Role of Arginine-163 and the ^{163}REEK^{166} Motif in the Oligomerization of Truncated αA-Crystallins†

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ABSTRACT: To gain insight into the mechanism by which Arg-163 influences oligomerization of αA-crystallin, we prepared a series of truncated αA-crystallins with or without mutation of the Arg-163 residue. Expression of the proteins was achieved in *Escherichia coli* BL21 (DE3) pLysS cells, and αA-crystallin was purified by size-exclusion chromatography. Molecular mass was determined by molecular sieve HPLC, chaperone activity was assayed with alcohol dehydrogenase as the target protein, and structural changes were ascertained by circular dichroism (CD) measurements. With an increasing number of residues deleted, there was about a 3% decrease in oligomeric size per residue, until 10 residues were deleted. When 11 residues, including Arg-163, were deleted, the oligomeric size decreased 85%. Mutation of Arg-163 to Gly (R163G) did not affect the molecular mass in the full-length αA-crystallin. However, R163G mutants of all the truncated αA-crystallins showed a decrease in oligomeric size, those lacking 8, 9, and 10 residues showing 60−80% decrease and those lacking 5, 6, and 7 residues showing only a 7−14% decrease as compared to the corresponding truncated αA-crystallin. These data suggest that R163, E164, E165, and K166 in the REEK motif are also relevant to αA-crystallin oligomerization. The molecular masses of αA_{1-163} and αA_{1-163} (R163K) were nearly the same, which suggests that the role of Arg-163 is to provide a positive charge for intersubunit electrostatic interactions in the C-terminal domain. In αA_{1-162} (S162R), recovery of the molecular mass to the level in αA_{1-163} has not occurred; this shows that the actual position of R163 is important.

αA-Crystallin is a predominant protein of the eye lens. The two subunits of α-crystallin, αA and αB, have molecular masses of about 20 kDa, share 57% sequence homology, and both belong to the family of small heat shock proteins (sHsp's)[1] having a monomeric mass between 15 and 30 kDa (*1*, *2*). In its native state, α-crystallin exists as large hetero-oligomeric complexes having an average molecular mass of 360−800 kDa (*3*−*5*) and having molecular chaperone function (*6*). The tertiary structure of αA- and αB-crystallin subunits consists of an N-terminal region and a conserved α-crystallin domain followed by the C-terminal extension of 140−173 residues including 8−10 amino acids that form an unstructured flexible tail at the C-terminus (*7*−*12*) (Figure 1). The α-crystallin domain and the N-terminal region are believed to control oligomerization; however, studies support the concept that the α-crystallin domain alone is not sufficient for the formation of higher order structure (*7*). The role of the C-terminal extension, the flexible tail in particular, has not been fully delineated. By mass spectrometric analysis of the soluble form of αA-crystallin from rat eye lenses, we have shown the presence of several truncated αA-crystallins with varying numbers of C-terminal residues cleaved (*13*). These were identified as αA_{1-168}, αA_{1-163}, αA_{1-162}, αA_{1-157}, and αA_{1-151}. Interestingly, the levels of these truncated mutants are significantly increased in diabetic lenses, and αA_{1-162} is present only in diabetic lenses (*13*).

Our recent studies provide clues about a possible role for the C-terminal extension, the Arg-163 residue in particular, and the C-terminal flexible tail in the oligomerization of αA-crystallin (*14*). Deletion of 10 C-terminal residues, forming αA_{1-163}, results in an oligomeric size of 520 kDa, whereas deletion of 11 residues (the 11th residue from the C-terminus is Arg-163), forming αA_{1-162}, produces a mutant with an oligomeric size of 150 kDa as compared to 600 kDa for intact αA-crystallin (*14*). On the basis of these findings, we have speculated that Arg-163 may play a role in forming high molecular mass aggregates of αA-crystallin. However, the mechanism by which Arg-163 can influence αA-crystallin oligomerization is unknown. Moreover, it is unclear whether Arg-163 exerts its effect on the full-length native αA-crystallin or only when a certain number of C-terminal residues are deleted. It is also not known whether other neighboring charged residues like Glu-164, Glu-165, and Lys-166 play a role in αA-crystallin oligomerization.

EXPERIMENTAL PROCEDURES

Cloning, Ttruncation, Site-Directed Mutagenesis, Expression, and Purification of αA-Crystallin and Its Mutants.

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[1] Abbreviations: kDa, kilodalton; CD, circular dichroism; HPLC, high performance liquid chromatography; ADH, alcohol dehydrogenase; EDTA, ethylenediamine tetra acetic acid; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; sHsp, small heat shock protein.

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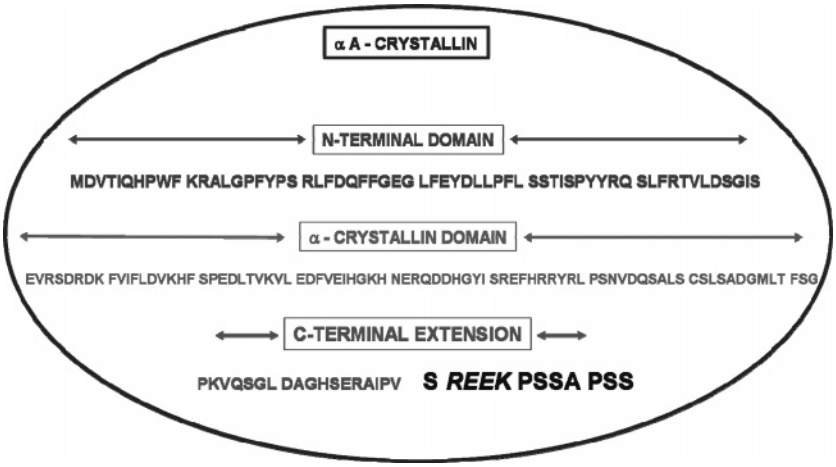

FIGURE 1: Amino acid sequence of rat αA-crystallin. N-terminal domain, α-crystallin domain, and the C-terminal extension including the REEK motif are highlighted.

