

Interaction of C-Terminal Truncated Human α A-Crystallins with Target Proteins

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Abstract

Background: Significant portion of α A-crystallin in human lenses exists as C-terminal residues cleaved at residues 172, 168, and 162. Chaperone activity, determined with alcohol dehydrogenase (ADH) and β L-crystallin as target proteins, was increased in α A₁₋₁₇₂ and decreased in α A₁₋₁₆₈ and α A₁₋₁₆₂. The purpose of this study was to show whether the absence of the C-terminal residues influences protein-protein interactions with target proteins.

Methodology/Principal Findings: Our hypothesis is that the chaperone-target protein binding kinetics, otherwise termed subunit exchange rates, are expected to reflect the changes in chaperone activity. To study this, we have relied on fluorescence resonance energy transfer (FRET) utilizing amine specific and cysteine specific fluorescent probes. The subunit exchange rate (k) for ADH and α A₁₋₁₇₂ was nearly the same as that of ADH and α A-wt, α A₁₋₁₆₈ had lower and α A₁₋₁₆₂ had the lowest k values. When β L-crystallin was used as the target protein, α A₁₋₁₇₂ had slightly higher k value than α A-wt and α A₁₋₁₆₈ and α A₁₋₁₆₂ had lower k values. As expected from earlier studies, the chaperone activity of α A₁₋₁₇₂ was slightly better than that of α A-wt, the chaperone activity of α A₁₋₁₆₈ was similar to that of α A-wt and α A₁₋₁₆₂ had substantially decreased chaperone activity.

Conclusions/Significance: Cleavage of eleven C-terminal residues including Arg-163 and the C-terminal flexible arm significantly affects the interaction with target proteins. The predominantly hydrophilic flexible arm appears to be needed to keep the chaperone-target protein complex soluble.

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Introduction

The major proteins in the vertebrate eye lens are α -, β -, and γ -crystallins the predominant one being the α -crystallin. α -Crystallin consists of two nearly homologous subunits, namely, α A- and α B-crystallins and both having a molecular mass of 20 kDa in the monomer form and contain 173 and 175 amino acid residues respectively [1–3]. Both α A- and α B-crystallins belong to the class of small heat shock proteins [4] and function as molecular chaperones having the ability to prevent aggregation of partially unfolded proteins [5–7]. The model structure of α -crystallin consists of a globular N-terminal domain and a C-terminal domain containing an exposed C-terminal arm rich in hydrophilic amino acids, whereas the C-terminal stretch of 80–100 residues known as the ‘ α -crystallin/sHsp domain’ are highly conserved [8].

C-terminal cleavage of α A-crystallin at residues 162, 168, and 172 has been reported earlier [9–15]. The major post-translational modification which occurs in human α A-crystallin is the loss of the C-terminal serine residue [9,14,15]. Enhanced cleavage of the C-terminal residue of α A-crystallin in diabetic human lenses has been reported in our earlier study, the average level of the truncated α A-crystallin increased from 30% to 50% [15]. Aziz *et al* [16] have recently reported modification in the oligomeric structure and chaperone function of the various truncated human α A-crystallins. Interestingly, the truncated α A₁₋₁₇₂ exhibited significant increase in its oligomeric size as well as chaperone activity. The oligomeric

size of α A₁₋₁₆₈ was similar to that of α A-wild type (α A-wt) whereas the chaperone activity was moderately decreased. α A₁₋₁₆₂, on the other hand, showed substantial decrease in the oligomeric size as well as the chaperone activity. If indeed chaperone to target protein binding is an essential step for α A-crystallin to operate as a molecular chaperone, characterization of the interaction of the truncated α A-crystallins with target proteins should show why their chaperone function is altered as a result of the truncation. In this study, we have used fluorescence resonance energy transfer (FRET) to study chaperone - target protein interaction using ADH and β L-crystallin, two widely used target proteins [16,17], and recombinant α A-wt and C-terminal truncated α A-crystallins.

Results

Levels of fluorescence labeling of human α A-wt, C-terminal truncated α A-crystallins and the target proteins

The level of subunit exchange between two different proteins was determined by FRET. In order to determine the *in vitro* FRET level two fluorescent dyes with overlapping fluorescence spectra are necessary. SITS and LYI are widely used fluorescent dyes with spectral overlaps and safe from structural alterations due to fluorescent tags [18–20]. So, in the present study the amine specific fluorescent probe SITS was attached to human α A-wt and the C-terminal truncated α A-crystallins and the cysteine specific LYI fluorescent probe was attached to the target proteins ADH and β L-

crystallin. The level of labeling was determined spectrophotometrically using molar extinction coefficients of $47,000 \text{ mol}^{-1} \text{ cm}^{-1}$ at 336 nm for SITS and $11,000 \text{ mol}^{-1} \text{ cm}^{-1}$ at 426 nm for LYI. The level of labeling was about 0.8, 1.12, 1.17, 1.04 and 7.1 and 1.56 moles for α A-wt, α A₁₋₁₇₂, α A₁₋₁₆₈, α A₁₋₁₆₂, ADH and β L-crystallin respectively. Differences in the level of labeling of the two different fluorophores were taken into account while computing the data.

