Mini-αB-Crystallin: A Functional Element of αB-Crystallin with Chaperone-like Activity†

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ABSTRACT: α-Crystallin is a member of the family of small heat-shock proteins (sHSP) and is composed of two subunits, αA-crystallin and αB-crystallin, which exhibit molecular chaperone-like properties. In a previous study, we found that residues 70−88 in αA-crystallin can function like a molecular chaperone by preventing the aggregation and precipitation of denaturing substrate proteins [Sharma, K. K., et al. (2000) *J. Biol. Chem. 275*, 3767−3771]. In this study, we show that the complementary sequence in αB-crystallin, residues 73−92 (DRFSVNLDVKHFSPEELKVK), is the functional chaperone site of αB-crystallin. Like the mini-αA-crystallin chaperone, the mini-αB-crystallin chaperone interacts with 1,1′-bi(4-anilino) naphthalene-5,5′-disulphonic acid (bis-ANS) and also possesses significant *β*-sheet and random coil structure. Deletion of four residues (DRFS) from the N-terminus or deletion of C-terminus LKVK residues from the 73−92 peptide abolishes the chaperone-like activity against denaturing alcohol dehydrogenase. However, removal of DRFS or HFSPEELKVK is necessary to completely abolish the antiaggregation property of the peptide in insulin reduction assay. Substitution of Asp at a site corresponding to D80 in αB-crystallin with D-Asp or *β*-Asp results in a significant loss of chaperone-like activity. Kynurenine modification of His in the peptide abolishes the antiaggregation property of the mini-chaperone. These data suggest that the 73−92 region in αB-crystallin is one of the substrate binding sites during chaperone activity.

α-Crystallin, the most abundant protein in the lens belongs to the group of small heat-shock proteins (sHSPs).[1] α-Crystallin exists as a polydisperse aggregate with an average molecular mass of 800 kDa and is composed of αA- and αB-subunits, each with a molecular mass of 20 kDa (*1*, *2*). The sequences of the subunits of α-crystallin have high sequence homology between them (*1*) and are highly conserved among species (*3*). Although αA-crystallin is primarily expressed in the lens, αB-crystallin is found in other tissues as well (*4*−*7*). Extensive studies have not yet delineated the quaternary structure of α-crystallin or its subunits. Available data suggest that the α-crystallin subunits have high amounts of *β*-sheet structure. Like all sHSPs, α-crystallin displays chaperone-like activity by preventing the aggregation of substrate proteins (*8*−*11*). This property has been implicated in the maintenance of lens transparency. Both A and B subunits in α-crystallin display chaperone-like activity independent of each other and as heteroaggregates (*12*).

Because the chaperone-like function of α-crystallin may have an integral role in lens transparency, identification of the critical residues required for the chaperone-like function of α-crystallin subunits has been the focus of numerous studies. The C-terminal region of αA-crystallin (*13*, *14*), Asp^{69} of αA-crystallin (*15*), Phe^{71} of αA-crystallin (*16*), the most hydrophobic N-terminal phenylalanine-rich region in αB-crystallin (*17*), and Arg^{120} of αB-crystallin (*18*) have all been suggested as necessary for chaperone-like activity.

Hydrophobic sites in α-crystallin have been hypothesized to be responsible for the chaperone-like activity (*19*, *20*), as in the case of molecular chaperone GroEL (*21*). We have previously determined that residues 50−54 and 79−99 in αA-crystallin and residues 75−103 in αB-crystallin are hydrophobic sites recognized by the environment-sensitive probe 1,1′-bi(4-anilino)*n*-naphthalene sulfonic acid (bis-ANS) (*22*). Because we found that prior binding of bis-ANS to α-crystallin diminishes chaperone-like activity (*23*), we concluded that hydrophobic sites participate in α-crystallin chaperone-like activity. A similar observation has been made for isolated αA- and αB-crystallins (*24*). We have also shown that residues 57−69 and residues 93−107 in αB-crystallin are involved in binding with ADH (*12*). In addition, we have determined that a 19-amino acid residue from αA-crystallin (residues 70−88, mini-αA-crystallin), which contains bis-ANS-binding sequences, possesses an antiaggregating property similar to that of αA-crystallin (*25*).

Here, we report that αB-crystallin residues 73−92 can independently prevent the aggregation of denatured substrate proteins, similar to the action of native αB-crystallin. We have coined the term "mini-αB-crystallin" to describe these specific residues. In this study, we describe the smallest

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[1] Abbreviations: sHSPS, small heat-shock proteins; bis-ANS, 1,1′-bi(4-anilino) naphthalene-5,5′-disulphonic acid; ADH, alcohol dehydrogenase; 1,5-AZNS, 1-azidonaphthalene 5-sulphonate.

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peptide sequence in αB-crystallin that shows an antiaggregation property and the critical residues required for such chaperone-like function.

