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Importance of Eye Lens α -Crystallin Heteropolymer with 3:1 α A to α B Ratio: Stability, Aggregation, and Modifications

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Summary

Chaperone-like activity (CLA) of α -crystallin is essential not only for the maintenance of eye lens transparency but also in the biology of other tissues. Eye lens α -crystallin is a heteropolymer composed of two homologous subunits, α A and α B, and in most vertebrates they are present in a ratio of 3:1. The structural and functional significance of this specific ratio of α -crystallin subunits is of considerable interest in understanding its role in the eye lens transparency. Previously, we have shown that although at physiologically relevant conditions α B-crystallin has greater CLA, under stress conditions such as elevated temperatures α -crystallin heteropolymer with 3:1 α A to α B ratio displayed higher CLA (Srinivas et al., *Biochem. J.*, 2008, 414, 453 – 460). Herein, we provide a rationale for the existence of α -crystallin heteropolymer with 3:1 α A to α B ratio in terms of structural stability, aggregation pattern, and susceptibility to posttranslational modifications that could explain the importance of the heteropolymer of α -crystallin in the eye lens. We demonstrate that α A-crystallin is not only more stable but also imparts stability to the heteropolymer by preventing the aggregation of α B-crystallin at higher temperatures by using differential scanning calorimetry, size-exclusion chromatography, and denaturant-induced unfolding methods. Further, the physiological significance of heteropolymer with higher proportion of α A subunit is substantiated by using a heteropolymer with mutant (F71L) α A-crystallin and the susceptibility of 3:1 heteropolymer to glycation-induced modifications. Thus, the existence of 3:1 heteropolymer might be vital for the eye lens transparency under diverse conditions to prevent cataract.

Keywords

α -crystallin; heteropolymer; 3:1 ratio; stability; unfolding; aggregation; differential scanning calorimetry

INTRODUCTION

Crystallins are the major structural proteins in the eye lens that account for up to 90% of its total soluble proteins (TSP). They belong to distinct families: α -, β -, and γ -crystallins, the short order arrangement of which helps in maintaining transparency of the eye lens (1, 2). Aging causes damage to lens proteins, as a result of conformational changes and modifications such as oxidation, deamidation, or cleavage that alter protein-protein interactions and result in aggregation, affecting the lens transparency (2, 3). Impaired lens function due to partial or complete loss of transparency is called cataract, a leading cause of blindness worldwide (3). α -Crystallin, by virtue of its unique structural features and high concentration, contributes to the flawless transmission of light with minimal scatter and spherical aberration through the lens (1, 2, 4). α -Crystallin belongs to the family of small heat-shock proteins (sHsp), characterized by the presence of a conserved “ α -crystallin domain” at C-terminal region (1, 2, 4, 5). Like other members of sHsp family, α -crystallin functions as a molecular chaperone, preventing or suppressing the aggregation of client proteins (mainly β - and γ -crystallins and enzymes of the eye lens) caused by heat or other stress conditions (1, 4–8).

Studies demonstrate that the chaperone-like activity (CLA) of α -crystallin is instrumental in maintaining lens transparency and prevention of cataract [reviewed in (4, 5)]. α -Crystallin has been shown to prevent precipitation of aggregation-prone proteins by forming a stable, soluble high-molecular-mass complex with the “substrate” or “client” protein. Intrinsic and surface elements in α -crystallin impart specificity to its interaction with the substrates that are on an off-folding pathway destined for aggregation and precipitation (4, 5, 9–11). The mechanism of action of α -crystallin CLA and the role of surface hydrophobicity, charge distribution, oligomeric size, and intrinsic stability are the subject of extensive study.

