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Temperature-dependent Chaperone Activity and Structural Properties of Human αA- and αB-crystallins*

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The chaperone activity and biophysical properties of recombinant human αA- and αB-crystallins were studied by light scattering and spectroscopic methods. While the chaperone function of αA-crystallin markedly improves with an increase in temperature, the activity of αB homopolymer appears to change very little upon heating. Compared with αB-crystallin, the αA-homopolymer is markedly less active at low temperatures, but becomes a more active species at high temperatures. At physiologically relevant temperatures, the αB homopolymer appears to be modestly (two times or less) more potent chaperone than αA homopolymer. In contrast to very similar thermotropic changes in the secondary structure of both homopolymers, αA- and αB-crystallins markedly differ with respect to the temperature-dependent surface hydrophobicity profiles. Upon heating, αA-crystallin undergoes a conformational transition resulting in the exposure of additional hydrophobic sites, whereas no such transition occurs for αB-crystallin. The correlation between temperature-dependent changes in the chaperone activity and hydrophobicity properties of the individual homopolymers supports the view that the chaperone activity of α-crystallin is dependent on the presence of surface-exposed hydrophobic patches. However, the present data also show that the surface hydrophobicity is not the sole determinant of the chaperone function of α-crystallin.

α-Crystallin, a major lens protein, plays an important role in maintaining the transparency and refractive properties of the eye lens (1–3). The lenticular α-crystallin consists of two 20-kDa polypeptide chains, αA and αB, that share about 60% sequence homology. These chains self-associate, forming large oligomeric complexes that contain 30–50 subunits at the αA to αB ratio of approximately 3:1. Although the high resolution structure of α-crystallin is currently not known, recent cryo-electron microscopy data shows that the recombinant αB-crystallin assembles into spherical particles with a diameter of 8–18 nm and a central cavity (4).

For many years it was believed that the expression of α-crystallin was restricted to the ocular lens. More recently, αB-crystallin has been shown to be expressed in a number of non-lenticular tissues such as heart, skeletal muscle, kidney, and brain (3, 5), suggesting that it may have a more general cellular function. Furthermore, overexpression of αB-crystallin has been associated with various disease states (3, 5). In contrast to αB-crystallin, αA chains are believed to be largely lens-specific. However, low levels of the latter protein have been detected in spleen, thymus, and retina (6, 7). The rapidly growing data on non-lenticular expression of αA and αB chains was accompanied by the finding that α-crystallin belongs to the family of small heat shock proteins (3, 5, 8). A new light on the potential physiological role of α-crystallin and related small heat shock proteins was shed by the discovery that these proteins act as molecular chaperones by preventing the aggregation of other proteins denaturated by heat or other stress conditions (2, 3, 9–13). The chaperone function of α-crystallin is likely to be of considerable importance *in vivo*. In particular, the above function was suggested to be instrumental in the prevention of cataract formation in the ocular lens (9, 14). In nonlenticular tissues, the role of small heat shock proteins may be to maintain substrate proteins in a folding-competent state (15, 16). Although the mechanism of the chaperone action of α-crystallin is at present not fully understood, it was shown that α-crystallin specifically recognizes aggregation-prone non-native structures that occur early on the denaturation pathway of proteins (17–19). Furthermore, a number of studies suggest that the chaperone activity of the protein is dependent on the presence of surface-exposed hydrophobic patches (11, 20–25).

The apparent difference in the tissue specificity of αA- and αB-crystallins strongly suggests that these two proteins might have evolved to play distinct physiological functions. Therefore, it is of considerable importance to understand how these differences correlate with the physicochemical properties of αA- and αB-crystallins. In the present study, we present a detailed comparison of the recombinant human αA- and αB-crystallins, with a special emphasis on the chaperone activity and the surface hydrophobicity of both proteins.

MATERIALS AND METHODS

Proteins and Reagents—Insulin, yeast alcohol dehydrogenase, citrate synthase, bovine α-lactalbumin (type III), lysozyme, DNase, protease inhibitor mixture, and DTT[1] were purchased from Sigma. ANS and bis-ANS were obtained from Molecular Probes.