EXPERIMENTAL PROCEDURES

Materials. γ-Crystallin was isolated from young bovine lenses and purified as described previously (*8*). αB-Crystallin was purified according to the procedure described elsewhere (*12*). All peptides used in this study were synthesized in the peptide synthesis core facility at the University of Missouri. The synthetic peptides were purified by reversed-phase HPLC, and their masses were confirmed by mass spectrometry prior to use. Biuret method was used to estimate the peptide concentrations used in various assays. Bis-ANS was obtained from Molecular Probes, Inc. (Junction City, OR). A stock solution of bis-ANS was prepared in 95% ethanol, and the concentration was determined by absorbance at 385 nm using an extinction coefficient, $\epsilon_{385} = 16\,790\ cm^{-1}\ M^{-1}$ (*26*). Insulin and yeast ADH were purchased from Sigma and Biozyme Laboratories, respectively. All other chemicals were of the highest grade commercially available.

Assay of Chaperone-like Activity. The capacity of the different peptides to prevent DTT-induced insulin aggregation (*27*), oxidation-induced γ-crystallin aggregation (*28*), and heat-induced ADH aggregation (*12*) was determined. Insulin and γ-crystallin assays were performed at 37 °C, whereas ADH assay was conducted at 48 °C. All chaperone assays were carried out in 1 mL reaction volumes that contained 50 mM sodium phosphate buffer, pH 7.2, and 0.1 M NaCl. Aggregation of denaturing proteins was monitored by measuring the apparent absorption at 360 nm for all the substrate proteins as a function of time in a Shimadzu spectrophotometer equipped with a multicell transporter and a Peltier temperature regulator.

CD Spectroscopy of Peptides. The far-UV CD spectra of purified peptides were recorded at 25 °C over a range of 190−250 nm on an Aviv 62DS spectropolarimeter using 0.1 mg/mL concentration of the peptide in 10 mM phosphate buffer, pH 7.2. A quartz cell of 0.1-cm path length was used, and five spectra for each sample were taken and averaged.

Bis-ANS Binding Studies. Fluorescence of bis-ANS bound to peptides was measured using a Jasco FP750 spectrofluorimeter. Bis-ANS solution, 16 μmol, was added to 0.1 mg/mL peptide solution in 0.05 M phosphate buffer, pH 7.2. The mixture was thoroughly mixed and then incubated for 15 min. Fluorescence emission spectra were then recorded at 400−600 nm using an excitation wavelength of 390 nm. The excitation and emission slits were set at 5 nm.

Modification of Mini-αB-crystallin with Kynurenine. Purified peptide was incubated with kynurenine (1:5 ratio of peptide to kynurenine) at 37 °C for 6 days. The modified peptide was purified by HPLC fitted with a Vydac C18 column and 0−80% acetonitrile gradient containing 0.1% trifluoroacetic acid. The kynurenine modification of the peptide was confirmed by mass spectrometric analysis. The chaperone-like activity of the modified peptide was measured using insulin reduction assay.

Size-Exclusion Chromatography and HPLC Analysis. Biogel P-30 gel filtration chromatography was performed to isolate the complex formed between denaturing ADH and mini-αB-crystallin, as described previously for mini-αA-crystallin and ADH studies (*25*). Following gel filtration, the complex was treated with urea and filtered through a 10 kDa filter, and the filtrate was analyzed in a C18 column (*25*). Sephadex G-75 chromatography was used to determine retropeptide aggregation.

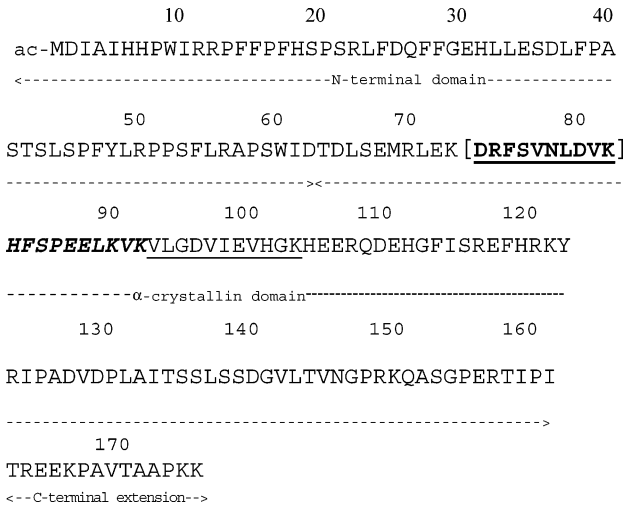

FIGURE 1: αB-Crystallin sequence showing the mini-αB-crystallin chaperone site. The bis-ANS-binding region is underscored and the mini-chaperone sequence is shown in bold. The mellitin-binding region is within the brackets. The 1,5-AZNS binding sequence is shown in italics. The ligand binding sites are taken from ref *22*.