The eye lens α -crystallin is a large heteropolymer composed of two subunits, αA and αB , each of 20 kDa and encoded by *CRYAA* and *CRYAB* genes, respectively (4, 5). Native and recombinant αA and αB subunits form heteropolymers according to the proportion of mixing of the individual subunits (12–15). Homopolymers of αA - and αB -crystallin are known to possess similar structural and functional integrity as that of native α -crystallin, although their relative CLA might not be identical. However, in nonlenticular tissues, α -crystallin is present either as αA - or αB -homopolymer (4, 5, 16). Although only traces of the αA -crystallin are found in some nonlenticular tissues, the distribution of αB is ubiquitous (16). The apparent difference in the tissue-specific distribution of αA - and αB -crystallins indicates that these two proteins might have evolved to play distinct physiological functions. αB -Crystallin is overexpressed in some neurological and degenerative diseases, and mutation(s) in αB -crystallin is associated with pathological conditions such as desmin-related myopathy [reviewed in (1, 4, 16–19)]. Moreover, certain point mutations in αA - and αB -crystallin genes are reported to cause various types of human cataracts, and most of these mutations are known to affect structure and CLA of α -crystallin (4, 20). Recently, we have identified a novel point mutation (F71L) in αA -crystallin associated with early onset of age-related cataracts due to defective CLA (21).

The lenticular α -crystallin heteropolymer exists as a polydisperse oligomer with an average molecular mass of 600–800 kDa and composed of 30–50 subunits (1). In most vertebrate lens, the molar ratio of αA to αB is 3:1, though this varies among species, from 1:3 in dogfish, 9:1 in kangaroo, and 19:1 in catfish (22–24). Studies from our laboratory and elsewhere have demonstrated that αA and αB subunits, either recombinantly expressed or reconstituted from native source, form heteropolymers in the proportion they are mixed, due to subunit or intermolecular exchange (13–15). The existence of a heteropolymeric complex of HspB2/B3 with a fixed subunit ratio but rather with a poor chaperone-like and

thermoprotective activity in muscle has been reported recently (25). Further, despite high sequence homology, α A- and α B-crystallins behave differently with respect to their CLA, hydrophobicity, structure, and other physicochemical properties, particularly at higher temperatures (8, 26–28). However, the significance of the existence of α -crystallin as a heteropolymer with a lens-specific subunit ratio has not been addressed comprehensively. Earlier, we had demonstrated that although under physiological conditions α B-homopolymer has a relatively higher CLA, at elevated temperatures ($>50^{\circ}\text{C}$) and upon structural perturbation, the CLA of α A-homopolymer or the heteropolymer with a higher α A proportion (3:1 ratio) was higher (15). Structural perturbation in the case of α -crystallin refers (but not exclusively) to treatment of the protein to mild/low concentrations of denaturants or heating to high temperatures followed by cooling to ambient temperature. These findings have necessitated further studies to understand how the 3:1 heteropolymer is bestowed with the optimal chaperone ability and its relevance to lens biology. To understand the significance of α -crystallin (3:1) heteropolymer in the eye lens, we studied the differences in structural and thermodynamic stability and susceptibility to aggregation and modification of heteropolymers (with varying ratios of α A to α B). Use of differential scanning calorimetry (DSC), denaturant-induced unfolding, and size-exclusion chromatography (SEC) allowed us to depict the landscape of diversity in structural stability that exists in the α -crystallin homo- and reconstituted heteropolymers.

EXPERIMENTAL PROCEDURES

Materials

Guanidine hydrochloride (GdmCl), dithiothreitol (DTT), methyl glyoxal (MGO), and molecular weight markers (thyroglobulin, γ -globulin, and BSA) were purchased from Sigma Chemical, and TSKG4000 SWXL 7.8 mm $\times$ 300 mm column was purchased from Tosoh (Tokyo, Japan). All other chemicals used were of the highest purity available.

Cloning, Expression, and Purification of Recombinant Wild-Type α A-, α B-Crystallins and Mutant α A-Crystallin

Cloning and expression of human α A-, α B- and mutant (F71L) α A-crystallins in the BL21/pET23d cells were described earlier (8, 21). Proteins from 1-L cultures were extracted and purified according to the procedures described previously (8). Purity and homogeneity of the recombinant crystallins were found to be more than 95% as analyzed by SDS-PAGE. The concentration of α A-, α AF71L-, and α B-crystallin was calculated using respective molar extinction coefficients (8).

