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Arginine hydrochloride enhances the dynamics of subunit assembly and the chaperone-like activity of α-crystallin

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Purpose: α-Crystallin, a major eye lens protein, bears homology with small heat shock proteins (sHsps) and exhibits molecular chaperone-like activity. Structural perturbation by temperature or low concentrations of denaturants leads to enhancement of its chaperone-like activity. We have earlier demonstrated similar enhancement of chaperone-like activity using biologically compatible solutes such as arginine hydrochloride and aminoguanidine. The purpose of the present study is to get an insight into the mechanism of the arginine induced enhancement of chaperone-like activity of α-crystallin.

Methods: The effect of arginine hydrochloride on the chaperone-like activity of α-crystallin at 25 °C was studied using DTT induced aggregation of insulin as a model system. Changes in the accessibility of the thiol group near the end of the α-crystallin domain in the absence and the presence of arginine hydrochloride were studied using dithiobisnitrobenzoic acid. Fluorescence resonance energy transfer studies were performed to investigate changes in the dynamics of the subunit assembly. Urea induced denaturation studies of α-crystallin were carried out to investigate structural destabilization of α-crystallin, if any, in the presence of arginine hydrochloride.

Results: Arginine hydrochloride increases the chaperone-like activity of α-crystallin several fold towards DTT induced aggregation of insulin at room temperature. Our study shows that both the extent and the rate of accessibility of the thiol group are increased in the presence of arginine. Fluorescence resonance energy transfer experiments show that arginine hydrochloride significantly increases the subunit exchange between the oligomers of α-crystallin. Arginine induced structural perturbation and loosening of subunit assembly of α-crystallin leads to overall destabilization of the protein as reflected by the urea denaturation study.

Conclusions: Arginine perturbs the tertiary and quaternary structure of α-crystallin and enhances the dynamics of the subunit assembly leading to enhanced chaperone-like activity. Thus, in addition to size, surface hydrophobicity, and charge distribution, the dynamics of the subunit assembly appears to be one of the critical factors that can modulate the chaperone activity.

Molecular chaperones are a class of proteins that are known to interact with partially unfolded states of other proteins and prevent off pathway reactions leading to aggregation and inactivation, thus keeping them in a folding competent state. α-Crystallins (αA- and αB-crystallin), major constituents of the eye lens, are known to share sequence homology with small heat shock proteins [1,2] and to exhibit molecular chaperone-like activity in preventing aggregation of other proteins [3-7], in protecting enzyme activity upon heat stress [8-11], and in helping some enzymed to refold [11-14]. αB-crystallin is also present in tissues such as brain, kidney, heart, and muscle, and its expression is inducible under stress and disease conditions [15]. Mutation of a conserved arginine residue to glycine (R120G) in αB-crystallin and to cysteine (R116C) in αA-crystallin leads to desmin related myopathy and congenital cataract, respectively [16,17], and also leads to decrease in the chaperone-like activity of these proteins [18-20].

Our earlier studies [4,21,22] and subsequent studies from other laboratories [23,24] have shown that structural perturbation of α-crystallin by temperature leads to increase in its chaperone-like activity. Low concentrations of denaturants such as urea [4,21,22] or guanidine hydrochloride (Gdn•HCl) [25] have also been shown to perturb the structure and enhance the chaperone-like activity of α-crystallin and that of another small heat shock protein (Hsp16.3) from *Mycobacterium tuberculosis* [26]. It is important to understand the "induced increase in chaperone activity" especially by small molecules that are biologically compatible. We have earlier shown that arginine hydrochloride (Arg•HCl) and aminoguanidine hydrochloride, can perturb the structure and increase the chaperone-like activity of α-crystallin [27]. In the present study we have probed the mechanism.