RESULTS AND DISCUSSION

Chaperone Site and Concept of Mini-Chaperone. The A and B subunits of α-crystallin contain an α-crystallin domain, an N-terminal domain, and a C-terminal extension. Figure 1 shows the amino acid residues contributing to these domains in αB-crystallin. The hydrophobic sites on this highly conserved protein have been implicated in playing a role in the chaperone-like activity of αB-crystallin (*2*, *19*, *20*, *23*, *29*−*31*). Bis-ANS-binding and cross-linking studies have shown that hydrophobic sites in α-crystallin subunits are involved in binding of substrate proteins to αA- and αB-crystallin during chaperone action (*22*). In a separate experiment, the 83−90 region in αB-crystallin was identified as another hydrophobic site-specific probe 1,5-AZNS-binding site (*22*). The 1,5-AZNS-labeled peptide sequence was sandwiched between the two bis-ANS-binding sequences in αB-crystallin. The structural differences between bis-ANS and 1,5-AZNS may contribute to the difference in the site of photoincorporation of these two probes to αB-crystallin. In a related study, we observed that one (i.e., FSVNLDV) of the two regions in αB-crystallin identified as bis-ANS-binding sequences also contributes to the mellitin-binding region (*12*). These studies, taken together, suggest that the hydrophobic mellitin-binding site in αB-crystallin subunit is part of the chaperone site. Additional insight into the role of hydrophobic sites in chaperone activity of α-crystallin subunits came from our novel finding that a peptide consisting of a hydrophobic sequence in αA-crystallin, which also includes the residues from the substrate binding site, can mimic the antiaggregation properties of the parent protein (*25*). We previously reported that a hydrophobic melittin-binding sequence, KFVIFLDVKHFSPEDLTVK, in αA-crystallin (residues 70−88) by itself displays chaperone-like antiaggregation activity (*25*). Because the A and B subunits

of α-crystallins have significant sequence homology, we hypothesized that an αB-crystallin peptide that shows significant sequence homology to αA-crystallin chaperone-like peptide will also demonstrate chaperone-like activity. Therefore, we investigated whether residues 73−92 of αB-crystallin (DRFSVNLDVKHFSPEELKVK; mini-αB-crystallin) prevent the aggregation of denatured substrate proteins. This region (i.e., residues 73−92) is known to be a highly conserved sequence in αB-crystallin of various species.

Chaperone-like Activity of Mini-αB-crystallin. Figure 2 shows the antiaggregating property of mini-αB-crystallin against DTT-induced aggregation of insulin at 37 °C (Figure 2A), oxidation-induced aggregation of γ-crystallin at 37 °C (Figure 2B), and thermal aggregation of ADH at 48 °C (Figure 2C). As Figure 2 shows, only a portion of αB-crystallin, represented as mini-αB-crystallin, is sufficient to prevent the aggregation of proteins denatured by heat or chemical methods. The antiaggregation activity of the peptide was found to be concentration-dependent. The data show that 40 μM of the peptide suppresses aggregation of 2.7 μM of ADH by 80%, comparable to the activity of mini-αA-crystallin reported previously (*25*). The formation of an ADH−mini-αB-crystallin complex was confirmed by gel filtration and HPLC analysis of the reaction mixture (data not shown). The mini-αB-crystallin was found to suppress insulin B chain aggregation very efficiently. A 1:1 molar ratio of mini-αB-chaperone and insulin nearly completely suppressed aggregation-induced light scattering. However, the mini-αB-peptide activity was lower than native αB-crystallin activity when ADH or γ-crystallin were used as substrates and resembled the pattern of mini-αA-crystallin (*25*). The αA-crystallin was 8-fold more effective in suppressing aggregation-induced light scattering than the mini-αA-crystallin on molar basis when ADH was used as the substrate (*25*). The different efficiency of native αA- and αB-crystallin toward different proteins has been previously observed by us (*32*) and others (*33*, *34*). Therefore, it can be concluded from these observations that the different chaperone-like activity of crystallins reported previously applies to mini-αB-crystallin as well.

Suppression of heat-induced aggregation of ADH by mini-αB-crystallin was tested by adding the peptide after the start of aggregation (Figure 3). The addition of mini-αB-crystallin after 20 and 40 min of heat-induced ADH aggregation significantly impeded further aggregation. The aggregated proteins were not solubilized, as in the case of α_s-casein (*35*). Therefore, this property of mini-αB-crystallin is consistent with that of other molecular chaperones: the chaperones bind to aggregation-prone proteins and do not solubilize preformed aggregates (*36*).

After the antiaggregation property of mini-αB-crystallin was confirmed, we investigated the ability of a retrosequence of mini-αB-crystallin (KVKLEEPSFHVDLNVSFRD) to suppress heat-induced aggregation of ADH. The retrosequence was found not to possess antiaggregation activity (Figure 4). Gel chromatograpy on Sephadex G-75 (profile not shown) did not show any evidence of retropeptide aggregation, suggesting that aggregation of the peptide is not the reason for the loss of activity. Further, the retropeptide did not form light-scattering aggregates during the assay (Figure 4). A similar result was obtained with mini-αA-crystallin and its retropeptide (*27*). Therefore, the amino acid sequence, rather than the composition in mini-αB-crystallin, appears to determine its chaperone-like activity.

