THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 49, Issue of December 3, pp. 34773–34778, 1999

Printed in U.S.A.

Differential Temperature-dependent Chaperone-like Activity of αA- and αB-crystallin Homoaggregates*

(Received for publication, June 8, 1999, and in revised form, July 22, 1999)

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α-Crystallin, a heteromultimeric protein made up of αA- and αB-crystallins, functions as a molecular chaperone in preventing the aggregation of proteins. We have shown earlier that structural perturbation of α-crystallin can enhance its chaperone-like activity severalfold. The two subunits of α-crystallin have extensive sequence homology and individually display chaperone-like activity. We have investigated the chaperone-like activity of αA- and αB-crystallin homoaggregates against thermal and nonthermal modes of aggregation. We find that, against a nonthermal mode of aggregation, αB-crystallin shows significant protective ability even at subphysiological temperatures, at which αA-crystallin or heteromultimeric α-crystallin exhibit very little chaperone-like activity. Interestingly, differences in the protective ability of these homoaggregates against the thermal aggregation of β_L-crystallin is negligible. To investigate this differential behavior, we have monitored the temperature-dependent structural changes in both the proteins using fluorescence and circular dichroism spectroscopy. Intrinsic tryptophan fluorescence quenching by acrylamide shows that the tryptophans in αB-crystallin are more accessible than the lone tryptophan in αA-crystallin even at 25 °C. Protein-bound 8-anilinonaphthalene-1-sulfonate fluorescence demonstrates the higher solvent accessibility of hydrophobic surfaces on αB-crystallin. Circular dichroism studies show some tertiary structural changes in αA-crystallin above 50 °C. αB-crystallin, on the other hand, shows significant alteration of tertiary structure by 45 °C. Our study demonstrates that despite a high degree of sequence homology and their generally accepted structural similarity, αB-crystallin is much more sensitive to temperature-dependent structural perturbation than αA- or α-crystallin and shows differences in its chaperone-like properties. These differences appear to be relevant to temperature-dependent enhancement of chaperone-like activity of α-crystallin and indicate different roles for the two proteins both in α-crystallin heteroaggregate and as separate proteins under stress conditions.

α-Crystallin is a major protein of the mammalian lens and constitutes as much as 50% of its dry weight. Studies over the past few years have shown that α-crystallin is expressed in several nonlenticular tissues such as heart, brain, and kidney, and its expression is enhanced severalfold during stress and disease conditions (1–6). α-Crystallin is shown to have homology with small heat shock proteins (7–10). Horwitz (11) shows that α-crystallin can prevent the thermal aggregation of β- and γ-crystallins and a few other proteins like a molecular chaperone. Demonstration of chaperone-like activity of α-crystallin has provided an excellent opportunity to investigate the mechanistic aspects of chaperone function in general and the role of α-crystallin under stress conditions in particular. It is possible that, in the lens, α-crystallin may chaperone the formation of the transparent and appropriately refracting ensemble and may also keep it that way by interacting with damaged proteins. α-Crystallin may have a similar function of interacting with aged or damaged proteins in Creutzfeldt-Jakob disease brain (12) and ischemic heart tissue (13). αB-crystallin may even play a regulatory role in cytomorphological rearrangements during development (14). A mutation in αA-crystallin is known to lead to cataract (15). Recently a missense mutation (R120G) in αB-crystallin was shown to cause desmin-related myopathy (16). To address the mechanistic aspects of the function of α-crystallin, we have used a nonthermal aggregation system and found that the chaperone-like activity of α-crystallin is temperature-dependent (17). Our studies with photoaggregation of γ-crystallin (17), thermal aggregation of β-crystallin, and DTT[1]-induced aggregation of insulin (18) together with the rapid refolding of crystallins (19) and the role of α-crystallin in these processes resulted in a hypothesis that sheds some light on the chaperone-like activity of α-crystallin. These studies show that α-crystallin prevents the aggregation of nonnative structures by providing appropriately placed hydrophobic surfaces. A structural transition above 30 °C enhances the protective ability perhaps by increasing or reorganizing hydrophobic surfaces. We have recently shown that tertiary structural changes precede quaternary structural changes (20, 21). α-Crystallin is a heteroaggregate of two gene products, αA- and αB-crystallin. Both the subunits can homoaggregate and function as chaperones, albeit to different extents (11, 22). The roles of the two proteins and their contribution to the structural and functional properties of α-crystallin are not well understood. αA- and αB-crystallin have nearly 40% sequence homology with the heat shock proteins (9) and have 57% sequence homology among themselves (23). Small heat shock proteins such as Hsp25, Hsp27, as well as α-crystallin have been shown to have a similar function in refolding citrate synthase and β-glucosidase *in vitro* (24). The expression of αB-crystallin can be induced by heat shock (7), osmotic stress (25), or mechanical stress (26). We set out to investigate the physiochemical properties of the individual subunits to understand their structural and functional contributions in α-crystallin. We have isolated αA- and αB-crystallins and generated the individual homoaggregates. We have investigated temperature-induced structural changes, chaperone-like activity as well as its temperature dependence, of the individual homoaggregates and the