Cloning of rat αA-crystallin and subsequent subcloning into the expression vector pET-23d(+) have been described previously (*15*). The QuickChange site-directed mutagenesis kit (Stratagene) was used to generate truncated αA-crystallins lacking 5 (αA_{1-168}), 6 (αA_{1-167}), 7 (αA_{1-166}), 8 (αA_{1-165}), 9 (αA_{1-164}), 10 (αA_{1-163}), and 11(αA_{1-162}) C-terminal residues by introducing stop codons at the positions of interest. The same kit was also used to generate single site-directed mutants R163G, R163K, and S162R. R163G was introduced in the full-length as well as in all the truncated αA-crystallins containing the Arg-163 residue, R163K only in αA_{1-163}, and S162R only in αA_{1-162}. Coding sequences of the truncated and single site-directed mutants were confirmed by automated DNA sequencing. Expression of the proteins was achieved in *Escherichia coli* BL21 (DE3) pLysS cells. The procedure followed for the expression and purification of the wild-type and the mutant proteins was described in an earlier communication (*15*). The expressed proteins were subjected to chromatography on Sephacryl S-300 HR size exclusion columns and repurified by molecular sieve HPLC. The purity of the various αA-crystallin preparations was examined by SDS−PAGE according to Laemmli (*16*).

Conformational Studies by Circular Dichroism (CD) Measurements. To assess the structural changes due to truncation and/or mutagenesis, CD spectra were measured at room temperature with a Jasco 715 spectropolarimeter. Protein solutions in 50 mM phosphate buffer, pH 7.4 at concentrations of 1.0 and 0.1 mg/mL were used for recording near-UV and far-UV spectra, with 1.0 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. The program PROSEC derived from Yang et al. (*17*) was used for estimation of secondary structure parameters.

Chaperone Activity Measurements. For chaperone activity assay, alcohol dehydrogenase (ADH) was used as the target protein at two different proportions (1:1 and 1: 5) of αA-crystallin to ADH. EDTA induced aggregation of ADH was monitored as light-scattering at 360 nm for 30 min at 37 °C in a Shimadzu UV 160 spectrophotometer (*18*).

Determination of Molecular Masses. Molecular masses of the αA-wt and the mutants were estimated using a 600 × 7.8 mm BIOSEP-SEC 4000 column (Phenomenex) with Beckman HPLC system Gold. The mobile phase was 150 mM TRIS, 50 mM NaCl, and 10 mM EDTA, and the flow rate was 0.5 mL/min. One hundred micrograms of the purified protein samples were loaded onto the column, and absorbance was monitored at 280 nm. Molecular masses were calculated from molecular mass versus retention time calibration curve generated with molecular mass standards from Sigma.

RESULTS

Secondary Structure of Truncated αA-Crystallins with and without Arg-163 Mutation. To study the effect of truncating different number of C-terminal residues and the combined effect of truncation and mutation of Arg-163 on the secondary structure of αA-crystallin, far-UV spectra were measured. Figure 2 A,B gives the far-UV spectra of the various truncated proteins with and without R163 mutation. It appears from the spectra that the secondary structure varies in different mutants. Comparison of the spectra of the various truncated mutants with those of the corresponding R163G mutants shows significant differences. For instance, the spectra of αA_{1-173} (R163G), αA_{1-168} (R163G), and αA_{1-165} (R163G) are significantly altered from those of αA-wt, αA_{1-168}, and αA_{1-165}, respectively (Figure 2A). Other pairs (e.g., αA_{1-163} (R163G) and αA_{1-163}; αA_{1-164} (R163G) and αA_{1-164}) of truncated vs truncated/ R163G mutants (Figure 2B) also show additional effect on secondary structure due to R163G mutation. Calculation of the secondary structure elements shows specific changes in the content of α-helix, β-sheet, and β-turn (data not given). The spectrum of αA_{1-165} (R163G) (Figure 2A) suggests a high level of α-helix, and the actual level is 12% as compared to 4% for αA_{1-165}. Likewise, αA_{1-163} (R163G) (Figure 2B) has an α-helix content of 11% as compared to 5% for αA_{1-163}. As shown in an earlier study (*14*), removal of R163 (αA_{1-162} in Figure 2B) results in relatively high level of α-helix. There is a steady increase in the β-sheet content (from 39% in αA-wt to 66% in αA_{1-163}) with increasing number of C-terminal residues deleted, R163G mutation showing an additional increase (an average of 4 percentage points higher) in the level of β-sheet. The β-turn content, at the same time, shows a concomitant decrease. Thus, it is apparent that the secondary structure of the truncated αA-crystallins is different from that of αA-wt, while the truncated/R163G mutants have additional secondary structural changes.