The dynamic nature of subunit assembly appears to be one of the properties of sHsps that is important for their activity [28-31]. It is evident from earlier studies that temperature induced increase in the rate of subunit exchange in α-crystallin [32-35] parallels the temperature induced increase in its chaperone-like activity [4,21,22]. *Methanococcus jannaschii* Hsp16.5 freely and reversibly exchanges subunits at temperatures of 68 °C and above, which are physiologically relevant to the organism, and also exhibits enhanced chaperone-like activity at these temperatures [36]. In order to understand the mechanism of the arginine induced enhancement of chaper-

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one activity of α-crystallin, we have probed the effect of Arg•HCl on the quaternary structure and dynamics of α-crystallin. Our present study demonstrates that Arg•HCl enhances the subunit exchange and hence the dynamic nature of the quaternary structure of α-crystallin.

METHODS

Materials: Lucifer Yellow Iodoacetamide dipotassium salt (LYI), 4-[acetamido-4'-(iodoacetyl)amino]stilbene-2,2'-disulfonic acid disodium salt (AIAS) and 2-(4'-maleimidylanilino) naphthalene-6-sulfonic acid sodium salt (MIANS) were purchased from Molecular Probes (Eugene, OR). Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid; DTNB), arginine monohydrochloride (Arg•HCl), lysine monohydrochloride (Lys•HCl) and glycine were obtained from Sigma (Sigma Chemical Company, St. Louis, MO).

Preparation of α-crystallin: α-Crystallin was purified from bovine eye lenses as described earlier [4]. Recombinant human αA-crystallin was prepared by cloning and over expressing the protein in *Escherichia coli*, as described previously [19].

Assay for chaperone-like activity of α-crystallin: The chaperone-like activity of bovine α-crystallin in the absence or the presence of Arg•HCl, Lys•HCl, or glycine was measured at 25 °C against the DTT induced aggregation of insulin. All assays were carried out in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl. Minor changes in the pH of the buffer upon addition of Arg•HCl or Lys•HCl (<0.2 units) were not found to affect the aggregation assays. Aggregation of insulin was initiated by reducing the disulfide bonds as described below. Buffer alone or buffer containing α-crystallin (0.2 mg/ml) and the required amount of the appropriate amino acid was taken in a cuvette and incubated at 25 °C for 3 min with constant stirring using a Julabo thermostated water bath. Insulin (0.2 mg/ml) was then added to the cuvette and reduction of insulin initiated by the addition of 20 µl of 1 M DTT to 1.2 ml of the sample. The extent of aggregation was measured by monitoring 90° scattering at 465 nm using a Hitachi F-4000 Fluorescence Spectrophotometer. The excitation and emission band passes were set at 3 nm.

Accessibility of thiol groups of bovine α-crystallin and recombinant human αA-crystallin: DTNB (final concentration of 40 µM) was added to a sample of bovine α-crystallin (0.46 mg/ml) or human αA-crystallin (0.2 mg/ml) in 50 mM Tris HCl buffer (pH 7.6) containing 100 mM NaCl in the absence and in the presence of required concentrations of additives such as Arg•HCl, Lys•HCl, and glycine. The samples were equilibrated either at 25 °C or 37 °C before adding the DTNB reagent. To measure the accessibility of the thiol groups to the reagent, the optical density of the samples was measured at 412 nm as a function of time. Fractional accessibility of the thiol groups was calculated using the molar extinction coefficient of 14150 at 412 nm [37].

MIANS labeling: Recombinant human αA-crystallin (6.4 mg/ml) in 50 mM Tris HCl buffer (pH 7.4) containing 100 mM NaCl and 1 mM EDTA was incubated with MIANS (1.83 mM) at 37 °C for 2 h and the excess label was removed using a PD-10 desalting column. MIANS labeled αA-crystallin (0.45 mg/ml) was incubated with various concentrations of Arg•HCl in 50 mM Tris HCl buffer (pH 7.4) containing 100 mM NaCl and 1 mM EDTA and the fluorescence spectra recorded with excitation wavelength of 313 nm using excitation and emission band passes of 3 nm, respectively.

Subunit exchange studies: The cysteine residues in αA-crystallin were covalently labeled with the fluorescence probes, AIAS and LYI, separately by incubating the protein samples (1 mg/ml) in 20 mM MOPS buffer (pH 7.9) containing 100 mM NaCl with 250 µM of the probes at 37 °C for 18 h. The unreacted probes were removed by passing the samples through a desalting column (PD10) and eluted using 50 mM sodium phosphate buffer (pH 7.5) containing 100 mM NaCl and 1 mM DTT. The void volume fractions containing the labeled protein were pooled and their concentrations determined.