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[1] The abbreviations used are: DTT, dithiothreitol; ANS, 8-anilinonaphthalene-1-sulfonate; λem max, λ emission maximum.

native α-crystallin heteroaggregate. Interestingly, we find that αA- and αB-crystallin homoaggregates do not differ at all in preventing the thermal aggregation of β_L-crystallin but show a significant difference in protection against the nonthermal DTT-induced aggregation of insulin. Circular dichroism and fluorescence spectoscopy, used to investigate the temperature-dependent structural changes in the homoaggregates, show interesting differences and explain the differential chaperone-like behavior. We believe these results indicate that relative stability or exposure of αA- or αB-crystallin subunits could modulate the chaperone-like activity of α-crystallin, either directly or by inducing global changes in the arrangement/packaging of subunits. It is possible that structural alteration by temperature forms a part of a general mechanism of chaperone function, since chaperones function more effectively at nonpermissible temperatures.

EXPERIMENTAL PROCEDURES

Isolation and Purification of α-Crystallin—Calf lens α-crystallin was isolated and purified as described earlier (18). The fractions corresponding to α-crystallin were pooled and concentrated at 4 °C using an Amicon ultrafiltration unit with an M_r 30,000 cutoff. The concentrated solution of α-crystallin was stored in Tris-HCl buffer at 4 °C. α-Crystallin was used as the heteroaggregate of αA-and αB-crystallin. The ratio of αA-, to αB-crystallin is 3:1 (w/w) in this heteroaggregate.

Separation of αA- and αB-crystallins and Generation of Homoaggregates—The subunits of α-crystallin can be separated by a variety of methods (11, 27, 28). The subunits were separated on a C4 reverse phase column using a water-acetonitrile gradient containing 0.08% trifluoroacetic acid. The peaks corresponding to αA- and αB-crystallin were pooled, lyophilized, and stored at −20 °C. The lyophilized samples were dissolved in 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, and 8 M deionized urea and left overnight at 4 °C. αA- and αB-crystallin homoaggregates were generated from these samples by extensive dialysis against 300 volumes of the 50 mM Tris-HCl buffer without urea, with 6 changes over a period of 36 h. The dialyzed proteins were concentrated using an Amicon ultrafiltration setup and then checked for any high molecular weight aggregate formation on a Superose-6 column. Their purity was checked by horizontal isoelectric focusing in the pH range 5–8.

Assay of Chaperone-like Activity—Insulin at a concentration of 0.2 mg/ml (in 10 mM phosphate buffer pH 7.4, 100 mM NaCl) in the presence or the absence of different amounts of α-, αA-, or αB-crystallin was equilibrated at the required temperature for 10 min with constant stirring in the cuvette using a Julabo thermostated water bath. The actual temperature in the cuvette was monitored with a Physitemp microthermocouple thermometer system. The reduction of insulin was initiated by the addition of 30 μl of 1M DTT to 1.5 ml of sample. The extent of aggregation was monitored by measuring the scattering at right angle in a Hitachi-4000 fluorescence spectrometer with both the excitation and emission monochromators set at 465 nm and excitation and emission bandpasses at 1.5 nm.

Thermal aggregation of β_L-crystallin was monitored in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl at 60 °C. The buffer, containing α-, αA-, or αB-crystallin, was preincubated at 60 °C for 10 min before the addition of 60 μl of β_L-crystallin to make a final concentration of 0.2 mg/ml. Aggregation was monitored by measuring the light scattering as described above. Results are expressed as percentage protection. Percentage protection is calculated as, $(I_t - I_{t+\alpha})/I_t$, where I_t is the intensity of scattered light for target protein insulin or βL-crystallin, and $I_{t+\alpha}$ is the intensity of scattered light in the presence of αA-, αB-, or α-crystallin.

