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The IXI/V motif in the C-terminal extension of α-crystallins: alternative interactions and oligomeric assemblies

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Purpose: α-Crystallin, a hetero-oligomer of αA- and αB-crystallin, is involved in maintaining eye lens transparency, primarily by its structural packing and chaperone activity. αA- and αB-crystallin share significant sequence homology, which is almost exclusively restricted to the central, conserved "αA-crystallin domain". The flanking N-terminal domain and C-terminal extension are highly variable both in sequence and length. Mutations and age-related post-translational modifications of these proteins are associated with congenital and age-onset cataracts. Interestingly, most mutations or truncations in the C-terminal extensions of the α-crystallins and other α-sHsps like Hsp27 lead to pathology. It is therefore important to understand the structure/function relationship of this region. Sequence alignment of the C-terminal extensions of αA- and αB-crystallin with other homologues shows a conserved IXI/V motif. The purpose of this study was to investigate the role of this conserved motif, IPV in αA-crystallin and IPI in αB-crystallin (corresponding to residues 159-161 in both crystallins), in the structure and chaperone activity.

Methods: The isoleucine/valine residues in the IPV motif of αA-crystallin and the IPI motif of αB-crystallin were mutated to glycine and studied the secondary and tertiary structure of the mutant proteins using circular dichroism and fluorescence spectroscopy, and the quaternary structure using glycerol density gradient centrifugation and dynamic light scattering. Chaperone activity was studied at 37 °C and 25 °C using DTT induced aggregation of insulin as a model system. We have performed fluorescence resonance energy transfer (FRET) experiments to investigate the interactions of this motif in homo- and hetero-oligomers. Since αB-crystallin is devoid of Cys residues, we have introduced a Cys residue flanking the IPI motif (T162CαB-crystallin) to facilitate fluorescence labeling studies.

Results: Unlike in other homologues from plants or prokaryotes, mutation of the isoleucine/valine residues in α-crystallins does not result in oligomer dissociation or loss of chaperone activity. On the contrary, the mutant proteins retain their capacity to oligomerize and show enhanced chaperone activity at 37 °C. The mutants also exhibit significantly higher chaperone-like activity at 25 °C. FRET experiments show that the region spanning the IPI/V motif comes in proximity either to the β-strands of the "α-crystallin" domain or the corresponding IPI/V region of another subunit.

Conclusions: Our mutational studies show that the IPI/V motif has a propensity to participate in inter-subunit interactions, either with regions in the α-crystallin domain or with the corresponding IPI/V region on another monomer. These interactions are important in the structure and function of α-crystallins. This motif also appears to be important in the temperature dependent chaperone-like activity of the α-crystallins. The propensity of the IPI/V motif to form multiple inter-subunit interactions may contribute to the diversity in structure and function seen in the α-crystallin/sHsp family.

α-Crystallin is isolated as a polydisperse, hetero-oligomeric complex of about 800 kDa from the mammalian eye lens [1]. In the oligomer, the 2 gene products, αA- and αB-crystallin are present in a 3:1 M ratio [2]. αA-Crystallin is mainly lenticular and is not stress inducible. αB-Crystallin is found in several other tissues and is induced under stress and disease conditions [3-6]. Both proteins have been implicated in several cellular processes such as maintaining the refractile properties of the lens, development, controlling cytoskeletal protein dynamics, and inhibiting apoptosis (See review [7]).

αA- and αB-crystallin show chaperone-like activity in preventing protein aggregation [8-11]. They interact with partially unfolded, molten globule-like intermediates of proteins via appropriately placed hydrophobic binding sites [12-14]. The α-crystallins also protect enzymes from heat induced inactivation [15-18]. Their differential and reversible interaction with early unfolding and refolding-competent intermediates of target proteins decreases their partitioning into aggregation-prone late unfolding intermediates [14,15,19]. The chaperone activity of the α-crystallins is temperature dependent, a structural transition at 30-40 °C leads to enhanced chaperone activity by increasing or favorably reorganizing the hydrophobic substrate-binding surfaces [10,11,20-23]. Such transitions at physiological heat-shock temperatures may be important for its function in vivo. It is believed that in the cell, the soluble α-crystallin-substrate complexes may serve as reservoirs that "hold" non-native substrates in a folding-competent state to be refolded by other chaperone systems (See review [24]). The chaperone activity of the α-crystallins appears to be important for its function in vivo as mutations in these genes (R116C in αA-crystallin and R120G in αB-crystallin) lead to congenital cataract and desmin-related myopathy, respectively [25,26]. These mutations result in altered protein structure and significant loss of chaperone activity [27,28].