Purification of α - and β -Crystallin From Goat Eye Lens

Goat eyeballs were obtained from a local abattoir, and the lenses were dissected out. Preparation of the TSP and purification of α - and β -crystallins from goat eye lens were carried out essentially as described earlier (15).

α -Crystallin Variants Used in the Study

Recombinant α -crystallin heteropolymers (with α A to α B subunit ratios of 3:1, 1:1, and 1:3 were obtained by mixing recombinant α A- (or F71L) and α B-crystallins in appropriate proportions) and α A- and α B-crystallin homopolymers were used in this study. Native α L-crystallin isolated from goat lens served as the native control for the heteropolymer having 3:1 α A to α B ratio and henceforth is referred to as α L. Formation of heteropolymers of α -crystallin upon mixing in the desired ratios of subunits was confirmed by immunoprecipitation followed by immunoblotting as described previously (15).

Differential Scanning Calorimetry

Studies to investigate the thermal unfolding of α -crystallin variants were carried out using a DSC (VP-DSC-Microcal LLC Northampton, MA). Protein samples (1 mg/mL) were extensively dialyzed at 4°C against 0.02 M potassium phosphate buffer, pH 7.4, containing 0.1 M potassium chloride and 0.001 M DTT. Both the dialysate and protein solutions were thoroughly degassed under vacuum at room temperature before the scans. Differential scanning calorimetric thermograms were recorded from 10 to 100°C at a scan rate of 60°C/h (29). Thermograms were also obtained at a scan rate of 30 and 90°C/h to check for the scan rate dependence of thermal unfolding. The data were analyzed using the Origin DSC software provided by the manufacturer.

Size-Exclusion Chromatography

Oligomeric profile of α -crystallin variants (0.02 mg) was performed on a Shimadzu HPLC system (Kyoto, Japan) connected to TSKG4000 SWXL 7.8 mm $\times$ 300 mm column equilibrated with 0.1 M sodium phosphate buffer, pH 6.7, containing 0.1 M sodium sulfate and 0.05% sodium azide (30). Thyroglobulin (669 kDa), γ -globulin (160 kDa), and BSA (67 kDa) were used as molecular weight standards. The oligomeric profile of pre-heated crystallins was also obtained. Preheating of α -crystallin variants was done by heating samples at 60°C for 15 min followed by cooling to 25°C.

Guanidinium Chloride-Induced Unfolding Studies

Denaturant-induced unfolding experiments were carried out by incubating varying concentrations of GdmCl (0–6 M) with α -crystallin variants for 15 h at 25°C to ensure that equilibrium was attained before measurements were made (31).

Tryptophan Fluorescence—The intrinsic tryptophan fluorescence intensity of α -crystallin variants (0.1 mg/mL, in 0.02 M sodium phosphate buffer, pH 7.2) at 335 nm and maximum emission wavelength (λ_{max}) was plotted as a function of GdmCl concentration by excitation at 280 nm and following the emission spectra from 300–400 nm in a spectrofluorimeter (Jasco FP-6500, Kyoto, Japan). Respective buffer blanks were corrected for the GdmCl concentration.

Circular Dichroism—Far- (0.1 mg/mL in 0.2-cm pathlength cuvette) and near-UV CD (1.0 mg/mL in 0.5-cm pathlength cuvette) spectra of α -crystallin variants (at equilibrium unfolding state in 0.02 M sodium phosphate buffer, pH 7.2) were recorded using a spectropolarimeter (Jasco J-810, Kyoto, Japan) (31). Each spectrum was the average of five accumulations and corrected with respective buffer blanks of GdmCl concentration.

Modification of α -Crystallins With Methyl Glyoxal

α -Crystallin variants (3.0 mg/mL) in 0.1 M sodium phosphate buffer, pH 7.2, were incubated with 0.005 M MGO in dark under sterile conditions for 72 h. At the end of incubation, the samples were thoroughly dialyzed against the same buffer to remove unbound MGO (30). Protein estimation was done by the Lowry method. Secondary and tertiary structure of modified crystallins was monitored by far-UV CD and intrinsic tryptophan fluorescence as described above.