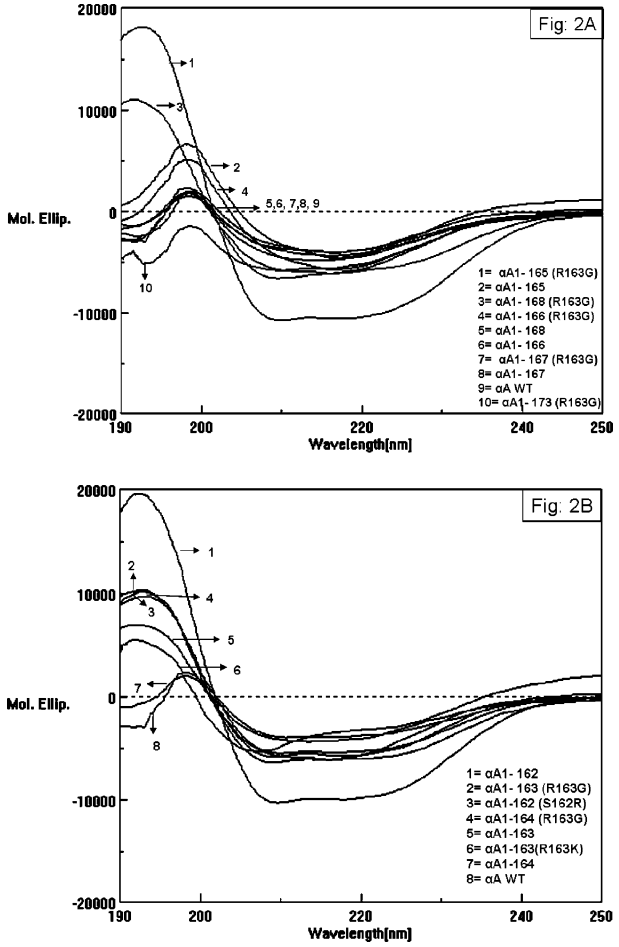

FIGURE 2: (A, B) Far-UV spectra of αA-wt and truncated αA-crystallins with and without R163G mutation, R163K mutation only in αA_{1-163}, and S162R mutation only in αA_{1-162}. Protein solutions of 0.1 mg/mL were prepared in 50 mM phosphate buffer, pH 7.4. A cylindrical quartz cell with a path length of 1.0 mm was used. Five scans were made and averaged for each sample. Identification of each curve is provided in the inset.

Tertiary Structure of Truncated αA-Crystallins with and without Arg-163 Mutation. 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 (*14*, *15*, *18*), the near-UV CD spectrum of αA-wt exhibits five distinct wavelength maxima and five distinct wavelength minima (Figure 3A,B). In the truncated αA_{1-168}, αA_{1-167}, αA_{1-166}, and αA_{1-165} with and without additional mutations at Arg-163, the far-UV spectra are similar to that of the αA-wt having all the spectral characteristics except that the molar ellipticity values are more positive (Figure 3A). As the number of C-terminal residues cleaved increases, molar ellipticity becomes increasingly positive indicating tertiary structural changes due to truncation. In every instance, R163G mutation results in a further increase in positive molar ellipticity suggesting additional changes in tertiary structure. In αA_{1-164} and αA_{1-163}, obvious spectral changes are seen due to truncation as well as due to R163G mutation. For instance, αA_{1-164} shows partial loss in the Trp, Tyr, and Phe signals (Figure 3B). The R163G mutant of this truncated αA-crystallin, on the other hand, lacks all the spectral characteristics due to Trp, Tyr, and Phe, which suggests substantial alterations in the tertiary structure. The spectra

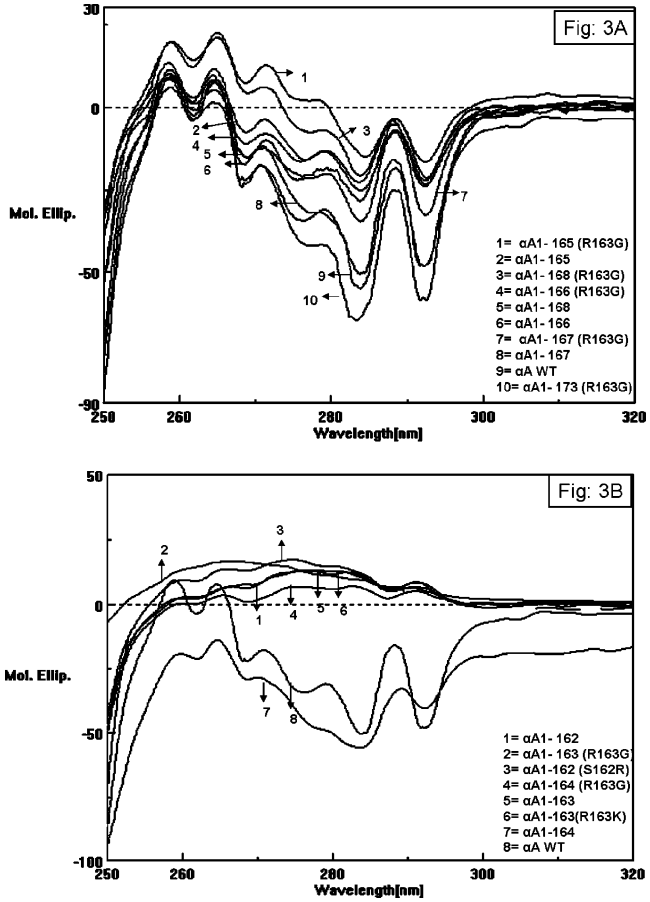

FIGURE 3: (A, B) Near-UV CD spectra of αA-wt and truncated αA-crystallins with and without R163G, R163K, and S162R mutations. Protein solutions of 1.0 mg/mL were prepared in 50 mM phosphate buffer, pH 7.4. A cylindrical quartz cell with a path length of 1.0 cm was used. Five scans were made and averaged for each sample. Identification of each curve is provided in the inset.

of αA_{1-162} and αA_{1-162} (S162R), and αA_{1-163} (R163K) lack distinct wavelength maximas and minimas, which suggest that they all have substantially modified tertiary structure.