Cloning, Overexpression, and Purification of Human α-Crystallins—Complementary DNA fragments encoding human αA- and αB-crystallin were cloned into the pET23d expression plasmid (Novagen). Cloning of human αA-crystallin cDNA was described previously (26). Human αB-crystallin cDNA was obtained as an IMAGE clone (GenBank number N35834) from Genome Systems, Inc. (St. Louis. MO). Coding regions in expression constructs were completely sequenced to verify their structures. For overexpression, plasmids were introduced into *Escherichia coli* strain BL21, and 1-liter cultures were grown as described previously (26). Cells were collected by centrifugation and proteins extracted by the DNase/lysozyme method as described by Andley *et al.*

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[1] The abbreviations used are: DTT, dithiothreitol; FTIR, Fourier-transform infrared; bis-ANS, 1,1′-bi(4-anilino)naphtalene-5,5′-disulfonic acid.

(26), with the exception that different DNase treatment conditions were used in the present study (1 h incubation at 37 °C with 20 μg/ml of the enzyme). The above conditions allowed us to completely remove nucleic acid contamination of α-crystallin preparations (see below). The extracted proteins were dialyzed against a large volume of 25 mM Tris-HCl buffer, pH 8.0, containing 0.5 mM EDTA, and 0.5 mM DTT. The precipitated material was removed by centrifugation, and the soluble lysate was applied onto a Q-Sepharose anion exchange column (Amersham Pharmacia Biotech) equilibrated with the dialysis buffer. The proteins were eluted using a linear 0–0.5 M gradient of NaCl in the same buffer. The fractions containing αA- or αB-crystallins were pooled, concentrated, and further purified by size exclusion chromatography using a Sephacryl S-300 HR column (Amersham Pharmacia Biotech). The purified recombinant α-crystallins showed on SDS-polyacrylamide gel a single band corresponding to protein with a molecular mass of approximately 20 kDa. Protein concentration was determined spectrophotometrically using the molar extinction coefficient, ϵ_{280}, of 16,500 and 19,000 M^{-1} cm^{-1} for αA- and αB-crystallin, respectively (27). The lack of nucleic acid contamination in the final protein preparations was verified by agarose gel electrophoresis and absorption spectroscopy: the A_{280}/A_{260} ratio for all samples was higher than 1.5 (27).

Fourier-transform Infrared Spectroscopy—α-Crystallin samples for FTIR were prepared in deuterium oxide buffer (50 mM phosphate, pH 7.2) at a protein concentration of approximately 10 mg/ml and placed between two calcium fluoride windows separated by a 50-μm thick spacer. Infrared spectra were recorded on a Bruker FTS 66 instrument equipped with a thermostated cell holder as described previously (28). The spectra in the 1500–1800 cm^{-1} region were corrected for the weak absorption of the buffer and for the residual water vapor signal. The technique of Fourier self-deconvolution was used to resolve the overlapping infrared bands (28). The secondary structure content was estimated by curve fitting of amide I band contours (28).

Bis-ANS Binding Studies—Fluorescence of ANS and bis-ANS in the presence of α-crystallin was measured on SLM 8100 spectrofluorometer using the excitation and emission wavelengths of 390 and 490 nm, respectively. All measurements were performed in 50 mM phosphate buffer, pH 7.2. To determine dye-α-crystallin binding constants, small aliquots of a concentrated bis-ANS solution (250 μM) were successively added to protein solution in buffer (0.1 mg/ml). After each addition of the dye the mixture was thoroughly mixed and left to incubate for 5 min (such an incubation period was found to be sufficient to establish equilibrium). Fluorescence intensity was then measured and the readings were corrected for buffer blanks and dilution. The calibration factor that relates the change in fluorescence intensity to the amount of bis-ANS bound to the protein was determined by a reverse titration method as described previously (29). To probe the temperature dependence of dye-protein binding, α-crystallin (0.1 mg/ml) was incubated with 25 μM bis-ANS for 10 min at each temperature, and the fluorescence of the dye was measured at 490 nm (20).

Chaperone Activity Assays—Chaperone activity of αA- and αB-crystallin was probed by measuring the ability of these proteins to prevent the aggregation of substrate proteins denatured by reduction of disulfide bonds (insulin, α-lactalbumin) or heat (alcohol dehydrogenase, citrate synthase). The aggregation of proteins upon denaturation was monitored by measuring the apparent absorption at 360 nm as a function of time. The relative chaperone activity of αA- and αB-crystallin was calculated as a percentage of protection against aggregation using the formula: % protection = $((A_o - A)/A_o) \times 100$, where A_o and A represent the apparent saturation absorption (usually after 1 h) in the absence and presence of α-crystallin, respectively (11). The aggregation assays with different target proteins were performed essentially as described by Horwitz *et al.* (27).

