystallin. Electrospray mass spectrometry analysis of the peptides revealed that the molecular weight(s) of the peptides ranged from 1400–1800. Tandem mass spectrometry and a database search revealed that two of the peptides originated from βA4-crystallin (LTIPEQENFLGR, residues 121–132) and β\textsubscript{B3}-crystallin (AINGTWGYEFPFGYR, residues 153–167) respectively. Oxidized synthetic peptides representing the same sequence were also found to enhance the aggregation of β\textsubscript{L}-crystallin in a manner similar to oxidized lens β\textsubscript{L}-crystallin peptides. These data suggest that the polypeptides generated after oxidation and proteolysis of β\textsubscript{L}-crystallins interact with denaturing proteins and facilitate their aggregation and light scattering, thus behaving like anti-chaperones.

Lens crystallins undergo several changes during aging and cataractogenesis (1–3). Oxidation is a key factor in cataract formation (2, 4). The ocular lens is under constant oxidative stress. Elevated H\textsubscript{2}O\textsubscript{2} levels have been measured in ocular tissues during senile cataract (4). Damage to lens crystallins appears to be largely attributable to the effects of UV radiation and/or various active oxygen species such as oxygen radicals, O\textsubscript{2} or H\textsubscript{2}O\textsubscript{2} (3). Previous studies on the relationships among oxidation, cataract formation, and proteolysis in cultured rat lens suggest that calpain may be activated in H\textsubscript{2}O\textsubscript{2}-treated lens (5). Based on the observation that calpain II increased the insolubilization of β-crystallin polypeptides (6), it was hypothesized that accumulation of crystallin fragments in vivo may lead to increased scattering and cataractogenesis.

Proteolysis is associated with cataract and aging, and increased amounts of crystallin fragments are present in cataract lenses (7–9). Studies of cataract lenses from animal model systems have suggested that lens polypeptides may also be cleaved into lower molecular weight components during lens opacification (10, 11). The presence of increased amounts of free amino acids (11) and low molecular weight polypeptides in cataract lenses (10) have confirmed that proteolysis occurs during cataract formation in rats and mice, respectively. Barber (12) reported that in human lens the quantity of degraded proteins increased with the severity of the cataract. Srivastava et al. (13) have reported that the amount of degraded crystallins in aged/cataract lenses from human eye can be as much as 20–25% of the total protein. As many as 13 polypeptide species with molecular masses between 8 and 17 kDa, originating from α, β, and γ-crystallins, have been isolated and characterized from human lenses (13).

Protein aggregation, which increases with age, results in the accumulation of high molecular weight aggregates that directly scatter light. While it has been hypothesized that improper interaction of crystallin fragments generated by proteolysis may be responsible for the development of cataract (7), how the crystallin fragments initiate or influence the process of lens protein aggregation to form high molecular weight aggregates is poorly understood.

To simulate aging and proteolysis we oxidized bovine β\textsubscript{L}-crystallins with H\textsubscript{2}O\textsubscript{2} in the presence of CuSO\textsubscript{4} (14) and then treated them with trypsin to generate peptide fragments. The resulting β\textsubscript{L}-crystallin fragments were tested for their ability to influence the aggregation of thermally denaturing β\textsubscript{L}-crystallin. We also investigated whether the aggregation kinetics of denaturing proteins are altered in the absence or presence of oxidized peptides and α-crystallin. This investigation shows that the peptides derived from oxidized β\textsubscript{L}-crystallin can enhance the aggregation of denaturing proteins. We also report the origin, molecular weights, and sequence of the polypeptides that bind to the aggregating β\textsubscript{L}-crystallin.

EXPERIMENTAL PROCEDURES

Enzymes and Reagents—Sequence-grade trypsin was purchased from Cooper Biomedicals. Yeast alcohol dehydrogenase (ADH) was procured from Sigma Chemical Company. All other chemicals were of analytical grade.

Purification of Crystallins—a-Crystallin was isolated from young bovine lens cortex by gel filtration on a Sephadex G-200 and ion-exchange chromatography on a TMAE Fractogel column (EM Separations) as described earlier (15). The β\textsubscript{L}-crystallin was isolated from bovine lens extracts following Sephadex G-200 chromatography as described earlier (16).