Molecular Masses of Truncated αA-Crystallins With and Without Additional Mutations at Positions 163 and 162. From earlier studies, we have predicted that with increasing loss of C-terminal residues molecular mass of αA-crytallin is expected to decrease. However, the combined effect of C-terminal truncation and R163 mutation was not predictable. To investigate this, molecular masses of αA-wt, C-terminal truncated mutants and truncated cum R163 mutated αA-crystallins were determined by molecular sieve HPLC after performing column calibration with high molecular mass standards. Figure 4 A,B provides the chromatograms of αA-wt and all the truncated mutants with and without additional mutation of R163 or S162. With some exceptions (αA_{1-168} and αA_{1-166} (R163G) in Figure 4A; αA_{1-164} in Figure 4B) all the peaks are symmetric. So, the molecular masses determined on the basis of peak heights are expected to provide reliable values. Figure 5 summarizes the molecular mass values, arranged in the order of increasing number of C-terminal residues deleted. In all the truncated mutants, without R163G mutation, there is gradual decrease in molecular masses, which averages about 3% per residue, with increasing number of residues deleted, until 10 residues are

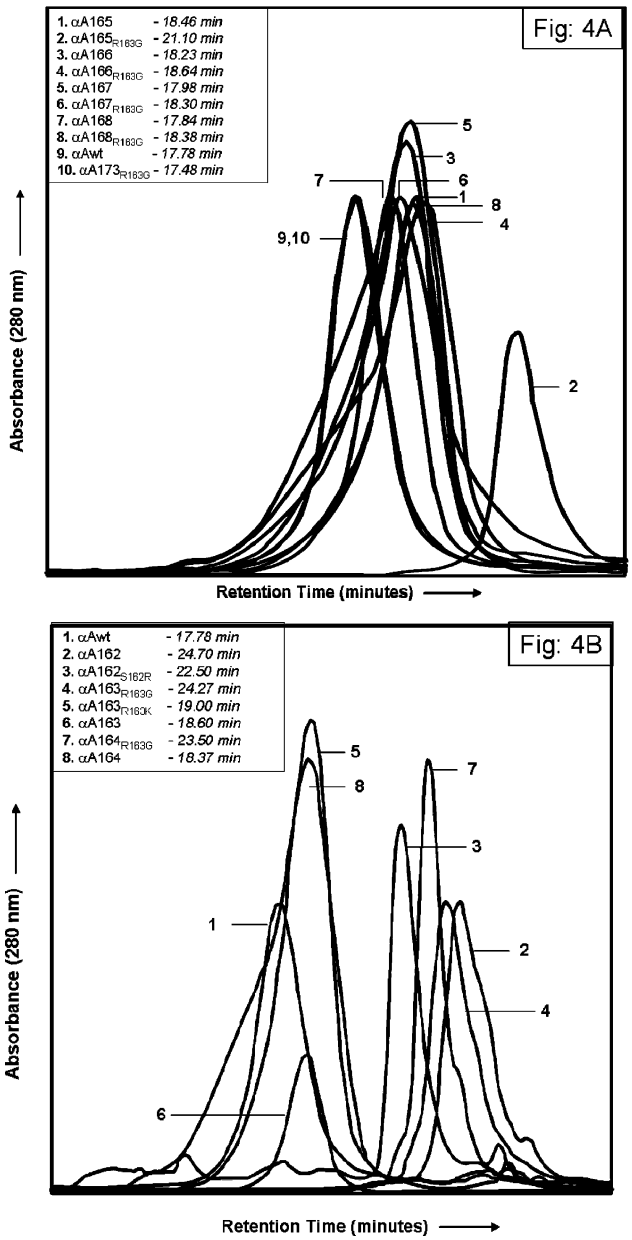

FIGURE 4: (A, B) Molecular sieve HPLC separations of αA-wt and the various C-terminal truncated αA-crystallins with and without additional mutations at positions 163 or 162. One hundred micrograms of the purified protein samples were loaded on to a BIOSEP-SEC 4000 HPLC column, and absorbance was monitored at 280 nm. Retention times in minutes are provided in the inset.

deleted. However, when 11 residues are deleted, the oligomeric size decreases by 85% to 110 kDa. The most significant finding of the present study is the varying degree of influence the R163G mutation has on the oligomeric size of the truncated αA-crystallins. The R163G mutation in αA-wt actually shows an unexpected small increase in the molecular mass whereas in αA_{1-168}, αA_{1-167}, and αA_{1-166} the effect is 15, 7, and 9% decrease, respectively. On the other hand, the decreases in molecular masses due to R163G mutation are 60, 79, and 80%, respectively, in αA_{1-165}, αA_{1-164}, and αA_{1-163}. Thus, the influence of R163G mutation on the oligomerization of αA-crystallin is dictated by the number of C-terminal residues deleted. To show whether the role of R163 is to provide a positive charge for interpolypeptide electrostatic interactions, R163 was mutated to a Lys in αA_{1-163}. The molecular mass of αA_{1-163} (R163K) (560 kDa) is nearly the same as that of αA_{1-163} (600 kDa) having Arg in the 163 position. In addition, the C-terminal serine in αA_{1-162} was mutated to an Arg. The purpose was to show whether an Arg at the 162 position will have the same effect as the one at the 163 position. Because of Arg at the 162 position, the molecular mass is increased from 110 to 180 kDa (60% increase), but full recovery of the oligomeric size to the level in αA_{1-163} (Arg in the 163 position) is not achieved. This suggests that the position of Arg is important to have a full impact on oligomerization.

