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Distinct roles of αA- and αB-crystallins under thermal and UV stresses☆

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Abstract

α-Crystallin, a major protein of all vertebrate lenses, consists of two subunits, αA and αB, which form polymeric aggregates with an average molecular mass of about 800 kDa. In this study, we have employed various biophysical methods to study aggregate sizes and conformational properties of purified αA, αB subunits, and cloned recombinant αB subunit. From far- and near-UV CD spectra, native α-, αA-, αB-, and recombinant αB-crystallins from porcine lenses all show similar β-sheet conformation to that from bovine and human lenses as reported previously. By means of gel-filtration chromatography and dynamic light scattering, we have found that the molecular sizes of all four crystallin aggregates are polydispersedly distributed in the following order of aggregate sizes, i.e., native α > αA > αB ≈ recombinant αB. To investigate the structural and functional relationships, we have also compared the chaperone activities of all four α-crystallin aggregates at different temperatures. From the results of chaperone-activity assays, ANS (8-anilinonaphthalene-1-sulfonic acid) binding and thermal stability studies, there appeared to be at least two factors playing major roles in the chaperone-like activity of these lens proteins: one is the hydrophobicity of the exposed protein surface and the other is the structural stability associated with each protein. We showed that αA-crystallin is a better chaperone to protect γ-crystallin against UV irradiation than αB-crystallin, in contrast to the observation that αB is generally a better chaperoning protein than αA for enzyme protective assays at physiological temperatures.

Keywords: α-Crystallins; Porcine eye lenses; Chaperone activity; Thermal stability; Dynamic light scattering

The major classes of lens proteins in all mammalian eye lenses can be divided into two families, α-crystallin and β/γ-crystallin superfamily. There are two α-crystallin genes (αA and αB), encoding two corresponding α-crystallin subunits being approximately 55–60% identical in amino acid sequence for most mammalian species [1,2]. α-Crystallin shows structural and functional similarities to small heat-shock proteins [3]; similarly, its expression can also be induced by thermal [4] and osmotic stresses [5]. In consistence with its close structural relationship to heat-shock protein, α-crystallin has also been shown to prevent thermal aggregation of crystallins and other proteins like a molecular chaperonin [6,7]. αA-Crystallin is highly specialized for expression in the lens [8]; but αB-crystallin is shown to be a functional small heat-shock protein, which is present in various tissues [9,10].

Owing to its large size and no crystallographic data available, the detailed molecular packing in the quaternary structure of α-crystallin has not been established. Therefore, various proposed packing models [11–13] for the association of α-crystallin subunits in vivo remain still a matter of controversy. In the absence of high-resolution structural data, one way in which structural information could be obtained is through probing the conformation and microenvironments of various amino acids or the location of hydrophobic areas by various biophysical methods.

Recent studies [14–17] on the chaperone activity and conformational properties of αA- and αB-crystallins from human and bovine lenses have shown some distinct differences between these two homoaggregates of α-crystallin subunits with a high degree of sequence homology. αB-Crystallin showed protective chaperone activity at

☆ *Abbreviations:* αA- or αB-crystallins, homoaggregates formed by association of αA- or αB-crystallin subunit; CD, circular dichroism; DLS, dynamic light scattering; ANS, 8-anilinonaphthalene-1-sulfonic acid.

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subphysiological temperatures, at which αA exhibited very little chaperone activity. However, αB-crystallin is more susceptible than αA to heat-induced conformational change and aggregation. Previously, we have also studied the effect of heat-induced structural changes in secondary and tertiary structures on the chaperone activity of porcine α-crystallin in vitro [18]. In this study, to resolve some discrepancy among the structural differences between native α- and the homoaggregates of its subunit chains αA and αB from the porcine eye lens, we have extended further our study on the conformational and chaperone properties of porcine native α-, αA-, αB-, and cloned recombinant αB-crystallins by dynamic light scattering (DLS), circular dichroism (CD), and fluorescence spectroscopy. The results obtained from these combined biophysical approaches should provide some insight into the structural basis of crystallin aggregation underlying the chaperone activity of α-crystallin.

Materials and methods

Isolation of lens crystallins. Porcine lenses were decapsulated and homogenized in the buffer containing 50 mM Tris–HCl, 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol, and 0.02% sodium azide, pH 8.0. After centrifugation at 27,000*g* for 30 min, the supernatant was applied to a column of TSK HW-55(F) and eluted at 25 ml/h. Five well-resolved peaks were obtained and identified as HMα-, α-, βH-, βL-, and γ-crystallins, based on their subunit compositions as revealed by SDS–PAGE. The pooled α-crystallin was further separated into αA- and αB-crystallin subunits by reverse-phase HPLC. After lyophilization, the αA- and αB-crystallins were separately dissolved in 8 M urea and applied again to a column of TSK HW-55(F) eluted with the buffer containing 50 mM Tris–HCl, 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol, and 0.02% sodium azide, pH 8.0 at 25 ml/h. The αA- and αB-crystallins were thus separated from urea, refolded, and reassociated to their respective homomultimeric aggregates. The cloned αB-crystallin was expressed and prepared according to our previous report [19]. The protein concentrations were determined by absorbance measurements using extinction coefficients that are calculated from amino acid sequence data of each protein [20].