Chaperone-Like Activity Assay

CLA of heteropolymer with 3:1 ratio of wild-type α A to α B or mutant F71L- α A to α B was assessed by measuring the ability of these combinations to suppress the aggregation of β L-crystallin at 65°C. Aggregation of β L-crystallin (0.2 mg/mL) in 0.05 M sodium phosphate buffer, pH 7.2, containing 0.1 M NaCl was studied in the absence and presence of crystalline

variants (0.05 mg/mL) in a UV-vis spectrophotometer (Perkin-Elmer Lambda-35, MA) set to 360 nm (15).

RESULTS AND DISCUSSION

Earlier, we had reported that the heteropolymer with a higher αA proportion (3:1) functioned as a better chaperone at elevated temperatures (above 50°C) when compared with αB or the heteropolymer with a higher αB proportion (1:3) (15). In this investigation, we provide a basis for the significance of existence of α -crystallin as a heteropolymer with an exclusive ratio of subunits, in terms of stability, aggregation, susceptibility to modifications, and the importance of αA in heteropolymer.

Differential Scanning Calorimetry

DSC was used to study the thermodynamic stability of the α -crystallin variants. In a typical DSC study, unfolding of the native structure of a protein is accompanied by an endothermic transition. DSC scans of all the variants of α -crystallin considered in this study exhibited a positive endotherm between 55 and 65°C (Fig. 1). Although scans for the αL , αA , and the 3:1 variant could be performed up to 100°C without any precipitation in the protein solution, αB and the 1:3 variants precipitated between 70 and 75°C (Fig. 1). Moreover, the unfolding transitions of the 3:1, αL , and αA variants were largely reversible and their rescans in the 10–100°C range yielded thermograms that were essentially identical with only a slight decrease in the enthalpy (data not shown). Although there was no significant difference in the transition temperatures of the different α -crystallin variants for scan rates between 30 and 90°/h scan rate (data not shown), the tendency of some of the α -crystallin variants (αB and the 1:3) to aggregate just above the unfolding transition affected the baseline of the thermograms, precluding accurate quantitative determination of thermodynamic parameters (32, 33). Thermodynamic data for αB and 1:3 were obtained by cutting the scale at the temperature at which these variants got precipitated.

In particular, the aggregation and precipitation of αB and the 1:3 heteropolymer seemed to be kinetically controlled due to which considerable variation was observed in the unfolding enthalpies obtained at different scan rates. Hence, in this investigation, we described the thermal unfolding of α -crystallin variants in qualitative terms. Best fitting of DSC thermograms to a single transition model indicates simultaneous dissociation of subunits and unfolding of polypeptides. The minimal cooperative unit of the α -crystallin variants appears to be the monomeric subunit (32–34). Although the thermal unfolding of α -crystallin variants could not be interpreted in quantitative terms based on the thermodynamic data obtained from DSC for the reasons mentioned above (that the aggregation of some of the α -crystallin variants after the unfolding transition precluded accurate determination of thermodynamic parameters), DSC profiles indicate that 3:1 heteropolymer and αA -crystallin homopolymer are more stable than αB homopolymer and the 1:3 heteropolymer, in the sense that they do not precipitate upon thermal unfolding whereas the latter two precipitated in the first heating (Fig. 1). These observations indicate that the αA moiety seems to impart stability to αB -crystallin in the heteropolymer.

Although αB and 1:3 have a higher unfolding temperature (T_m , 63 and 62°C) compared with the other α -crystallin variants (αA , 3:1 and 1:1; 58–61°C) their relative thermal stability is low, that is., they precipitate from solution because of the formation of large aggregates. This may be attributed to the fact that αB might require a substantial or complete unfolding process before it gets aggregated. Formation of larger insoluble aggregates until complete unfolding of all the molecules may be attributed to a higher exothermic or precipitation temperature in the case of αB and 1:3. However, in spite of relatively lower T_m , 3:1, αA , and αL have shown no signs of precipitation. Similar results

were obtained in the SEC where there was no significant change in the 3:1, α L, and α A upon preheating in contrast to α B-homopolymer.

