Influence of the C-Terminal Residues on Oligomerization of αA-Crystallin†

Prajitha Thampi and Edathara C. Abraham*

Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Received May 23, 2003

ABSTRACT: Earlier studies have shown that the chaperone activity of α-crystallin is significantly affected in diabetic rat and human lenses. Subsequently, mass spectrometric analysis showed diabetic lenses having high levels of the αA-crystallins in which different numbers of C-terminal residues were deleted. The present study was aimed to show whether cleavage of these residues influences protein structure, oligomerization, and chaperone function. For generation of various mutants, a stop codon was introduced at the positions of interest, proteins were expressed in BL21(DE3)pLys S *E. coli*, and the truncated αA-crystallins were purified by size-exclusion chromatography. The molecular masses, as determined by molecular sieve HPLC, of mutants with deletions of 1, 5, and 10 C-terminal residues (group-1) were 519−602 kDa, and those of mutants with deletions of 11, 16, and 22 C-terminal residues (group-2) were 148−152 kDa, as compared to 607 kDa for αA-wild type. On the basis of circular dichroism measurements, the α helix content was 2-fold higher and the tertiary structure was significantly altered in the group-2 mutants. Chaperoning abilities, as determined by the ADH assay and the βL-crystallin heat denaturation assay, of the group-1 mutants, with the exception of αA_{1-163}, were slightly improved or unchanged, that of αA_{1-163} was moderately affected, and those of the group-2 mutants were severely affected. Most strikingly, cleavage of 11 C-terminal residues including Arg-163 showed a substantial decrease in oligomeric size and chaperone function and significant changes in protein structure whereas cleavage of 10 residues had either a small effect or no effect at all. This points to an important role for the C-terminal extension, Arg-163 in particular, and no significant role for the C-terminal flexible tail in the oligomer assembly of αA-crystallin.

α-Crystallin, one of the predominant proteins of the eye lens, belongs to the family of small heat shock proteins (sHsps) (*1*) having monomeric mass between 15 and 30 kDa (*2*). In its native state α-crystallin forms large oligomeric complexes of ∼800 kDa (*3*−*5*), a property that relates to the unique functional feature that it shares with sHsps, which is its ability to function as a molecular chaperone (*6*). The two subunits of α-crystallin, αA and αB, have molecular masses of about 20 kDa each and share 57% sequence homology. The tertiary structure of αA-crystallin consists of an N-terminal region and a conserved α-crystallin domain followed by the C-terminal extension (residues 140−173) including the unstructured, flexible, polar, and solvent exposed C-terminal tails of nine amino acids (*7*−*12*). Earlier studies have established a critical role for the N-terminal region in the assembly of sHsps into functional high molecular masses (*13*−*15*). However, such a role has not been attributed to the α-crystallin domain or the C-terminal extension. Disparity in the sequences of the N-terminal region and the C-terminal extension may provide for the diversity of the sHsps. In spite of considerable variations in the sequence of the C-terminal extension of sHsps, a conserved motif "IXI/V" (IPV in the case of αA-crystallin) is present in the C-terminal extension (*12*).

Flexibility of the C-terminal tail, a feature shared by mammalian sHsps, is essential for the chaperoning and thermostability functions (reviewed in refs *16* and *17*). Incubation of rat α-crystallin with caplain II cleaving the 11−12 residues from the C-terminal end of αA-crystallin caused a loss in chaperone activity (*18*). Limited tryptic digestion of α-crystallin from fetal bovine lens too showed that the chaperone activity was affected upon removal of 16 C-terminal residues in αA-crystallin (*19*). The product of deletion of 17 C-terminal amino acids of αA-crystallin had spectroscopic properties similar to those of the wild type with marked reduction in the solubility as well as molecular chaperone activity (*20*). Insertion of a hydrophobic tryptophan residue altered the flexibility of the C-terminal extension and affected the functional and structural integrity of α-crystallin (*21*). Yet another study involving either substitution or addition of residues at the C-terminal extension of αA-crystallin, resulting in alteration of length/polarity/charge, showed that the molecular chaperoning ability was still maintained (*22*). Swapping of the C-terminal extensions of αA- and αB-crystallins lead to detectable changes in structure and function (*23*). The structural and functional properties of αA-crystallin from blind mole rat were compared to those of rat αA-wild type. Over the course of

† This work was supported by Grant EY11352 from the National Institutes of Health.

* To whom correspondence should be addressed. Phone: (501) 526-6088. Fax: (501) 686-8169. E-mail: abrahamedatharac@uams.edu.

[1] Abbreviations: CD, circular dichroism; TNS, 2-(*p*-toluidino)-naphthalene-6-sulfonic acid; ADH, alcohol dehydrogenase; HPLC, high performance liquid chromatography; EDTA, ethylenediamine tetraacetic acid; kDa, kilodalton; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; sHsp, small heat shock protein.