Oxidation and Proteolysis of Bovine Lens β\textsubscript{L}-Crystallins—The β\textsubscript{L}-crystallins isolated from bovine lens (30 mg) were oxidized with 10 μM

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† The abbreviations used are: ADH, alcohol dehydrogenase; HPLC, high pressure liquid chromatography; ESIMS, electrospray ionization mass spectrometry; MS, mass spectrometry.
H2O2 in the presence of 0.1 mM CuSO4 (14) and 0.05 mM sodium phosphate buffer, pH 7.0, in a final volume of 30 ml. The mixture was incubated for 12 h at 25 °C. The extent of protein oxidation was monitored by amino acid analysis and tryptophan fluorescence measurement. The oxidized solution was extensively dialyzed (Mw cut-off 10,000 Da) against 0.05 mM phosphate buffer, pH 7.0, for 12 h. The dialyzed protein samples were treated with trypsin (50:1 w/w) for 12 h at 37 °C to generate peptides. Following this, 1 mM N-tosyl lysine chloromethyl ketone (TLCK) was added to inactivate the trypsin, and the solution was filtered through a 10-kDa centrifugal filter (Amicon). The low molecular weight peptides were recovered and designated as the oxidized and proteolyzed fraction. As a control, native β-crystallin was digested with trypsin without oxidation as mentioned above.

**Tandem Mass Spectrometry (MS2)—**MS2 analysis of peptides was performed to determine the sequences of the peptides. Automated analysis of CID spectra to determine the isolated and fragmented to give a series of fragment ions, due to backbone cleavage of the peptide. Automated analysis of CID spectra to determine the amino acid sequence of peptides was performed on computer as described (17) using SEQUEST software from ThermoFinnigan.

**Other Methods**—The protein measurements were made at 280 nm by the Bicinchoninic acid method (18). The amino acid composition of the native and oxidized β-crystallin was determined by amino acid analysis at the Experimental Station Chemical Laboratories of the University of Missouri according to the method described by Gehrke et al. (19). The fluorescence measurements were made in a Jasco spectrophotofluorometer. The fluorescence spectra of the native and oxidized β-crystallin (0.1 mg/ml) in 50 mM phosphate buffer, pH 7.0, were taken using an excitation wavelength of 295 nm and an emission scan between 300–400 nm. Synthetic peptides LITFEQENVFLGR (P-1), AINGTWGVYEFPGY (P-2), and DRRFWSLRSAPGA (P-3) were prepared in the Department of Chemistry, University of Missouri. These peptides were oxidized with CuSO4 and H2O2 as described above for 6 h, and their aggregation-promoting effect on denaturing β-crystallin was studied essentially as mentioned earlier. Control peptides without oxidation were also processed simultaneously. The extent of tryptophan modification in the oxidized synthetic peptides (P-2 and P-3) was quantified by spectrometric method (20). Peptide (P-1) oxidation was confirmed by amino acid analysis.

**RESULTS**

Exposure of Proteins to H2O2 and Cu2+ results in extensive oxidation. To analyze the extent of H2O2 and CuSO4 induced oxidation of β1-crystallin, we monitored the change in the fluorescence characteristics of the protein as the tryptophan residues were damaged during the oxidation process. Native β1-crystallin shows a fluorescence emission peak at 338 nm when excited at 295 nm. Under the oxidation conditions we employed there was a decrease (60%) in the intrinsic tryptophan fluorescence of the oxidized β1-crystallin protein compared with the native protein, indicating a significant oxidation of tryptophan residue(s). Furthermore, amino acid analysis indicated a decrease in the levels of several amino acids in oxidized protein relative to that of the native β1-crystallin (Table I). SDS-PAGE (Fig. 1) analysis under reducing conditions revealed the formation of both high and low molecular weight components. These changes can be attributed to the free radicals generated by the H2O2 and Cu2+ mixture, known to cause protein oxidation, degradation as well as cross-linking.

**Oxidized β1-Crystallin Fragments Facilitate Aggregation of Denaturing Proteins**—Thermal denaturation of β1-crystallin resulted in aggregation and light scattering. Addition of the oxidized β1-crystallin fragments (50 μg) increased scattering of denaturing β1-crystallin compared with one without β1-crystallin fragments as measured by the increase in absorbance (Fig. 2a). This effect was dependent on the concentration of the oxidized crystallin fragments. For instance, increasing the concentration of the oxidized β1-crystallin fragments from 50 to 100 μg resulted in further increased absorbance due to more scattering elements (Fig. 2a). However, the increased scattering elements were not due to the aggregation of β1-peptides since the peptides by themselves did not scatter light under the experimental conditions (data not shown). Further, oxidation was also required for the increased scattering, since non-oxidized β1-crystallin fragments had no effect on protein aggregation.