Chaperone Activities of Truncated αA-Crystallins With and Without Additional Mutations at 163 and 162 Positions. To show whether changes in the oligomeric structure result in concomitant changes in chaperone function, chaperone activities were determined with ADH as the target protein at two different proportions (1:1 and 1:5) of αA/ADH. The data are given in two different formats; Figure 6A−D gives the ADH denaturation curves (chaperone assay curves) measured as turbidity at 360 nm for 30 min, and Figure 7 gives the data as the percentage protection calculated from the turbidity readings at 30 min and by taking the protection by αA-wt as 100%. Figure 6A,B gives the chaperone assay curves for αA-wt and four truncated αA-crystallins lacking 5, 6, 7, and 8 C-terminal residues and their respective R163G mutants at 1:1 proportion (Figure 6A) and 1:5 proportion (Figure 6B) of αA/ADH. Figure 6C,D gives the similar curves for the remaining mutants at 1:1 (Figure 6C) and 1:5 (Figure 6D) proportions. On the basis of the data summarized in Figure 7, with increasing number of C-terminal residues deleted, chaperone activity decreases, deletion of 11 residues including Arg-163 having the maximum effect. Mutation of R163G in αA-wt actually shows a slight increase in chaperone activity, whereas about 5% decrease in chaperone activity is seen due to R163G mutation in each of αA_{1-168}, αA_{1-167}, and αA_{1-166} and 12% decrease in αA_{1-165}. Because of R163G mutation in αA_{1-164} and αA_{1-163}, 30 and 54% decrease in chaperone activity, respectively is observed, over and above the decrease due to truncation alone. αA_{1-163} and αA_{1-163} (R163K) have the same chaperone activity, which indicates Arg or Lys at the 163 position has the same effect on the chaperone function. The chaperone activity of αA_{1-162} is decreased 65% as compared to αA-wt. But the substitution of Ser in the 162 position with an Arg does not show any effect on the chaperone activity, which confirms that the position of R163 is critical for influencing chaperone function.

DISCUSSION

In the present study, we have focused on the role of Arg-163 in the oligomerization of αA-crystallin. An earlier study has implicated this residue in αA-crystallin oligomerization because the deletion of 11 C-terminal residues including this residue (Arg-163 is the 11th residue from the C-terminus) decreases the oligomeric size by 75%, whereas deletion of 10 residues, excluding Arg-163, reduces the size by only 15% (*14*). However, several questions about the actual role of Arg-163 in the αA-crystallin oligomerization still remain unanswered. For instance, does Arg-163 exert its influence on the full-length αA-crystallin or does the flexible tail have to be fully or partially deleted for the effect of Arg-163 to become evident? Preliminary studies actually showed Arg-

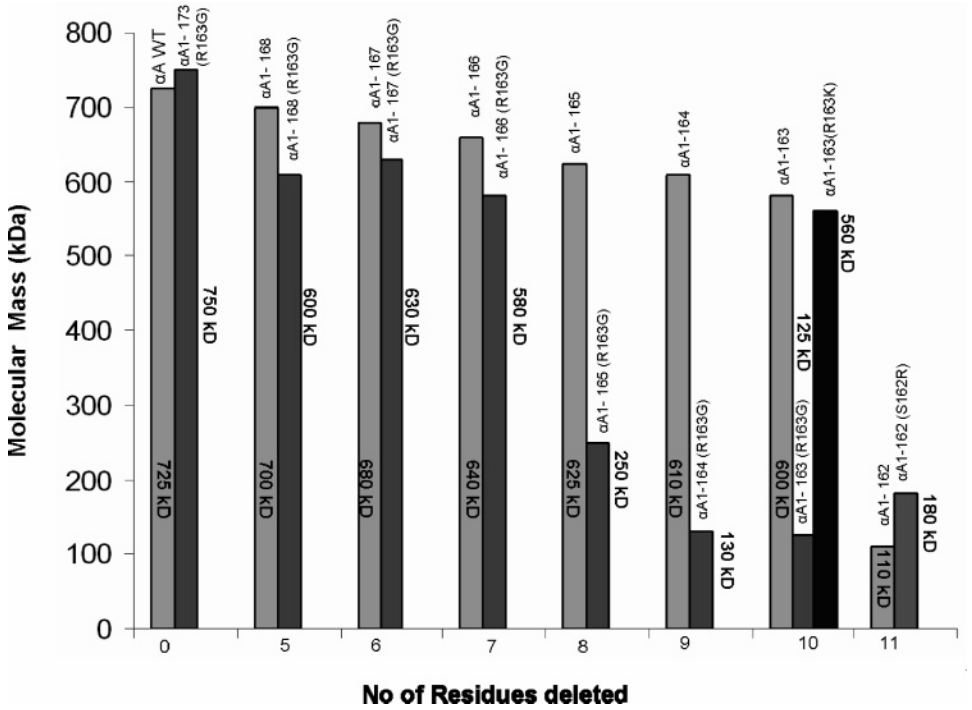

FIGURE 5: Molecular masses of αA-wt and the various truncated mutants compared with those having additional mutations at 163 and 162 positions. Molecular masses were calculated from molecular mass vs retention time calibration curve generated with molecular mass standards.