Subunit exchange experiments were performed by mixing the AIAS labeled and the LYI labeled αA-crystallin at equal concentrations (total protein concentration was 0.7 mg/ml) in 50 mM sodium phosphate buffer (pH 7.5) containing 100 mM NaCl and 1 mM DTT either in the absence or the presence of the required concentrations of Arg•HCl, Lys•HCl, or glycine. The samples were incubated at the indicated temperatures in a Julabo thermostated water bath. At different time intervals, 20 µl of sample was withdrawn and diluted to 0.4 ml with the same buffer and the fluorescence spectra recorded at room temperature using a Hitachi F4000 Fluorescence Spectrophotometer in corrected spectrum mode. The excitation wavelength was set at 332 nm and the excitation and emission band passes were both set 5 nm.

Urea induced denaturation of α-crystallin: The effect of Arg•HCl on the urea induced denaturation of bovine α-crystallin was studied by monitoring the change in its intrinsic tryptophan fluorescence as a function of urea concentration. Bovine α-crystallin (0.2 mg/ml) was incubated for 1 h at 25 °C in the absence or the presence of 300 mM Arg•HCl in 50 mM phospahte buffer, pH 7.4. Urea was added to each of the samples to obtain final concentrations ranging from 0-7 M urea and the samples incubated overnight at 25 °C. The samples were excited at 295 nm and fluorescence spectra recorded on a Hitachi F-4000 fluorescence spectrophotometer with excitation and emission band passes set at 5 nm.

RESULTS & DISCUSSION

Arg•HCl enhances the chaperone-like activity of α-crystallin at room temperature: Structural perturbation, either by temperature or by low concentrations of some denaturants, is known to increase the chaperone-like activity of α-crystallin [4,21-25] and some other sHsps [26,38-41]. Our earlier study showed that arginine hydrochloride and amino guanidine hydrochloride increase the chaperone-like activity of α-crystallin at 37 °C [27]. To understand the mechanism of the enhancement of chaperone-like activity and whether Arg•HCl induced changes mimic temperature induced changes in α-crystallin, we investigated Arg•HCl induced enhancement of

the chaperone-like activity of bovine α-crystallin (hetero-oligomer of αA- and αB-crystallin subunits in a ratio of about 3:1 [42]) and human recombinant αA-crystallin at 25 °C. Figure 1 shows the chaperone-like activity of α-crystallin and the effect of various concentrations of Arg•HCl towards the DTT induced aggregation of insulin. At equal weight ratio, α-crystallin prevents this aggregation of insulin only marginally (about 13%). Arg•HCl increases the chaperone-like activity of α-crystallin in a concentration dependent manner. At 300 mM Arg•HCl the percentage protection reaches almost 95% (Figure 1). On the other hand, Lys•HCl or glycine enhances the observed aggregation either in the absence or in the presence of α-crystallin (data not shown).

Arg•HCl increases the accessibility of the thiol group of αA-crystallin: We have shown earlier that Arg•HCl significantly decreases the oligomeric size of α-crystallin [31]. We have further probed the quaternary structural changes of bovine α-crystallin in the presence of Arg•HCl. α-Crystallins and other sHsps contain a conserved stretch of 80-100 amino acids called the "α-crystallin domain" flanked by an N-terminal domain and a C-terminal region called the "C-terminal extension" [43]. The bovine αA-subunit contains a single cysteine residue at position 131, towards the end of the "α-crystallin domain," whereas αB-crystallin subunits do not contain any cysteine residue. Only a fraction of the thiol groups in the oligomeric assembly of α-crystallin is accessible to thiol-modifying reagents and the accessible fraction increases upon perturbing the assembly by denaturants or the pH of the medium [44,45].