Circular Dichroism Measurements—The CD measurements were carried out using a JASCO J-715 spectropolarimeter. Sample concentrations were 1.0 mg/ml in 10 mM sodium phosphate buffer, pH 7.4, containing 100 mM NaCl (moderate salt), except for temperature-dependent changes in the far UV-CD, where 30 mM sodium phosphate buffer without NaCl (low salt) was also used. The near and far UV-CD spectra were recorded using 1 cm and 0.01-cm path length cells, respectively. For the temperature-dependent CD measurements, water-jacketed sample cells of the mentioned path lengths were used, and the temperature was maintained using a Julabo thermostated water bath. The sample temperature was monitored using a Physitemp microthermocouple thermometer.

Fluorescence Studies—Aliquots (7 μl) from a 7 M acrylamide stock were added to the protein (0.2 mg/ml) in 10 mM phosphate buffer, pH 7.4, with 100 mM NaCl, and maintained at the required temperatures. The drop in fluorescence intensity was measured on a Hitachi-F4000 fluorimeter. The excitation and emission monochromators were set at 295 nm (5 nm bandpass) and 340 nm (3 nm bandpass), respectively. For the measurement of the temperature-dependent change in acrylamide quenching, the proteins were incubated with 0.35 M acrylamide at the lowest starting temperature for 20 min, and the drop in fluorescence was measured at every subsequent temperature after a 5-min incubation period. Instrument parameters were as given above. The fluorescence intensity values were corrected for dilution and inner filter effect.

For the temperature-dependent 8-anilinonaphthalene-1-sulfonate (ANS) fluorescence measurements, 10 μl of a 10 mM methanolic stock of ANS was added to 1 ml of 0.2 mg/ml protein solution and incubated at the starting temperature for 2 h. The temperature was maintained and monitored as mentioned above. The excitation monochromator was set at 365 nm(3 nm bandpass), and the emission monochromator was scanned from 400 to 530 nm(1.5 nm bandpass) in the correct spectrum mode to monitor the emission maximum of ANS.

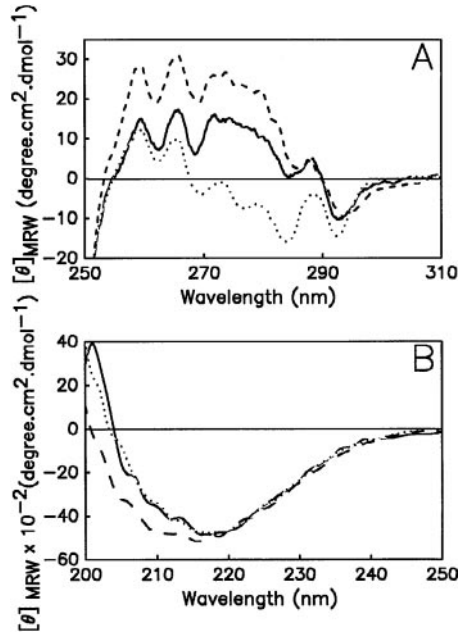

FIG. 1. **Near (*A*) and far (*B*) UV-CD spectra of α-crystallin (*solid line*), αA-crystallin (*dotted line*), and αB-crystallin (*dashed line*).** The spectra of 1 mg/ml protein solutions in 10 mM sodium phosphate, 100 mM NaCl, pH 7.4, were recorded using 1-cm and 0.01-cm path length cells for near and far UV-CD, respectively. *MRW*, mean residue weight.

RESULTS AND DISCUSSION

We showed earlier that the chaperone-like activity of α-crystallin can be enhanced severalfold upon structural perturbation. To gain an insight into the chaperone-like activity and its enhancement with structural perturbation, we investigated the constituent subunits separately. The subunits were separated by reverse phase high performance liquid chromatography on a C4 column and checked for purity by isoelectric focusing in the pH range 5–8. αA- and αB-crystallin homoaggregates were generated by dialyzing individual crystallins from 8 M urea against 50 mM Tris-HCl buffer, pH 7.4, 100 mM NaCl. Such a slow refolding by removing the denaturant by dialysis (29) or rapidly by dilution (18) results in native α-crystallin. The molecular mass of the refolded α-crystallin, however, may vary depending on conditions like ionic strength, pH, and temperature (30). The size of the homoaggregates thus obtained are comparable to refolded heteromultimeric α-crystallin (31, 32). αB-crystallin homoaggregates obtained in this way have a similar molecular mass as αB-crystallin homoaggregates isolated from nonlenticular tissue such as the heart (10, 33, 34).