RESULTS

FTIR Measurements—The conformation-sensitive amide I band contours of αA- and αB-crystallin at room temperature exhibit a maximum at 1632 cm^{-1}, a feature characteristic of proteins rich in β-sheet structure (Fig. 1*A*). The spectra of both proteins also show a shoulder between approximately 1645 and 1660 cm^{-1}. However, the relative intensity of the 1632 cm^{-1} maximum with respect to the above shoulder is significantly higher for αB- than for αA-crystallin, suggesting that the former protein is characterized by a higher proportion of β-sheet structure. This qualitative conclusion has been confirmed using Fourier self-deconvolution and band fitting analysis of the spectra (28, 30). The deconvolved spectra of both proteins are

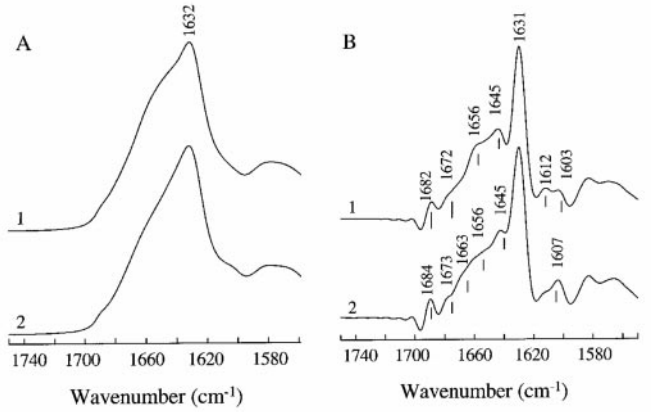

FIG. 1. *Panel A,* infrared spectra of αA- (*top*) and αB-crystallin (*bottom*) at 25 °C. *Panel B,* same spectra (*top*, αA-crystallin; *bottom,* αB-crystallin) after band narrowing by Fourier self-deconvolution using a Lorentzian line shape of 15 cm^{-1} half-width and a resolution enhancement factor of 2.2.

dominated by a β-sheet band at 1631 cm^{-1} (Fig. 1*B*). The weaker bands at 1645, 1656, and 1663 cm^{-1} are usually assigned to unordered structure, α-helices and turns, respectively, whereas the components at 1672/1673 and 1682/1684 cm^{-1} may contain contributions from both β-sheets and turns (28, 30–32). Quantitative band fitting analysis shows that the fractional area of the β-sheet band at 1631 cm^{-1} amounts to 43% for αA-crystallin and 48% for αB-crystallin. The respective values for the α-helical band at 1656 cm^{-1} are 18 and 12%. The conclusion that αB homopolymer contains a higher proportion of β-structure and lower content of α-helix is valid despite the ambiguity in the assignment of the 1672/1673 and 1682/1684 cm^{-1} bands since the fractional area of these weak components in the spectra of both proteins is very similar.

Infrared spectroscopy was also used to probe the thermal stability of αA and αB homopolymers. Upon heating of αA-crystallin, the "native" β-sheet peak at 1632 cm^{-1} was gradually replaced by a broad band with a maximum at 1647–1649 cm^{-1} (Fig. 2*A*). Similar spectral changes were observed for αB-crystallin (spectra not shown). To further analyze the thermotropic changes in the secondary structure of α-crystallin homopolymers, the intensity ratio of the amide I band contour at 1632 cm^{-1} to that at 1649 cm^{-1} was plotted as a function of temperature (Fig. 2*B*). The above plots indicate that both proteins undergo a relatively minor conformational change between approximately 40 and 55 °C, followed by a major transition in the backbone conformation with a midpoint around 62 °C.

Surface Hydrophobicity—Bis-ANS is a hydrophobic molecule that becomes highly fluorescent upon binding to protein hydrophobic sites. This dye has been widely used for probing the surface hydrophobicity of proteins (20, 25, 33). Fig. 3 shows the fluorescence titration curves for bis-ANS binding to αA- and αB-crystallins at 23 °C. At a saturating dye concentration, the fluorescence intensity of bis-ANS associated with αB-crystallin is approximately 50% higher than that of the dye bound to αA-crystallin. A very similar difference between the saturation fluorescence intensity of the dye in the presence of αA and αB homopolymers was also seen when protein hydrophobicity was probed using a related monomeric dye, ANS (data not shown). The Scatchard analysis of the titration data for bis-ANS (Fig. 3, *inset*) yields the dissociation constants of 1.4 and 1.1 μM and a number of binding sites per protein monomer of 0.44 and 0.55 for αA- and αB-crystallin, respectively. The above dissociation constants are similar to those reported previously for the mixed chain α-crystallin from bovine lens (25). Overall, this data