We have, therefore, investigated the accessibility of the thiol group of α-crystallin to the Ellman's reagent, DTNB, in the absence and in the presence of Arg•HCl, Lys•HCl, and glycine both at 25 °C and 37 °C. Figure 2A shows the accessibility of the thiol group of bovine α-crystallin at 37 °C. The fractional accessibility of the thiol group in buffer alone at 37 °C was 0.4 at 60 min, which increased marginally (to 0.45) in the presence of 200 mM Lys•HCl, whereas it decreased to 0.32 at 60 min in the presence of glycine. Arg•HCl increased both the rate and the extent of the accessibility of the thiol group in a concentration dependent manner (Figure 2A). At 200 mM Arg•HCl, for example, the fractional accessibility increased to a value of 0.6 in 60 min. An earlier study by Siezen et al. [44] found three classes of sulfhydryl groups in the oligomeric structure of bovine α-crystallin. About 25% of the sulfhydryl groups were surface exposed, the accessibility increasing to about 57% in the presence of 6 M urea, with the remaining sulfhydryl groups being inaccessible even in the presence of urea. Our results show that Arg•HCl can perturb the structure and increase the accessibility of even the second class of sulfhydryl groups in the oligomeric structure of bovine α-crystallin. Similar to the observation made at 37 °C, we also observed that Arg HCl increases the rate and the extent of accessibility of the thiol groups of bovine α-crystallin at 25 °C. The fractional accessibility of the thiol groups in buffer alone (0.19) increased to 0.31 in the presence of 200 mM Arg. HCl. Lys•HCl was comparatively less effective, whereas glycine did not significantly affect either the rate or the extent of accessibility of the thiol groups of α-crystallin (data not shown).

Thus our result shows that the accessibility of the single thiol residue of the bovine αA-subunits in α-crystallin and hence the segment towards the end of the "α-crystallin domain" is increased significantly in the presence of Arg•HCl. We have tested this phenomenon in the case of the homo-oligomer, human recombinant αA-crystallin. Human αA-crystallin, in contrast to bovine αA-crystallin, contains two cysteine residues at positions 131 and 142, which are also present towards the end of the "α-crystallin domain." Sequence based secondary structure prediction shows that the two cysteine residues may lie on β-strands of the α-crystallin domain [43]. Thus, a study of thiol accessibility would provide information regarding the exposure of these β-strands in the Arg•HCl induced changes. Figure 2B shows the effect of various additives on the fractional accessibility of the thiol groups of human αA-crystallin at 37 °C as a function of time. In buffer alone, the fractional accessibility reaches a value of approximately 0.8, indicating that the thiol groups of human αA-crystallin are more accessible compared to that of bovine α-crystallin. Whereas glycine decreases the accessibility, Lys•HCl seems not to affect the accessibility of the thiol groups. As observed in the case of bovine α-crystallin, Arg•HCl increases both the rate and the extent of accessibility of the thiol residues of human αA-crystallin to DTNB suggesting that the two putative β-strands in the "α-crystallin domain" become exposed in the presence of Arg•HCl. All the sulfhydryl groups become accessible to DTNB in the presence of 300 mM Arg•HCl (fractional accessibility of 1.0). Augusteyn et al. [45] found that in the presence of 4 M urea, all the sulfhydryl groups in human α-crystallin become accessible to DTNB [45].

Arg•HCl increases the dynamics of the subunit assembly of α-crystallin: There are two possible mechanisms for the