10.1021/bi030129w CCC: $25.00
Published on Web 09/16/2003

time, mole rat αA-crystallin differed from its wild type counterpart in having nine amino acid replacements, five in the N-termini, one in the C-terminal domain, and three in ~15 C-terminal residues of which 2 were at residues 172 and 173 (*24*). These substitutions at the C-terminal tail made it more hydrophobic, which resulted in a reduction in the chaperone activity (*24*). Truncation of 10 amino acids from the C-terminal end of αA-crystallin did not significantly effect its oligomerization (*25*).

αA- and αB-crystallins undergo varied posttranslational modifications, and one such modification is truncation of the C-terminal residues (*7*). By mass spectrometric analysis of αA-crystallin from human lenses, Miesbauer et al. (*26*) have reported cleavage of the Ser 172−Ser 173 bond. Takemoto (*27*) has identified and quantified cleavage of this peptide bond and showed that it occurs predominantly during the first 12 years of life. In a recent study done in our laboratory, both the αL and αH fractions of the water-soluble proteins from diabetic and age-matched nondiabetic human as well as rat lenses were analyzed by electrospray ionization mass spectrometry (*28*). In these analyses, the human lens αA-crystallin showed only the C-terminal Ser truncated product, αA_{1-172}, but no αB-crystallin truncated product. The level of αA-crystallin missing the C-terminal serine was elevated in diabetic human lenses. In the rat lenses, however, αA-crystallin had multiple cleavage sites that generated αA_{1-168}, αA_{1-163}, αA_{1-162}, αA_{1-157}, and αA_{1-151} whereas αB-crystallin had only one truncated product, which is αB_{1-170} (*28*). The levels of all the truncated α-crystallins were significantly increased in diabetic rat lenses. Previous studies have reported loss of chaperoning ability of the α-crystallin in lenses from streptozotocin diabetic rats and diabetic humans (*29*, *30*). This observation can be explained if indeed these truncated α-crystallins have decreased chaperone function. It is not known whether cleavage of a specific number of residues from the C-terminal extension including the C-terminal tail affects the oligomeric structure, oligomer assembly in particular, which is expected to affect the chaperone function. So, the focus of this study is on the structure, oligomerization, and chaperone function of the multiple truncated αA-crystallins that are formed in vivo.

EXPERIMENTAL PROCEDURES

Cloning, Site-Directed Mutagenesis, Expression, and Purification of Rat αA-Crystallin and the C-Terminal Truncated Mutants. Cloning of rat αA-crystallin and subsequent subcloning into the expression vector pET-23d(+) have been described previously (*31*). To generate the different C-terminal truncated αA-crystallins lacking 1 (it was included in this study because it is a major product in human lens although not in rat lens), 5, 10, 11, 16, and 22 residues, stop codons were introduced at the positions of interest using the QuickChange site-directed mutagenesis kit (Stratagene). Coding sequences for the αA-wild type (αA-wt) and its C-terminal truncated mutants were confirmed by automated DNA sequencing. Expression of the αA-wt and the truncated mutants (all of them still in the pET vector) was achieved in *E. coli* BL21(DE3) pLysS cells. The procedures followed for the expression and purification of the wild type and mutant proteins were described in an earlier communication (*31*). The expressed proteins were purified by Sephacryl S-300 HR size exclusion chromatography, the peak fractions collected were concentrated and repurified by molecular sieve HPLC using a 600 mm × 7.8 mm BIOSEP-SEC 4000 column (Phenomenex). The purity of the wild type and mutant αA-crystallins was examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (*32*).

Molecular Sieve HPLC for Determination of Molecular Masses. Oligomeric sizes of the rat αA-wt and the mutants were estimated using a 600 mm × 7.8 mm BIOSEP-SEC 4000 column (Phenomenex) with Beckman HPLC System Gold. The mobile phase consisted of 150 mM Tris, 50 mM NaCl, and 10 mM EDTA, and the flow rate was maintained at 1 mL/min. One hundred micrograms of the "double-purified" protein samples were loaded on to the column, and absorbance was measured at 280 nm. Molecular masses were obtained by calibrating the column with molecular mass standards from Sigma.

Circular Dichroism (CD) Measurements. CD spectra were measured at room temperature with a Jasco 715 spectropolarimeter. Protein concentrations of 1 and 0.1 mg/mL in 50 mM phosphate buffer (pH 7.4) were used for recording near-UV and far-UV spectra, with 1 and 0.1 cm path length quartz cells, respectively. The reported spectra are the average of five accumulations, which were smoothed and corrected for buffer blanks. Secondary structure parameters were estimated with the program PROSEC derived from Yang et al. (*33*).

Chaperone Activity Measurements. Chaperone activity was assayed as described before (*34*) by measuring the ability of the αA-wt and its mutants to prevent (a) EDTA-induced aggregation of alcohol dehydrogenase (ADH) and (b) heat-induced aggregation of βL-crystallin. The aggregation of the target proteins was monitored as light scattering at 360 nm as a function of time in a Shimadzu UV160 spectrophotometer (Columbia, MD) equipped with a temperature-regulated cell holder. ADH assay was done at 37 °C, and βL assay, at 62 °C, with the αA/target protein ratios being 1:1 and 1:5 for both the systems.