Subunit exchange between labeled α A-crystallins and the target protein ADH

The interaction between the SITS-labeled human α A-wt and the C-terminal truncated α A-crystallins and the LYI-labeled target protein ADH was initiated by mixing equimolar concentration of the proteins in 20 mM MOPS buffer with 100 mM NaCl and 10 mM EDTA at 37°C. EDTA was used for unfolding ADH so that α A-crystallin will bind to ADH under the same condition as used for the chaperone assay. The rate of subunit exchange with ADH was determined by FRET analysis. Figure 1 shows the fluorescence spectra showing the time dependent decrease in SITS emission intensity at 426 nm and a concomitant increase in LYI fluorescence at 515 nm. After 30 min at 37°C, there was no remarkable change in the emission intensity at 515 nm due to the achievement of stable equilibrium (data not shown). The fluorescence emission spectra illustrate that the highest increase in the acceptor spectrum at 515 nm was observed in α A-wt when compared to the C-terminal truncated α A-crystallins (Fig. 1). We have calculated the rate of

subunit exchange from the increase in acceptor fluorescence intensity after taking into account differences in the levels of tagging of the various proteins by the probes. Figure 2A shows the plot of F_t/F_0 of LYI at 515 nm as a function of time, where F_t and F_0 are the emission intensities at time t and zero respectively. The rate constant was obtained by fitting the data to the exponential function $F_t/F_0 = A_1 + A_2 e^{-kt}$, where A_1 and A_2 are constants and k is the rate constant for subunit exchange. The increase in the relative fluorescence intensity at 515 nm is due to fluorescence resonance energy transfer from donor SITS-labeled human α A-wt and C-terminal truncated α A-crystallins' fluorophore to the acceptor LYI-labeled ADH during the interaction. The maximum relative fluorescence intensity was seen with α A-wt whereas α A₁₋₁₇₂ had lower fluorescence intensity, α A₁₋₁₆₈ showed further decrease in the fluorescence intensity, and α A₁₋₁₆₂ showed the lowest. The subunit exchange rates or the k values are summarized in Table 1 which is in agreement with the above observation. For instance, α A-wt had the highest k value (2.073) whereas the k values of α A₁₋₁₇₂, α A₁₋₁₆₈, and α A₁₋₁₆₂, respectively, were 6, 24, and 43% lower. Figure 2B illustrates the decrease in the relative fluorescence at 426 nm when the fluorescence resonance energy is transferred to the acceptor target protein. The rate constant (k) values (Table 1) were higher when collected and calculated at donor energy (at 426 nm) than when calculated at acceptor energy (at 515 nm). This is believed to be due to fluorescence quenching because no direct transfer of fluorescence energy occurs where there is no sufficient contact between the residues carrying the two probes. The amount of loss