Size-Exclusion Chromatography

SEC was used to determine the oligomeric masses of normal and preheated crystallin variants as oligomeric size/mass has been shown to influence CLA of α -crystallin. Consistent with the reported studies, the apparent oligomeric mass of the native α L-crystallin is ~800 kDa (1, 2), the recombinant homo- and heteropolymers are ~650 kDa (8, 14, 28, 35) (Fig. 2A). However, with preheating (heating at 60°C for 15 min followed by cooling to 25°C) all the variants, except α B-crystallin, eluted ahead of the respective unheated variants indicating an increase in the aggregate molecular mass upon structural perturbation supporting the increased CLA for heteropolymers upon preheating (Fig. 2A). These results are consistent with many previous studies that reported a reorganization of oligomer with an increased size upon preheat treatment (5, 8, 9, 28). Consistent with previous studies (28), α B-homopolymer got precipitated upon preheating. SDS-PAGE analysis of α -crystallin variants upon heating indicates that although α B-homopolymer was present mostly in the insoluble fraction, rest of the crystallin variants remained in solution (Fig. 2B). These results are in tune with the DSC data where there was no significant change in the 3:1, α L, and α A upon preheating in contrast to α B-homopolymer. Further, these observations substantiate the DSC results that the α A moiety might impart stability to α B-crystallin.

Unfolding Studies

Tryptophan fluorescence as a function of GdmCl concentration indicates that all the α -crystallin variants are almost completely unfolded by 3.0 M GdmCl (Fig. 3). Tryptophan fluorescence of α A, α B, and 3:1 at 335 nm for a given GdmCl concentration is depicted in (Figs. 3A–3C). Crystallin variants 1:3, 1:1, and α B start unfolding at a lower concentration of GdmCl compared with 3:1 and α A (Fig. 3D). Apparent C_m (concentration of GdmCl required for 50% unfolding) of α A (1.6 M), α L (1.5 M), and 3:1 (1.5 M) is relatively higher compared with 1:1 (1.1 M), α B, and 1:3 (0.9 M).

Consistent with results of tryptophan fluorescence, significant changes in tertiary structure were observed at 1.0 M GdmCl in the near-UV CD intensity of α -crystallin variants, and a substantial loss of tertiary structure was observed by 1.5 M GdmCl for all the variants (data not shown). In contrast to 3:1, 1:1, and α L, the extent of loss in tertiary structure for α B and 1:3 was higher at 1.0–1.5 M GdmCl consistent with the tryptophan fluorescence as a function of GdmCl. However, there were subtle but no significant differences in GdmCl-induced unfolding pattern of secondary structure between the α -crystallin variants as monitored by CD (Fig. 4). However, secondary structure of all the α -crystallin variants is relatively more stable than tertiary structure to denaturant-induced unfolding as no substantial loss of secondary structure was observed even at 3.0 M GdmCl (Fig. 4).

It is too complicated to analyze the unfolding curves of the large heteropolymer such as α -crystallin; the same could not be fitted to a simple two- or three-state transition model by other workers (26). The apparent C_m of tryptophan fluorescence in GdmCl unfolding for α A, α L, and 3:1 was significantly higher compared with α B, 1:3, and 1:1 variants, indicating higher stability for 3:1 and α A. Nevertheless, the unfolding patterns observed in the two methods used (tryptophan fluorescence and CD) in this study imply the existence of equilibrium unfolding intermediates. Further, it also indicates that the protein has more than one domain, because the critical unfolding steps and the existence of folding intermediates for large proteins are thought to be related to protein domains (36, 37). However, dissociation and unfolding appear to proceed simultaneously in thermal unfolding as

revealed by DSC. These results further suggest that the thermal and chemical unfolding pathways could be different. However, the data obtained with denaturant-induced unfolding studies indicate a gradual increase in stability between the heteromeric complexes of αA and αB with a ratio from 1:3 to 3:1 and highlight the robustness of the 3:1 heteropolymer. A higher ratio of αA to αB (6:1 and 10:1) showed similar chaperone function and stability as that of 3:1 heteropolymer (PNBS Srinivas and GB Reddy, unpublished data), further implying the heteropolymer with 3:1 might be better suited for lens.