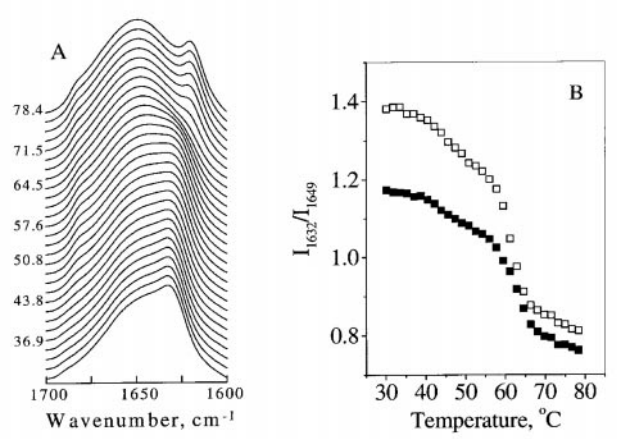

FIG. 2. *A,* temperature dependence of the amide I band contour of αA-crystallin. *Numbers* at every third spectrum indicate temperature in degrees centigrade. *B,* ratio of the amide I band intensity at 1632 cm^{-1} to that at 1649 cm^{-1} as a function of temperature for αA- (*closed square*) and αB-crystallin (*open square*).

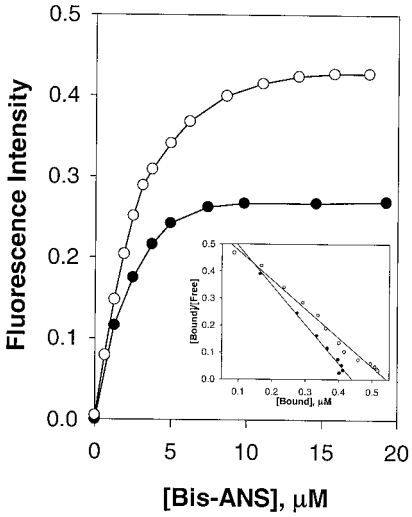

FIG. 3. **Fluorescence titration of αA- (*closed circle*) and αB-crystallin (*open circle*) with bis-ANS at 23 °C.** Protein (0.1 mg/ml) was titrated with small aliquots of 250 μM bis-ANS solution and the fluorescence intensity was monitored at 490 nm. *Inset,* Scatchard plots for the binding of bis-ANS to αA- (*closed circle*) and αB-crystallin (*open circle*). The concentration of protein-bound bis-ANS was determined from the titration curves as described under "Materials and Methods."

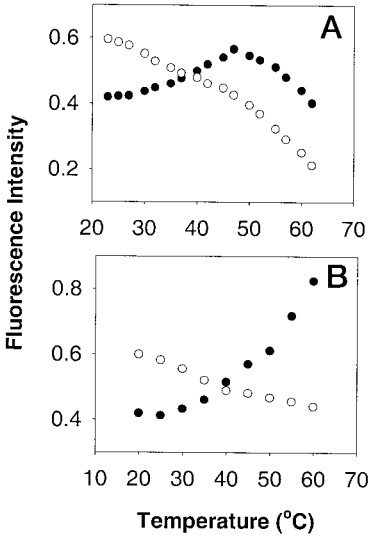

FIG. 4. *A,* temperature dependence of the fluorescence intensity at 490 nm of bis-ANS (25 μM) in the presence of 0.1 mg/ml αA- (*closed circle*) and αB-crystallin (*open circle*). *B,* fluorescence emission intensity at 23 °C of bis-ANS in the presence of αA- (*closed circle*) and αB-crystallin (*open circle*) as a function of the preincubation temperature. Samples of αA- or αB-crystallin (0.1 mg/ml) were preincubated at different temperatures for 15 min and cooled down to 23 °C. Bis-ANS (25 μM) was then added and fluorescence intensity measured at 490 nm.