Molecular mass analysis. Native purified α-, αA-, and αB-crystallins were dissolved in 50% acetonitrile containing 1% acetic acid to make a final concentration of 0.1 μM. The sample was then analyzed in an LCQ mass spectrometer (Finnigan, San Jose, CA) at an infusion rate of 5 μl/min. The spectra were analyzed with a software program (LCQ BioWorks) supplied from the manufacturer.

Circular dichroism. Circular dichroic spectra were performed on a JASCO J-715 spectropolarimeter. Protein concentrations were 1.9×10^{-5} M in Tris buffer with a 0.1 cm light path for far-UV CD measurements and with a 1.0 cm light path for near-UV measurements. All spectra reported are the average of five scanning accumulations.

Gel-permeation FPLC. Multimeric sizes of α-, αA-, αB-, and cloned αB-crystallins were evaluated on an analytical Superose-6 HR 10/30 prepacked column. High molecular mass standards (Amersham Pharmacia Biotech AB, Uppsala, Sweden) were used for calibration. The concentrations of native α-, αA-, αB-, and cloned αB-crystallins were adjusted to the same concentration of 7.17×10^{-5} M and 1 ml of each sample was applied to the column. The flow rate was 0.5 ml/min.

Dynamic light scattering. Scattering experiments were performed on a DLS-700 dynamic light-scattering spectrophotometer (Otsuka Electronics, Tokyo, Japan). A He–Ne laser operating at a wavelength of 632.8 nm was used as the light source. Protein samples in the buffer containing 50 mM Tris–HCl, 0.1 M NaCl, pH 8.0, were filtered through 0.45 μm filter membrane and incubated at 25 °C for 24 h. The concentration of protein samples was adjusted to a concentration of 7.17×10^{-5} M determined by the aforementioned method.

Assays of chaperone activity under thermal stress. The chaperone activity of purified native porcine α-, αA-, αB-, and cloned αB-crystallins was studied under three different conditions. Equine liver alcohol dehydrogenase (ADH), porcine βL-, and γ-crystallins were used as substrates at 37, 60, and 70 °C, respectively. Equine ADH (2.5 μM) was dissolved in the buffer of 50 mM NaH_2PO_4, 0.1 M NaCl, 2 mM EDTA, pH 7.0, at a molar ratio of 1:1 with porcine α-, αA-, or αB-crystallin and incubated at 37 °C, respectively. The turbidity or light scattering was measured at 360 nm every 5 s. Similarly, the porcine βL-crystallin (7.16 μM) in PBS buffer was also used as substrates for chaperone activity assays at 60 °C. Substrate and crystallins in various molar ratios were used to measure the chaperone activity of each crystallin. We have also used porcine γ-crystallin as the third substrate (at a final concentration of 4.9 μM in PBS buffer and in a molar ratio of 1:1) for assays at 70 °C.

Assays of chaperone activity under UV irradiation. Photoaggregation of γ-crystallin (10 μM) was monitored in a buffer of 50 mM Tris–HCl, 0.1 M NaCl, pH 8.0, in the absence or presence of porcine native α-, αA-, αB-, and cloned αB-crystallins. The molar ratio of γ-crystallin/chaperone was 1:1 for each sample. The sample solutions of 200 μl each were exposed to UV lamp (254 nm) of Spectrolinker XL-1000 (Spectronics, New York, NY). The extent of protein aggregation or turbidity was measured at OD 405 nm with an ELISA reader periodically.

Thermal stability of porcine α-, αA-, αB-crystallins, and cloned αB-crystallin. The assayed proteins were in a buffer containing 10 mM NaH_2PO_4, 2 mM KH_2PO_4, 3 mM KCl, and 0.1 M NaCl, pH 7.4. Each protein sample with the same concentration of 36 μM was heated at a temperature range of 20–80 °C, continuously measuring turbidity at OD 360 nm.

ANS binding assay. Surface hydrophobicity of porcine native α-, αA-, and αB-crystallins was measured using the fluorescent probe 8-anilinonaphthalene-1-sulfonic acid (ANS). For each sample, 10 μl ANS in methanolic stock solution (0.1 M) was added to 1 ml of 0.3 mg/ml protein solution and incubated at indicated temperatures for 1 h, respectively. The fluorescence spectra were recorded with a Hitachi F4010 fluorescence spectrophotometer by setting the excitation wavelength at 395 nm, a light slit of 5 nm for both excitation and emission modes.

Results and discussion

Crystallin purification and characterization of molecular masses

Crude lens extract of porcine eye lenses was separated into their respective crystallin fractions by gel-filtration chromatography on a TSK HW-55(F) column. The pooled and lyophilized fractions of α-crystallin were then separated into αA- and αB-crystallins by reverse-phase HPLC. The molecular masses of αA- and αB-crystallins were analyzed by electrospray mass spectrometry and confirmed to possess correct molecular masses of these subunits, i.e., 19,791, and 20,172 Da, respectively. After lyophilization, the homomultimeric αA- and αB-crystallins were dissolved in 8 M urea, refolding in a column of TSK HW-55(F). The homomultimeric αA- and αB-crystallins were thus reassociated and refolded after removal of urea.