Susceptibility to MGO-Induced Glycation

Previously, MGO modification has been shown to increase the negative ellipticity in the far-UV CD spectrum of bovine α -crystallin (30). Similarly, in this study, MGO modification induced significant alterations in secondary and tertiary structure of α -crystallin variants. The αA , 3:1, and αL variants were relatively less susceptible to MGO-induced alterations in secondary structure compared to that of αB , 1:1, and 1:3 as indicated by percentage change in the negative ellipticity in far-UV CD over the unmodified crystallin variants (Fig. 5). Similarly, αA , 3:1, and αL have shown least alterations compared with αB , 1:1, and 1:3 in tertiary structure upon MGO modifications as monitored by intrinsic tryptophan fluorescence (data not shown).

Chaperone Activity

We have recently reported a point mutation (F71L) in αA -crystallin with decreased CLA in heat-induced aggregation assays (21). Hence, we studied the CLA of 3:1 heteropolymer, in which wild-type αA was replaced with F71L- αA . In heat-induced βL -crystallin aggregation assay, the 3:1 heteropolymer with wild-type αA to αB displayed about 80% protection, whereas 3:1 heteropolymer with mutant $\alpha AF71L$ to αB displayed significantly reduced (35%) protection (Fig. 6). αA -Homopolymer exhibited almost similar CLA to that of 3:1 heteropolymer; αB - and F71L- αA -homopolymers have shown much lesser protection (Fig. 6). Results with a mutant αA subunit wherein CLA of the 3:1 heteropolymer (with mutant F71L- αA) was reduced to more than 50% compared with the 3:1 heteropolymer (with wild-type αA) suggest the importance of αA subunit in the structure and function of α -crystallin. However, it should be noted that experimental data obtained under extreme conditions do not constitute the physiological conditions of the eye lens. Nevertheless, temperature-mediated or chaotropic agent-induced changes are generally used to derive the conclusions on structure and function of proteins under extreme conditions. More so, temperature-dependent chaperone assays have been routinely used for α -crystallin.

It is well established that during aging, lens crystallins including α -crystallin undergo various post-translational modifications such as nonenzymatic glycation, phosphorylation, and truncation. Glycation of lens proteins is believed to be one of the mechanisms responsible for both age-related and diabetic cataracts (3, 30, 38). Further, glycation is known to affect CLA of α -crystallin (30, 38, 39). Hence, we have studied the effect of MGO-induced glycation on α -crystallin variants. Our data on susceptibility to nonenzymatic glycation gain merit with regard to the physiological significance of 3:1 heteropolymer in the eye lens as we demonstrated that 3:1 ratio of αA to αB was less susceptible to MGO-induced secondary and tertiary structural changes. Earlier, we have reported an increased expression of αA - and αB -crystallins both at transcript and protein levels albeit to varied extent under certain pathophysiological conditions like streptozotocin-induced diabetic cataract in rats (18). A drastic increase in αB -crystallin in the lens is consistent with the fact that αB -crystallin is a Hsp and is associated with diabetic cataract. In addition, results of 2D gel electrophoresis followed by immunoblotting with αA and αB antibodies indicate alteration in the relative quantities of αA and αB in cataractous rat eye lens when compared with control (PNBS Srinivas and GB Reddy, unpublished data). This is indicative of an

alteration or disturbance in the 3:1 stoichiometry of αA to αB in the eye lens, which may affect the lens transparency. Taken together, these findings highlight the physiological importance of αA on the overall stability and chaperone potential of the α -crystallin heteropolymer.