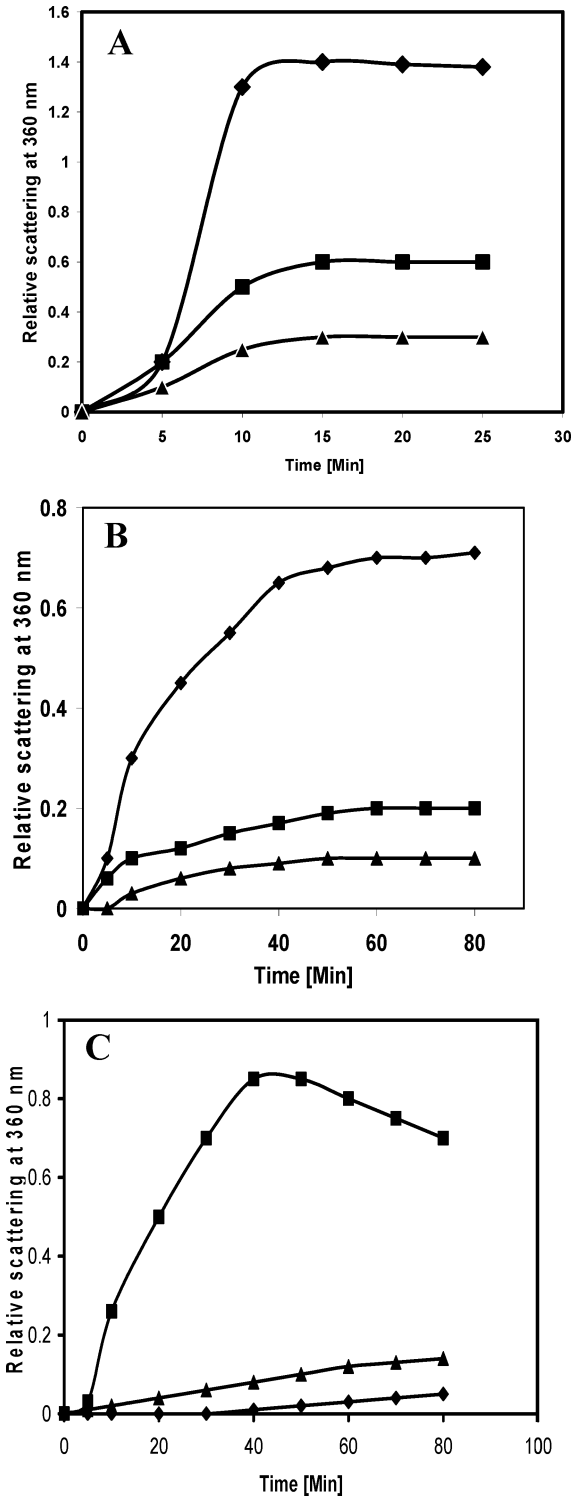

FIGURE 2: Aggregation of denaturing proteins in the presence of mini-αB-crystallin. Light scattering at 360 nm of 0.4 mg/mL insulin and 20 mM DTT (A), 0.25 mg/mL γ-crystallin (B), and 0.3 mg/mL ADH (C) in the absence and presence of mini-αB-crystallin. Insulin and γ-crystallin assays were done at 37 °C, whereas ADH assay was performed at 48 °C. (A) Diamond, insulin; square, insulin and 0.05 mg/mL mini-αB-crystallin; triangle, insulin and 0.1 mg/mL mini-αB-crystallin. (B) Diamond, γ-crystallin; square, γ-crystallin and 0.05 mg/mL mini-αB-crystallin; triangle, γ-crystallin and 0.1 mg/mL mini-αB-crystallin. (C) Square, ADH; triangle, ADH and 0.05 mg/mL mini-αB-crystallin; diamond, ADH and 0.1 mg/mL mini-αB-crystallin.

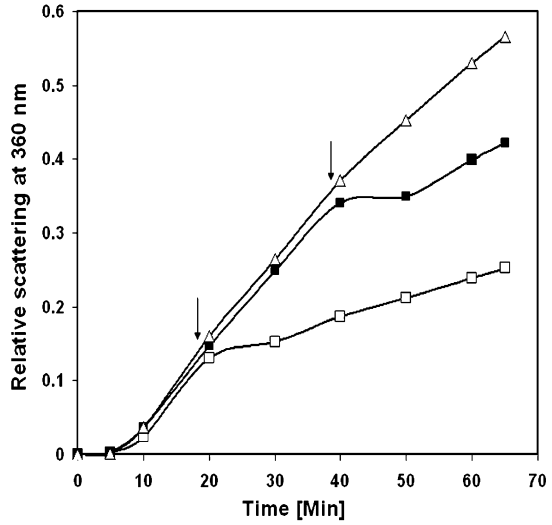

FIGURE 3: Effect of the addition of mini-αB-crystallin on ADH aggregation. The aggregation of 0.3 mg of ADH at 48 °C in 50 mM phosphate buffer containing 0.1 M NaCl was measured in the presence and absence of mini-αB-crystallin. Mini-αB-crystallin, 0.1 mg, was added 20 and 40 min after initiation of heat-induced aggregation (shown by arrows), and the assay was continued for 65 min. (Open triangle, ADH; filled square and open square, ADH with mini-αB-crystallin added.)