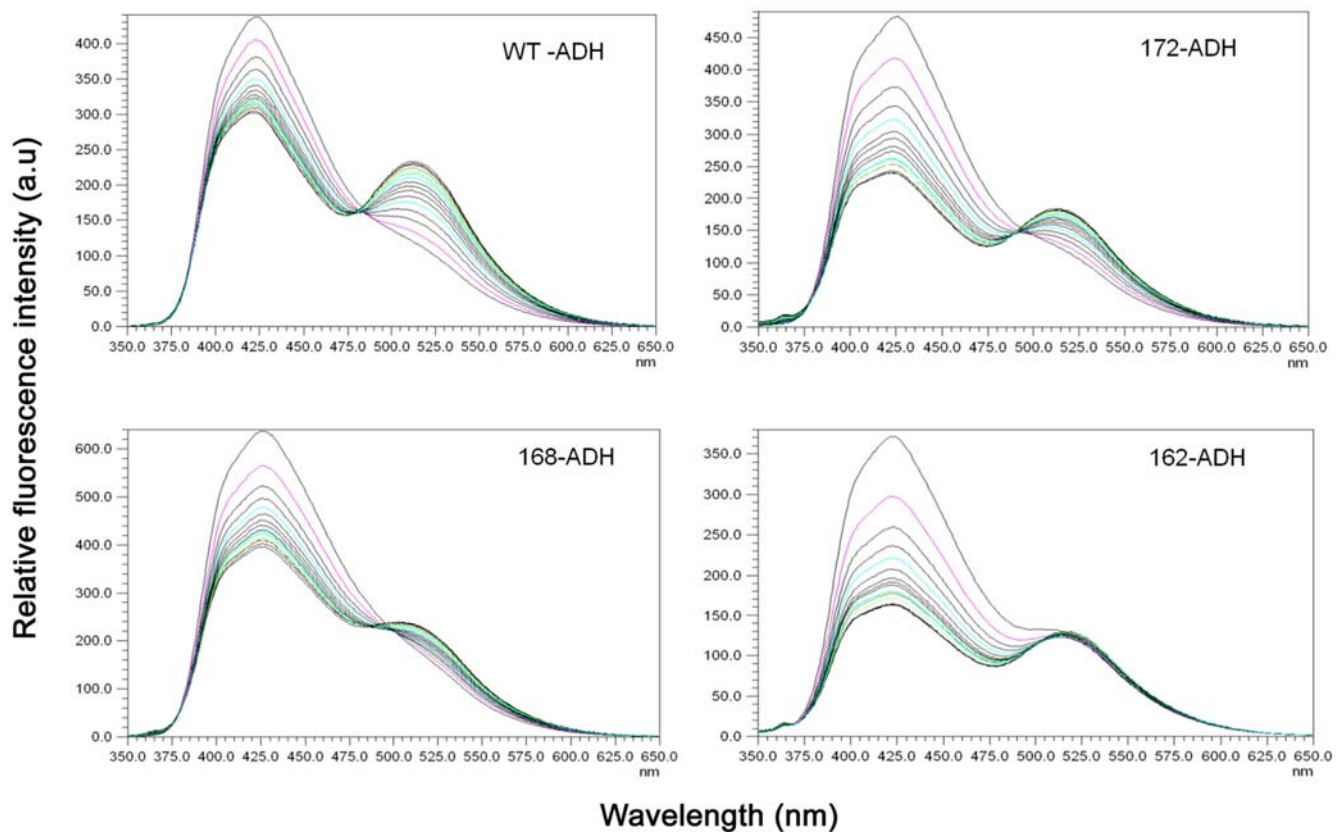


Figure 1. Time-dependent spectral changes in the emission spectra of SITS-labeled α A-crystallins interacting with LYI-labeled ADH. The emission spectra of fluorescence-labeled α A crystallin excited at 336 nm were recorded at every 2 min intervals at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 minutes after mixing of SITS-labeled α A-wt and its C-terminal truncated α A₁₋₁₇₂, α A₁₋₁₆₈, and α A₁₋₁₆₂ with LYI-labeled ADH in 1:1 ratio, with a final protein concentration of 1 mg/ml at 37°C. The decrease in fluorescence intensity at 426 nm of SITS-labeled α A-crystallins and concomitant increase in fluorescence intensity at 515 nm of LYI-labeled ADH is indicative of energy transfer. doi:10.1371/journal.pone.0003175.g001

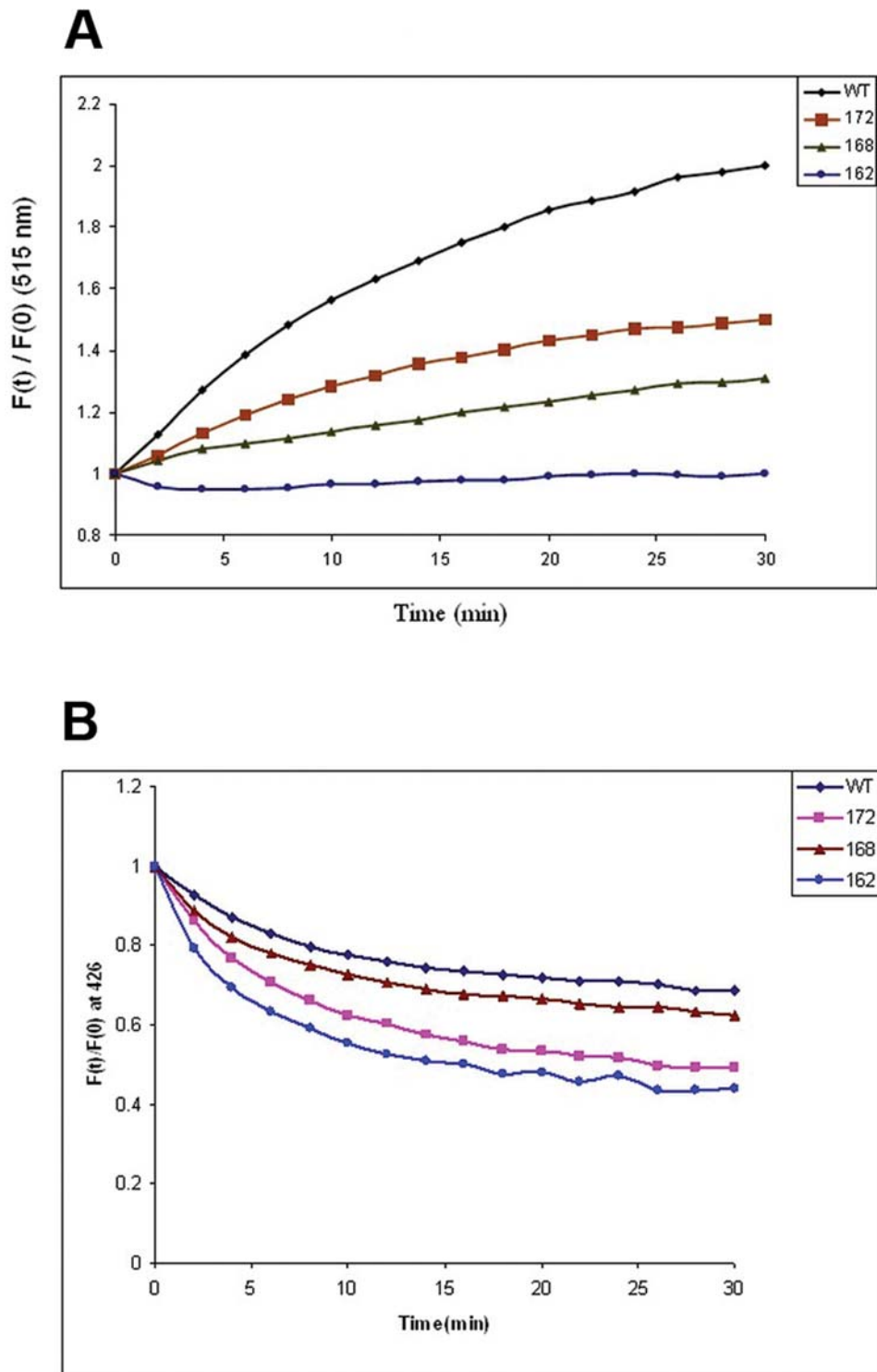


Figure 2. A: Relative fluorescence intensity at 515 nm due to subunit exchange of SITS – labeled α A-crystallins with LYI – labeled ADH. Time – dependent increase in acceptor fluorescence emission intensity is due to subunit exchange as evident from energy transfer from the SITS – labeled protein to the LYI – labeled protein. SITS – labeled α A wt (\blacklozenge), αA_{1-172} (\blacksquare), αA_{1-168} (\blacktriangle) and αA_{1-162} (\circ) was incubated with LYI – labeled ADH. Each curve was analyzed with the best curve fit of the data to the exponential function $F_t/F_0 = A_1 + A_2 e^{-kt}$. **B: Relative fluorescence intensity at 426 nm due to subunit exchange of SITS – labeled α A-crystallins with LYI – labeled ADH.** Time – dependent decrease in donor fluorescence emission intensity is due to subunit exchange as evident from energy transfer from the SITS – labeled protein to the LYI – labeled protein. SITS – labeled α A wt (\blacklozenge), αA_{1-172} (\blacksquare), αA_{1-168} (\blacktriangle) and αA_{1-162} (\circ) was incubated with LYI – labeled ADH. Each curve was analyzed with the best curve fit of the data to the exponential decay function $F_t/F_0 = A_1 + A_2 e^{-kt}$.
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Table 1. Subunit exchange rate constant (k) of αA-wt and its C-terminal truncated forms interacting with ADH when increase and decrease respectively (Mean ± SE).