Circular dichroism spectroscopy

The far-UV CD spectra of refolded porcine αA- and αB-crystallin homoaggregates are similar to those of native α-crystallin (data not shown). αA- and αB-crystallins showed a minimum at 217 and 215 nm, respectively. Based on the CD spectra as analyzed by the popular algorithm program [21], the results of secondary structural estimation for α-, αA-, and αB-crystallins showed mainly β-sheet structure, which were consistent with the prevailing evidence that α-crystallin consists mostly of β-sheet secondary structure [22]. The near-UV CD spectra of porcine αA- and αB-crystallins showed some difference in the range of 270–290 nm. The near-UV CD spectra which reflect the microenvironments of bulky tryptophan and tyrosine residues of these two protein aggregates of α-crystallin subunits are significantly different.

Analytical gel filtration chromatography

The aggregate sizes of porcine αA- and αB-crystallin aggregates are slightly smaller than that of native α-crystallin, as estimated by gel-permeation chromatography on Superose-6 HR FPLC column (Fig. 1). The molecular masses of native α-, αA-, and αB-crystallins were estimated to be about 860, 700, and 690 kDa, respectively. From the chromatographs in Fig. 1, it is evident that native α-crystallin forms a broad molecular-mass distribution, somewhat more dispersed than αA- and αB-crystallins. In our analysis, the native α-, αA-, and αB-crystallins of the same concentration and sample volume were assayed under identical conditions. The elution profile of the native α-crystallin shows the broadest band shape and lowest peak intensity, whereas that of αB-crystallin shows the sharpest band shape and highest peak intensity. Quantitative comparison of the half bandwidths of elution peaks from gel-permeation chromatography gives an estimate of the distribution of their apparent molecular masses for each crystallin aggregate. It clearly indicates that the bandwidth observed for α-crystallin is larger than those for αA- and αB-crystallins, with the latter showing the lowest bandwidth and molecular size. It should be noted that a slight variation in the half bandwidth was observed when different porcine lenses were used, but the half bandwidth of αA-crystallin is always found to be larger than that of αB-crystallin. A previous report [23] also showed a similar result that recombinant bovine αA-crystallin has a larger half bandwidth than recombinant human αB-crystallin. The wider half-bandwidth of an elution peak generally reflects a more size-dispersed system of an eluting protein; therefore, the polydisperse property of porcine α-crystallin and its subunit homoaggregates should follow the order $\alpha > \alpha A > \alpha B$.