indicates that at room temperature αB-crystallin is characterized by a higher surface hydrophobicity than αA-crystallin. However, the above difference is not preserved at elevated temperatures. As shown in Fig. 4*A*, the temperature dependence of the surface hydrophobicity for αA- and αB-crystallin is dramatically different. For αA-crystallin, the fluorescence intensity of bis-ANS is relatively stable up to approximately 30 °C and increases rapidly upon further increase in temperature, reaching a maximum at approximately 48 °C. This phase is followed by a gradual decrease in bis-ANS fluorescence upon further heating of the protein. The increase in the emission intensity between approximately 30 and 48 °C indicates that in this temperature range αA-crystallin undergoes a conformational transition associated with the exposure of additional hydrophobic sites. No such conformational transition is detectable for αB-crystallin. In contrast, the fluorescence intensity of bis-ANS in the presence of the latter protein shows a small gradual decrease upon heating in the entire temperature range between 20 and 60 °C. Overall, data of Fig. 4*A* shows that the relative surface hydrophobicity of αA- and αB-crystallins is strongly temperature dependent: compared with αA homopolymer, αB-crystallin appears to be more hydrophobic at low temperatures, but becomes less hydrophobic at high temperatures. Importantly, at physiological temperatures the hydrophobicity properties of the two proteins are very similar.

To further characterize the thermotropic behavior of αA- and αB-crystallins, the proteins were preincubated for 15 min at temperatures between 22 and 60 °C. After cooling down to 23 °C, the samples were incubated for 3 h with bis-ANS and the fluorescence intensity of the dye was measured. As shown in Fig. 4*B*, when αA-crystallin was preincubated at temperatures above approximately 30 °C, upon cooling it was characterized by a markedly increased affinity for bis-ANS. This indicates that, once exposed to elevated temperatures, the protein does not return to its original conformational structure but adopts a new conformation that is characterized by an increased exposure of hydrophobic surfaces. No such "activation" of hydrophobic surfaces was observed for αB-crystallin.

Chaperone Activity—The chaperone activity of αA- and αB-crystallin was studied by monitoring the ability of these proteins to prevent the aggregation of substrate proteins denatured by a reducing agent or heat. The application of complementary assays allowed us to compare the chaperone activity of αA and αB homopolymers over the wide range of temperature.

Reduction of the disulfide bond connecting the insulin A and B chains leads to the unfolding and aggregation of the B chain. The aggregation reaction can be suppressed by α-crystallin which binds the non-native conformer of the B-chain (12, 20). Fig. 5*A* shows the percentage protection at two different temperatures as a function of the concentration of αA- and αB-crystallin. From these plots, one can estimate the concentration of αA and αB homopolymers required to reduce the aggregation of insulin B-chain by 50%. The above parameter provides an empirical measure of a relative chaperone activity of both proteins. At 23 °C, the αB homopolymer is approximately 4.5 times more active than αA-crystallin. However, the two proteins respond differently to temperature changes: the chaperone activity of αA-crystallin markedly increases when the temperature

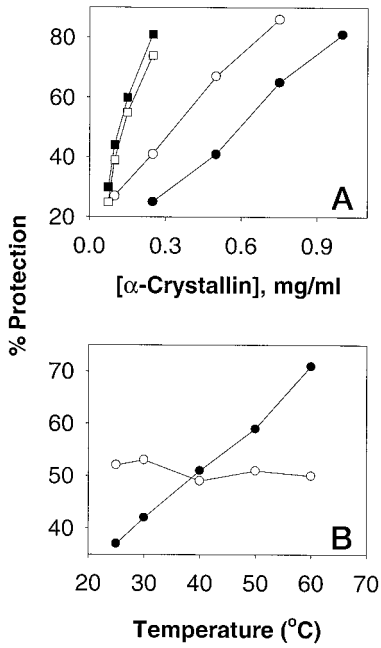

FIG. 5. *A,* the chaperone activity of α-crystallin homopolymers as assessed by the suppression of the aggregation of insulin B-chain. The protective effect of αA-crystallin at 23 °C (*closed circle*) and 37 °C (*open circle*) and that of αB-crystallin at 23 °C (*closed square*) and 37 °C (*open square*). Insulin (0.4 mg/ml in 50 mM phosphate buffer, pH 7.2) was reduced with 20 mM DTT and the aggregation of the B-chain in the absence and presence of αA or αB homopolymers was monitored by measuring the apparent absorption at 400 nm. *B,* the effect of the preincubation temperature on chaperone activity of αA- (*closed circle*) and αB-crystallin (*open circle*). α-Crystallin samples were preincubated for 15 min at different temperatures and then cooled down to room temperature. The chaperone activity of each protein sample was assessed by measuring the protection against the aggregation of insulin B-chain as described above. The concentration of αA and αB homopolymers was 0.4 and 0.15 mg/ml, respectively.