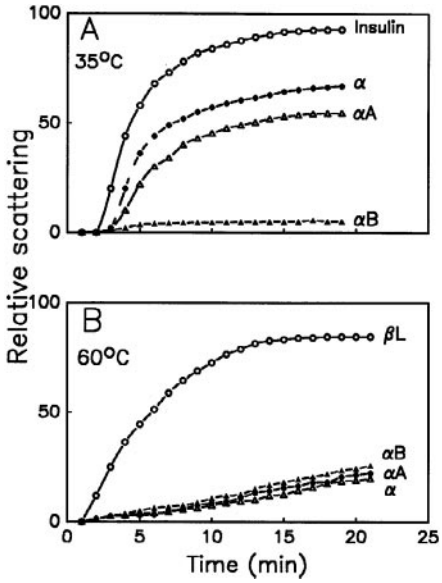

FIG. 2. **Chaperone-like activity of αA- and αB-crystallin homoaggregates and α-crystallin heteroaggregate against nonthermal DTT-induced insulin aggregation at 35 °C (insulin, 0.2 mg/ml; α-crystallins, 0.1 mg/ml) (*A*) and thermal aggregation of β_L-crystallin at 60 °C (β_L-crystallin 0.2 mg/ml; α-crystallins 0.04 mg/ml) (*B*).**

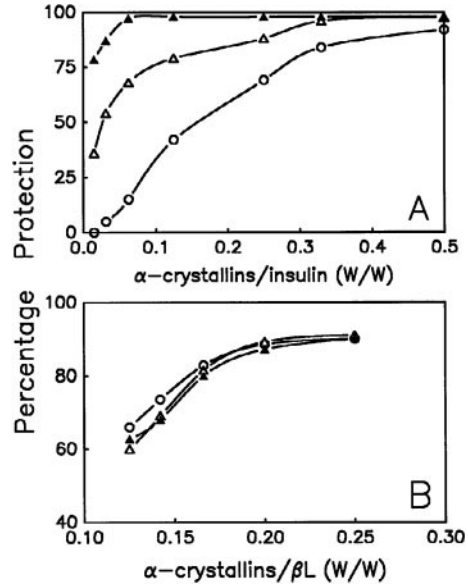

FIG. 3. **The concentration dependence of the protective ability of α-crystallin (○), αA- (△), and αB-crystallin (▲) homoaggregates against DTT-induced insulin aggregation at 40 °C (*A*) and thermal aggregation of β_L-crystallin at 60 °C (*B*).** The concentrations of insulin and β_L-crystallin were 0.2 mg/ml.

Fig. 1, *A* and *B*, compare the near and far UV-CD spectra of αA- and αB-crystallin homoaggregates with those of α-crystallin. The far UV-CD of αA- and αB-crystallin homoaggregates (Fig. 1*B*) are similar with minima at 217-nm and 214-nm, respectively. The near UV-CD spectra of αA- and αB-crystallin homoaggregates, however, show differences in the 270–290-nm region, where Trp and Tyr contribute to the observed circular dichroism. The characteristic signals at 259 and 266 nm, contributed by phenylalanine are common to both αA- and αB-crystallin homoaggregates although different in intensity. The significant differences in the 270–290-nm region suggest differences in the packaging of aromatic chromophores. Both the near and far UV-CD spectra of αA- and αB-crystallin homoaggregates are comparable to those of the corresponding human recombinant proteins (21). The molecular weights of the αA- and αB-crystallin homoaggregates and α-crystallin were determined by gel filtration on a Superose-6 column, calibrated as per the manufacturer's instructions, and found to be 600 kDa, 450 kDa, and 800 kDa, respectively.