| Crystallin | Subunit exchange rate constant at 515 nm ($\times 10^{-4} \text{ S}^{-1}$) | Subunit exchange rate constant at 426 nm ($\times 10^{-4} \text{ S}^{-1}$) |
|--------------------------|--|--|
| αA wt+ADH | 2.073 ± 0.169 | 8.035 ± 0.373 |
| αA ₁₋₁₇₂ +ADH | 1.947 ± 0.149 | 7.549 ± 0.327 |
| αA ₁₋₁₆₈ +ADH | 1.585 ± 0.112 | 7.170 ± 0.462 |
| αA ₁₋₁₆₂ +ADH | 1.190 ± 0.111 | 5.737 ± 0.403 |

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due to quenching is comparatively lower in both αA-wt and αA₁₋₁₇₂ than in αA₁₋₁₆₈ and αA₁₋₁₆₂.

Subunit exchange between αA-crystallins and the target protein βL-crystallin

The interaction between the SITS- labeled human αA-wt and the C-terminal truncated αA-crystallins and the LYI- labeled target protein βL-crystallin was initiated by mixing equimolar concentration of the proteins in 20 mM MOPS buffer with 100 mM NaCl at

62°C. Higher temperature was necessary to unfold βL-crystallin. Figure 3 shows the fluorescence spectra and Figure 4A and 4B show the increase and decrease in relative fluorescence intensity or Ft/F0 at 515 nm and 426 nm, respectively as a function of time. The k values which reflect the data in Figure 4A and 4B were summarized in Table 2. Interestingly, the acceptor gain rate constant (k) value for αA₁₋₁₇₂ was maximal at 2.422 and it was about 8% higher than the value for αA-wt and about 41% higher than the values for αA₁₋₁₆₈ and αA₁₋₁₆₂. The same trend was noticed in donor k values determined at 426 nm also. The C-terminal truncated αA₁₋₁₇₂ was maximal at 6.391 which was about 24% higher than that of αA-wt and 34% and 54% higher than those of αA₁₋₁₆₈ and αA₁₋₁₆₂ respectively. As mentioned above, here also at the time of energy transfer from donor fluorophore to the acceptor fluorophore some energy loss was noticed. The amount of energy lost was about 54–66%. The subunit exchange rate constant (k) values clearly showed that in αA-crystallin and the counterparts with ADH at both emission intensities (at 426 & 515 nm) the αA-wt showed the highest interaction compared to the C-terminally truncated αA-crystallins and among the C-terminally truncated αA-crystallins, αA₁₋₁₇₂ showed higher k value followed by αA₁₋₁₆₈ and αA₁₋₁₆₂ in that order. However, the subunit exchange rate constant (k) values in αA-crystallin and counterparts with βL crystallin were higher in αA₁₋₁₇₂ followed by αA-wt, αA₁₋₁₆₈ and αA₁₋₁₆₂. Nevertheless, the loss of energy (due to quenching) was least in αA-wt with both target