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αA- and αB-Crystallin show the presence of a central conserved "α-crystallin domain" Proteins having this domain are referred to as the α-crystallin small heat shock proteins (α-sHsps) [29]. The "α-crystallin" domain is involved in inter-subunit contacts, mainly responsible for forming lower order oligomers like dimers and trimers, which are devoid of chaperone activity [30,31]. The functional entities of most α-sHsps, however, are large oligomeric assemblies. The regions flanking the α-crystallin domain are highly variable in sequence and length. The N-terminal domain is responsible for assembly into higher order functional oligomers and the charged C-terminal extensions are thought to keep the α-sHsp-target protein complex in solution [30-32]. However, a recent study from our laboratory shows that swapping the C-terminal extensions between αA- and αB-crystallin resulted in alteration of overall structure and chaperone activity [33], suggesting an additional role(s) for the C-terminal extensions.

Several earlier reports have shown that truncations in the C-terminal extensions of α-sHsps are mainly responsible for causing myofibrillar myopathy and cataract [34-36]. Comparison of the sequences of the C-terminal extensions of several α-sHsps shows a motif, IXI/V, that appears to be highly conserved [29]. Mutations of the isoleucine residues in the IXI/V motif and truncations spanning this region in some bacterial and plant α-sHsps have been shown to result in dissociation of the subunits and loss of chaperone-like activity [37,38]. In order to understand the role of the IPI/V motif of αA- and αB-crystallin, we have mutated the isoleucine and valine residues to glycine in both proteins. The mutant proteins, I159G-V161GαA- and I159G-I161GαB-crystallin, are referred to as αAgxg and αBgxg, respectively. We have studied the effect of these mutations on subunit interactions, structure, and chaperone-function. Interestingly, in contrast to other sHsps, mutations in the IPI/V motif of the α-crystallins do not result in oligomer dissociation or loss of chaperone-like activity. The mutant proteins retain their ability to oligomerize and show increased chaperone activity. FRET experiments indicate that the IPI/V motif can come proximal to regions in the α-crystallin domain, similar to those seen in crystal structures of *M. jannashii* Hsp16.5 and wheat Hsp16.9. It can also come in proximity to the IPI/V motif of another monomer suggesting its propensity to engage in additional or alternate interactions. Such multiple interactions of regions like the IXI/V motif with different parts of the α-crystallin oligomer may be responsible for the differences in oligomer size, polydispersity and diversity in function seen in human α-crystallins from those of the plant and bacterial α-sHsps.

METHODS

Materials: pET-21a(+), T7 promoter and terminator primers were obtained from Novagen (Madison, WI), pBS(II)SK from Stratagene (La Jolla, CA), and insulin and bis-ANS from Sigma Chemical Company (St. Louis, MO). Sephacryl HR-300, Q-Sepharose, and Phenyl Sepharose were purchased from AP-Biotech (Uppsala, Sweden). Dithiothreitol (DTT) was obtained from Sisco Research Laboratories (Mumbai, India). The disodium salt of 4-acetamido-4'-((iodoacetyl)amino)stilbene-2,2'-disulfonic acid (AIAS) and the dipotassium salt of lucifer yellow iodoacetamide (LYI) were purchased from Molecular Probes, Inc. (Eugene, OR).

Creating mutants of αA- and αB-crystallin: Recombinant human αA- and αB crystallin genes cloned in pET-21a(+) were used as templates to generate mutants using polymerase chain reaction (PCR). For αAgxg, two independent PCRs were performed using T7 promoter primer and the mutagenic primer 5'-CTC CCG CGA CCC GGG GCC GGC TCG CTC-3' as one primer pair and 5'-GAG CGA GCC GGC CCC GGG TCG CGG GAG-3' and T7 terminator primer as the second primer pair. The resulting fragments were ligated in the *Nde*I and *Hin*dIII sites of the pET-21(+) expression vector. The same strategy was employed for αBgxg using T7 promoter primer and 5'-CCT GAG CGC ACC GGT CCC GGC ACC CGT GAA GAG-3' and 5'-CTC TCA CGG GTG CCG GGA CCG GTG CGC TCA GG-3' and the T7 terminator primer and for T162CαB-crystallin using T7 promoter primer and 5'-CTT CTC TTC ACG GCA G AT GGG AAT GGT-3' and 5'-ACC ATT CCC ATC TGC CGT CGT GAA GAG AAG-3' and T7 terminator primer as the primer pairs. These constructs were verified by sequencing using a 3700 ABI automated DNA sequencer.