Increased light scattering in presence of oxidized peptides was accompanied by precipitation of greater quantity of protein. This was confirmed by measuring the amount of the proteins precipitated with and without oxidized peptides. Therefore, increased light scattering was likely due to an increased proportion of aggregated protein rather than a simple increase in aggregate size.

| Amino acid | Native | Oxidized | % decrease |
|------------|--------|----------|------------|
| Thr        | 100    | 93       | 7          |
| Met        | 100    | 95       | 5          |
| Tyr        | 100    | 10       | 90%        |
| His        | 100    | 25       | 75%        |

Only the amino acids affected by oxidation are listed. Other amino acids did not show any significant change. Increase in MetSO and cysteine sulfonic acid was observed in the oxidized sample. The data shown are the average of two samples analyzed.
peptides facilitate aggregation even in the presence of α-crystallin. Fig. 2b shows the aggregation kinetics of βL-crystallin in the presence of α-crystallin (15 μg) and/or oxidized βL-crystallin fragments. The concentration of α-crystallin (15 μg) used in these assays was such that it allowed us to observe the modulating effect of the oxidized βL-crystallin peptides. As expected, α-crystallin was able to suppress the aggregation of denaturing βL-crystallin (this is due to the chaperone-like activity of α-crystallin). Addition of oxidized βL-crystallin fragments (50 μg) facilitated light scattering by denaturing βL-crystallin in the presence of α-crystallin as well. Furthermore, increasing the βL-crystallin peptide fragments concentration in the system from 50 to 100 μg appeared to further increase the scattering and precipitation of the substrate protein. However, the peptide fragments derived from non-oxidized βL-crystallin did not have any influence on thermally denaturing βL-crystallin in the presence of α-crystallin (Fig. 2b). Similar results were obtained when ADH was used as a denaturing substrate protein (data not shown).

**Isolation of Oxidized βL-Crystallin Peptides That Facilitate Aggregation of Denaturing βL-Crystallin**—A representative HPLC chromatogram depicting the elution profile of the precipitate obtained after heat treatment of a mixture of native βL-crystallin and oxidized βL-crystallin peptides is shown in (Fig. 3). Four peaks were observed eluting between 47 and 57 min, followed by βL-crystallin. Analysis of the βL-crystallin precipitate obtained after heat treatment under similar conditions showed only βL-crystallin profile (data not shown). These results suggest that some of the oxidized βL-crystallin derived peptides bind to the aggregating βL-crystallin. The peptides eluting between 47 and 57 min during HPLC (Fig. 3) were pooled and designated as fractions A (peak nos: 1 + 2) and B (peak nos: 3 + 4).

**Molecular Mass Determination of HPLC-fractionated Peptides**—The pooled peptide fractions (A and B) were analyzed by electrospray mass spectrometry. The spectra of peptide fraction A is depicted in Fig. 4a. There were peaks at mass charge (m/z) 1730.1 for the singly charged form (MH⁺) and at m/z 865.1 for the doubly charged form (MH²⁺) of a single peptide. The 881.2 m/z ion is 32-dalton higher than the doubly charged parent ion,
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Fig. 3. Reverse-phase HPLC chromatogram of the precipitate obtained after heating a mixture of β-lens crystallin and oxidized β-lens crystallin peptide fragments. An aliquot of the precipitate dissolved in 6 M urea (see “Experimental Procedures”) was analyzed by HPLC to isolate peptides that co-precipitate with β-lens crystallin. Peptide peaks (1–4) eluted with a retention time between 47–57 min. Peaks (1, 2) and (3, 4) were pooled separately and designated as fractions A and B respectively. Peaks labeled 5–7 correspond to β-lens crystallins.