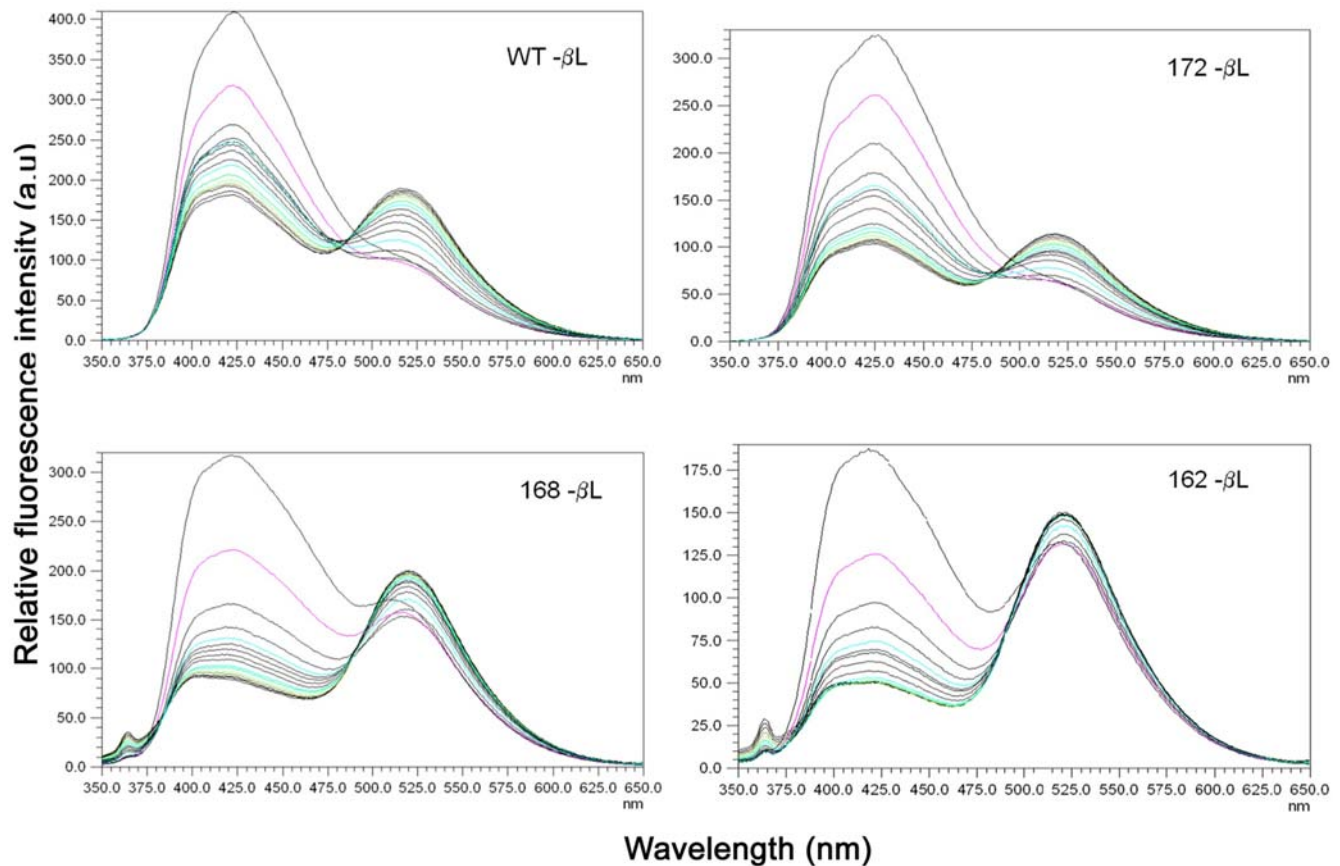


Figure 3. Time –dependent spectral changes in the emission spectra of the SITS-labeled αA- crystallins interacting with LYI- labeled βL- crystallin. The emission spectra of fluorescence- labeled αA crystallin excited at 336 nm were recorded at every 2 min intervals at 0, 2,4,6,8,10,12,14,16,18,20,22,24,26,28 and 30 minutes after mixing of SITS – labeled αA - wt and C-terminal truncated αA-crystallins with LYI – labeled βL- crystallin in a 1:1 ratio, with a final protein concentration of 1 mg/ml at 62°C. The decrease in fluorescence intensity at 426 nm of SITS-labeled αA-crystallins and concomitant increase in fluorescence intensity at 515 nm of LYI- labeled ADH is indicative of energy transfer due to exchange of subunits between the two labeled proteins.

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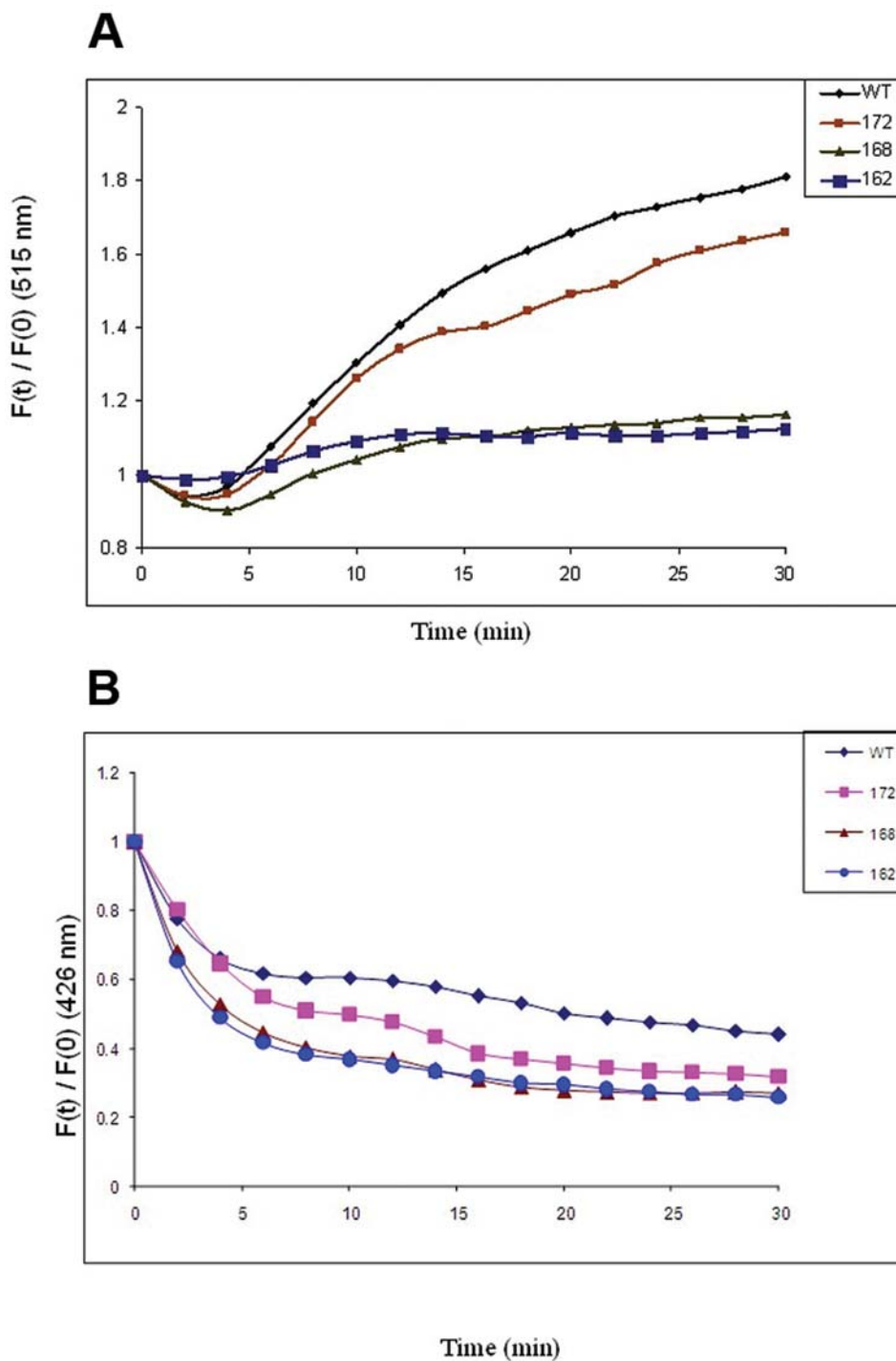