Expression and purification of the recombinant wild-type and mutant proteins: The wild type and the mutant recombinant proteins were over expressed in *Escherichia coli* BL21 (DE3) cells and purified essentially as described earlier [33]. All bacterial lysates except that of αAgxg were sonicated to shear the genomic DNA. αAgxg precipitated out on sonication, therefore, after cell lysis by lysozyme, 7 μg/ml DNaseI was used to fragment the genomic DNA. The wild type proteins and T162CαB-crystallin precipitate with 30-60% saturated ammonium sulfate, while both gxg mutant proteins precipitated with 15-35% saturated ammonium sulfate. The protein pellets thus obtained were dissolved in 50 mM Tris-HCl, pH 7.2, containing 100 mM NaCl and 1 mM EDTA (TNE) buffer. The T162CαB-crystallin ammonium sulfate pellet remained insoluble in TNE buffer and was solubilized in the presence of 5 mM DTT. DTT was not included in all further steps of purification. Except T162CαB-crystallin, all ammonium sulfate fractionated proteins were loaded on to a Sephacryl HR-300 gel filtration column (1.8x130 cm). The fractions containing α-crystallin were pooled and subjected to ion exchange chromatography using Mono Q. T162CαB-crystallin was directly subjected to ion exchange chromatography. αAgxg was further purified using hydrophobic interaction chromatography on Phenyl Sepharose in the same TNE buffer, and eluting it with 50% ethylene glycol. The purified proteins were dialyzed against the TNE buffer and concentrated by ultra filtration. The purity of the wild type and deletion mutant proteins was checked by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and the proteins were found to be homogeneous. The concentrations of the protein samples were determined by the method described by Pace, et al. [39].

Chaperone assays: The chaperone-like activity of the wild type and mutant proteins was studied using insulin as the target protein as described earlier [20].

Fluorescence studies: Intrinsic tryptophan fluorescence spectra of the wild type and the mutant α-crystallins (0.15 mg/ml) in 10 mM sodium phosphate buffer (pH 7.4) containing 100 mM NaCl were recorded using a Hitachi F-4000 fluorescence spectrophotometer with excitation and emission band passes set at 5 nm and 3 nm, respectively at an excitation wavelength of 295 nm.

Subunit exchange studies were performed essentially as described earlier [40]. The cysteine residues in αA- and T162CαB-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 and the probes (250 μM) at 37 °C for 18 h. Excess probes were removed by passing the samples through a desalting column (PD10), 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 protein and the LYI labeled protein at equal concentration (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 and incubating at 37 °C in a Julabo thermostated water bath. Sample (20 μl) was withdrawn at different time intervals and diluted to 0.4 ml with the same buffer and the fluorescence spectrum was recorded at 25 °C with the excitation wavelength of 332 nm and excitation and emission band passes of 5 nm each. All fluorescence spectra were recorded in the corrected spectrum mode.

Circular dichroism studies: Near and far UV circular dichroism (CD) spectra of the wild type and the mutant α-

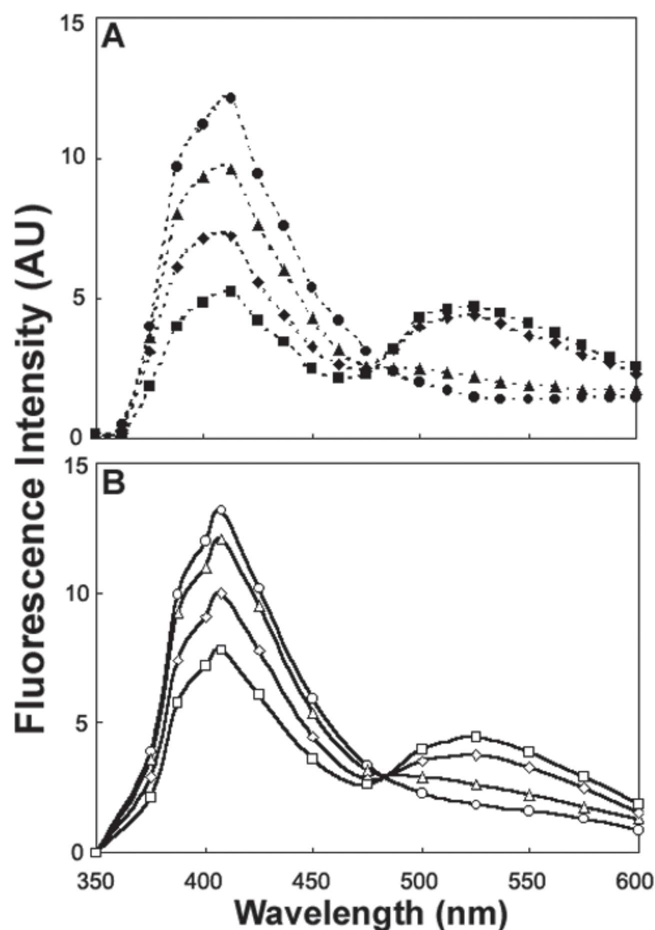

Figure 1. Subunit-exchange as monitored by FRET. **A**: Subunit-exchange between AIAS (donor) labeled T162CαB-crystallin and LYI (acceptor) labeled αA-crystallin at 37 °C. The dashed line spectra (filled circle) was recorded immediately after mixing, the filled triangle was after 10 min incubation, the filled diamond was after 60 min incubation, and the filled square was after 90 min incubation. **B**: Subunit-exchange between AIAS labeled and LYI labeled T162C αB-crystallin. The thick solid line spectra was recorded (open circle) immediately after mixing, the open triangle was after 10 min incubation; the open diamond was after 30 min incubation; and the open square was after 60 min incubation.