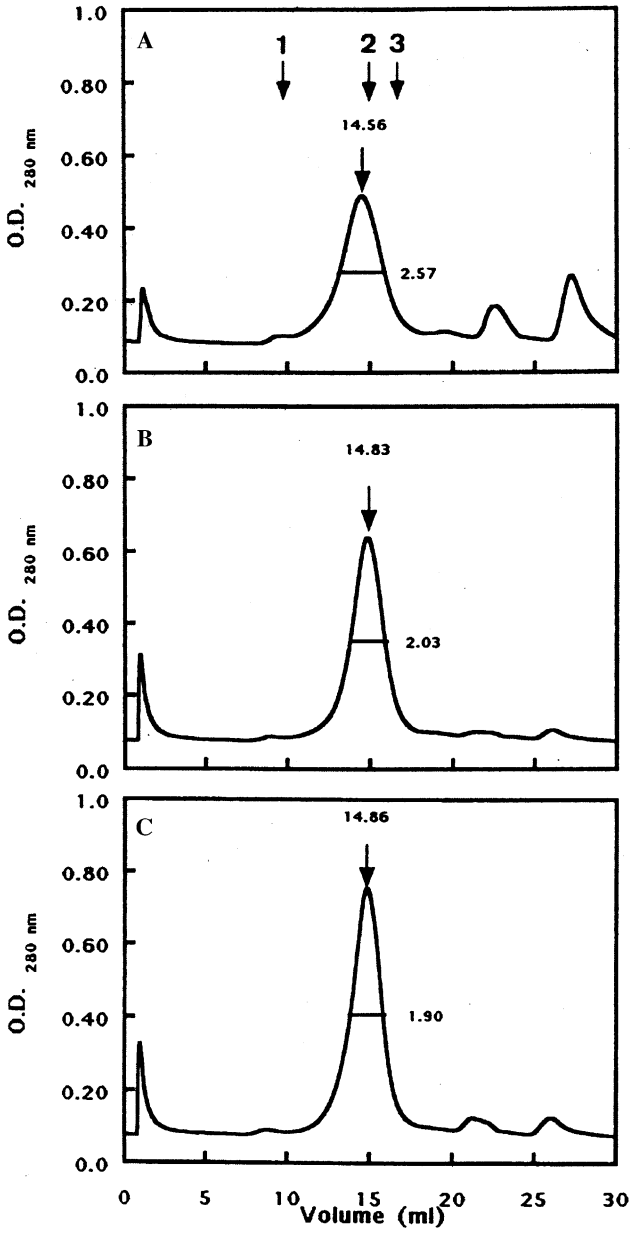

Fig. 1. Analytical gel-filtration analysis of α-crystallin and its subunit aggregates. Elution profiles for each crystallin aggregate are shown: (A) porcine native α-crystallin, (B) αA-crystallin, and (C) αB-crystallin. The system used was a Pharmacia FPLC system with a Superose 6HR 10/30 column. The sample concentration and the injected volume were 72 μM and 1 ml each, respectively. The elution buffer was 50 mM Tris–HCl, 0.1 M NaCl, pH 8.0. The flowrate was set at 0.5 ml/min. All samples were chromatographed under identical conditions at room temperature. The elution times are indicated at the top of the major peaks and the half-height volumes are also shown next to the half-width of each eluted peak. Arrows 1, 2, and 3 indicate the elution volumes of molecular-size markers, blue dextran (>10^3 kDa), thyroglobulin (660 kDa), and aldolase (160 kDa), respectively.

Dynamic light scattering (DLS) measurements

In this experiment, α-crystallin samples were filtered through a 0.45 μm filter membrane and stored at 25 °C for 24 h to validate the fact that the crystallin aggregates

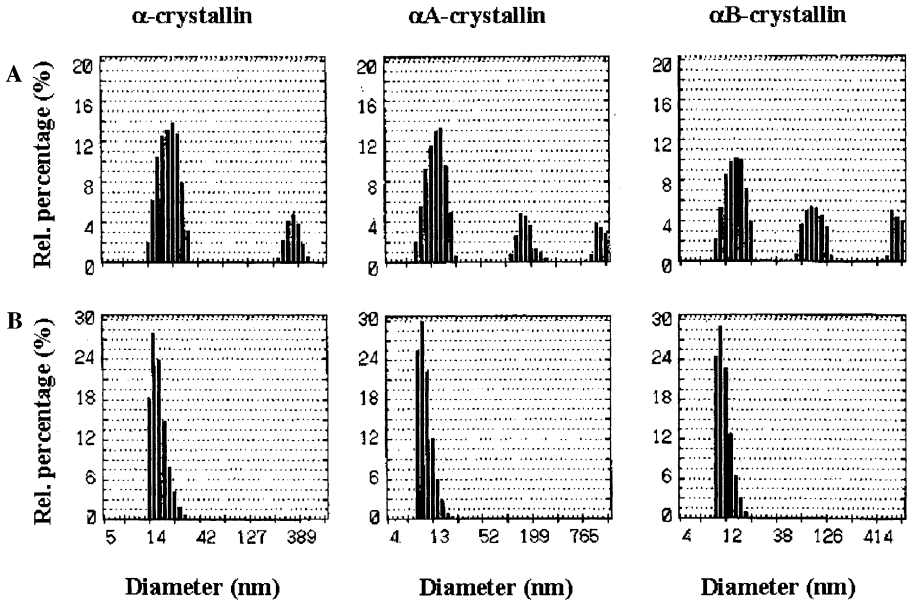

Fig. 2. Dynamic light-scattering measurements for α-crystallin and its subunit aggregates. The data are shown in bar representations with (A) the scattering intensity distribution and (B) the converted particle-number distribution for each crystallin aggregate. The vertical axis shows the scattering intensity in relative percentage (%) and the horizontal axis shows the distribution range of particle size in diameter (nm). All samples were in the buffer of 50 mM Tris–HCl, 0.1 M NaCl, pH 8.0, and equilibrated at 25 °C for 24 h. The measurements of dynamic light scattering were carried out at 20 °C.

had reached their equilibrium. For each sample, at least three measurements were performed and the hydrodynamic diameters for native α-, αA-, and αB-crystallin were calculated to be 22.2, 15.8, and 14.9 nm (mean of triplicate measurements), respectively. All three multimeric aggregates also showed polydisperse characteristics. A pivotal DLS analysis for scattering intensity and particle number distributions of three aggregates are shown in Fig. 2A. In the plots of intensity distribution, some particles with larger diameter values were found, indicative of the existence of higher aggregates in the samples. Because our samples were filtered carefully before all DLS measurements, the effect of dust could be ruled out. Therefore, the tendency of α-crystallin to associate into aggregates of higher sizes may account for this spurious observation. However, from the particle number distribution (Fig. 2B), the percentage of the population of higher aggregate particles appears to be relatively low in the samples. The mean particle diameters calculated from the scattering intensity distribution were shown to be 22.3 ± 5.0, 15.7 ± 4.0, and 14.7 ± 3.0 nm for α-, αA-, and αB-crystallins, respectively; whereas the particle diameters analyzed from particle number distribution were 17.2 ± 3.0, 11.3 ± 2.0, and 11.3 ± 2.0 nm, respectively. The difference between these two sets of data pointed to the fact that aggregates of smaller sizes constitute the major fractions among total particle-size distribution. It is worth mentioning that the size order of the three proteins was also found to be $\alpha > \alpha A > \alpha B$ in each DLS measurement. However, the size difference between αA and αB may be too small to detect by DLS measurements, owing to the inherent tendency of α-crystallin subunits to associate into complex polydisperse systems.