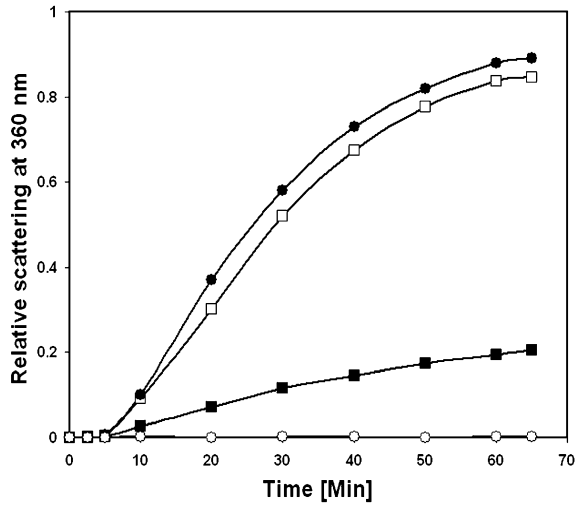

FIGURE 4: ADH aggregation in the presence and absence of retro-mini-αB-crystallins. The aggregation of 0.3 mg of ADH at 48 °C in 50 mM phosphate buffer containing 0.1 M NaCl was measured in the absence and presence of 0.1 mg of mini-αB-crystallin and retro-mini-αB-crystallin. (Filled circle, ADH; filled square, ADH and mini-αB-crystallin; open square, ADH and retro-mini-αB-crystallin; open circle, retro-mini-αB-crystallin.)

Effect of Truncation or Substitution on the Chaperone-like Activity of Mini-αB-crystallin. Since the mini-chaperone sequence is highly conserved in several species, we prepared several peptides that are truncated forms of the active peptide chaperone to determine the minimum sequence required for chaperone-like activity. Table 1 shows the relative antiaggregation activity of truncated forms of mini-αB-crystallin against DTT-induced insulin aggregation at 37 °C and against ADH aggregation at 48 °C. The data show that deletion of two residues—DR from the amino terminal end and VK from the C-terminal end of the peptide—partially reduced chaperone-like activity of the peptide against both the substrates. Deletion of eight residues from the C-terminal end of the peptide yielded a peptide that showed significant protection against insulin aggregation but no ability to suppress aggregation of denaturing ADH. In contrast, deletion of four residues (DRFS) from the N-terminal end of the peptide completely abolished the antiaggregation property of the peptide against both substrates, but removal of DR residues had no significant effect. Furthermore, substitution of F with A in the N-terminal region resulted in complete loss of activity. These results suggest that, like the N-terminal region F of mini-αA-crystallin (*25*), the F in the N-terminal region of mini-αB-crystallin may be essential for chaperone-like activity of the peptide. The critical role of F of mini-αA-crystallin (corresponding to F71 of αA-crystallin) was confirmed by a site-directed mutagenesis study in which the mutant αA-crystallin was found to not display chaperone activity at physiological temperatures (*16*).

Table 1: Effect of Truncation and Substitution on Antiaggregation Property of Mini-αB-crystallin[a]

peptides	percent protection: ADH assay	percent protection: insulin assay	bis-ANS binding
DRFSVNLDVKHFSPEELKVK (peptide1)	84	86	32 ± 2
DRFSVNLDVKHFSPEELK (peptide 3)	64	70	11 ± 0.5
DRFSVNLDVKHFSPEE	0	52	ND[b]
DRFSVNLDVKHFSP	0	63	ND[b]
DRFSVNLDVKHF	0	64	ND[b]
DRFSVNLDVK	0	0	ND[b]
FSVNLDVKHFSPEELKVK (peptide 4)	70	74	8 ± 0.4
VNLDVKHFSPEELKVK	0	0	ND[b]
FSVNLAVKHFSPEELKVK (peptide 5)	24	67	27 ± 1.5
FSVNLDVKAFSPEELKVK (peptide 6)	69	72	9 ± 0.5
DRASVNLDVKHFSPEELKVK (peptide 7)	0	ND[b]	ND[b]

[a] Relative activity of the truncated forms of mini-αB-crystallin against ADH aggregation at 48 °C and DTT-induced insulin aggregation at 37 °C was measured as described under Experimental Procedures. The fluorescence intensity of bis-ANS bound to peptides was recorded, as described under Experimental Procedures. [b] ND, not determined.