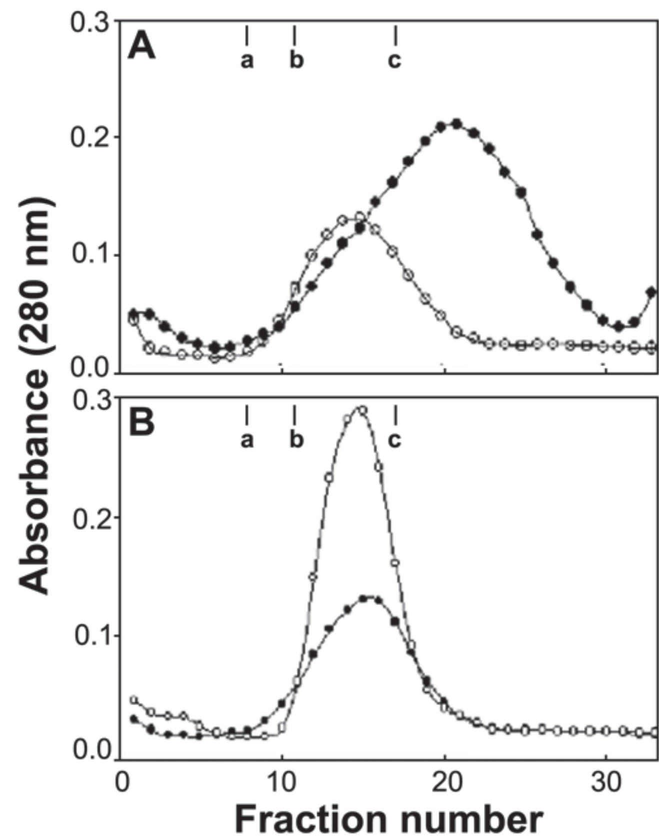

Figure 2. Glycerol density gradient centrifugation of wild type α-crystallins and the gxg mutants. The proteins were sedimented through a linear 10-40% gradient of glycerol. **A**: αA-crystallin (open circle) and αAgxg-crystallin (filled circle). **B**: αB-crystallin (open circle) and αBgxg-crystallin (filled circle). The positions of proteins used for standard molecular masses are also indicated. The marker band at "a" indicates aldolase (158 kDa), the marker band at "b" indicates catalase (232 kDa), and the marker band at "c" indicates thyroglobulin (669 kDa).

crystallins were recorded using a JASCO J-715 spectropolarimeter. Experiments were performed with 1.0 mg/ml of protein in TNE buffer using a 1 cm path length cell for near UV region and 0.01 cm path length cell for far UV region. All spectra reported are the average of 5 accumulations.

Sedimentation through glycerol gradient: αA- or αB-Crystallin and the deletion mutants (1 mg) in TNE buffer were loaded on a linear gradient of glycerol (10-40%). Density gradient centrifugation was carried out essentially as described earlier [41,42]. Proteins with defined molecular masses such as thyroglobulin (669 kDa), catalase (232 kDa) and aldolase (158 kDa) were used as standards.

Dynamic light scattering studies: The hydrodynamic radii of the wild type and the mutant α-crystallins measured using a DynaPro MS/X Dynamic Light Scattering Instrument from Protein Solutions Inc. (Charlottesville, VA). The α-crystallin samples (2 mg/ml) were filtered through a 0.22 μ membrane and measurements made at 22 °C. The data were analyzed using graphical size analysis software (Dynamics; Protein Solutions Inc.), provided with the instrument.

RESULTS & DISCUSSION

The C-terminal extension of several α-sHsps shows the presence of a conserved IXI/V motif [29]. Truncations and mutations in the C-terminal extension of some α-sHsps have been shown to cause myopathies and cataract [34-36]. In fact, mutation of the Proline residue in this motif of human Hsp27 leads to Charcot-Marie-Tooth disease and distal hereditary motor neuropathy [36]. Crystal structures of *Methanococcus jannaschii* Hsp16.5 and wheat Hsp16.9, both belonging to α-sHsp family, show this motif to be in a β-strand. The isoleucine and valine residues of this β-strand participate in specific inter-subunit contacts with a hydrophobic groove formed by the β4 and β8 strands on a monomer of the interacting dimer. The orientation of the C-terminal extension relative to the α-crystallin domain in the two assemblies however is different, resulting in different oligomeric symmetries [43,44]. Since mammalian sHsps differ considerably in their oligomeric assemblies from those of plants and prokaryotes [37,38,45], we have investigated the possible interaction(s) of the IPI/V motif in αA- or αB-crystallin and their effect on oligomeric assembly and chaperone-like activity.