Figure 4. A: Relative fluorescence intensity at 515 nm of SITS - labeled α A-crystallins interacting with LYI - labeled β L- crystallin. Time - dependent increase in acceptor fluorescence in emission intensity is due to subunit exchange. Increase in the relative fluorescence intensity at 515 nm is due to energy transfer from the SITS - labeled protein to the LYI - labeled protein. SITS - labeled α A- wt (\blacklozenge), αA_{1-172} (\blacksquare), αA_{1-168} (\blacktriangle) and αA_{1-162} (\bullet) were incubated with LYI - labeled β L- crystallin. Each curve was analyzed with the best curve fit of the data to the exponential function $F_T/F_0 = A_1 + A_2 e^{-kt}$. **B: Relative fluorescence intensity at 426 nm of SITS - labeled α A-crystallins interacting with LYI - labeled β L-crystallin.** Time - dependent decrease in donor fluorescence in emission intensity is due to subunit exchange. Decrease in the relative fluorescence intensity at 426 nm is due to energy transfer from the SITS - labeled protein to the LYI - labeled protein. SITS - labeled α A- wt (\blacklozenge), αA_{1-172} (\blacksquare), αA_{1-168} (\blacktriangle) and αA_{1-162} (\bullet) were incubated with LYI - labeled β L- crystallin. Each curve was analyzed with the best curve fit of the data to the exponential function $F_T/F_0 = A_1 + A_2 e^{-kt}$.
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Table 2. Subunit exchange rate constant (k) of αA-wt and its C-terminal truncated forms interacting with βL-crystallin when increase and decrease respectively (Mean ± SE).

| Crystallin | Subunit exchange rate constant at 515 nm (×10 ⁻⁴ S ⁻¹) | Subunit exchange rate constant at 426 nm (×10 ⁻⁴ S ⁻¹) |
|------------------------------------|---|---|
| αA wt+βL-crystallin | 2.233±0.010 | 4.826±0.876 |
| αA ₁₋₁₇₂ +βL-crystallin | 2.422±0.039 | 6.391±0.501 |
| αA ₁₋₁₆₈ +βL-crystallin | 1.419±0.008 | 4.183±0.287 |
| αA ₁₋₁₆₂ +βL-crystallin | 1.435±0.004 | 3.462±0.267 |

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proteins (ADH & βL crystallin) followed by αA₁₋₁₇₂, αA₁₋₁₆₈ and αA₁₋₁₆₂.

Chaperone activity of αA-wt and the C-terminal truncated αA-crystallins

Although the chaperone activity of the truncated human αA-crystallins were reported earlier [16] the chaperone activity assay was repeated in the present study using the ratio of 1:1 which is the same ratio as used in the FRET analysis. With ADH as the target protein, αA₁₋₁₇₂ showed about 18% better chaperone activity than αA-wt, αA₁₋₁₆₈ had nearly the same chaperone activity as αA-wt and αA₁₋₁₆₂ showed nearly 80% loss (Fig. 5). With βL-crystallin as the protein, both αA₁₋₁₇₂ and αA₁₋₁₆₈ showed normal chaperone activity whereas αA₁₋₁₆₂ showed nearly 60% loss in chaperone activity (Fig. 6).

Materials and Methods

Fluorescence probes

Lucifer yellow iodoacetamide (LYI) and 4-acetamido-4'-isothiocyanatostilbene-2,-2'-disulfonic acid (SITS) were obtained

from Molecular Probes (Eugene, OR, USA). Alcohol dehydrogenase (ADH) and βL-crystallin were purchased from Sigma-Aldrich (St. Louis, USA).

Cloning, site-directed mutagenesis, expression and purification of human αA-crystallin wild type (αA-wt), and C-terminal truncated αA-crystallins

Cloning of human αA-crystallin and subsequent subcloning into the pET-23d(+) expression vector has been described previously [21]. To generate the different C-terminally truncated human αA-crystallins lacking 1 (αA₁₋₁₇₂), 5 (αA₁₋₁₆₈), and 11(αA₁₋₁₆₂) residues, stop codons were introduced at desired sites as described previously [16]. The pET-23d(+) expression vector harboring DNA constructs of human αA-wt and C-terminally truncated αA₁₋₁₇₂, αA₁₋₁₆₈, and αA₁₋₁₆₂ were expressed in BL21 (DE3) PLYS *E. coli* cells. 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 protein preparations was examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [22].