TNS Binding Studies. The surface hydrophobicity of αA-wt and the truncated mutants was studied by using the specific hydrophobic probe TNS as described before (35). The fluorescence was measured using an excitation wavelength of 320 nm and an emission range of 350−550 nm.

Determination of Protein Stability. The stability of αA-wt and the mutants was determined at 37 and 62 °C, the temperatures at which chaperone assays were done. One hundred micrograms of αA-crystallin in 50 mM phosphate buffer was incubated in the absence of target protein, and absorbance at 360 nm was monitored for 30 min.

RESULTS

Molecular Masses of Rat αA-wt and the Truncated Mutants. The oligomeric size of the rat αA-wt and the various truncated mutants was obtained by molecular sieve HPLC of purified proteins (Figure 1). High molecular mass standards were used to calibrate the column. The estimated molecular mass of rat αA-wt was 607 kDa. As for the truncated mutants αA_{1-172}, αA_{1-168}, and αA_{1-163} (group-1), the observed molecular masses were 602, 598, and 519 kDa, respectively (Figure 1 inset), with the decrease in oligomeric size being 1.0, 2.5, and 14.5% respectively. For αA_{1-162}, αA_{1-157}, and αA_{1-151} (group-2), the values were 152, 152,

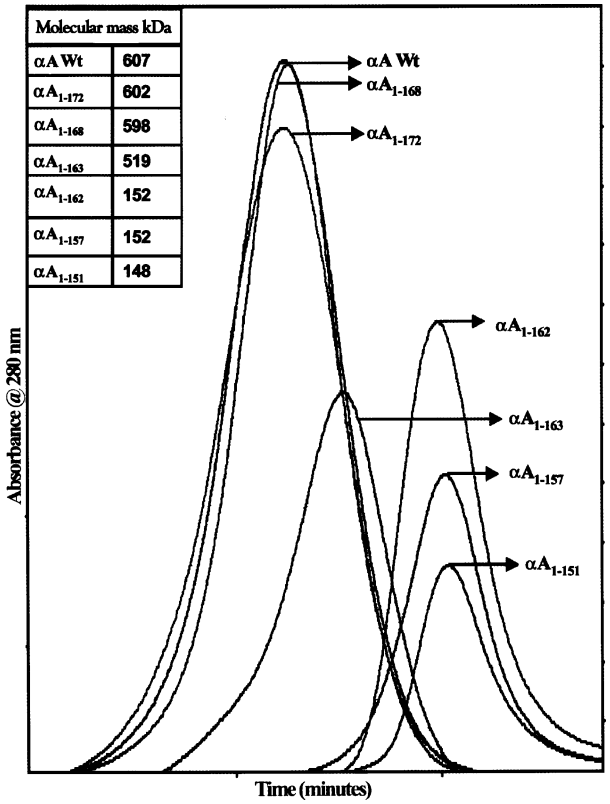

FIGURE 1: Determination of the oligomeric size of αA-wt and its truncated mutants by molecular sieve HPLC. The molecular masses, calculated from the retention times of the molecular mass protein standards and of the various samples, are given in the inset.

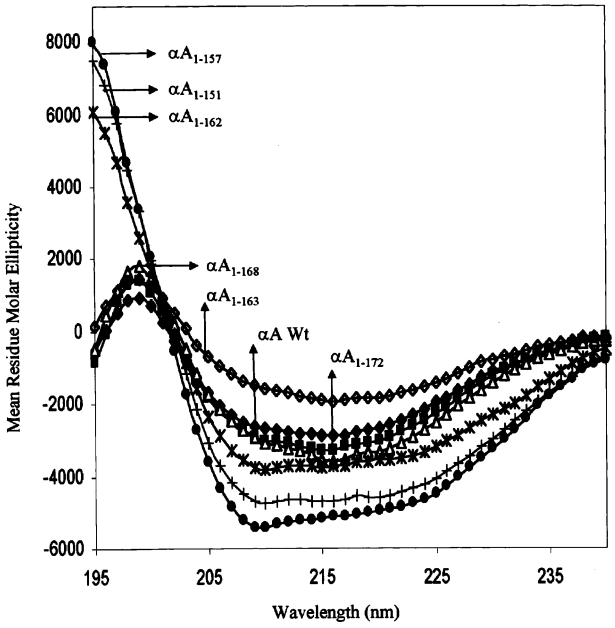

FIGURE 2: Far-UV CD spectra of αA-wt and the truncated mutants recorded at room temperature. The protein concentration was 0.1 mg/mL. (◆) αA-wt; (■) αA_{1-172}; (△) αA_{1-168}; (◇) αA_{1-163}; (∗) αA_{1-162}; (●) αA_{1-157}; (+) αA_{1-151}.

and 148 kDa, respectively (Figure 1 inset), with the decrease in oligomeric size being 75%.