Earlier studies point out the differential roles of αA and αB crystallin with respect to lens transparency. For example, development of lens transparency is not affected in αB -crystallin knockout mice (40), but the deletion of αA -crystallin in mice has resulted in cataract (41). Although it was reported that the double knockout (of αA and αB) animals develop cataract (42), the physiological role of 3:1 heteropolymer has not been highlighted. Similarly, the *in vitro* (8, 14, 26, 27) and cell culture studies (43, 44) that reported the differential properties of αA - and αB -crystallins have not emphasized or discussed the importance of 3:1 ratio.

Both αA and αB had originated from a common ancestral gene that diverged about 450 million years ago to serve different physiological functions. In general, αB -crystallin appears to be a better chaperone than αA -crystallin under physiological conditions (8, 26, 27). However, the lower stability and aggregation behavior at elevated temperatures (14, 26, 28, 45) may not be advantageous for αB -crystallin to function under diversified conditions to which eye lens is exposed. On the other hand, αA -crystallin being more stable and having better chaperone activity at high temperatures makes it a versatile chaperone. In an environment of low protein turnover, any compromise in CLA and stability would be detrimental to the eye lens. Thus, the lens may have evolved to possess a specific combination of the two proteins in the eye lens, which will not only compliment each other functionally but also provide an enduring chaperoning apparatus. The findings reported in this article together with those of our previous study provide a basis for the significance of α -crystallin heteropolymer with αA to αB in the 3:1 ratio, which may possibly be an evolutionary adaptation to serve the biological demands of the eye lens.

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Abbreviations

CD	circular dichroism
DSC	differential scanning calorimetry
GdmCl	guanidinium hydrochloride
sHsp	small heat-shock protein
SEC	size-exclusion chromatography
TSP	total soluble protein

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Figure 1.

Differential scanning calorimetric (DSC) profiles of α -crystallin variants. Unfolding endotherms of α -crystallin variants scanned from 10 to 100°C at a scan rate of 60°C/h. Continuous line represents experimental data, and dashed line is the best fit of the experimental data to a two-state transition model using Microcal Origin software. Arrows indicate the temperature at which αB and 1:3 precipitated.

Figure 2. Size-exclusion chromatography (SEC). Panel A: SEC profiles of unheated (continuous line) and preheated (dashed line) α -crystallin variants. Elution positions of standard molecular weight markers, thyroglobulin (650 kDa; position-1), ovalbumin (150 kDa; position-2), and BSA (67 kDa; position-3), are indicated on the top. Panel B: SDS-PAGE profile of α -crystallin variants in the order of unheated, preheated (soluble), and preheated (insoluble) samples.

Figure 3.

Unfolding of α -crystallin variants monitored by tryptophan fluorescence. Panels A–C: Representative tryptophan emission fluorescence profiles of αA , αB , and 3:1, respectively, as a function of GdmCl concentration. Panel D: Tryptophan fluorescence intensity ratio of α -crystallin variants at 335 nm as a function of GdmCl concentration. Data are average of four experiments.

Figure 4. Secondary structure. Far-UV CD spectrum of α -crystallin variants (0.1 mg/mL) monitored as a function of GdmCl concentration. Each spectrum is an average of five accumulations.

Figure 5. Secondary structure of MGO-modified α -crystallin variants. Panel A: Representative far-UV CD of normal and modified 3:1 and 1:3 variants. Panel B: Percent increase in the negative ellipticity (217 nm) of α -crystallin variants upon MGO modification over respective unmodified crystalline variants. Results are mean $\pm$ SD ($n = 4$).

Figure 6.

Chaperone-like activity (CLA). CLA of homo- and heteropolymers of α A, F71L- α A, and α B. Panel A: Representative profile of the suppression of heat-induced aggregation of β L-crystallin (0.2 mg/mL) in the absence and presence of 0.05 mg/mL of α A, F71L- α A, α B, 3:1, and F71L-3:1 (w/w ratio of α A to α B). Panel B: Relative chaperone activity (percentage protection) was calculated by taking the aggregation of β L-crystallin alone as 100%. Results are mean $\pm$ SD ($n = 4$).