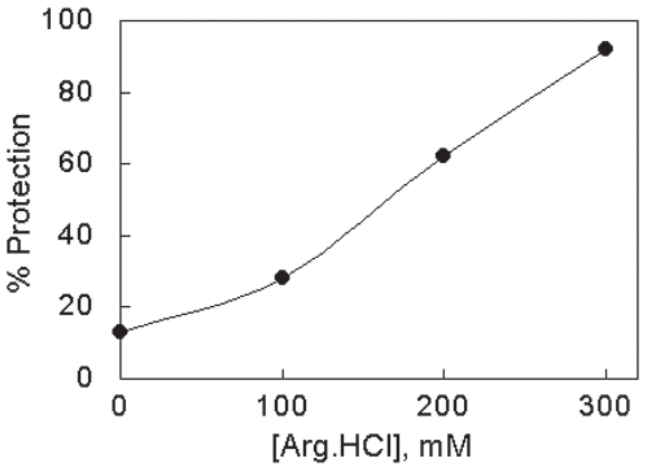

Figure 1. Effect of arginine hydrochloride on the chaperone-like activity of α-crystallin. The percentage protection is the suppression of DTT-induced insulin aggregation (at 25 °C) by bovine lens α-crystallin with respect to the aggregation of insulin in buffer alone or in the presence of indicated concentrations of argining hydrochloride.

observed increase in the rate and accessibility of the thiol groups to DTNB in the presence of Arg•HCl: (i) Arg•HCl induces a conformational change around the region containing the two cysteine residues present near the end of the "α-crystallin domain" in αA-crystallin or (ii) Arg•HCl loosens the quaternary structural arrangement and increases the dynamic property of the oligomeric assembly. In order to investigate these aspects, we have labeled the thiol groups of human αA-crystallin with the polarity sensitive fluorescent probe, MIANS. Free MIANS exhibits little or no fluorescence, but upon covalently linking to a thiol group its fluorescence increases dramatically [46]. The fluorescence property of the ANS moiety is sensitive to the polarity of its microenvironment. Its emission maximum blue shifts, accompanied by an increase in fluorescence intensity, in a less polar environment [46]. We found that the fluorescence spectra of MIANS labeled αA-crystallin in the absence and in the presence of 300 mM Arg•HCl differ only marginally. The emission maximum of the spectrum in buffer alone is 430.6 nm, whereas in the presence of Arg•HCl it is red shifted to 432.8 nm without significantly affecting the fluorescence intensity. This result indicates only marginal (yet detectable) change in the polarity of the microenvironment