Conformational Studies. The far-UV CD spectra (Figure 2) suggested that the secondary structure of the group-2 mutants is different from that of the wild type and the group-1 mutants. This was confirmed when the secondary structural

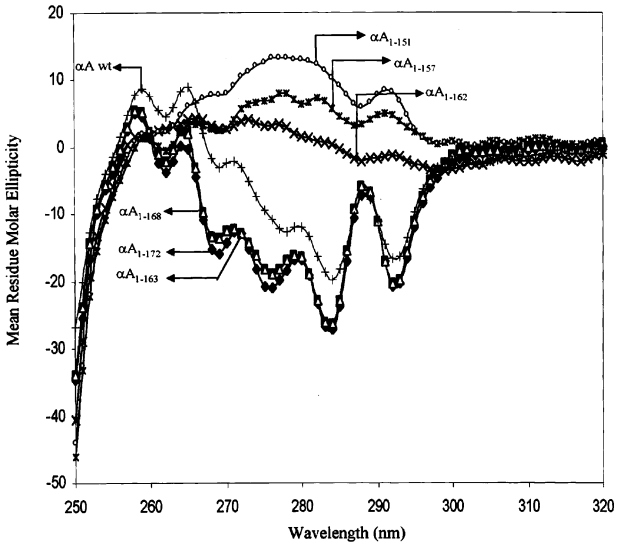

FIGURE 3: Near-UV CD spectra of αA-wt and its mutants recorded at room temperature. The protein concentration was 1 mg/mL. (+) αA-wt; (◆) αA_{1-172}; (■) αA_{1-168}; (△) αA_{1-163}; (×) αA_{1-162}; (∗) αA_{1-157}; (○) αA_{1-151}.

elements were estimated by the program PROSEC. The α helix content increased about 2-fold from 4% in the wild type to 7−11% in the group-2 mutants at the expense of the β turn content, which decreased from 14% to about 11% (data not given). The β sheet content, on the other hand, remained nearly the same between 60 and 64%. The CD spectrum of a protein in the near-UV region indicates changes in the tertiary structure reflective of the local environment of the aromatic residues Trp, Tyr, and Phe. As expected from earlier studies (*31*, *34*−*36*), the near-UV CD spectrum of αA-wt exhibited five distinct wavelength maxima and five distinct wavelength minima (Figure 3). The three mutants in group-1 also showed these wavelength maxima and minima, indicating tertiary structure similarity to that of the wild type. The vibronic signals at 292 and 284 nm minima are due to Trp residues. The maxima at 258 and 264 nm are due to Phe fine structure. The remaining transitions between 271 and 288 nm arise from Tyr and/or Trp. The mutants in group-2, however, lack distinct wavelength maxima and minima and have significantly positive ellipticity, which indicate altered tertiary structure in these mutants. Figure 4 shows the fluorescence spectra of TNS bound to αA-wt and the different truncated mutants. αA-wt and the group-1 mutants had the highest level of fluorescence intensities whereas the group-2 mutants showed significantly decreased fluorescence intensities. The data suggest the following order of the relative availability of surface hydrophobic sites: αA_{1-168} > αA-wt = αA_{1-172} > αA_{1-163} > αA_{1-151} > αA_{1-157} > αA_{1-162}. It is unclear why αA_{1-168} had about 30% higher fluorescence intensity than αA-wt or the other group-1 mutants.

Chaperone Activity of aA-wt and the Truncated Mutants. The chaperone activity assays were done by monitoring the ability of αA-crystallin and the truncated mutants to prevent the denaturation and aggregation of target proteins, ADH at 37 °C and βL-crystallin at 62 °C at two different ratios (1:1 and 1:5) of αA/target protein. At a 1:1 ratio, the αA-crystallin and the mutants in group-1 were able to completely suppress the aggregation of ADH at 37 °C, with the group-1 mutants being slightly better chaperones than αA-wt (Figure 5A).

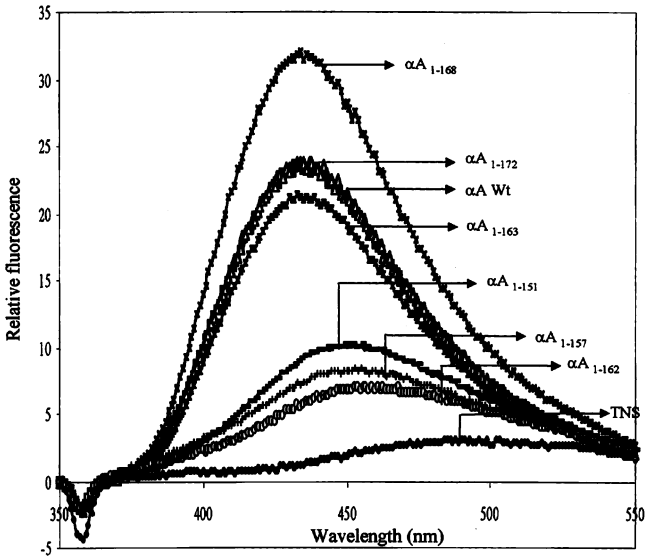

FIGURE 4: Fluorescence emission spectra of the TNS-bound form of αA-wt and the mutants recorded using an excitation wavelength of 320 nm and an emission range of 350−550 nm. The protein concentration was 0.1 mg/mL. (△) αA-wt; (■) αA_{1-172}; (×) αA_{1-168}; (∗) αA_{1-163}; (○) αA_{1-162}; (+) αA_{1-157}; (−) αA_{1-151}; (◇) TNS.