163 mutation in the full-length αA-crystallin having no effect on the oligomeric size (*19*). So, we speculated that there are other residues in the C-terminal extension that may also play a role in αA oligomerization. It is possible that ^{163} REEK^{166} containing four charged amino acid residues, common to αA- and αB-crystallins may constitute a motif contolling the oligomeric size. To investigate these possibilities, we have studied recombinant full-length αA-crystallin (αA-wt) and a series of truncated mutants lacking varying number of C-terminal residues with and without additional mutations of Arg-163 residue.

The effect of deletion of the C-terminal residues of αA-crystallin on the oligomeric size is expected from an earlier study (*14*). In the present study, we have included additional truncated αA-crystallins for the purpose of dissecting the role of the C-terminal extension, the flexible tail, the Arg-163 residue, and the REEK motif in particular, in αA-crystallin oligomerization. The effect of deletion of 10 C-terminal residues (αA_{1-163}) and 11 C-terminal residues (αA_{1-162}) are 17 and 85% decrease, respectively, in the oligomeric size (Figure 5). The molecular mass (110 kDa) reported here for αA_{1-162} is significantly lower than the value (150 kDa) reported before (*14*) but closer to the molecular mass (88 kDa for αA_{1-162} versus 720 kDa for αA-wt) as determined by the dynamic light scattering method (Abraham and Cao, unpublished). Thus, we can categorically conclude that deletion of 11 C-terminal residues, the 11th residue being Arg-163, causes a substantial decrease in the oligomeric size of rat αA-crystallin. In the full-length αA-crystallin (αA_{1-173} or αA-wt), mutation of Arg-163 to Gly (R163G) does not affect the molecular mass of αA-crystallin (Figures 4A and 5). On the contrary, results from the R163G mutants of all the truncated αA-crystallins show a decrease in oligomeric size, R163G mutants of αA-crystallins lacking 8, 9, and 10 residues showing a 60−80% decrease, and those lacking 5, 6, and 7 residues showing only a small decrease. The fact that the molecular masses of αA_{1-163} and αA_{1-163} (R163K) are nearly the same suggests that Arg-163 can be replaced by a lysine without significantly affecting the oligomeric size and that the role Arg-163 is to provide a positive charge needed for maintaining intersubunit electrostatic interactions in the C-terminal domain. Arg-163 is absent in αA_{1-162}, the result being a substantial decrease in oligomeric size. So, it was speculated that if Ser in the 162 position is mutated to an Arg it may behave like αA_{1-163}, i.e., Arg in the 163 position. Although αA_{1-162} (S162R) shows about 60% increase in molecular mass (from 110 to 180 kDa), complete recovery of the molecular mass to the level seen in αA_{1-163} (600 kDa) has not been accomplished. Thus, the position of Arg-163 is critical in influencing oligomerization. However, a 60% increase in the oligomeric size due to an Arg in the 162 position cannot be ignored.

Changes in chaperone activities, consistent with the changes in oligomeric size, are also seen in the present study (Figures 7 and 8). Both in the truncated as well as in the truncated mutated αA-crystallins, a strong relationship between oligomeric size and chaperone activity is shown in Figure 8 (correlation coefficient = 0.923, $P = 0.001$). As seen with the oligomeric size data (Figures 4A and 5), R163G mutation in the full-length αA-crystallin actually shows a slight increase in chaperone activity. In the truncated αA-crystallins lacking 5, 6, 7, and 8 C-terminal residues, R163G mutation has only a small effect, whereas in those lacking 9 and 10 residues there is a much larger effect. Lys or Arg in the 163 position has the same effect on the chaperone activity. However, an Arg introduced into the 162 position has no influence on the chaperone function, although it has improved the oligomeric size.

Although a good correlation between oligomeric size and chaperone activity has been demonstrated (Figure 8), the impact of changes in protein structure on protein oligomerization or chaperone function cannot be ignored. Examination