is increased from 23 to 37 °C, whereas the activity of αB-crystallin remains essentially unchanged (Fig. 5*A*). At 37 °C, αB-crystallin still appears to be more effective at suppressing insulin B chain aggregation. However, the ratio of the apparent potency of the αB and αA species is reduced from 4.5 at room temperature to approximately 2 at 37 °C. The insulin assay was also used to probe the relationship between the chaperone activity and structural reorganization of α-crystallin upon activation at high temperatures. As discussed above (see data of Fig. 4 B), upon exposure to elevated temperatures and subsequent cooling, αA-crystallin (but not αB-crystallin) adopts a conformation characterized by substantially increased exposure of hydrophobic surfaces. Fig. 5*B* shows that high temperature preincubation also results in a markedly increased chaperone activity of the αA-homopolymer. This contrasts with αB-crystallin which shows no improvement of the chaperone function upon preincubation at elevated temperatures.

Another protein suitable for studying the chaperone action of α-crystallin is bovine apolactalbumin. Reduction of disulfide bonds with DTT results in a rapid aggregation of this protein (27). Both αA- and αB-crystallin suppress this nonspecific aggregation. At 23 °C, the αB homopolymer shows approximately three times higher activity than the αA species (Fig. 6). However, the ability of αA-crystallin to suppress the aggregation of α-lactalbumin markedly improves when the temperature is raised to 37 °C. This contrasts with the behavior of αB homopolymer, the activity of which is unaffected by the temperature. Importantly, at temperatures around 37 °C, the αA and αB homopolymers appear to have essentially the same ability to prevent the aggregation of α-apolactalbumin.

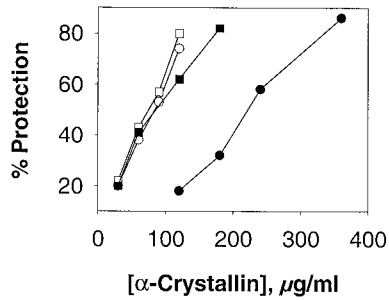

FIG. 6. **The chaperone activity of α-crystallin homopolymers as assessed by the suppression of the aggregation of reduced α-lactalbumin.** The protective effect of αA-crystallin at 23 °C (*closed circle*) and 37 °C (*open circle*) and that of αB-crystallin at 23 °C (*closed square*) and 37 °C (*open square*). α-Lactalbumin (0.2 mg/ml in 50 mM phosphate, 2 mM EDTA, 100 mM NaCl, pH 6.2) in the absence and presence of αA or αB homopolymers was reduced with 20 mM DTT and the kinetics of the aggregation was followed by measuring the apparent absorption at 360 nm.

Due to thermal instability of DTT, the chaperone assays described above could not be performed at temperatures exceeding 40 °C. However, the chaperone function of αA- and αB-crystallin at high temperatures could be studied by measuring the ability of these homopolymers to prevent the aggregation of thermally denatured proteins. The substrates suitable for such studies are alcohol dehydrogenase and citrate synthase (27, 34). As shown in Fig. 7, the relative potency of αA and αB homopolymers in suppressing the thermal aggregation of alcohol dehydrogenase is strongly temperature dependent. At 40 °C αB-crystallin is about two times more potent than αA-crystallin. However, at 50 °C the two proteins become almost equally active and, upon further increase in temperature to 55 °C, the chaperone activity of αA-crystallin exceeds that of the αB homopolymer. A very similar temperature dependence of the relative chaperone activity of αA and αB homopolymers was observed using citrate synthase as a substrate protein (Fig. 8).

DISCUSSION

One of the unresolved issues in α-crystallin research is the significance of the apparently different tissue specificity of the αA and αB chains. While the lens protein contains both αA and αB subunits (1–3), non-lenticular distribution of α-crystallin is clearly dominated by the αB chain (3, 5). Furthermore, elevated expression of the αB gene has been correlated with a number of neurological disorders (3, 5). The above differences suggest that αA- and αB-crystallins may play distinct physiological functions. Based on very limited data, it was recently proposed that the broader tissue distribution of αB-crystallin may be rationalized by the greater hydrophobicity, and thus higher chaperone activity, of the αB-homopolymer as compared with αA-crystallin (35). The present results provide new insight into the relationship between the surface hydrophobicity and the chaperone function of αA- and αB-crystallins.