at 865.1 m/z. The addition of two oxygen atoms to one of the amino acids in a peptide would result in a 32-Da mass shift. When we determined the sequence of singly charged 1730.1 m/z ion by MS/MS analysis it was found to match the sequence of residues 153–167 (AINGTGWGYEEFPFYR) in bovine β3-crystallin. The oxidation of Trp to N-formylkynurenine in AINGTGWGYEEFPFYR peptide (P-2) could result in a doubly charged 881.2 ion found during the initial analysis (Fig. 4a). This was confirmed by MS/MS analysis of the peptide with m/z 881.2 ion from Fig. 4a, and the data are shown in Fig. 4b. During MS/MS analysis, several of the b and y ions produced showed 32 dalton mass shifts compared with the unoxidized peptide spectra (not shown). Since the masses of b3, y3, and y5 ions do not reflect any oxidation, we feel that the 32-dalton mass shift in b6 and y11 ions were likely due to the oxidation of the most susceptible amino acid in the peptide, Trp. The 881.2 m/z ion has the sequence AINGTWoxVGYEEFPFYR (P-2), which matched with residues 153–167 of bovine β3-crystallin. The other ion peaks between m/z 1000–1500 in Fig. 4a could be contributed by more than one oxidized β-lens crystallin fragment present in the sample. These ions were not analyzed to determine their identity.

Analysis of fraction B showed peaks at m/z 1466.4 (MH+), and m/z 734.0 (MH+2) (Fig. 5a). The peak at m/z 749.5 is 32 Da higher than the doubly charged parent ion, which indicates the addition of two oxygen atom (32 Da) to the peptide. In the same fraction, other peaks at m/z 1774.5 (MH+) and m/z 888.1 (MH+2) were also observed during MS analysis (data not shown). The doubly charged parent ions in all cases were additionally analyzed by MS/MS to confirm the sequence of the peptide. An example of an MS/MS of a peptide with m/z 734.0 (MH+ 1466.4) is shown in Fig. 5b. The spectrum shows the fragments formed by collisional activation of the peptide, which has the sequence LTIFEQENFLGR (P-1). Sequence matching of this peptide was performed to determine the identity of the parent crystallin. The P-1 peptide sequence was similar to the amino acid residues 123–132 of the bovine βA4-crystallin. In this case the 749.1 ion was not further analyzed. MS/MS analysis of another peptide with m/z 888.1 showed a sequence ENIQINADNQYVLK. We were unable to confirm the identity of the parent protein for this peptide as it did not match any of the known β-, crystallin protein sequences.

Aggregation-promoting Effect of Oxidized Synthetic Peptides—Peptides (1 and 2) corresponding to the sequences of P-1 and P-2 were synthesized and tested to confirm their role in facilitating aggregation of denaturing proteins. These peptides were oxidized by H2O2 and Cu2+. Amino acid analysis of peptide P-1 indicated a decrease in the following amino acids. The percent decrease was (Phe, −37%, Arg, −40%, Thr, −36%), respectively, compared with the unoxidized peptide. Spectrometric assay suggested that the amino acid tryptophan in the peptide-2 (P-2) was oxidized (60–80%). Enhanced aggregation of denaturing β-lens crystallin was observed in the presence of oxidized P-2 (Fig. 6a). When 25 μg of oxidized peptide was used, the enhanced aggregation was nil or only marginally higher with respect to the control β-lens crystallin without peptide. However, further addition of the peptide (100 μg) showed a significant increase (70–80%) in the aggregation of β-lens crystallin as evidenced by the increased light scattering. It should be noted that, with 100 μg of peptide in the system (Fig. 6a), maximum aggregation and precipitation occurred at the midpoint of the assay duration (20 min), after which the aggregates started settling down as precipitate. Interestingly, when unoxidized peptide-2 (P-2) was added, no increase in aggregation of the β-lens crystallin was observed (Fig. 6b). Additionally, the peptides by themselves did not precipitate under the experimental conditions. These results suggest that oxidation plays a key role in the aggregation-promoting effect of this peptide.

Addition of the unoxidized peptide P-2 (100 μg) to a α-crystallin chaperoning system did not show any effect (Fig. 6b). However, when the oxidized P-2 peptide was included in the assay an increased aggregation was found (Fig. 6b). A similar observation was made with oxidized peptide P-1 but the enhancement in aggregation was less compared with that seen with peptide-2 (data not shown). Also, when these two peptides (P-1 and P-2) were added together after oxidation to an assay, the increase in light scattering of denaturing β-lens crystallin was greater than after separate additions. While addition of 50 μg of either of the peptides to the assay showed less than a 10% increase in the absorbance by light scattering particles, the assays with 50 μg of each peptide added together resulted in a 30% increase in absorbance.