Comparison of the chaperone activity under thermal stress

In Fig. 3, native α-, αA-, and αB-crystallins were used to protect equine liver ADH from thermal aggregation at 37 °C. At a molar ratio of 1:1 (ADH:αB-crystallin), αB-crystallin suppressed the aggregation of ADH by about 80%. In contrast, α- and αA-crystallins suppressed the aggregation of ADH by about 60% and 50%, respectively. Therefore, porcine αB-crystallin showed better chaperone activity than both α- and αA-crystallins at physiological temperature. In Figs. 4A–C, porcine βL-crystallin was used as the target substrate protein and various combinations of βL-crystallin to α-, αA-, and αB-crystallins in different molar ratios were tested in chaperone activity assays at 60 °C. All three crystallins completely suppressed βL-crystallin aggregation at a molar ratio of 1:1. However, it was found that αB-crystallin had less chaperone activity than both α- and αA-crystallins at a molar ratio of 5:1 (Fig. 4C). In Fig. 5, porcine γ-crystallin was used as the substrate for the assays at 70 °C. At this high temperature and a molar ratio of 1:1, αA-crystallin possessed the best chaperone activity among these three crystallins. It is noteworthy that αB-crystallin showed little or no chaperone-like activity at this temperature. Severe aggregation was found when αB-crystallin was assayed at 70 °C after 10 min.

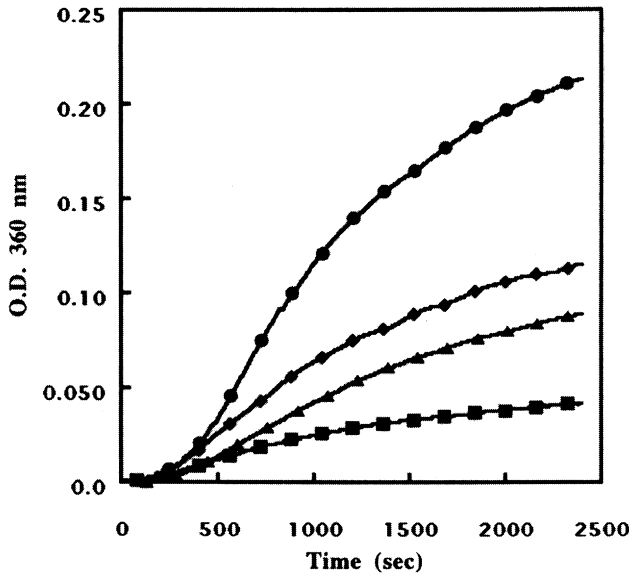

Fig. 3. Comparison of chaperone activities for various α-crystallin aggregates against thermal aggregation of ADH incubated at 37 °C. The scattering curves at 360 nm in the presence of chaperoning crystallins are shown with different symbols as follows: control solution without crystallin (circle), porcine native α-crystallin (triangle), αA-crystallin (rhombus), and αB-crystallin (square). The concentration of ADH in each curve was 0.2 mg/ml. Equine liver ADH in a buffer of 50 mM $NaH_2PO_4 \cdot H_2O$, 0.1 M NaCl, 2 mM EDTA, pH 7.0, was used as protein substrate at a molar ratio of 1:1 for the chaperone activity assays.

Thermal stability of α-, αA-, and αB-crystallins

In Fig. 6, we study the protein stability by incubating native α-, αA-, αB-, and cloned αB-crystallin solutions of the same concentration in a temperature range between 20 and 80 °C. The solutions of α- and αA-crystallins remain clear, even after heating up to 80 °C for 10 min whereas the solution of αB-crystallin becomes turbid when temperature is higher than 60 °C.

Chaperone activity assay under UV irradiation

In Fig. 7, native α-, αA-, and αB-crystallins were used to protect γ-crystallin from UV-induced protein aggregation at 25 °C. For each sample, at least eight measurements were carried out to obtain more accurate values of light scattering. We have reported that α-crystallin can protect γ-crystallin from UV-induced aggregation previously [24]. Here, at a molar ratio of 1:1, αB-crystallin can suppress only 29% of the aggregation of control γ-crystallin under UV-irradiation for 4 h. However, α- and αA-crystallins suppressed up to 62% and 69% of the aggregation of γ-crystallin under similar conditions. It is of interest to note that even native α-crystallin shows less protective activity than αA-crystallin alone. Contrary to the results of most chaperone-like activity assays under thermal stress, our