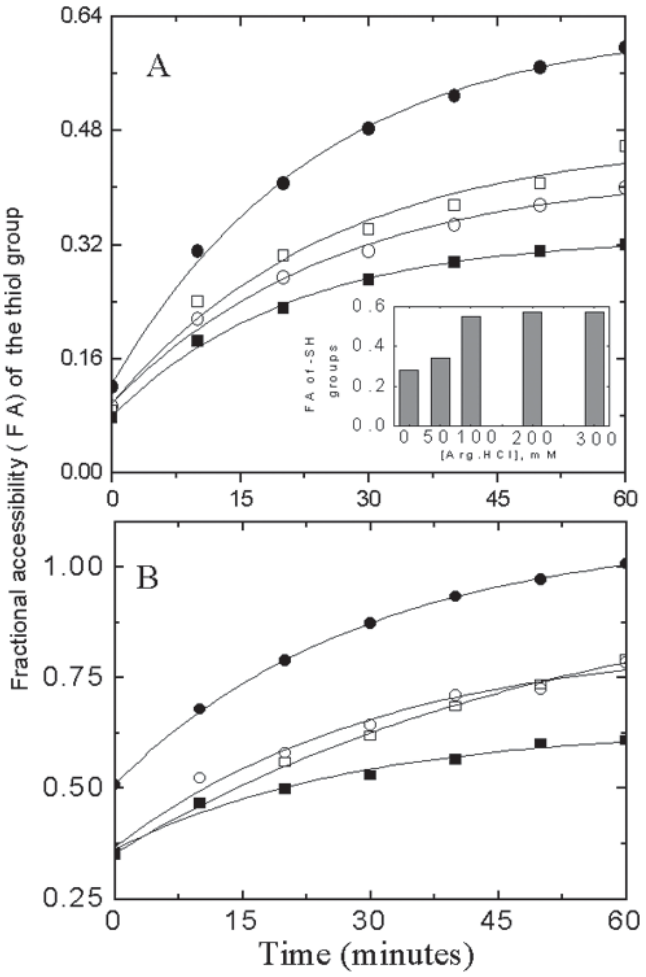

Figure 2. Effect of arginine hydrochloride on thiol group accessibility. Accessibility or reactivity of the thiol group of the cysteine residue of bovine α-crystallin (**A**) and human recombinant αA-crystallin (**B**) to the Ellman's reagent (DTNB) at 37 °C. In both the panels the accessibility of the protein thiol group in buffer alone (open circle) in buffer containing 200 mM Arg•HCl (closed circle), 200 mM Lys•HCl (open square) and 200 mM glycine (closed square). The inset in **A** shows the effect of various concentrations of Arg•HCl on the accessibility of the thiol group of bovine α-crystallin at 37 °C. The fractional accessibility of thiol in bovine α-crystallin was calculated using a 3:1 molar ratio of the αA-crystallin and αB-crystallin subunits in the hetero assembly [42].

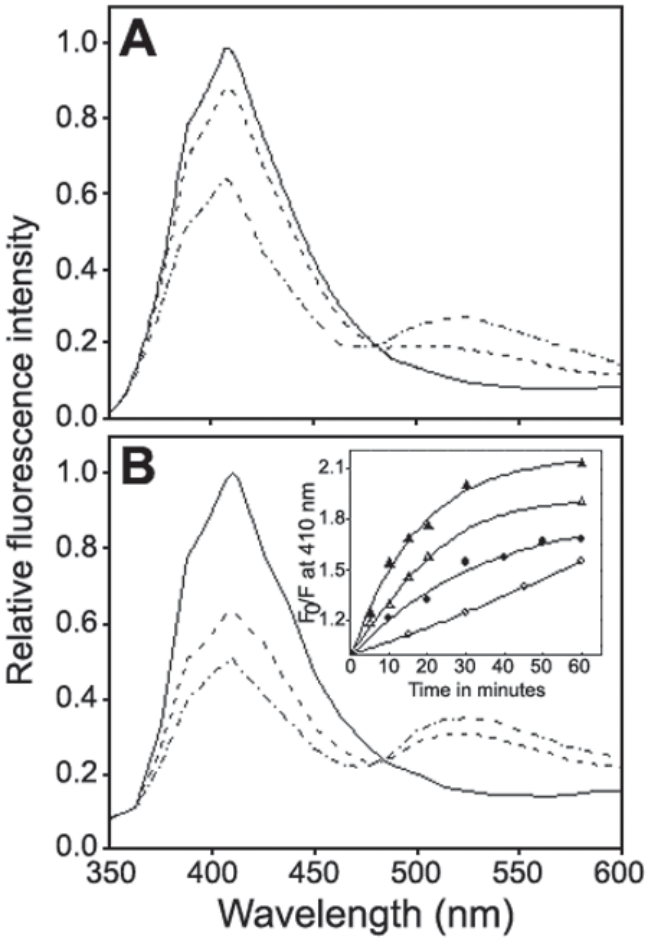

Figure 3. Effect of Arg•HCl on the dynamic property of the subunit assembly of recombinant human αA-crystallin at 37 °C. Subunit exchange between the oligomers of the protein was monitored by Forster Resonance Energy Transfer (FRET) between the donor fluorescent probe, AIAS labeled αA-crystallin and the acceptor fluorescent probe, LYI labeled αA-crystallin. The sample was excited at the donor excitation wavelength of 332 nm and the emission spectrum was recorded using the excitation and emission band passes of 3 nm in corrected spectrum mode. Subunit exchange was observed in buffer alone (**A**) and in the presence of 300 mM Arg•HCl (**B**). In both the panels, fluorescence spectra recorded immediately (within 2 min) after mixing the donor and acceptor probe labeled αA-crystallin (solid line); after 30 min, (dashed line); and after 60 min, (dash-dot line) incubation. Inset shows the plot of F_0/F as a function of time where F_0 and F are the initial fluorescence of the donor and that at a given time at 410 nm in buffer alone (open circle) and in buffer containing 100 mM Arg•HCl (closed circle), 200 mM Arg•HCl (open triangle), and 300 mM Arg•HCl (closed triangle).

around the thiol group, and hence the region towards the end of the "α-crystallin domain." However, this minor change alone cannot account for the drastic change in the accessibility of the thiol group.