Structural Features of Native and Substituted Mini-αB-crystallin. The emission maximum of bis-ANS, a hydrophobic site-specific probe, shifts to a lower wavelength when it interacts with proteins or peptides that have significant hydrophobicity (*26*). Binding of bis-ANS to the peptide chaperone resulted in a shift in the emission maximum of the fluorophore to 499 nm from 520 nm in aqueous media (data not shown). The far-UV CD spectra of the peptide in buffer showed significant amounts of β-sheet and random coil structure (Figure 5). Compared with mini-αA-crystallin (*25*, *27*), mini-αB-crystallin demonstrated a slightly greater, noncompact conformation, which may be due to its random coil content being greater than its β-sheet content. The conformation and affinity of mini-αB-crystallin and its substituted forms to hydrophobic probe were determined by far-UV CD spectra and bis-ANS binding studies (Figure 5 and Table 1). The studies were performed to identify any correlation between the peptide hydrophobicity and chaperone-like activity. When compared to mini-αB-crystallin (peptide 1), the peptides 3−6 (Table 1) showed a decrease in the β-sheet content and an increase in the random coil content (Figure 5). Other truncated or Ala-substituted mini-αB-peptides were also mostly in random-coiled form, with negligible β-sheet content, and did not bind bis-ANS (data not shown). All mutated or truncated peptides, with the exception of peptide 5, were found to bind significantly less

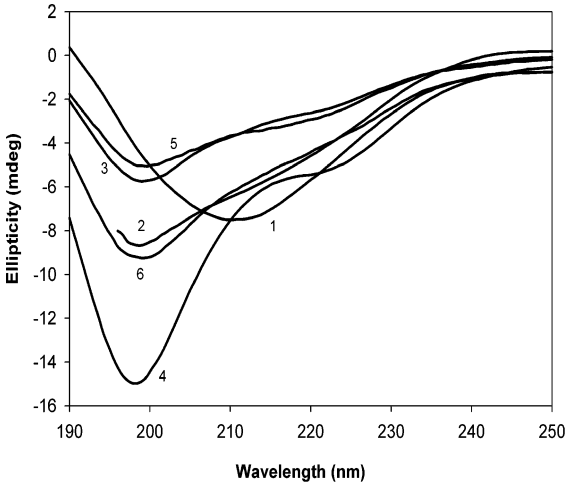

FIGURE 5: Far-UV CD spectra of native, mutant, and heat-treated mini-αB-crystallins. The sequences of peptides 1 and 3−6 are shown in Table 1, and the experimental conditions are described under Experimental Procedures. Trace 2 is the spectra for peptide 1 after heat treatment.

bis-ANS than mini-αB-crystallin (Table 1). The binding of bis-ANS to peptide 5 was comparable to that of mini-αB-crystallin (peptide 1).

Previously, we reported that mini-αA-crystallin regains most of its secondary structure upon refolding from its heat-denatured conformation and that the chaperone-like activity of the native and heat-treated mini-αA-crystallin was not significantly different (*27*). To investigate the effect of heat treatment on mini-αB-crystallin, a known amount of the peptide was heated to 60 °C in assay buffer. After 30 min at 60 °C, the peptide solution was cooled to room temperature and subjected to CD spectroscopy. Compared to the far-UV CD spectrum of native mini-αB-crystallin, the spectrum of mini-αB-crystallin after thermal unfolding and refolding showed a more random-coiled structure (trace 2 in Figure 5). Surprisingly, the chaperone-like activity of the refolded peptide was comparable to that of the native peptide. The activities of the native and heat-treated peptides were comparable during DTT-induced insulin reduction assay (Figure 6A) and temperature-induced ADH denaturation assay (Figure 6B). Similar data were obtained when citrate-synthase was used in place of ADH (not shown). Therefore, it appears that, although the secondary structure of refolded mini-αB-crystallin is more disrupted than that of its native state, the structural differences do not affect the chaperone-like activity of the peptide. In this aspect, the peptide behaves like a major random-coiled protein α-casein, which has been reported to possess antiaggregation properties against denatured substrate proteins (*35*). Therefore, a combination of hydrophobicity and ionic interactions stemming from the amphipathic nature of the peptide seems to determine the activity. This conclusion is also based on the observations that (a) removal of charged K-x-K residues from the C-terminus of the peptide resulted in the loss of activity, (b) substitution of D with A (peptide 5) reduced the chaperone-like activity, and (c) substitution of hydrophobic F with less hydrophobic A at the N-terminal region of the peptides abolished the chaperone-like activity.

Effect of Substitution with D*-Asp or β-Asp on Chaperone-like Activity*. Studies of the effects of substitutions at various locations in the peptide chaperone revealed that the substitution of hydrophillic amino acid residues D80 and H83 with Ala preserved most of the chaperone-like activity of mini-αB-crystallin (Table 1). However, when substitution was with D-Asp or *β*-Asp at a position corresponding to D80 in αB-crystallin, the peptide lost its ability to suppress aggregation of denaturing ADH (Figure 7), and both forms of the peptide had also lost the *β*-sheet content when examined by CD spectroscopy (data not shown). A modest (about 10%) increase in the ADH aggregation in the presence of D-Asp peptides was observed. This effect is most likely due to the interaction of D-Asp-peptide with denaturing ADH. In the past, we have observed that modified crystallin peptides increase the aggregation of denaturing ADH (*37*). The presence of D-Asp or *β*-Asp likely alters the peptide structure in such a way that it becomes less favorable for the interaction. The presence of D-Asp or *β*-Asp in peptides is known to contribute to the structural change (*38*, *39*).