When the facilitated aggregation was tested in the presence of a control non-lentilicate peptide P-3, (DRIIFWSSLR-SAPGA; 100 μg), the facilitated aggregation of the denatured protein was <10% higher compared with the aggregation of β-lens crystallin by itself (Fig. 7). However, in the presence of peptide P-2 the facilitated aggregation of β-lens crystallin was 90% higher (the percent increase in absorbance was due to more light scattering particles) compared with P-3. This indicates that the facilitated aggregation is significantly higher in the presence of lenticular peptide P-2, compared with a non-lentilicate peptide.

DISCUSSION

β-Crystallin is the most heterogenous of the lens crystallins. Earlier workers have shown that βA3/A1 crystallin is cleaved in vivo in human lenses and contributes to the formation of 4- and 5-kDa polypeptides (22). One of the contributing factors for the generation of low molecular weight peptides from partially denatured or oxidatively modified crystallins in vivo is proteolysis (23–28). The rate of oxidized protein degradation is also dependent on the concentration of proteases that preferentially degrade oxidized proteins (29). Shang et al. (29) reported that bovine lens epithelial cell extracts contain proteases which selectively degrade oxidized crystallin. Earlier studies indicated that proteins oxidized by hydrogen peroxide are susceptible to hydrolysis by acylpeptide hydrolase (30). Acylpeptide
hydrolase activity has also been found in lens tissue (31). Therefore, it is likely that, in vivo, the oxidatively modified lens proteins are selectively degraded by specific enzyme systems, resulting in the generation of low molecular weight polypeptides. Additionally, non-enzymatic mechanisms are also likely to generate crystallin fragments (32, 33). Further degradation of the peptides to release amino acids depends upon the aminopeptidase activity (34–36) in vivo. Since the peptidase activity is lower in the inner cortex and nucleus compared with the outer cortical region of the lens (24), a decrease in peptidase activity may be contributing to an increased accumulation of the degraded polypeptides in aging lens (36, 37).

Previous reports indicated that in vivo proteolysis of lens crystallins might lead to opacification in the human lens (38, 39). In vitro incubation of relatively high concentrations of rat lens water-soluble proteins (50 mg/ml) with calpain results in proteolysis of β1-crystallin and turbidity (8), suggesting that proteolysis may lead to lens opacification. However, in our experiments, addition of low concentrations of β1-crystallin fragments (100–200 µg/ml) derived from proteolysis of non-oxidized proteins did not result in increased aggregation of denaturing protein or modulation of the anti-aggregation property of β-crystallin (Fig. 2, a and b). Surprisingly, the presence of the same concentrations of oxidized β1-crystallin fragments in assays led to increased aggregation, light scattering, and precipitation of denaturing proteins. Therefore, oxidation of the protein prior to proteolysis is likely to be a critical event in the peptide induced process. Our studies with oxidized synthetic peptides (Fig. 6a) further confirm a plausible role for oxidation and proteolysis in lens protein aggregation and cataractogenesis.

The light scattering process is the primary factor responsible for the observed opacity in a cataractous lens. The temporal process of aggregation of crystallins and other enzymes is characterized by an initial period of low light scattering before a

**Fig. 4. Analysis of oxidized peptides recovered from thermally aggregated β1-crystallin and oxidized peptide mixture by mass spectrometry.** a, ESMS analysis of the HPLC fractions. Fraction A from HPLC showed a singly charged (m/z 1730.1) and doubly charged (m/z 865.1) peptide. Information regarding other ions is provided in the results section. b, tandem mass spectrum analysis of peptide with MH2+ 881.2. The b and y series ions identified as fragments generated during CID of the oxidized peptide are labeled. The unlabeled ions in the spectrum represent the internal fragments. The sequence of βB3 crystallin 153–167, with N-formylkynurenine designated as Wox and masses of the predicted fragment ions are shown as an inset.
steep increase in scattering that slows as the aggregation process is saturated (40, 41). Facilitated aggregation of denaturing \( \beta_L \)-crystallin in the presence of increasing concentrations ofoxidized \( \beta_L \)-crystallin fragments suggests that oxidized \( \beta_L \)-crystallin fragments function like anti-chaperones. (The term anti-chaperone is used only with reference to the aggregation-promoting property of the peptides as opposed to the aggregation-suppressing effect of \( \alpha \)-crystallin or mini \( \alpha \)-crystallin, Ref. 42.) A possible mechanism for this process is that the denaturing proteins self-aggregate, and the \( \beta_L \)-crystallin fragments