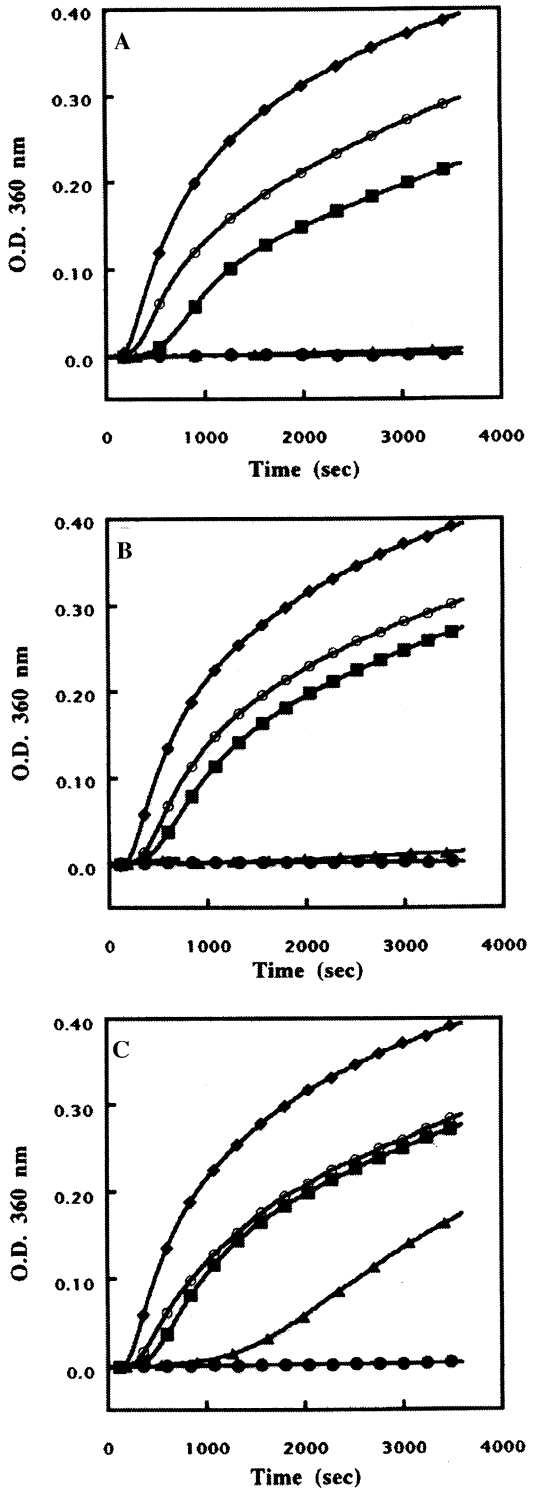

Fig. 4. Comparison of chaperone activities for various α-crystallin aggregates against thermal aggregation of porcine βL-crystallin at 60 °C. Porcine βL-crystallin was used as substrate for chaperone-activity assays of (A) α-crystallin, (B) αA-crystallin, and (C) αB-crystallin in various molar ratios (chaperone: βL-crystallin) of 1:1 (open circle), 1:5 (triangle), 1:25 (square), and 1:50 (open circle). The sample which contained βL-crystallin only (rhombus) was used as control. The concentration of βL-crystallin was 7.16 μM in PBS. It is noticeable that at a molar ratio of 1:5, porcine αB-crystallin lost part of its activity to prevent βL-crystallin from thermal aggregation when compared with α-crystallin and αA-crystallin.

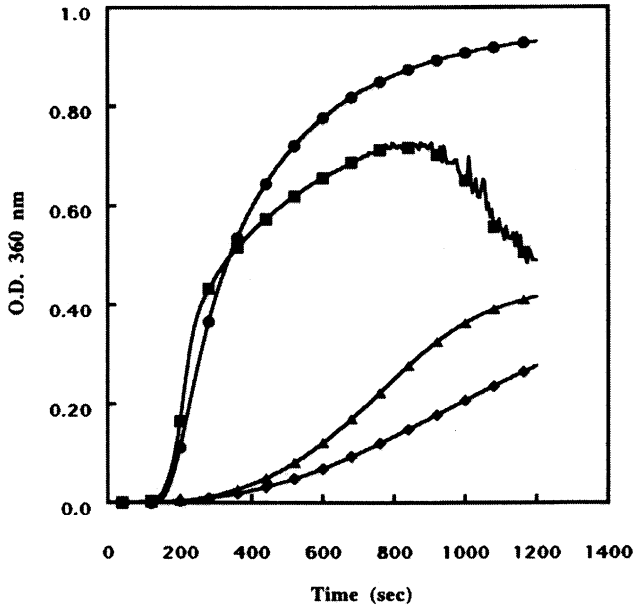

Fig. 5. Comparison of chaperone activities for various α-crystallin aggregates against thermal aggregation of porcine γ-crystallin at 70 °C. Porcine γ-crystallin (4.9 μM) was used as substrate at a molar ratio (chaperone: γ-crystallin) of 1:1. The sample which contained γ-crystallin only (circle) was used as control. Three crystallin aggregates were used for chaperone-activity assays: porcine α-crystallin (triangle), αA-crystallin (rhombus), and αB-crystallin (square). Note that porcine αB-crystallin failed to protect γ-crystallin from thermal aggregation and form precipitate in less than 10 min.