FRET is useful in studying proximal interaction of regions from different part(s) of molecules. Human αA-crystallin contains two cysteine residues in its "α-crystallin" domain at positions 131 and 142 present in a region predicted to have a propensity to form β-strands. As αB-crystallin does not contain cysteine residues in its sequence, we have introduced a cysteine residue in place of the threonine residue at position 162 (T162C), adjacent to the IPI motif, to study interactions with (i) regions around the cysteine residues in the "α-crystallin" domain using αA-crystallin and (ii) the region spanning the IPI motif using T162CαB-crystallin.

We have labeled the T162CαB-crystallin with the fluorescent probe, AIAS (donor), and αA-crystallin with LYI (acceptor). As the donor and acceptor fluorophores are on different crystallins, this study will only reflect on the hetero-intersubunit interactions, if any. Energy transfer would indicate

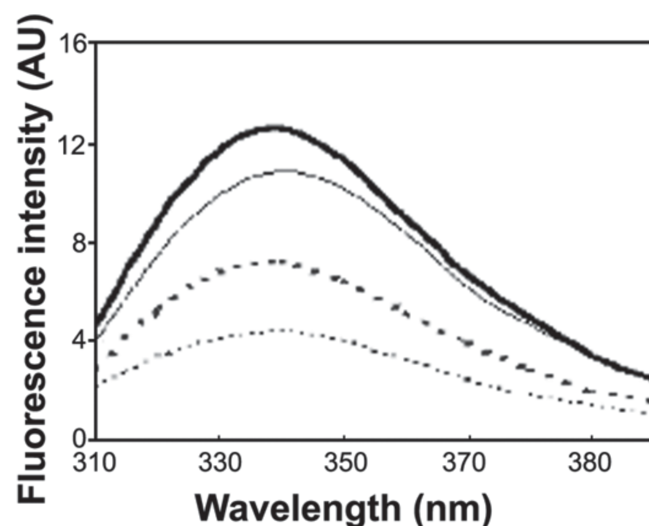

Figure 3. Fluorescence spectra of wild type and mutant α-crystallins. Intrinsic fluorescence spectra of the wild type and mutant α-crystallins at 0.15 mg/ml. αA-crystallin (thick dotted line), αAgxg-crystallin (thin dotted line), αB-crystallin, (thick solid line), and αBgxg-crystallin (thin solid line).

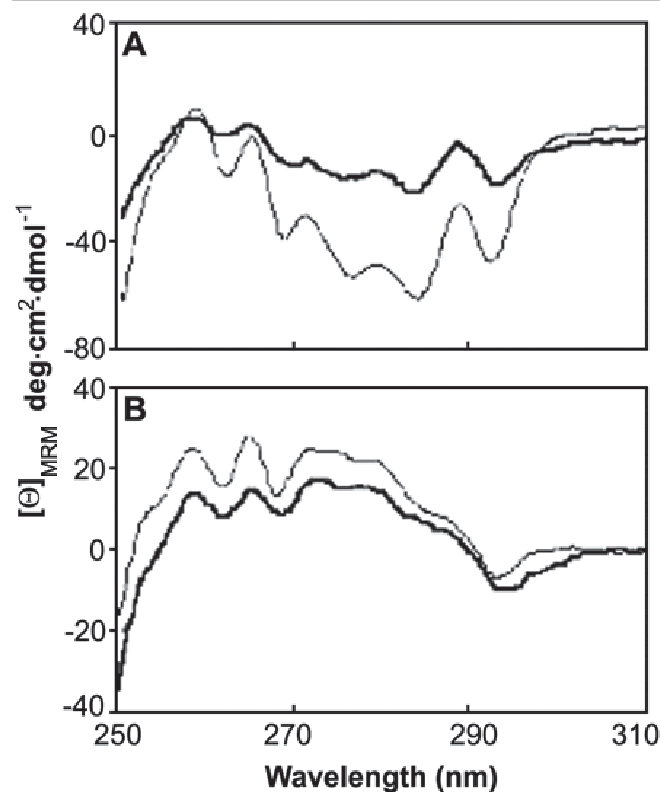

Figure 4. Near UV CD of wild type and mutant α-crystallins. **A**: Near UV CD of wild type αA-crystallin (thick line) with αAgxg (thin line). **B**: Near UV CD of wild type αB-crystallin (thick line) and αBgxg (thin line), respectively.