Group-2 mutants were only slightly less effective. At a 1:5 ratio, αA_{1-172} and αA_{1-168} still showed slightly higher chaperone activity and αA_{1-163} had slightly lower chaperone activity than αA-wt (Figure 5B). The three group-2 mutants, on the other hand, showed nearly 50−60% loss in chaperone activity (taking the absorbance reading at 1800 s of αA + ADH as 100% protection and the reading of ADH alone as 0%). The *β*L-crystallin assay at a 1:1 ratio showed that αA-wt, αA_{1-172}, and αA_{1-168} completely suppressed *β*L aggregation whereas αA_{1-163} showed about a 20% decrease in chaperone activity and αA_{1-162}, αA_{1-157}, and αA_{1-151} lacked any chaperone activity (Figure 6A). At a 1:5 ratio, αA-wt, αA_{1-172}, and αA_{1-168} became less effective, αA_{1-163} lost 90% activity, and αA_{1-162}, αA_{1-157}, and αA_{1-151} had no chaperone activity (Figure 6B).

Protein Stability of Truncated αA-Crystallins. The stability of all the mutants was determined by measuring light scattering of the proteins at 37 and 62 °C, the temperatures used in chaperone assays. At 37 °C, αA-wt and the mutants failed to show any light scattering or aggregation (data not given). At 62 °C, αA_{1-157} and αA_{1-151} showed some aggregation and increase in absorbance at 360 nm. However, the absorbance reached only about 0.015 absorbance units by 30 min, which is extremely small and thus did not influence the *β*L-crystallin thermal denaturation assay.

DISCUSSION

While the importance of an intact N-terminal domain is well established, the actual contribution of the C-terminal extension in the high-molecular-mass complex formation in sHsps, α-crystallin in particular, is still unknown. In this report, we present evidence in support of a dominant role for the C-terminal extension in the formation of multimeric αA-crystallin. We have cloned, expressed, and characterized rat αA-wt and five C-terminal truncated mutants of different lengths—αA_{1-168}, αA_{1-163}, αA_{1-162}, αA_{1-157}, and αA_{1-151}—which exist in rat lenses and an additional mutant—αA_{1-172}—