To gain insight into our earlier observation of temperature-dependent chaperone-like activity of α-crystallin, we investigated the chaperone-like activity against thermal (heat-induced β_L-crystallin aggregation) and nonthermal (DTT-induced aggregation of insulin) modes of aggregation. Fig. 2*A* shows the DTT-induced aggregation of insulin at 35 °C. Insulin starts aggregating, as seen by the increasing scattering, after the addition of DTT and levels off at about 20 min. α-Crystallin prevents this aggregation to a limited extent. αA-crystallin prevents this aggregation to a greater extent. Interestingly, αB-crystallin completely prevents this aggregation under similar conditions. It is interesting to note that both the constituent subunits individually display higher chaperone-like activity than the native heteroaggregate. The biological significance of this is not yet clear. Fig. 2*B* shows a similar protection experiment with β_L-crystallin. β_L-crystallin aggregates with time when kept at 60 °C. All the three proteins, αA- and αB-crystallin homoaggregates and native α-heteroaggregate, offer comparable protection against heat-induced aggregation. The differences seen at 35 °C (Fig. 2*A*) are absent at 60 °C (Fig. 2*B*). These experiments are carried out at a fixed concentration of α-crystallin, and the target protein. Fig. 3*A* shows the concentration dependence of protection against DTT-induced aggregation of insulin at 40 °C. αB-crystallin is able to completely prevent the DTT-induced aggregation of insulin even at a ratio of 0.1/1 (αB/insulin w/w), whereas αA- and α-crystallin protect to a comparable extent only beyond ratios of 0.3:1 and 0.5:1 (w/w), respectively. Thus αB-crystallin homoaggregate has a significantly higher protective ability than either αA-crystallin homoaggregate (about 3-fold higher) or α-crystallin (5-fold) at near physiological temperatures. Such a difference in the protective ability of αA- and αB-crystallin homoaggregates and α-crystallin is totally absent against a thermal mode of aggregation as shown in Fig. 3*B*. The percentage protection offered by αA-and αB-crystallin homoaggregates and α-crystallin against thermal aggregation of β_L-crystallin at 60 °C is comparable. Although at physiological temperatures αB-crystallin exhibits a substantially higher chaperone-like activity, at higher temperatures its protective ability is essentially the same as that of αA- and α-crystallin.

Earlier work from our laboratory suggested that α-crystallin undergoes a temperature-dependent structural perturbation, which results in an increase in its chaperone-like activity (16). It is possible that these changes are due to the reorganization of the subunits within the aggregate and/or small perturbation in the packing of domains within the subunits themselves. This structural perturbation above 30 °C and the resulting increase in chaperone-like activity seem to be physiologically relevant. We have therefore compared the temperature-dependent chaperone-like activity of αA- and αB-crystallin homoaggregates and α-crystallin between 25 °C and 42 °C. A fixed ratio of 1:0.5 w/w (insulin:chaperone) was used in these experiments. As Fig. 4 shows, under the given conditions, by about 40 °C all the three crystallins show almost complete protection against DTT-induced aggregation of insulin. At lower temperatures, however, the protective ability of αA- and α-crystallin declines rapidly between 35 °C and 30 °C, whereas αB-crystallin offers significant protection even at 25 °C. The data indicate that αB-crystallin displays significant chaperone-like activity at temperatures lower than physiological temperatures, unlike αA- or α-crystallin, which work efficiently only at or above

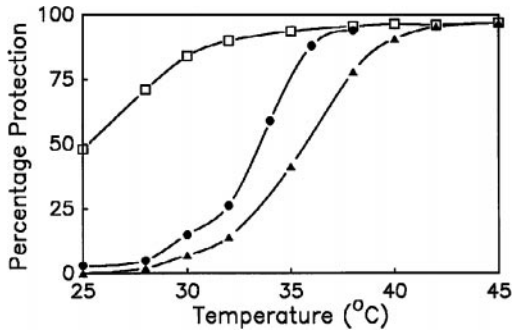

FIG. 4. **The temperature dependence of the chaperone-like activity measured as percentage protection of α-crystallin (▲), αA-crystallin (●), and αB-crystallin (□).** The concentrations of the proteins were: insulin, 0.2 mg/ml; αA-, αB-, and α-crystallin, 0.1 mg/ml.