proximity of the labeled regions, suggesting interaction of the IPI motif of αB-crystallin with the α-crystallin domain of αA-crystallin. Figure 1A shows that the fluorescence of the donor probe (350-470 nm region) progressively decreases while the fluorescence of the acceptor probe (470-600 nm region) increases upon incubating the mixture of the labeled αA-crystallin and T162CαB-crystallin at 37 °C. This result indicates that the IPI motif of αB-crystallin comes in proximity with the region around the cysteine residues located in β-strands towards the end of the "α-crystallin" domain of αA-crystallin, suggesting possible interaction. It is interesting to note that cysteine 131 of αA-crystallin lies in a β-strand corresponding to the β8-strand of sHsps 16.5 and 16.9 suggesting the possibility of a similar inter-subunit interaction in α-crystallins [43,44].

FRET experiments with AIAS and LYI labeled T162CαB-crystallin also show energy transfer upon incubating the mixture of the labeled T162CαB-crystallins at 37 °C as a function of time (Figure 1B), indicating that the residues in the β-strand containing the IPI motif in 2 subunits of αB-crystallin also come in proximity. Thus, in addition to the possible interactions with the α-crystallin domain, the IPI/V motif may also form alternative interactions with the corresponding IPI/V motif in the β-strand in the C-terminal extension of an adjacent monomer.

Additionally, our laboratory as well others using FRET [40,42] have shown that the region containing the cysteine residues (at positions 131 and 142) in the "α-crystallin" domain of αA-crystallin interacts with the corresponding region in another monomer. Using two-hybrid studies, Wotton, et al. [46] showed that the multimerization of Hsp42p of *Saccharomyces cerevisiae* also involves this conserved region towards the C-terminal end of the protein. Thus, such alternative interactions may be responsible for the observed differences in oligomer size, assembly and high polydispersity in α-crystallins compared to their plant and bacterial counterparts.

We have mutated the hydrophobic isoleucine and valine residues in the IPI/V motif to glycine. The resultant mutant proteins, αAgxg and αBgxg, interestingly, precipitated at lower ammonium sulfate saturation (15-35%) compared to the wild-type proteins (30-60%), suggesting considerable differences in columbic interactions and solubility.

Glycerol density gradient centrifugation studies show that αAgxg sediments faster than wild type αA-crystallin (Figure 2A), indicating that αAgxg forms significantly higher molecular mass species compared to αA-crystallin. The estimated

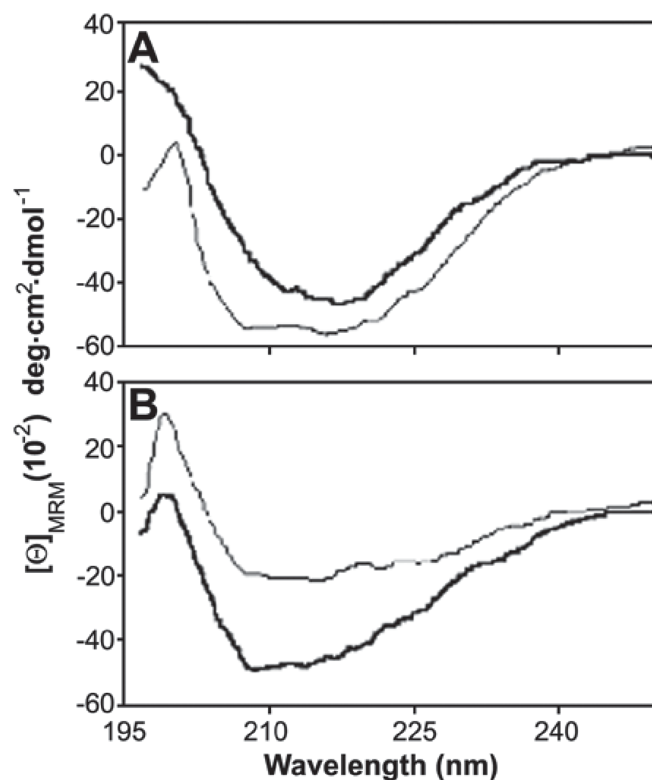

Figure 5. Far UV CD of wild type and mutant α-crystallins. **A**: Far UV CD of wild type αA-crystallin (thick line) with αAgxg (thin line). **B**: Far UV CD of Wild type αB-crystallin (thick line) and αBgxg (thin line), respectively.