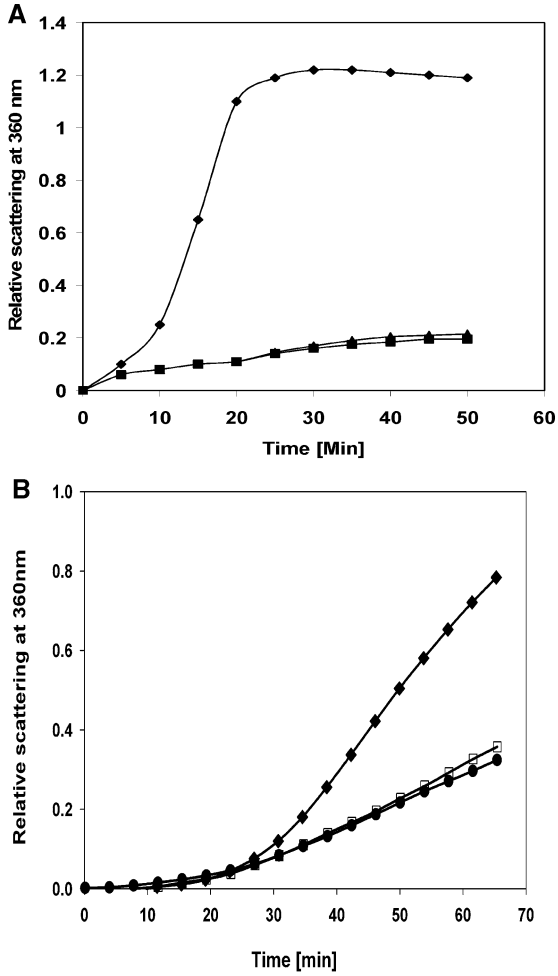

FIGURE 6: Effect of heat treatment on chaperone-like activity of mini-αB-crystallin. The peptide was heated to 60 °C in assay buffer. After 30 min at 60 °C, the peptide solution was cooled to room temperature and the residual chaperone-like activity was measured. (A) Light scattering was measured at 360 nm for 0.4 mg/mL insulin and 20 mM DTT at 37 °C in the absence (diamond) and presence of native 0.1 mg/mL mini-αB-crystallin (triangle) and 0.1 mg/mL refolded mini-αB-crystallin (square) after thermal unfolding. (B) Light scattering at 360 nm of 0.4 mg/mL ADH in the absence (diamond) and presence of 0.1 mg of native (open square) and heat-treated (circle) mini-αB-crystallin at 48 °C.

Effect of Modification of His with Kynurenine on Mini-αB-crystallin Chaperone-like Activity. The post-translational modification of α-crystallin subunits has been attributed to

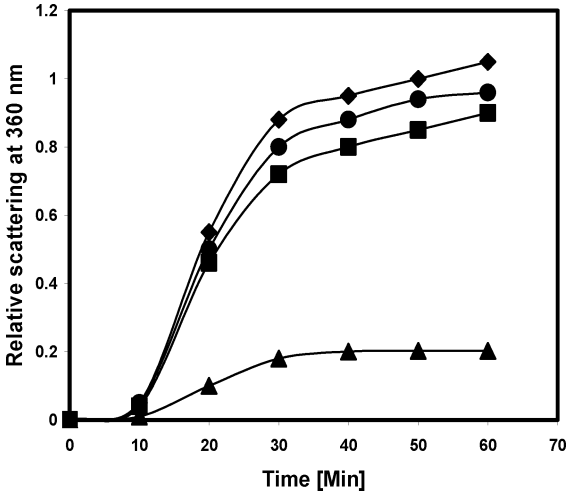

FIGURE 7: Effect of D-Asp or β-Asp substitution on chaperone-like activity of mini-αB-crystallin. DTT-induced aggregation of 0.3 mg of insulin in the presence and absence 0.1 mg of substituted and native mini-αB-crystallin was measured at 37 °C in 1 mL of 50 mM phosphate buffer, pH 7.0 (square, insulin; circle, insulin and β-Asp containing mini-αB-crystallin; diamond, insulin and D-Asp containing mini-αB-crystallin; triangle, insulin and mini-αB-crystallin).