Labeling of human αA-wt, C-terminal truncated αA-crystallins and target proteins ADH and βL-crystallin with fluorescence probes

Labeling of αA-crystallin and C-terminal truncated αA-crystallins with SITS and the target proteins ADH and βL-crystallin with LYI was done as described previously [20,21]. Briefly, 10 fold excess of solid SITS was added to 1 ml of a protein solution (3 mg/ml) in 20 mM MOPS buffer containing 100 mM NaCl (pH 7.9) and the reaction was allowed to proceed for about 16 h at room temperature (25°C) in the dark. The unlabeled fluorescent dye was removed from the fluorescently labeled protein

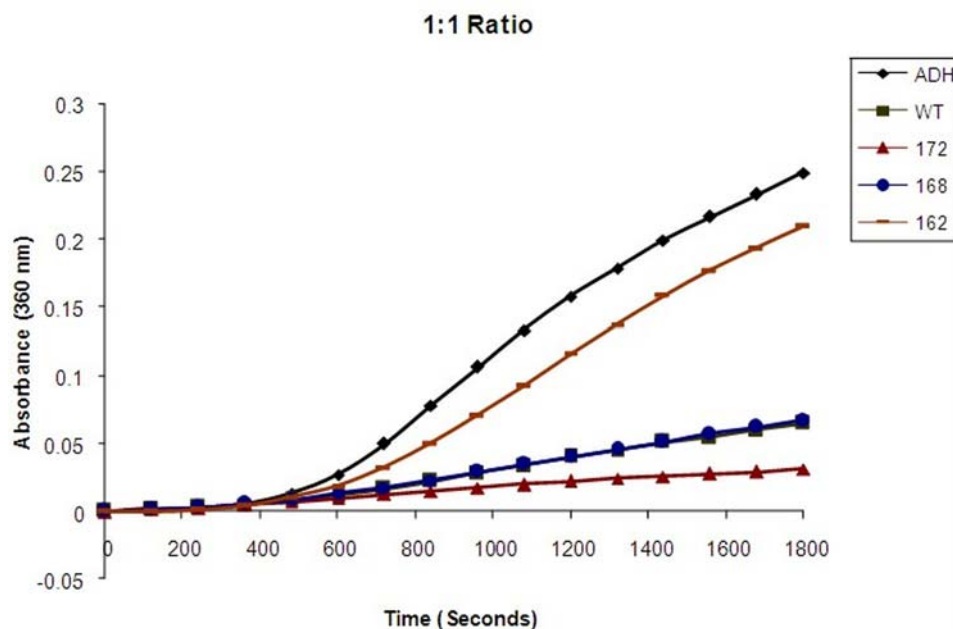


Figure 5. Effect of C-terminal truncation of αA-crystallin on its chaperone activity as measured with ADH as the target protein. The chaperone activity of αA-wt (■) and C-terminal truncated αA₁₋₁₇₂(▲) αA₁₋₁₆₈ (●) and αA₁₋₁₆₂ (◻) ADH alone (◆) determined with ADH as the target protein at 1:1 ratio (0.14 mg/ml) denatured with EDTA at 37°C.

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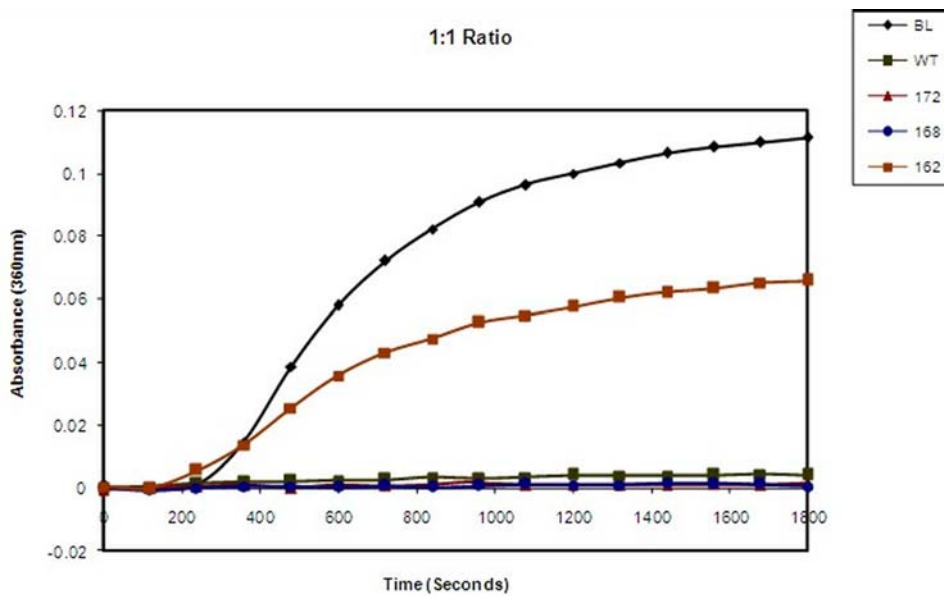


Figure 6. Effect of C-terminal truncation of α A-wt on its chaperone activity as measured with β L-crystallin as the target protein. The chaperone activity of α A-wt (■) and C-terminal truncated α A₁₋₁₇₂(▲) α A₁₋₁₆₈ (•) and α A₁₋₁₆₂ (–) β L-crystallin alone (◆) determined with ADH as the target protein at 1:1 ratio (0.14 mg/ml) at 62°C.
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on a Sephadex G-25 column equilibrated with 20 mM MOPS buffer containing 100 mM NaCl (pH 7.9). Elution was performed with the same buffer, pooled and concentrated. LYI-labeled protein was prepared as described above with the same buffer in 20 fold excess of the reagent. The extent of labeling was determined spectrophotometrically using molar extinction coefficients of 47,000 mol⁻¹ cm⁻¹ at 336 nm for SITS and 11,000 mol⁻¹ cm⁻¹ at 426 nm for LYI and corrected for the contribution of the dye at 280 nm.