We have, therefore, investigated the second possibility. Many sHsps including α-crystallin exhibit subunit exchange between oligomers [28,33-36,47]. We have monitored the effect of Arg•HCl on the subunit exchange of bovine α-crystallin and human αA-crystallin, following the method of Bova et al. [33] using fluorescence resonance energy transfer (FRET). When the AIAS labeled bovine α-crystallin was mixed with the LYI labeled bovine α-crystallin, we could only see a marginal reduction in the donor fluorescence and a slight increase in the acceptor fluorescence (data not shown) even after prolonged incubation at 37 °C, where subunit exchange is known to occur [28]. Absence of FRET, despite the subunit exchange, suggests that the labeled subunits are not proximal even after the exchange, perhaps due to the presence of the unlabelled αB-crystallin subunits in the assembly.

We have, therefore, labeled the thiol groups of human αA-crystallin with these fluorescent probes and investigated the subunit exchange at 37 °C. This system exhibits fluorescence resonance energy transfer similar to that observed by Bova et al. [33] with rat αA-crystallin. Figure 3A shows that the fluorescence band of the acceptor LYI in the 480-600 nm region is not observed immediately upon mixing of the AIAS labeled and LYI labeled αA-crystallin, but starts appearing as a function of incubation time, accompanied by a progressive decrease in the donor fluorescence. Figure 3B shows the changes in the fluorescence spectra of the donor and the acceptor probes in the presence of 300 mM Arg•HCl; the inset in the figure compares the subunit exchange kinetics, as monitored by FRET, in buffer alone and in the presence of increasing concentrations of Arg•HCl. As the exchange progresses, the donor fluorescence decreases and the intensity ratio F_0/F increases progressively. When the exchange reaction is performed in buffer alone, this value reaches about 1.5 at 60 min. As evident from Figure 3B, the rate and the extent of subunit exchange in the oligomeric assembly of human αA-crystallin is significantly enhanced in the presence of Arg•HCl. The F_0/F value reaches approximately 2.15 at 60 min in the presence of 300 mM Arg•HCl. On the other hand, we found that, whereas Lys•HCl only marginally increased the rate even at concentration as high as 300 mM (F_0/F is approximately 1.6 at 60 min), glycine did not significantly affect the subunit exchange rate (data not shown). Thus, our results clearly show that Arg•HCl enhances the rate and extent of subunit exchange and hence the dynamics of the subunit assembly of αA-crystallin.

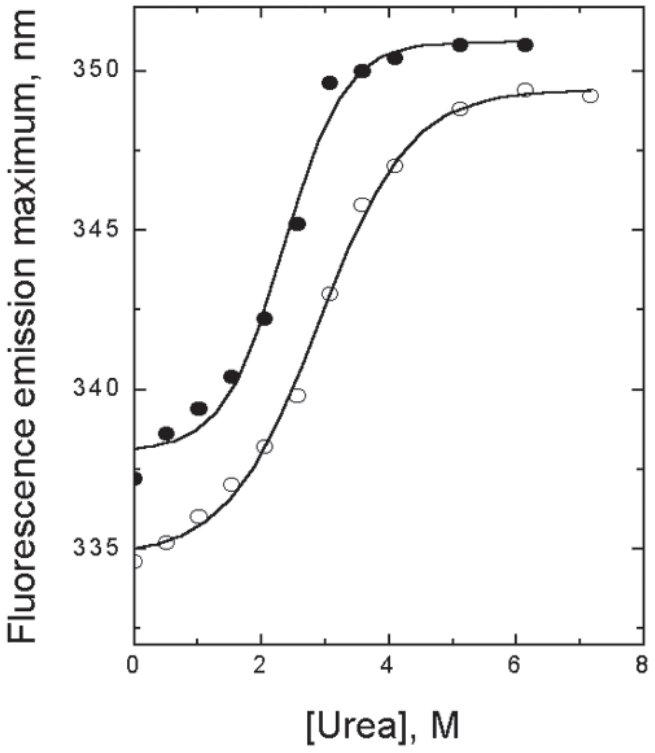

Figure 4. Effect of Arg•HCl on the denaturation of bovine α-crystallin by urea at 25 °C. The open circle indicates α-crystallin alone, the closed circle indicates α-crystallin plus 300 mM Arg•HCl. The α-crystallin concentration was 0.2 mg/ml, the excitation wavelength was 295 nm, and the excitation and emission band passes were each set at 5 nm.