One of the main findings of this study is that αA- and αB-crystallins fundamentally differ in the way they respond to temperature. The chaperone function of the αA homopolymer markedly improves with an increase in temperature, whereas the activity of αB-crystallin appears to change very little. As a result of this different response, the relative chaperone potency of αA- and αB-crystallins strongly depends on the temperature: αA homopolymer is markedly less active than αB at low temperatures, but becomes a more active species at high temperatures. Under physiologically relevant conditions (37–40 °C), the αB homopolymer is approximately two times more potent as a chaperone with respect to three out of four proteins tested, whereas with respect to one substrate the activity of both

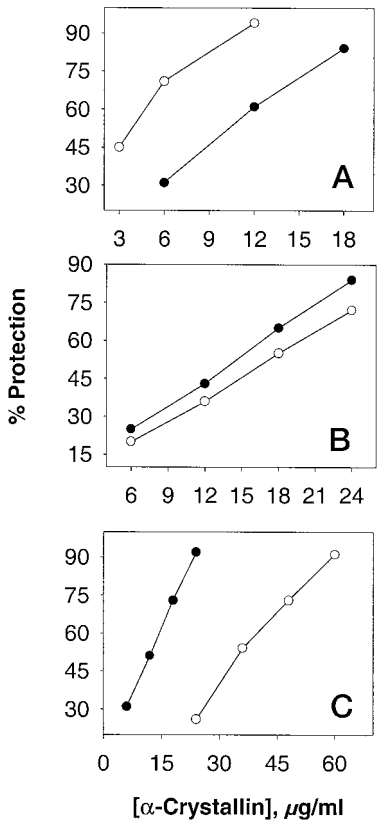

FIG. 7. **The chaperone activity of αA- (*closed circle*) and αB-crystallin (*open circle*) as assessed by the suppression of the aggregation of heat-denatured alcohol dehydrogenase at 40 °C (*panel A*), 50 °C (*panel B*), and 55 °C (*panel C*).** Yeast alcohol dehydrogenase (0.2 mg/ml in 50 mM phosphate, 2 mM EDTA, 150 mM NaCl, pH 7.2) was incubated at a given temperature in the absence and presence of αA- or αB-crystallin, and the kinetics of enzyme aggregation was followed by monitoring the apparent absorption at 360 nm.

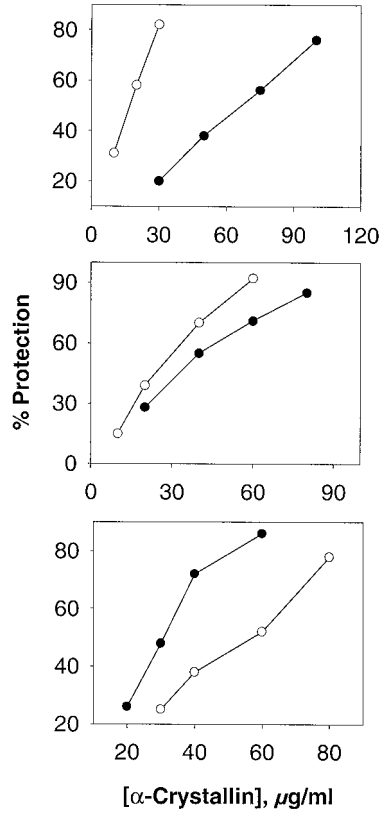

FIG. 8. **The chaperone activity of αA- (*closed circle*) and αB-crystallin (*open circle*) as assessed by the suppression of the aggregation of heat-denatured citrate synthase at 40 °C (*panel A*), 50 °C (*panel B*), and 55 °C (*panel C*).** The enzyme (0.05 mg/ml in 50 mM phosphate, 2 mM EDTA, 150 mM NaCl, pH 7.8) was incubated at a given temperature in the absence and presence of αA- or αB-crystallin, and the kinetics of enzyme aggregation was followed by monitoring the apparent absorption at 360 nm.

species is essentially identical. Based on the results of the "insulin assay," it was recently concluded that αB-crystallin has a dramatically higher chaperone activity than αA homopolymer (35). A direct comparison of this data with our results is hampered by the lack of information about the temperature used in the previous study. Nevertheless, the present data shows that at physiological temperatures the difference in the chaperone activity of αA and αB-crystallins is very modest.