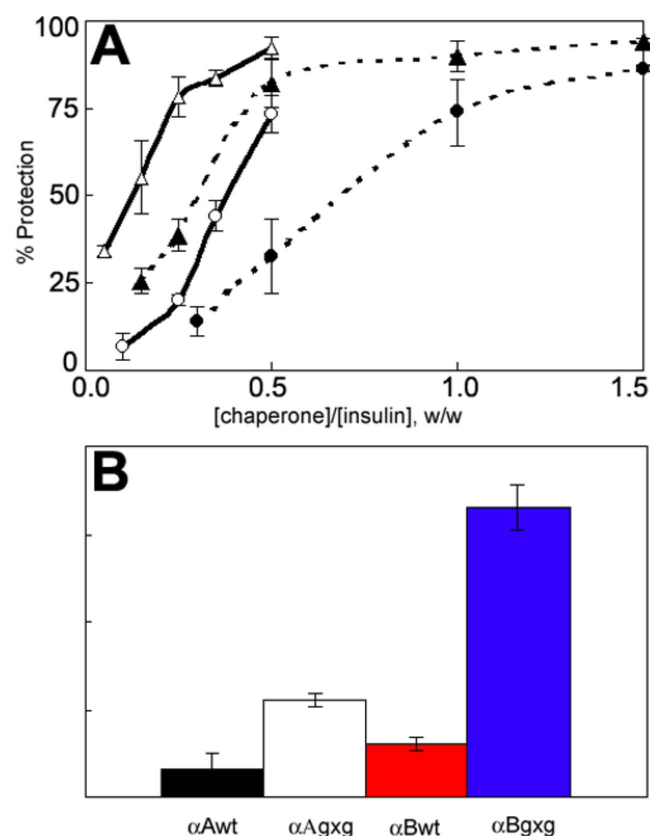

Figure 6. Chaperone activity of wild type and mutant α-crystallins.. **A**: Chaperone activity (represented as percent protection) was measured at different ratios of chaperone to insulin at 37 °C. Wild type αA-crystallin (closed circle, dashed line), αAgxg (open circle, dashed line), wild type αB-crystallin (closed triangle, solid line), αBgxg (open triangle, solid line). **B**: Chaperone activity was measured at a 1:1 ratio of chaperone to insulin (w/w) at 25 °C.

molecular masses of αAgxg and αA-crystallin are about 1,500 kDa and about 485 kDa, respectively. However, αBgxg sediments only marginally faster than wild type αB-crystallin, their estimated molecular masses being about 532 kDa and about 442 kDa, respectively (Figure 2B). The sedimentation profiles also indicate increased polydispersity for αAgxg and αBgxg. Our dynamic light scattering data show that the hydrodynamic radius (Rh) and polydispersity of αAgxg (Rh=51.28 nm, polydispersity=66.5%) are dramatically higher than those of αA-crystallin (Rh=8.88 nm, polydispersity=22.17%). αBgxg (Rh=10.06 nm, polydispersity=36.55%) is also larger and more polydisperse than αB-crystallin (Rh=7.85 nm, polydispersity=18.2%). These results clearly show that the IXI/V motif in αA- and αB-crystallin is an important determinant of the oligomer size and assembly and unlike in plant or bacterial sHsps, mutation of the IXI/V motif does not lead to dissociation of the oligomeric assembly suggesting that in αA- and αB-crystallin there are additional interactions, probably in the N-terminal region which still hold the oligomer together. Interactions of this motif in αA- and αB-crystallin may be involved in generating different type(s) of oligomeric assembly using different subunit-interfaces.

Tryptophan fluorescence spectra of the gxg mutants show considerable decrease in fluorescence intensity compared to their wild type proteins (Figure 3). The near UV CD spectra of αAgxg and αBgxg show enhanced ellipticity compared to wild type proteins (Figure 4), indicating increase in chirality or greater extent of tertiary packing, especially in the microenvironments around the aromatic amino acid residues. Since the near UV CD spectra indicate greater tertiary packing, the decrease in intrinsic fluorescence intensity observed in the gxg mutants (Figure 3) may be attributed to structural changes (other than flexibility) leading to fluorescence quenching. No aromatic amino acids are present in αA- and αB-crystallin in the C-terminal extensions around the IPI/V motif. Thus, mutations in the IPI/V motif appear to have long-range conformational consequences leading to significant changes in the microenvironment of the aromatic amino acid residues present in distal regions of the sequences. In addition to tertiary structural changes, the mutations lead to observable changes in secondary structure. Far UV circular dichroism spectroscopy shows αAgxg to have increased ellipticity, with a possibility of some increase in the α-helical content, compared to wild type αA-crystallin (Figure 5A). However, αBgxg shows significantly decreased ellipticity compared to wild type αB-crystallin (Figure 5B). Despite the sequence similarity, the effect of this mutation on the secondary structure of αA- and αB-crystallin is different. Thus, the IXI/V motif is an important determinant of the overall structure of the α-crystallin assemblies and mutations in this motif lead to considerable changes in secondary, tertiary, and quaternary structure.