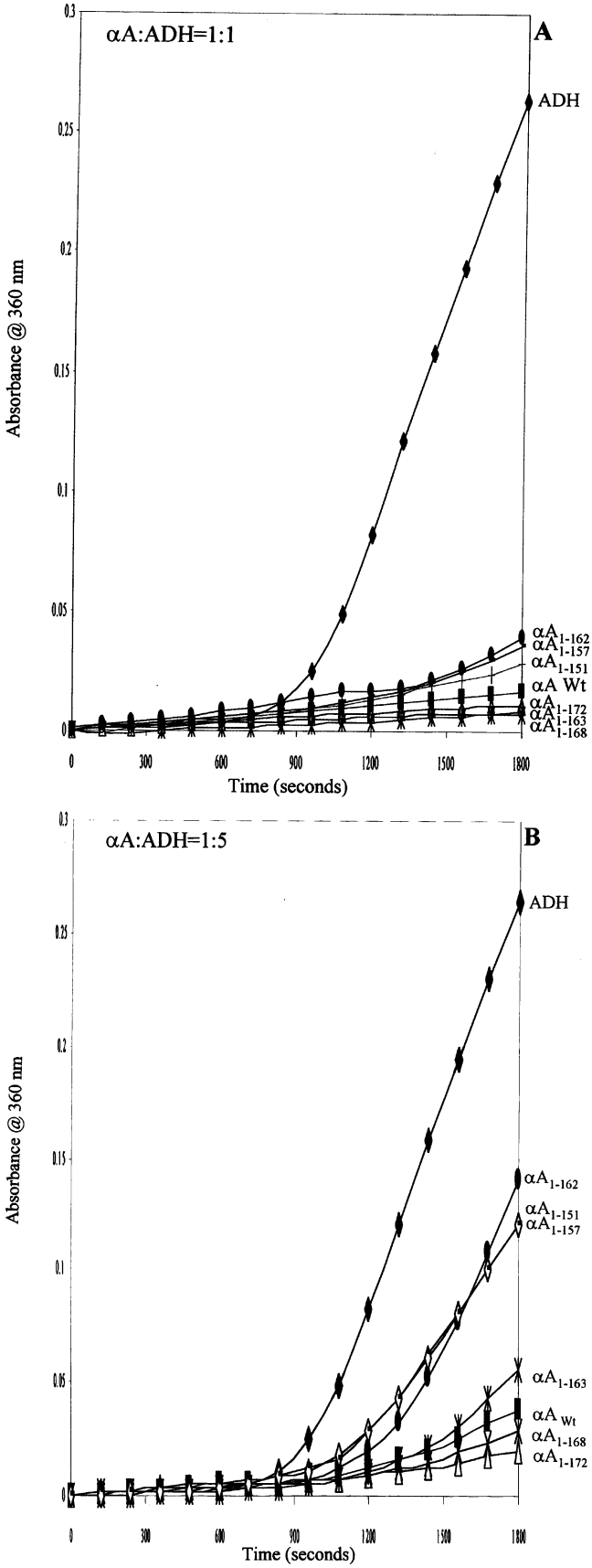

FIGURE 5: Chaperone activity of αA-wt and the mutants using ADH as the target protein at 37 °C, with the αA/ADH ratios being 1:1 [400 *μ*g of αA + 400 *μ*g of ADH in 1 mL (A): ■, αA-wt; △, αA_{1-172}; ∗, αA_{1-168}; ○, αA_{1-163}; ●, αA_{1-162}; ■, αA_{1-157}; +, αA_{1-151}; ◆, ADH] and 1:5 [80 *μ*g of αA + 400 *μ*g of ADH in 1 mL (B): ■, αA-wt; △, αA_{1-172}; ×, αA_{1-168}; ∗, αA_{1-163}; ●, αA_{1-162}; ■, αA_{1-157}; ◇, αA_{1-151}; ◆, ADH].

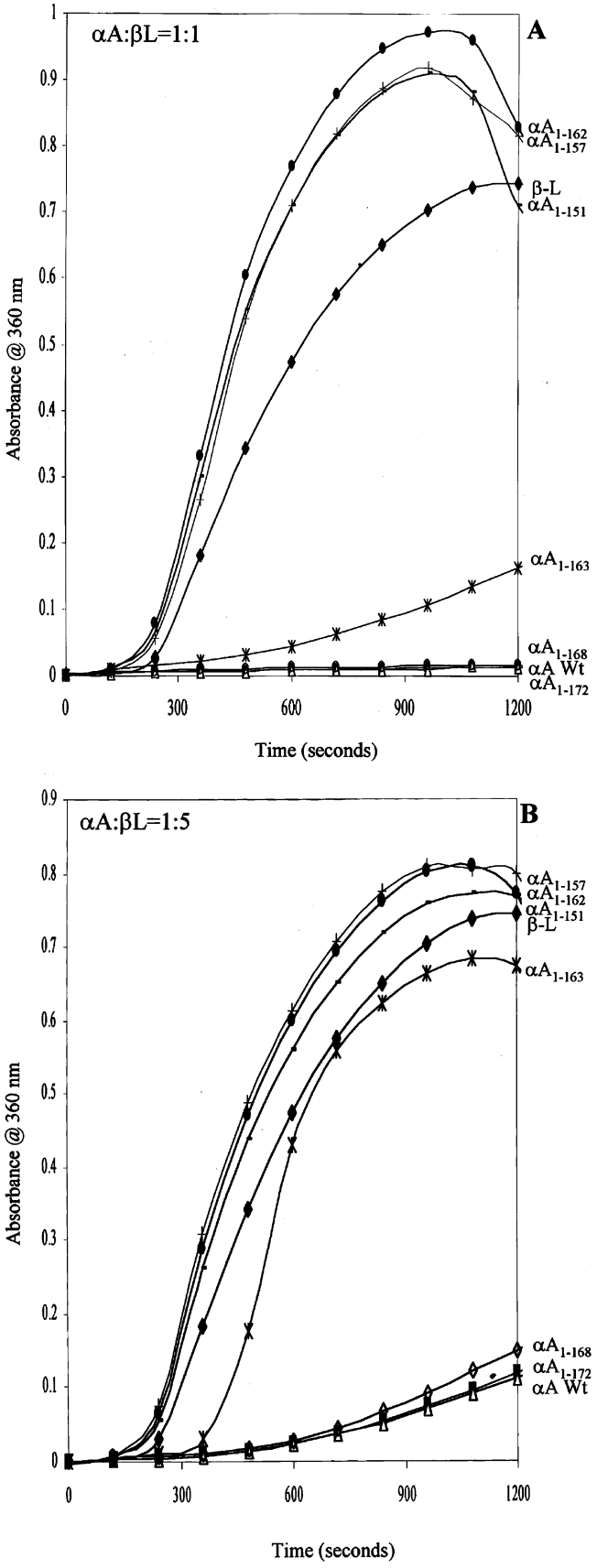

FIGURE 6: Chaperone activity of αA-wt and the mutants using βL-crystallin as the target protein at 62 °C, with the αA:βL ratios being 1:1 [250 μg of αA + 250 μg of βL in 1 mL (A): △, αA-wt; ■, αA_{1-172}; ○, αA_{1-168}; ∗, αA_{1-163}; ●, αA_{1-162}; +, αA_{1-157}; −, αA_{1-151}; ◆, βL] and 1:5 (50 μg of αA + 250 μg of βL in 1 mL (B): △, αA-wt; ■, αA_{1-172}; ◇, αA_{1-168}; ∗, αA_{1-163}; ●, αA_{1-162}; +, αA_{1-15}; −, αA_{1-151}; ◆, βL].

which is present only in human lenses. The data show that the cleavage of 1, 5, or 10 residues, which include the flexible C-terminal tail, did not significantly affect the oligomeric size, secondary and tertiary structures, and chaperone function whereas cleavage of the last 11, 16, or 22 residues resulted in substantial decrease in oligomeric size with concomitant changes in protein structure and loss in chaperone activity. The data show that deletion of 11 or more C-terminal residues results in improper folding of αA-crystallin. However, this may not be representative of a protein that is folded first and then cleaved in vivo. The most noteworthy finding in this study is the dramatic change in the oligomeric structure of αA_{1-162} as compared to αA_{1-163}, which suggests an important role for Arg-163 in αA-crystallin oligomerization. Moreover, the present study confirms that the decreased chaperone activity of α-crystallin from diabetic rat lenses (*29*, *30*) is, at least in part, due to increased presence of C-terminal truncated α-crystallins (*28*) having decreased chaperone activity.