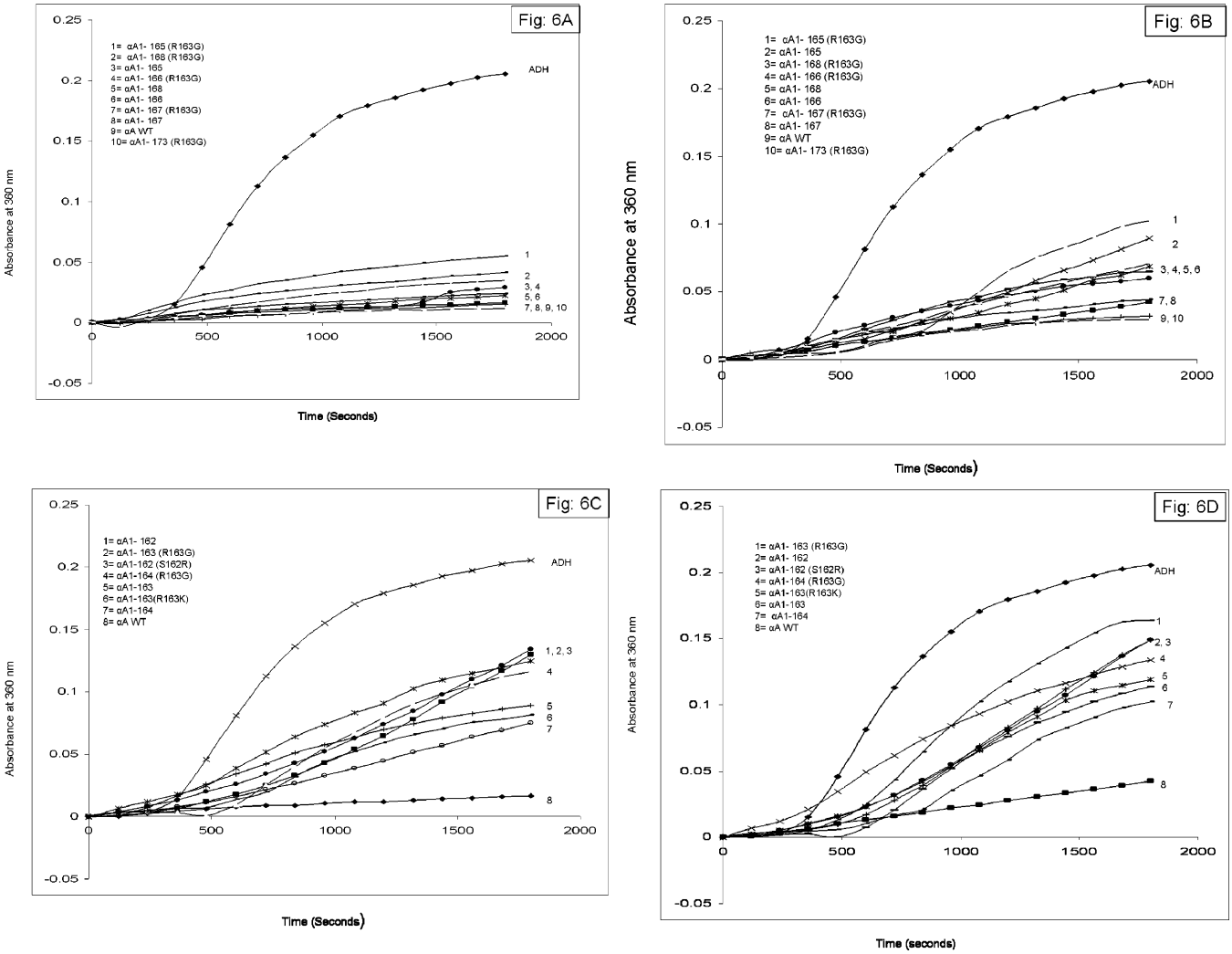

FIGURE 6: (A, B, C, D) Chaperone activities of αA-wt and the truncated αA-crystallins with and without additional mutations, using ADH as the target protein at 37 °C, the ratios of αA/ADH being 1:1 (A, C) and 1: 5 (B, D). EDTA induced aggregation of ADH was monitored as light scattering at 360 nm for 30 min (1800 s). Identification of the curves is provided in the inset.

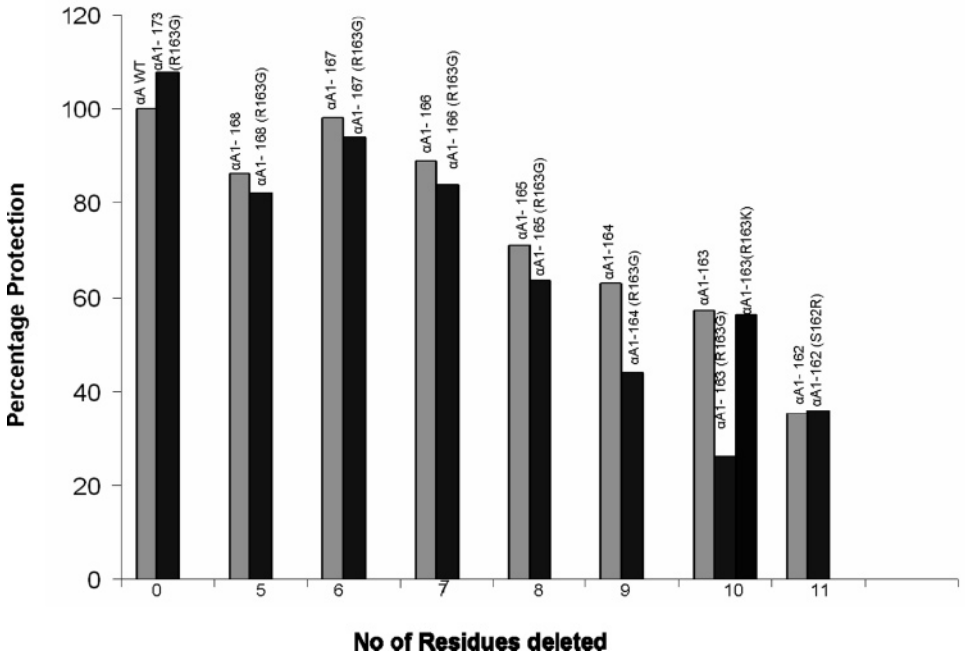

FIGURE 7: Chaperone activities of αA-wt and the various mutants, expressed as the percentage protection of ADH from aggregation, calculated from the turbidity readings at 360 nm at 30 min and by taking the protection by αA-wt as 100%.