Measurement of the rate of subunit exchange among α A-crystallins and the target proteins

The rate of subunit exchange was measured by the method of fluorescence resonance energy transfer (FRET). The subunit exchange reaction was initiated by mixing equal amounts (0.5 mg/ml) of SITS -labeled α A-wt and C-terminal truncated α A-crystallins (α A₁₋₁₇₂, α A₁₋₁₆₈ & α A₁₋₁₆₂) with the same amount of LYI-labeled target proteins: 1)ADH in 20 mM MOPS buffer containing 100 mM NaCl and 10 mM EDTA (pH 7.9) at 37°C and 2) β L-crystallin in 20 mM MOPS buffer containing 100 mM NaCl (pH 7.9) at 62°C. The fluorescence emission spectra were obtained with Shimadzu RF-5301PC spectrofluorophotometer (Columbia, MD) at an excitation wavelength of 336 nm. Decrease in SITS emission intensity at 426 nm and increase in LYI emission intensity at 515 nm were recorded at every 2 min intervals and the subunit exchange rate constant (k) was calculated by curve fitting an exponential function $F_t/F_0 = A_1 + A_2 e^{-kt}$ where A_1 and A_2 are constants and k is the rate constant for subunit exchange.

Determination of Chaperone activity

The ability of α A-wt and the C-terminal truncated α A-crystallins to prevent the extent of EDTA induced aggregation of ADH and thermal aggregation of β L-crystallin was determined as described before [16,17]. α A-wt and the C-terminal truncated α A-crystallins were mixed with equal amounts of target proteins (1:1 ratio) at a total concentration of 0.14 mg/ml. ADH was induced to unfold with 10 mM EDTA in 50 mM PBS buffer

(pH 7.9) at 37°C and the β L-crystallin unfolding was thermally induced at 62°C. The extent of aggregation was measured by monitoring the light scattering at 360 nm using Shimadzu UV 160 spectrophotometer equipped with a temperature regulated cell holder.

Discussion

We have shown earlier that cleavage of 1, 5, and 11 C-terminal residues affects or improves chaperone activity depending on the number of residues cleaved and the chaperone assay system [16]. Chaperone activity of the truncated α A-crystallins can vary by two major factors: accessibility to the chaperone sites which are responsible for the chaperoning process or due to enhanced or decreased affinity to the substrate which is being unfolded. The present study focused on the latter aspect by studying the binding of the wild-type and the truncated α A-crystallins with the putative target proteins. The data show that the binding kinetics (k value) reflect the chaperone activity differences with the exception that α A₁₋₁₇₂ showed higher chaperone activity, but, lower k value. Thus, we can conclude that the binding affinity to target proteins does not always concur with the chaperone activity data. However, it is noteworthy that all the three truncated α A-crystallins have shown decreased target protein binding.

In an earlier study, we have determined the oligomeric size, secondary and tertiary structures and chaperone activity of recombinant human α A-wt and the various C-terminal truncated α A-crystallins [16]. α A₁₋₁₇₂, which is the major form of the truncated human α A-crystallin, had a molecular mass of 866 kDa as compared to 702 kDa for α A-wt, when the molecular mass was determined by dynamic light scattering. The chaperone activity of α A₁₋₁₇₂ was higher than that of α A-wt, when ADH, insulin and β L-crystallin were used as target proteins and the α A-crystallin : target protein ratio varied between 1:1 and 1:20. In this study, we have used equal amounts of α -crystallin and target protein, ie, 1:1 ratio, in all the assays to avoid artificially exaggerated differences between α A-wt and the truncated α A-crystallins. As shown in

Figures 5 and 6, αA_{1-172} had slightly better chaperone activity than αA -wt, which confirms our previous observation [16]. However, αA -crystallin-target protein binding studies gave conflicting results. For instance, the relative fluorescence intensity due to FRET and the k values were lower in αA_{1-172} (Figs. 2 & 4; Tables 1 & 2). Both the present study and the earlier study [16] have shown αA_{1-168} having nearly the same chaperone activity as αA -wt. however, the FRET studies gave contradicting results as the relative fluorescence intensity as well as the k values were significantly lower (Figs. 2 & 4; Tables 1 & 2). With both the ADH and the βL -crystallin target protein methods, αA_{1-162} showed the lowest chaperone activity (Figs. 5 & 6) as shown earlier. Interestingly, αA_{1-162} also showed the lowest level of relative fluorescence intensity and k value during FRET studies. Thus, the cleavage of 11 C-terminal residues of αA -crystallin, which is known to affect its secondary and tertiary structures [16], severely affects its binding to target proteins which in turn affect its ability to function as a molecular chaperone.

By using FRET analysis, we have recently investigated the effect of cleavage of the C-terminal residues of human αA -crystallin on

subunit exchange with αB -crystallin forming heteroaggregates [21]. The subunit exchange rate or the k value for αA_{1-172} interacting with αB -wt was decreased by 50% compared to αA -wt interacting with αB -wt. Likewise, the k value was decreased 40% when αA_{1-168} interacted with αB -wt, whereas interaction of αA_{1-162} with αB -wt showed 84% decrease in the k value. Thus, cleaving 11 residues including lysine-163 had shown the most effect. The importance of lysine-163 in maintaining the oligomeric structure of αA -crystallin has been demonstrated in a recent study [23]. In fact, in αA_{1-162} the secondary and tertiary structures were significantly altered and the molecular mass was substantially decreased [16]. Such changes affect binding to αB -crystallin and heterooligomerization [21] and also seem to affect binding to target proteins decreasing the chaperone activity.

Author Contributions

Conceived and designed the experiments: AK EA. Performed the experiments: AK. Analyzed the data: AK. Contributed reagents/materials/analysis tools: AK. Wrote the paper: AK.

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