physiological temperatures. α-crystallin in its native state is a hydrophobic yet highly soluble protein. It is known that a slight perturbation of its conformation by heat (16, 17) or chaotropic agents (16, 35) results in an increase in its hydrophobicity and, therefore, its substrate binding capacity. To check if the observed differences in protective abilities are due to differences in their hydrophobicities, we have probed the hydrophobic surfaces of αA- and αB-crystallin homoaggregates at 25 °C and 60 °C using the polarity-sensitive fluorescent dye ANS. ANS fluoresces weakly in aqueous solutions, and its fluorescence quantum yield increases in a hydrophobic environment; its λ emission maximum (λem max) is indicative of the apolarity of its environment. This property of ANS has been exploited to monitor the hydrophobic surfaces of proteins (36), polysaccharides (37), and folding/unfolding intermediates of proteins (38). At 25 °C, the fluorescence intensity of ANS bound to αB-crystallin is higher than that bound to αA- or α-crystallin, indicating a greater extent of hydrophobicity of αB-crystallin (Fig. 5*A*). At 60 °C the difference in fluorescence intensities is much less (Fig. 5*B*) compared with that seen at 25 °C. Fig. 5*C* shows the shift in the λem max of ANS bound to αA-, αB-, and α-crystallin as a function of temperature. The λem max of ANS bound to αB-crystallin is marginally red-shifted compared with αA- and α-crystallin, suggesting that the ANS-bound hydrophobic surfaces of αB-crystallin might be slightly more solvent-accessible at 25 °C. The λem max of ANS bound to all the proteins increases with temperature, indicating a further temperature-dependent exposure of the hydrophobic surfaces to the solvent. At lower temperatures, the shift in the λem max of fluorescence from αB-bound ANS is more compared with αA- or α-crystallin. However, at higher temperatures, gradually αA- and α-crystallin become comparable to αB-crystallin.

To further investigate the differences in the temperature-dependent structural changes of αA- and αB-crystallin, we recorded far (Fig. 6) and near UV-CD (Fig. 7) spectra between 25 °C and 65 °C. As can be seen from Fig. 6*A*, the secondary structure of αA-crystallin does not show significant changes with temperature, except an enhanced CD signal at 207 nm at higher temperature. αB-crystallin, on the other hand, shows a significantly larger change. The far UV-CD spectra show a gradual increase in ellipticity at 217 nm with temperature. This increase in ellipticity at 217 nm is more for αB-crystallin than αA-crystallin and is dependent on both the protein (data not shown) and salt concentrations (Fig. 6*C*). The observed change in ellipticity at 217 nm is larger at higher protein and salt concentrations. A similar increase in ellipticity has been observed for the native heteroaggregate of α-crystallin (39, 40). However the reports differ in the extent of this increase. These differences can be explained on the basis of our results on individual subunits. In the earlier reports, α-crystallin used

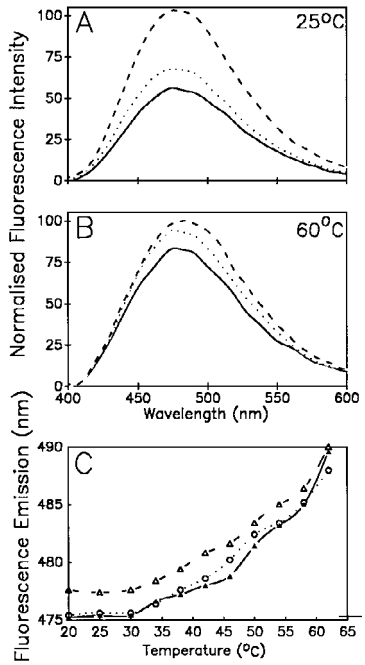

FIG. 5. **The normalized fluorescence emission spectrum of ANS bound to α-crystallin (*solid line*), αA-crystallin (*dotted line*), and αB-crystallin (*dashed line*) at 25 °C (*A*) and 60 °C (*B*).** The concentrations of ANS and the proteins were 100 μM and 0.1 mg/ml, respectively. *C*, the fluorescence emission maximum of ANS bound to α-crystallin (○), αA-crystallin (▲), and αB-crystallin (△) as a function of temperature. The excitation and emission bandpasses were 3 nm and 1.5 nm, respectively.