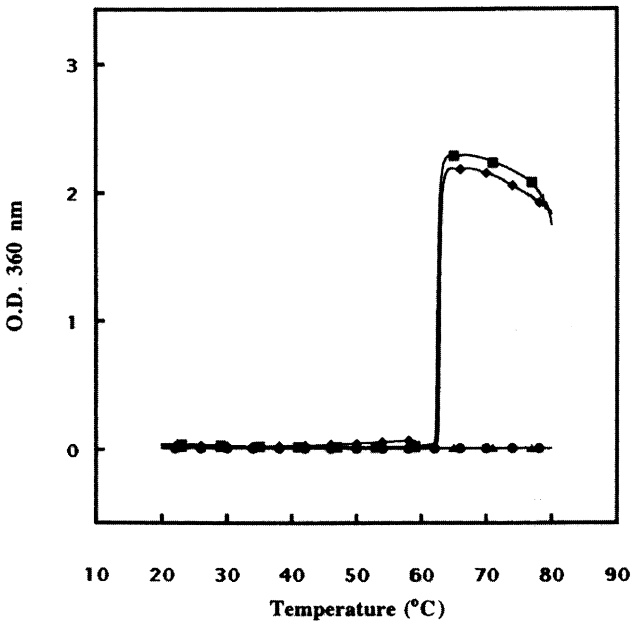

Fig. 6. Comparison of thermal stability for various α-crystallin aggregates and recombinant αB-crystallin. Four porcine α-crystallin aggregates with identical concentration (36 μM in 0.5 ml) were analyzed for their thermal stability by heating the samples from 20 to 80 °C. Turbidity changes for each crystallin solution were followed continuously by measuring OD (optical density) at 360 nm. Curves were shown for α-crystallin (circle), αA-crystallin (triangle), αB-crystallin (square), and recombinant αB-crystallin (rhombus). Note that αB-crystallin solution and its recombinant form turn turbid with a transition temperature of about 62 °C. α- and αA-crystallin solutions remain clear even up to 80 °C.

result attests to the important role of αA-crystallin in the protection of γ-crystallin from UV-induced aggregation at ambient temperatures under physiological conditions.

Surface hydrophobicity of α-, αA-, and αB-crystallins

ANS is basically a hydrophobic probe, essentially nonfluorescent in aqueous solution. It becomes fluorescent when bound to the hydrophobic area on the surface of various macromolecules. To investigate if there exists a correlation between surface hydrophobicity and chaperone activity of α-crystallins at different temperatures, we probed the surface hydrophobicities of α-, αA-, and αB-crystallins at 37 and 60 °C using ANS. At 37 °C, αB-crystallin aggregate showed the highest fluorescence intensity, whereas αA-crystallin showed the lowest among these three crystallin aggregates (Fig. 8A). When α-, αA-, and αB-crystallin solutions were incubated at 60 °C for 1 h, αB-crystallin became slightly turbid, resulting in the rapid decrease of ANS fluorescence (Fig. 8B). On the other hand, α- and αA-crystallin solutions remained clear and the ANS fluorescence intensity was slightly higher at 60 °C than at 37 °C. As expected, the more surface hydrophobic region exposed, the better chaperone activity these crystallin molecules possessed at 37 °C. At 60 °C, αB-crystallin lost part of its chaper-

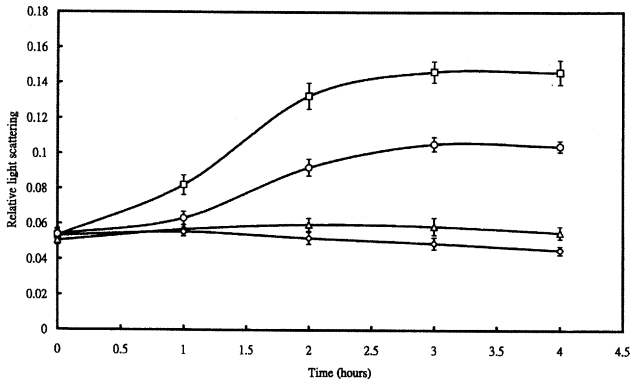

Fig. 7. Comparison of chaperone activities for various α-crystallin aggregates against photoaggregation of porcine γ-crystallin under UV-irradiation. Porcine γ-crystallin (10 μM) in 50 mM Tris–HCl, 0.1 M NaCl, 5 mM EDTA, 0.01% β-mercaptoethanol, and 0.02% sodium azide, pH 8.0, was used as substrate at a molar ratio (chaperone:γ-crystallin) of 1:1. The sample which contained γ-crystallin only (square) was used as control. Three crystallin aggregates were used for chaperone-activity assays against 254-nm UV-irradiation. Turbidity changes represented by relative light-scattering intensities after irradiation for various periods are shown for porcine α-crystallin (triangle), αA-crystallin (rhombus), and αB-crystallin (circle). The turbidity of each sample was measured at 405 nm by ELISA reader at 1-h periods and the bars denote ranges of variation from eight measurements.