It has been demonstrated that subunit exchange rates of α-crystallin are low at lower temperatures and increase with increasing temperatures [28,32]. This temperature induced increase in subunit exchange rate [28,32-35] correlates with the temperature induced increase in chaperone-like activity [4,21,22]. Since our study showed that Arg•HCl increased the chaperone-like activity of α-crystallin even at a low temperature (25 °C, Figure 1), we have investigated whether Arg•HCl also increased the rate of subunit exchange at this temperature. Subunit exchange of α-crystallin is significantly less at 25 °C compared to 37 °C. The F_0/F value at 410 nm is about 1.1 upon 90 min incubation. We found that Arg•HCl, indeed, enhances the dynamics of subunit exchange as the F_0/F value increases to about 1.4 in the presence of 300 mM Arg. HCl (data not shown).

Arg•HCl induced structural perturbation leads to destabilization of α-crystallin: We have also investigated whether the presence of Arg•HCl brings about structural destabilization of α-crystallin. Figure 4 shows the urea induced denaturation of bovine α-crystallin in the absence and in the presence of 300 mM Arg•HCl as monitored by change in the intrinsic tryptophan fluorescence of the protein. In the absence of urea, the fluorescence spectrum of α-crystallin is only marginally affected by 300 mM Arg•HCl with a 2-3 nm red shift in the emission maximum. It is seen from the urea induced denaturation profile (Figure 4) that in the presence of Arg•HCl, the transition is at a significantly lower concentration of urea (about 2.2 M) than in its absence (about 3 M), indicating that Arg•HCl can considerably destabilize the structure of α-crystallin.

Dynamics of subunit assembly and chaperone function of small heat shock proteins: Subunit exchange between homomultimers and between two different sHsps to form heteromultimers seems to have some functional significance. Heteromultimeric forms among αB-crystallin, αA-crystallin, Hsp27,

and Hsp22 have been found to occur [28,33,34,36,47,48]. The dynamic properties of subunit assembly in sHsps are important for their activity [28-31]. Reversible exchange of subunits at physiologically relevant temperatures appears to be important in the case of α-crystallin [28-31] and *Methanococcus jannaschii* Hsp16.5 [36]. Our present study shows that Arg•HCl increases the rate of subunit exchange in α-crystallin. Such a dynamic behavior also accompanied by a decrease in the multimeric size of the protein [27] similar to that observed at elevated temperatures [5,49]. One of the current hypotheses suggests that a dissociation mechanism may be involved in the subunit exchange of many members of the small heat shock family [36]. For instance, Hsp 16.3 from *Mycobacterium tuberculosis* is a nonamer at normal temperatures. It dissociates at elevated temperatures, accompanied by a greatly increased chaperone-like activity [38]. Similarly, Hsp 26 from *Saccharomyces cerevisiae*, which exists as a large oligomer at physiological temperatures, dissociates under heat shock conditions to smaller oligomers which are active in binding to unfolding proteins [31].

Based on our findings we propose that guanidinium compounds such as Arg•HCl, can perturb the structure of α-crystallin. This results in enhanced accessibility of its thiol group(s) and hence the region towards the end of the "α-crystallin domain," bringing about subtle changes in the tertiary structure, leading to increased exposure of hydrophobic surfaces and enhanced chaperone-like activity. More importantly, Arg•HCl destabilizes the multimeric assembly of α-crystallin and increases the rate and extent of subunit exchange. This suggests that altering the dynamics of the α-crystallin subunit assembly by Arg•HCl plays a vital role in the observed enhanced activity of the molecule. These studies show that small molecules such as Arg•HCl, which are biologically compatible, may find potential use in improving the chaperone function and possible therapeutic applications.

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