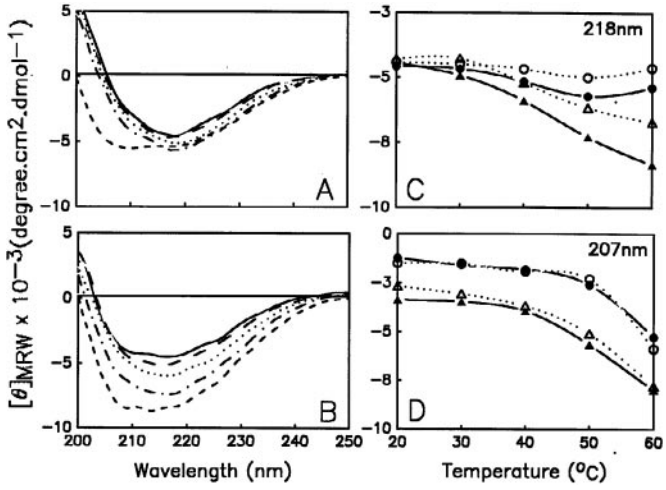

FIG. 6. **Temperature-dependent changes in the secondary structure of αA- and αB-crystallin.** *A*, αA-crystallin; *B*, αB-crystallin. Spectra shown at 25 °C (*solid line*), 30 °C (*long dashes*), 40 °C (*dotted line*), 50 °C (*long dashes and dots*), and 60 °C (*short dashes*). Change in $[\theta]_{MRW}$ at 218 nm (*C*) and 207 nm (*D*) of αA-crystallin (○, ●) and αB-crystallin (△, ▲) in 30 mM sodium phosphate, pH 7.4 (*dots*), and in 10 mM sodium phosphate, 100 mM NaCl, pH 7.4 (*solid line*) as a function of temperature.

was isolated from the lens cortex (39) or the whole lens (40). Since the composition of α-crystallin varies from the outer cortex to the nucleus, with αB-crystallin being higher in the cortex, differential composition of α-crystallin could lead to the observed differences. Above 60 °C, the signal at 207 nm increases. The extent of this increase at 207 nm is not dependent on salt concentration (Fig. 6*D*). The observed increase in signal at 207 nm around 60 °C appears to correspond to the transition observed for α-crystallin by Ramar and Rao (20), Surewicz and Olesen (40), and Walsh *et al.* (41). The changes in the tertiary

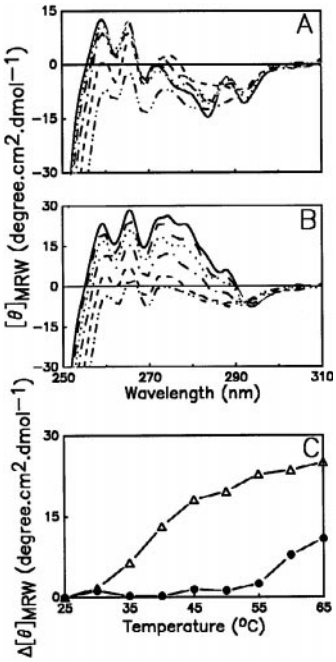

FIG. 7. **Temperature-dependent changes in the tertiary structure of αA-crystallin (*A*) and αB-crystallin (*B*) between 25 °C and 65 °C monitored by near UV-CD.** Spectra shown at 25 °C (*solid lines*), 30 °C (*long dashes*), 35 °C (*dots*), 40 °C (*long dashes and single dots*), 50 °C (*short dashes*), and 60 °C (*long dashes and double dots*). *C*, comparison of the change in $[\theta]_{MRW}$ of αA-crystallin (●) and αB-crystallin (△) at 272 nm with temperature.

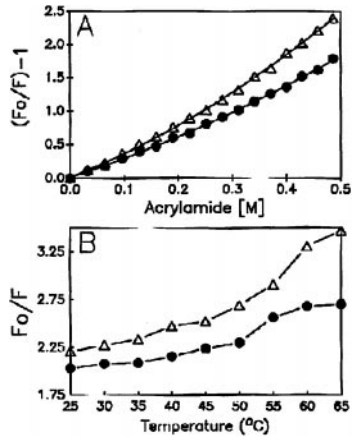

FIG. 8. **Stern-Volmer plot of acrylamide quenching of tryptophan fluorescence in αA-crystallin (●) and αB-crystallin (△) at 40 °C (*A*). F_0/F is shown as a function of temperature in the presence of 0.35 M acrylamide, αA-crystallin (●), and αB-crystallin (△) (*B*).** F_0 and F are the fluorescence intensities before and after the addition of acrylamide.

structure of αA- and αB- crystallin homoaggregates between 25 °C and 65 °C, monitored by near UV-CD, show interesting differences (Fig. 7). αA-crystallin homoaggregate (Fig. 7*A*) shows significant alteration of tertiary structure only above 50 °C, whereas αB-crystallin homoaggregate shows considerable loss by 45 °C. This is evident from Fig. 7*C*, which compares the change in chirality at 272 nm between 25 °C and 65 °C. The changes observed at 259 nm and 266 nm are similar but differ in intensity (data not shown). Taken together the near and far UV-CD spectra indicate that αB-crystallin loses its tertiary structure but retains significant secondary structure at about 50 °C, a characteristic of the molten globule state. At 65 °C, both the proteins exhibit extensive loss of tertiary structure but retain some secondary structure.