The α-crystallin domain and the N-terminal region are believed to be controlling oligomerization by providing extensive subunit−subunit contacts (*16*, *17*). However, a genetically engineered human αB-crystallin comprising only the α-crystallin domain exists as a dimer, suggesting that this domain alone is not sufficient for the formation of higher-order structures (*37*). Hsp 16.2, an α-crystallin member of *C. elegans* has been used as a model protein for studies of multimerization (*14*, *15*). The α-crystallin domain of this heat shock protein is flanked by a N-terminal region of 41 residues and a C-terminal extension of 22 residues. N-Terminally truncated protein lacking 15, 32, or 44 amino acids failed to form native complexes but existed as trimeric and tetrameric species with no chaperone activity (*14*, *15*). Human αA-crystallin lacking the first 20 amino acids retained its high-molecular-mass structure whereas truncation of 56 residues reduced the complex to trimers and tetramers (*25*). However, the N-termini of certain α-sHsps are insensitive to sequence extensions in response to multimerization (*15*, *38*). For instance, adding a tail of 4 kDa to the N-terminus of *C. elegans* Hsp 16.2 did not disturb its multimerization (*15*). Likewise, after a 42 kDa maltose-binding protein was added to the N-terminus of αB-crystallin, the fusion protein still associated into high-molecular-mass oligomers that displayed full chaperone activity (*38*).

The role of the C-terminal extension in the oligomerization of α-sHsps has not been delineated. Complete removal of the C-terminal extension of *C. elegans* Hsp 16.2 had little effect on oligomerization (*15*). However, it is noteworthy that all the complexes formed from this mutant readily precipitated, which suggests that one function of the C-terminal extension is to solubilize α-Hsp complexes (*15*). Mouse Hsp25 without its C-terminal extension also showed a normal oligomeric size (*39*). There are other instances where the oligomer status of α-Hsps is sensitive to alterations in their C-terminal extensions. Pea Hsp17.7 variants lacking 3, 5, or 10 amino acids of the C-terminus were unable to form full-size oligomers (*40*). C-Terminally truncated *B. japonicum* α-Hsps, Hsp B and Hsp C, lacking the conserved I−X−I motif formed only small complexes (*16*). Deletion of 25 amino acids from the carboxyl end of Xenopus Hsp30C resulted in reduced solubility and impaired chaperone activity (*41*). Recombinant human αA-crystallin without 17 C-

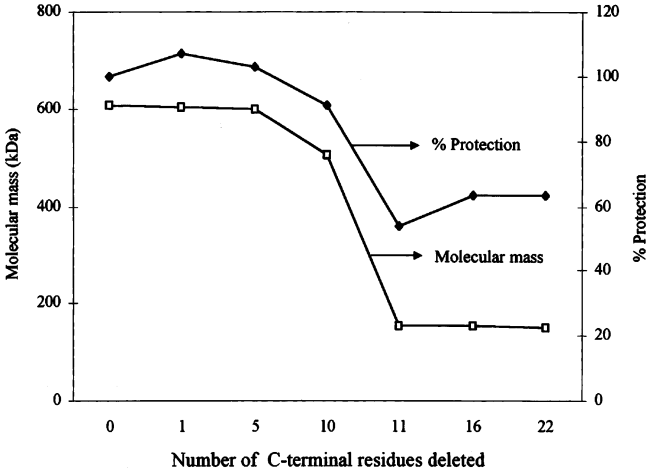

FIGURE 7: Relationship between oligomeric size and chaperone activity. Molecular mass values were taken from Figure 1, and chaperone activities expressed as percentage protection of ADH denaturation were taken from Figure 5B.