To gain insight into the molecular basis of the temperature-dependent functional properties of αA- and αB-crystallins, we compared selected structural properties of these two proteins. Previous circular dichroism experiments provided conflicting data regarding the secondary structure of αA- and αB-crystallins. Sun *et al.* (35) reported a higher content of β-sheet and lower proportion of α-helix for αA than αB-homopolymer, whereas data of Horwitz *et al.* (27) indicates a similar secondary structure of both species. Here, we analyzed the secondary structure of both proteins using FTIR spectroscopy. Unlike circular dichroism, the FTIR method is not prone to potential artifacts due to light scattering and, thus, it is well suited for studying large oligomeric proteins such as α-crystallin. The FTIR results indicate that the secondary structure of both homopolymers is similar, with a slightly higher content of a β-sheet structure (and a lower proportion of α-helix) in αB-crystallin. The above small difference in the secondary structure is, however, unlikely to account for the temperature-dependent differences in the chaperone activities of αA- and αB-crystallins. This conclusion is supported by the finding that the thermotropic changes in the secondary structure of αA and αB homopolymers are essentially identical. Furthermore, the notion that the secondary structure is not a key determinant of the chaperone activity of α-crystallin is consistent with the observation that the chaperone function is preserved at temperatures as high as 55–65 °C, *i.e.* under the conditions leading to a major loss of the native secondary structure of α-crystallin.

It is believed that binding of non-native proteins to α-crystallin is driven largely by hydrophobic interactions (11, 20–25). Therefore, there is considerable interest in the hydrophobicity properties of the chaperone. Sun *et al.* (35) recently reported that αB-crystallin is characterized by a much higher surface hydrophobicity than αA-crystallin, as indicated by a 3-fold stronger fluorescence intensity of the αB-bound ANS probe. However, our results do not concur with the previous study. While at room temperature αB indeed appears to be somewhat more hydrophobic than αA homopolymer, the hydrophobicity properties of the two proteins at physiologically relevant temperatures are essentially identical. The αA and αB homopolymers differ, however, with respect to the temperature dependence of their hydrophobicity profiles. Between approximately 30 and 48 °C, αA-crystallin appears to undergo a conformational transition resulting in the exposure of additional hydrophobic sites. Furthermore, once exposed to elevated temperature, upon cooling the protein adopts a new conformational state that is characterized by a markedly increased affinity for hydrophobic probes. No such effects occur for αB-crystallin. Its hydrophobicity properties appear to be remarkably resistant to

temperature changes. The structural basis for the "thermal activation" of the surface hydrophobicity and chaperone activity of αA-crystallin is at present unknown. However, our preliminary spectroscopic experiments with various single tryptophan mutants of αA-homopolymer suggest that these effects may be related to an irreversible thermal transition within the C-terminal portion of the protein.[2]

The assessment of the "intrinsic" chaperone activity of α-crystallin at different temperatures is complicated by a potential effect of temperature on the hydrophobicity of substrate proteins. However, under the present experimental conditions the latter effect appears to be not very significant since bis-ANS binding to two substrate proteins (non-reduced insulin and α-lactalbumin) was found essentially identical at 23 and 40 °C (data not shown). Furthermore, any potential temperature-dependent changes in surface hydrophobicity of target proteins would affect the interaction of these proteins with αA and αB homopolymers in the same way and, therefore, could not account for the observed differences in temperature-dependent chaperone function of αA and αB homopolymers. Given that, it is remarkable that the increase in the surface hydrophobicity of αA-crystallin invariably leads to an improved chaperone activity of this homopolymer. Furthermore, the temperature insensitivity of the chaperone function of αB homopolymer appears to correlate with the lack of thermal changes in the hydrophobicity of the latter protein. This apparent correlation provides strong support to the notion that the chaperone activity of α-crystallin is dependent on the presence of surface-exposed hydrophobic patches (11, 20–25). However, the present data also show that surface hydrophobicity is not the sole determinant of the chaperone activity of α-crystallin. For example, at physiologically relevant temperatures the two homopolymers are characterized by very similar hydrophobicity, whereas their ability to suppress the aggregation of some target proteins is significantly different. These findings provide a basis for future studies (*e.g.* using site-directed spectroscopic probes) on the structural correlates of the chaperone function of αA/αB-crystallin and related small heat shock proteins.

[2] G. B. Reddy, J. M. Petrash, and W. K. Surewicz, unpublished data.

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