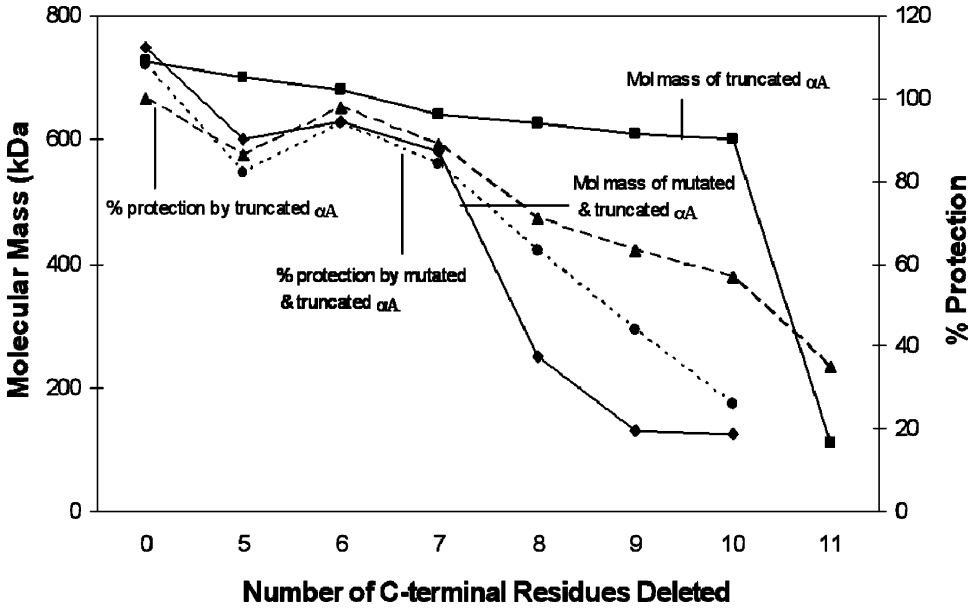

FIGURE 8: Relationship between oligomeric size and chaperone activity, expressed as percentage protection, in αA-wt and various truncated αA-crystallins with and without R163G mutation. Oligomeric size and chaperone activity values were plotted as a function of the number of the C-terminal residues deleted. Correlation analysis of oligomeric size versus chaperone activity was done with a Statistical Program SPSS for Windows, which gave a correlation coefficient = 0.923 and $P = 0.001$.

of the far-UV and near-UV CD spectra (Figures 2A,B and 3A,B) reveals that some of the mutated αA-crystallins showing 30−40% loss in chaperone activity have secondary and tertiary structures altered, but, the oligomeric size has decreased only by about 15%. There are truncated/R163G mutants having the structure, oligomeric size, and chaperone activity substantially affected. Here, the structural changes could have affected oligomerization and, thus, the chaperone activity. Chaperone activity may be correlated with protein conformational changes and oligomeric size, but it is not feasible to distinguish the effect of the former from the latter on chaperone activity.

Arg-163 occupies a unique position in the C-terminal extension of αA-crystallin. It is situated at the interface of the flexible tail and the nonflexible part of the C-terminal extension. On the basis of the studies of Carver and Lindner (*20*), the "tail" has been defined as the highly mobile amino acid residues at the C-terminus of mammalian sHsps. The final 8 and 10 residues, respectively, of αA-crystallin and αB-crystallin are known to be highly mobile (*20*). Likewise, the last 16 residues of human Hsp 27 and 18 residues of mouse Hsp 25 are mobile (*20*). Involvement of the C-terminal extension in oligomeric contacts can be predicted to be similar in most sHsps. The requirement of this region for oligomerization is supported by the present study as well as a large body of other mutagenesis data (*10*, *21*−*23*) and by the oligomeric structure differences in certain variant sHsps (*24*, *25*). A truncated mutant of mammalian Hsp 20, which lacks the C-terminal extension motif, forms primarily dimers (*24*). Likewise, in Hsp 12.6 from *Caenorhabditis elegans* the C-terminal extension is absent and the protein is monomeric (*25*).

The motif ^{163}REEK^{166} is common to both αA- and αB-crystallins. In addition to the well-documented role of Arg-163 in this study, it appears that Lys-166 and Glu-165 do play some role in the oligomerization of these truncated mutants, evident only in the absence of Arg-163. For instance, the molecular masses of αA_{1-168} (R163G), αA_{1-167} (R163G), and αA_{1-166} (R163G) are 600, 630, and 580 kDa, respectively. On the other hand, the molecular mass of αA_{1-165} (R163G), which lacks Lys-166, is only 250 kDa (>50% decrease). Likewise, there is about a 50% decrease in the oligomeric size (130 kDa) of αA_{1-164} (R163G), which lacks Glu-165, as compared to αA_{1-165} (R163G). Thus, in the absence of Arg-163, the presence or absence of Lys-166 or Glu-165 will have a significant impact on the oligomeric size. These findings provide evidence for a possible role for each amino acid residue in the REEK motif in the αA-crystallin oligomerization. Thus, it is apparent that the oligomeric mass remains unchanged even after R163G mutation in the full-length αA-crystallin and decreases only slightly in αA_{1-168}, αA_{1-167}, and αA_{1-166} because the remaining charged residues in the REEK motif provide the intersubunit electrostatic interactions in the C-terminal extension needed to form high-molecular-mass oligomers.

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