terminal amino acid residues showed significantly decreased solubility and chaperone activity (*20*). However, this mutant did not show any change in oligomeric size probably because it was purified from urea-solubilized proteins of the insoluble pellet of the *E. coli* cell lysate. In our study, αA_{1-162}, αA_{1-157}, and αA_{1-151} behaved similarly and a major portion of these mutants appeared in the insoluble pellet. However, no attempt was made to purify them from the insoluble fraction because sufficient amounts were present in the soluble form. Interestingly, these mutants had significantly decreased oligomeric size (Figures 1 and 7). The solubility of the group-1 mutants was not significantly affected, and their oligomeric size was only slightly decreased. Two of these mutants where one residue or five residues were deleted from the C-terminal tail showed 1.0 and 2.5% decreases, respectively, in the oligomeric size (Figures 1 and 7). These data confirm that the C-terminal tail does not play a role in αA-crystallin oligomerization. Earlier studies have shown that point mutations or amino acid insertions within the last four residues of bovine αA-crystallin had little effect on its molecular mass (*21*). The most noteworthy finding with respect to αA-crystallin oligomerization and the C-terminal extension is that the cleavage of the last 10 amino acids had only a small effect on the oligomer size whereas cleavage of 11 residues including Arg-163 resulted in an αA-crystallin showing an about 75% decrease in oligomeric size (Figures 1 and 7). This points to a critical role for Arg-163 in the oligomer assembly. We hypothesize that charge−charge interaction involving Arg-163 may be a critical factor in the formation of high-molecular-mass αA-crystallin. Moreover, Arg-163 is situated at the interface of the flexible tail and the nonflexible C-terminal extension and thus may play an additional role in enhancing inter- or intrasubunit interactions. A combination of hydrophobic interactions, provided mostly by the N-terminal residues, and ionic interactions, provided mostly by the C-terminal residues, is required for protein stability and oligomeric complex formation. Mutants αA_{1-162}, αA_{1-157}, and αA_{1-151} have significantly altered tertiary structure (Figure 3). The αA_{1-163} still retained its tertiary structure, which could also suggest that the charged Arg-163 participates in tertiary interactions, thereby helping oligomerization.

Chaperone activities of αA-wt and the six truncated mutants were determined at physiological temperature using ADH as the target protein and at 62 °C using βL-crystallin as the target protein at two different αA/target protein ratios (1:1 and 1:5). Chaperone assays generally give relative chaperone activity values which are influenced by temperature, by type of target protein, by method of target protein denaturation, and mostly by chaperone/target protein ratios. We have presented four sets of data (Figures 5 and 6), and the common finding is that αA-wt and the group-1 mutants, except αA_{1-163}, have similar chaperone activities, group-2 mutants have the lowest chaperone activity, and the chaperone activity of αA_{1-163} was generally in the middle. The ADH assay showed a maximum of 60% loss of activity in the group-2 mutants whereas the βL-crystallin assay showed no chaperone activity. In fact, in the case of the βL-crystallin assay, the absorbance of the group-2 mutants−βL complex was higher than that of the βL alone. Binding of unfolded target protein to αA-crystallin causes an increase in surface hydrophobicity of the complex, which in the case of αA-wt is compensated by the hydration effect of the polar C-terminal extension (*22*). In the case of the mutants lacking 10, 11, 16, and 22 C-terminal residues, the truncated polar extension in a gradation fails to compensate for the increased surface hydrophobicity and hence causes the target protein−αA-crystallin complex to become increasingly insoluble. It should be pointed out that cleavage of one or five residues from the C-terminus of αA-crystallin slightly improves chaperone activity (according to the ADH assay) (Figure 5). A similar observation has been made recently with αB-crystallin devoid of the last five residues (*42*, *43*). When the chaperone activities (derived from ADH assays at a 1:5 ratio; from Figure 5B) were compared with the molecular masses of the various mutants (from Figure 1), a strong relationship between chaperone activities and molecular masses emerged (Figure 7). The mutants with the lowest molecular masses (~150 kDa) had their chaperone activities mostly affected. The same mutants also had their secondary and tertiary structures significantly altered (Figures 2 and 3) and the surface hydrophobicity substantially decreased (Figure 4). Decreased surface hydrophobicity is expected to affect chaperone activity because chaperone to target protein binding involves hydrophobic interactions.

We have reported earlier that the levels of all the truncated αA-crystallins (with the exception of αA_{1-172}, which is present only in human lenses) used in this study are increased in diabetic rat lenses (*28*). αA_{1-162}, a m-calpain proteolysis product, is particularly interesting because this mutant appeared only in diabetic and not in normal rat lenses (*28*). From the present study, it can be concluded that the chaperone activity of α-crystallin from diabetic rat lenses is significantly affected (*29*, *30*) primarily due to increased levels of truncated mutants αA_{1-162}, αA_{1-157}, and αA_{1-151}. During an earlier study, Kelley et al (*18*) have shown that proteolysis of α-crystallin with m-calpain results in decreased chaperone activity. Interestingly, αA_{1-162} is the m-calpain proteolytic product and the observation of these authors can be explained by the formation of this truncated mutant whose chaperone activity is significantly decreased (Figures 5 and 6). αA_{1-172} is the major truncated α-crystallin present in human lenses (*27*, *28*). A recent study in our laboratory has shown this mutant having about 30% loss of chaperone

activity (*44*) whereas the rat αA_{1-172}, in this study, has shown either a slight improvement or no change in chaperone activity (Figures 5 and 6); the reason for this disparity is unclear at this time. The in vivo formed αA_{1-172} in human lens and all the truncated αA- and αB-crystallins in rat lens are concentrated mostly in the αH fraction as opposed to the αL fraction (*28*). The presence of these truncated α-crystallins with decreased chaperone activity seems to be the major cause of the reduced chaperone activity of the αH fraction seen in an earlier report (*29*).

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BI030129W