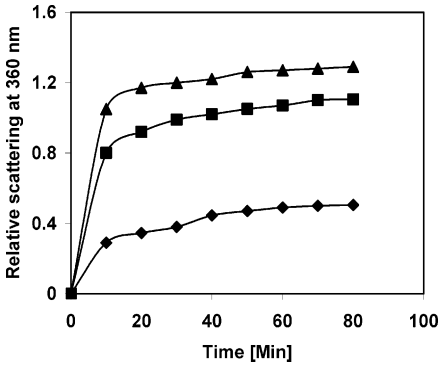

FIGURE 8: Effect of His modification in mini-αB-crystallin by kynurenine on chaperone-like activity. DTT-induced aggregation of 0.3 mg of insulin in the presence and absence 0.1 mg of kynurenine-modified mini-αB-crystallin was measured at 37 °C in 1 mL of 50 mM phosphate buffer, pH 7.0 (triangle, insulin; square, insulin and kynurenine-modified mini-αB-crystallin; diamond, insulin and 0.1 mg of mini-αB-crystallin).

the loss of chaperone-like activity (*40*). Truscot et al. (*41*) reported that His residues in αB-crystallin are susceptible to modification by the tryptophan metabolite kynurenine. Because H83 of αB-crystallin is at the chaperone site, we investigated whether the His present in mini-αB-crystallin chaperone is susceptible to modification by kynurenine and whether such a modification leads to a loss in chaperone-like activity of the peptide. As shown in Figure 8, the modification of mini-αB-crystallin chaperone by kynurenine resulted in more than an 80% loss of chaperone-like activity. Therefore, the age-related modification of αB-crystallin by kynurenine likely leads to diminished chaperone-like activity. Furthermore, the Lys in αB-crystallin is also susceptible to glycation. We have previously shown that glycation of α-crystallin decreases ANS binding and reduces chaperone-like activity of α-crystallin (*22*). Others have shown that Lys in the αB-crystallin chaperone site is susceptible to modification (*42*). Since Lys 92 is the C-terminal residue on mini-αB-crystallin chaperone, glycation likely affects the chaperone site and interferes with the binding of denaturing proteins. While we did not measure the chaperone-like activity of kynurenine-modified, glycated αB-crystallin, these two modifications are likely to significantly reduce the chaperone-like activity of the protein. On the basis of data from this study, the loss of chaperone activity we observed in an earlier study (*22*) can be attributed to the structural changes at the chaperone site. Deamidation of a residue, N78, at the chaperone site has been shown to diminish the ability of the protein to suppress aggregation of denaturing proteins (*43*).

CONCLUDING REMARKS

Both subunits of the sHSP α-crystallin display chaperone-like activity. Previous studies have shown that the hydrophobic region in the α-crystallin domain of these proteins is the binding site for denaturing proteins (*22*). Site-directed mutagenesis study and the demonstration of chaperone-like activity by a 19-mer peptide, DFVIFLDVKHFSPEDLTVK (residues 71−88 of αA-crystallin), have confirmed that in αA-crystallin the beginning part of α-crystallin domain is the chaperone site (*16*, *25*). The present study documents that the corresponding sequence in αB-crystallin, DRFS-VNLDVKHFSPEELKVK, is a chaperone site as well. During a pin array study, the DRFSVNLDVK sequence (residues 73−82 in αB-crystallin) was also identified as one of the chaperone sites (*44*). In a related study, the same authors reported that residues 75−82 in αB-crystallin are involved in subunit interaction (*45*). However, the mechanism of function of the same region as chaperone site and subunit interaction site are yet to be determined. The peptide array study (*44*) also identified sequences 9−20, 43−58, 113−120, 131−138, 141−148, and 157−164 in αB-crystallin as target protein binding sites. The same study also reported that synthetic peptides 73−85 and 131−141 inhibited thermal aggregation of βH-crystallin, ADH, and CS, and sequence 73−85 showed the maximal activity. The peptides identified after pin array study were less efficient in suppressing the aggregation of ADH compared to the activity of αB-peptide 73−92 (this study) or the αA-peptide that represented the chaperone site 70−88, as reported previously (*25*). It is yet to be determined whether the peptides corresponding to the remainder of the binding sites determined by pin array studies function independently as mini-chaperones. Earlier, we reported that peptide TSLSPFYLRPPSFLRA (sequence 42−57 in αB-crystallin), identified as αA-crystallin-interacting region and containing the chaperone interactive domain reported by Ghosh et al. (*44*), has no chaperone-like activity. Likewise, several other peptides representing other regions of αB-crystallin showed no chaperone-like activity (data not shown) during in vitro aggregation assays. We hypothesize that a number of mutations affecting the chaperone-like activity of αA- or αB-crystallins impact the structure of the mutant protein in such a way that the chaperone site 70−88 in αA-crystallin or 73−92 in αB-crystallin is masked partially or fully. Further studies are required to prove this hypotheses.

Mini-αA- and mini-αB-crystallins are the shortest sequences in sHSPs capable of displaying maximal antiaggregation activity. In a previous study, we found that a 21-mer peptide (residues 40−60) of yeast ADH also shows antiaggregation activity (*46*). However, the yeast ADH peptide was

less active than mini-αA- or mini-αB-crystallin. The demonstration of diminished activity following modification of the chaperone peptide with kynurenine may explain in part the decrease in the chaperone activity of α-crystallin isolated from the aged lenses.

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