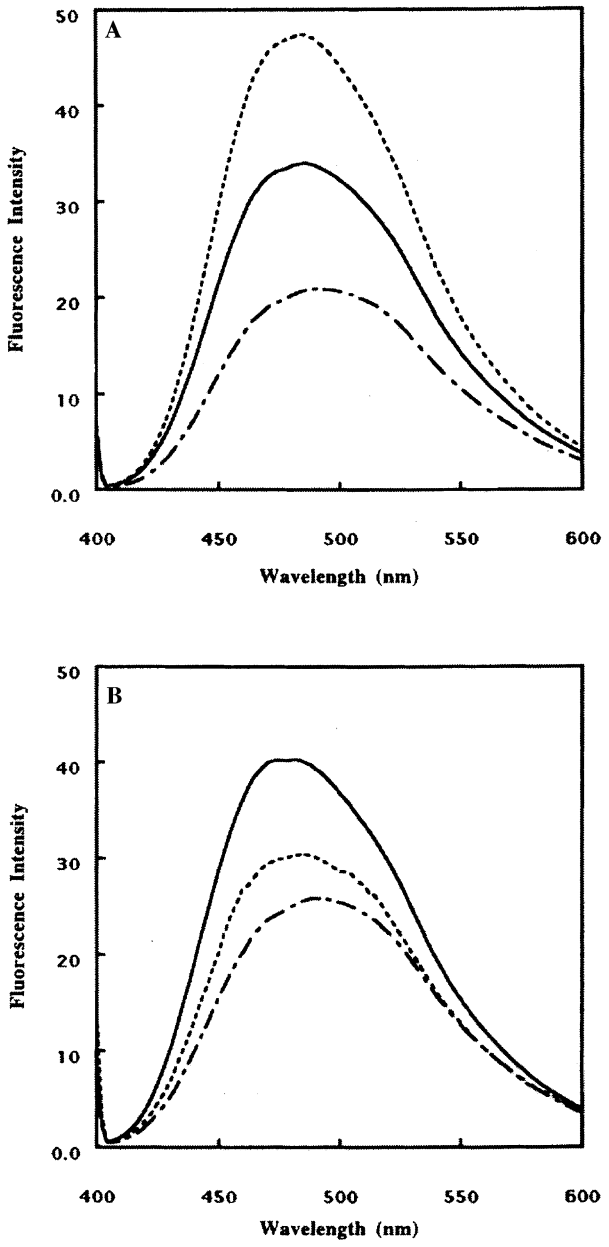

Fig. 8. Comparison of ANS fluorescence emission spectra for various α-crystallin aggregates. For each crystallin, 10 μl ANS in methanolic stock (0.1 M) was added to 1 ml crystallin solutions (0.3 mg/ml each) and incubated at (A) 37 °C or (B) 60 °C for 1 h before fluorescence emission measurements. Curves are shown for porcine α-crystallin (solid line), αA-crystallin (dashed line), and αB-crystallin (dotted line). αB-Crystallin solution turned turbid after 1-h incubation at 60 °C, resulting in the drastic change of the emission spectrum.

one activity to protect βL-crystallin from thermal aggregation (Fig. 4C). At this temperature, the exposed surface hydrophobicity of these three proteins did not match with their relative chaperone activities. This observation may shed some light on the mechanism underlying the finding that chaperone-like activity of αA-crystallin higher than that of αB-crystallin exists at temperatures above 60 °C.

Conclusion

There is considerable interest in the functional role of α-crystallin, especially after it was shown to have in vitro chaperone activity [6]. However, the mechanism of this chaperone-like action of α-crystallin remains elusive. We have employed various analytical methods to investigate detailed biochemical characteristics of porcine α-, αA-, and αB-crystallins in an endeavor to correlate their functional differences with physicochemical properties. Recently, the crystal structure of a homologous small heat-shock protein MjHSP16.5 (from *Methanococcus jannaschii*, a hyperthermophilic archaeon) chaperone protein was solved, revealing the fact that subunit association of this chaperone is arranged in octahedral symmetry [25]. Our elution profiles of gel permeation study coupled with dynamic light scattering for porcine α-, αA-, and αB-crystallins also indicated that these proteins possess an average molecular weight close to the symmetric aggregate of 24 MjHSP16.5 monomers, albeit with a less-defined and variable quaternary structure. Analytical gel-filtration chromatography and dynamic light scattering measurements all revealed the order of molecular mass and size as $\alpha > \alpha A > \alpha B$. We have also demonstrated that both surface hydrophobicity and structural stability play some roles in the molecular mechanism, underlying the chaperone-like activity of α-crystallin and its subunit aggregates. Owing to the significant difference of chaperone activity observed between αA- and αB-crystallins under thermal stress and UV-irradiation at physiological temperature, we propose that αA- and αB-crystallins may play distinct roles in lenses or other non-lens tissues. As a member of small heat-shock proteins, αB-crystallin may be a better chaperone under most thermal stress. On the other hand, αA-crystallin as a molecular chaperone that is present exclusively in the lens, which is far more abundant than αB, may play its major role as a chaperoning protein against lens insults from UV-irradiation or oxidative stress [26–29].

Acknowledgments

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