In a native protein individual amino acids occupy unique positions within the three-dimensional structure. Alterations in this structure could lead to a change in their accessibility. We have investigated the accessibility of tryptophan(s) in αA- and αB-crystallin to the neutral quencher acrylamide. Fig. 8*A* shows the Stern-Volmer plot of quenching of tryptophan fluorescence by acrylamide at 40 °C. The tryptophan fluorescence from αB-crystallin is quenched at a lower acrylamide concentration than that from αA-crystallin. The two tryptophans in bovine αB-crystallin are at positions 9 and 60. The single tryptophan of bovine αA-crystallin is at position 9.

As mentioned earlier, αA- and αB-crystallins undergo a change in structure with temperature. We have monitored the change in the accessibility of the tryptophans in αA- and αB-crystallin to a fixed amount of acrylamide as a function of temperature (see "Experimental Procedures" for details). The change in F_0/F as a function of temperature reflects a change in accessibility. Fig. 8*B* shows that even at 25 °C the accessibility of the tryptophans in αB-crystallin is more than that in αA-crystallin. Upto 45 °C the change in the accessibility of the tryptophans in both the proteins increases gradually. Above 45 °C the F_0/F value increases more significantly for αB-crystallin, indicating further change in structure. The F_0/F for αA-crystallin also increases above 50 °C, although not as sharply as in the case of αB-crystallin.

All the above results suggest that despite being evolutionarily related and having a high degree of sequence homology, αA- and αB-crystallin show some remarkable differences in their structural stability and chaperone-like activity. At physiological temperatures, αB-crystallin is a better chaperone-like molecule than αA-crystallin. This difference is even more prominent at temperatures below 30 °C, where αA- and α-crystallin do not offer any significant protection against aggregation of target proteins. This appears to be due to its higher hydrophobicity and a greater exposure of these hydrophobic patches to the solvent at this temperature. This is perhaps also reflected in the greater accessibility of the tryptophans in αB-crystallin to acrylamide compared with that in αA-crystallin. However, our study does not address the question if differences in charge distribution on the surfaces of αA- and αB-crystallin or differences in their aggregate sizes may have any role in this process. Flexibility calculations by Bloemendal and Bloemendal (42) show that αB-crystallin is more flexible than αA-crystallin. This property may indicate the easier loss of structure in αB-crystallin. Our results show that αB-crystallin is structurally less stable and shows significant structural alteration by 45 °C. In comparison, αA-crystallin shows significant changes above 55 °C. The thermal stability of α-crystallin can therefore be attributed to its subunit αA-crystallin. αB-crystallin has the greater chaperone-like activity but lower structural stability. αA-crystallin has lower chaperone-like activity but a greater structural stability. The properties of α-crystallin (heteroaggregate) is a compromise between structural stability and chaperone-like activity. Modulation of αA- to αB-crystallin ratio could shift the balance. The stability of αA-crystallin may be one of the reasons for its predominance in the eye lens, a tissue that does not show protein turnover. In fact, targeted disruption of the mouse αA-crystallin gene results in cataract and cytoplasmic inclusion bodies containing αB-crystallin (43). Since αB-crystallin is seen in many nonlenticular tissues and in pathological conditions, it may be this ability to offer greater protection and the ability to increase/up-regulate this level of activity under stress that makes it important. It is interesting to note that the composition of α-crystallin is different in the lens epithelial cells and the inner cortex. In the bovine lens, the ratio of αA- to αB-crystallin in the lens epithelial cells is 1:3, whereas that in the post-differentiated fiber cells is 3:1 (44). In

the lens, the presence of αB-crystallin in the α-crystallin heteroaggregate, by virtue of its sensitivity to structural change, could directly increase the chaperone-like activity or could do so by inducing global changes in the arrangement/packaging of subunits in the aggregate.

Acknowledgment— We thank Ritu Kanwar for useful discussions and suggestions and Dr. T. Ramakrishna Murthy for critical comments on the manuscript. S. Datta is thankful to the University Grants Commission for his student fellowship.

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