**FIG. 5.** Analysis of oxidized peptides recovered from thermally aggregated \( \beta_L \)-crystallin and oxidized peptide mixture by mass spectrometry. \( a \), ESMS analysis of fraction B. This fraction showed MH\(^+\) (\( m/z \) 1466.4) and MH\(^2+\) (\( m/z \) 734.0) forms. The ion peak at \( m/z \) 749.5 is 32 Da higher than the doubly charged parent ion, indicating the addition of two oxygen atoms to the amino acid(s) in the peptide. \( b \), tandem mass spectrum analysis of peptide with MH\(^+\) 1466.4. The doubly charged ion at \( m/z \) 734.0 was selected as a precursor ion. The inset shows the expected fragments from the sequence of peptide (LTIFEQENFLGR) found in the spectrum. When the charge on the peptide stayed with the N terminus, fragments from the b series were seen; when the charge stayed with the C terminus, fragments from the y series were seen.
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The high molecular weight protein-associated polypeptides were found to be a heterogeneous mixture derived from α-, β- and γ-crystallins (13). The accumulation of crystallin fragments in *vivo* is likely to be one of the causes for lens opacity, as shown by Horwitz et al. (45). By microdissection and analysis of the opaque and clear regions of the same lenses Horwitz et al. (45) have shown that low molecular weight crystallin fragments are present in the opaque region but the clear regions of the same lenses do not contain crystallin fragments. They also reported that brunescent lens nuclei, which are cataractous in nature and have significant quantities of oxidized protein, also contain crystallin fragments. From our experiments it is clear that the presence of oxidized β- crystallin peptides leads to increased aggregation of the denaturing proteins. Based on our results we hypothesize that the hydrolysis of oxidized proteins by lens proteases and the accumulation of peptides during aging could significantly contribute to *in vivo* light scattering by way of facilitated aggregation and insolubilization of the modified or partially denatured lens proteins.

The mechanism by which classical chaperones suppress protein aggregation is thought to involve binding of the chaperones to the denatured protein, which decreases the concentration of denatured substrate in solution (48). Previous studies suggest that binding of increasing amounts of partially denatured γ-crystallin to the α-crystallin decreased the ability of the latter to protect against heat-induced denaturation and aggregation of ADH (49). Since a significant percentage of the α-crystallin isolated from the high molecular weight aggregate of aged bovine lens was found to be complexed to either β- or γ-crystallin (50), it is possible that irreversible binding of lens proteins to the α-crystallin particle may decrease the ability of α-crystallin to prevent further aggregation of lens proteins *in vivo*. In the present study, enhancing the concentration of the oxidized peptides seemed to increase aggregation of denaturing proteins despite the presence of α-crystallin (Fig. 6b). While it is not clear whether the peptides bind to α-crystallin during the aggregation process, from our studies, it appears that oxidized β- crystallin fragments enhance the aggregation of denaturing proteins even when α-crystallin attempts to suppress their aggregation. This effect may involve the interaction of peptide...
and α-crystallin at different sites on the denaturing protein. From our in vitro study it is clear that the oxidized peptides bind to the denaturing β-crystallin. Alternately, an intermolecular competition for common binding sites in the denaturing proteins may be taking place between the oxidized β-crystallin fragments and α-crystallin. The relative concentrations of the protein and peptides may determine the end result. Further studies on the interaction of the peptide or α-crystallin with the denaturing proteins are required to unravel the molecular mechanisms behind the interactions.

It is difficult to quantitatively relate the effects of the oxidized peptides on the aggregation process in vitro to the protein aggregation and opacification in vivo without knowing the precise amount and location of the oxidized peptides in lens. However, it is of interest that improper interactions of the lens proteins and peptides generated after oxidation and subsequent proteolysis facilitated aggregation and increased light scattering. Further characterization of the chemical basis for the action of the oxidized peptides and their interaction with crystallins and other proteins in vitro will enable us to understand the importance of the facilitated aggregation process in suppressing lens transparency.

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