We studied the chaperone-like activity of the wild type and mutant proteins by comparing their ability to prevent the DTT induced insulin aggregation at 37 °C. Figure 6A shows that both αAgxg and αBgxg mutant proteins exhibit increased protective ability compared to their wild type proteins. As mentioned earlier, lens α-crystallin as well as homo-oligomers of αA- and αB-crystallin exhibit temperature dependent chaperone activity [10,11,20,21]. At 25 °C both αA- and αB-crystallin exhibit relatively poor chaperone-like activity [11]. The gxg mutants, however, showed considerable chaperone activity even at 25 °C (Figure 6B). These results suggest that mutations in this motif either increase the accessibility of chaperone sites or cause conformational changes leading to exposure of such sites in other regions, probably similar to those exposed at elevated temperatures.

Though the involvement of the N-terminal domain in multimeric complex formation has been demonstrated for αA- and αB-crystallin [30,31], the contribution of the C-terminal extension in forming inter-subunit contacts is debatable. C-terminally truncated pea Hsp17.7 and *Bradyrhizobium* HspH and HspF are defective in oligomer formation [37,38]. Also, mammalian Hsp20, which lacks the C-terminal extension, primarily forms dimers [47]. On the other hand, mutations in or truncations of the C-terminal extension of αA-crystallin, Hsp25 and *C. elegans* Hsp16.2 had little, if any, effect on oligomer assembly or size [32,48,49].

Our study shows that the region around the IXI/V motif in αA- and αB-crystallin can come proximal to the β-strand regions towards the end of the "α-crystallin domain", probably stabilizing local secondary or tertiary structural elements. The IXI/V motif also appears to interact with the corresponding motif in the C-terminal extension of a partner monomer. Packing induced alteration in folding and organization has been shown to occur in hetero-oligomer formation between αA- and αB-crystallin [50]. Such interactions may be altered in the gxg mutant proteins as they show significant changes in their quaternary, tertiary, and secondary structure. These results clearly show that the conserved IPI/V motif in αA- and αB-crystallin contributes to the quaternary structural organization in both proteins. Since mutation of the isoleucine and valine residues in αA- and αB-crystallin also leads to enhanced chaperone-like activity at 25 °C, this region may be one of the important determinants regulating the temperature dependence of chaperone activity in α-crystallins. Our results also reinforce the earlier observations from the crystal structures of Hsp16.5 and Hsp16.9 that the interactions of the IXI motif in different sHsps may be responsible for different oligomeric assemblies.

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REFERENCES

1. Groenen PJ, Merck KB, de Jong WW, Bloemendal H. Structure and modifications of the junior chaperone alpha-crystallin. From lens transparency to molecular pathology. Eur J Biochem 1994; 225:1-19.

2. Siezen RJ, Bindels JG, Hoenders HJ. The quaternary structure of bovine alpha-crystallin. Size and charge microheterogeneity: more than 1000 different hybrids? Eur J Biochem 1978; 91:387-96.
3. Bhat SP, Nagineni CN. alpha B subunit of lens-specific protein alpha-crystallin is present in other ocular and non-ocular tissues. Biochem Biophys Res Commun 1989; 158:319-25.
4. Dasgupta S, Hohman TC, Carper D. Hypertonic stress induces alpha B-crystallin expression. Exp Eye Res 1992; 54:461-70.
5. Klemenz R, Frohli E, Steiger RH, Schafer R, Aoyama A. Alpha B-crystallin is a small heat shock protein. Proc Natl Acad Sci U S A 1991; 88:3652-6.
6. Clark JI, Muchowski PJ. Small heat-shock proteins and their potential role in human disease. Curr Opin Struct Biol 2000; 10:52-9.
7. MacRae TH. Structure and function of small heat shock/alpha-crystallin proteins: established concepts and emerging ideas. Cell Mol Life Sci 2000; 57:899-913.
8. Horwitz J. Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci U S A 1992; 89:10449-53.
9. Sun TX, Das BK, Liang JJ. Conformational and functional differences between recombinant human lens alphaA- and alphaB-crystallin. J Biol Chem 1997; 272:6220-5.
10. Raman B, Rao CM. Chaperone-like activity and quaternary structure of alpha-crystallin. J Biol Chem 1994; 269:27264-8.
11. Datta SA, Rao CM. Differential temperature-dependent chaperone-like activity of alphaA- and alphaB-crystallin homoaggregates. J Biol Chem 1999; 274:34773-8.
12. Rajaraman K, Raman B, Rao CM. Molten-globule state of carbonic anhydrase binds to the chaperone-like alpha-crystallin. J Biol Chem 1996; 271:27595-600.
13. Das KP, Petrash JM, Surewicz WK. Conformational properties of substrate proteins bound to a molecular chaperone alpha-crystallin. J Biol Chem 1996; 271:10449-52.
14. Goenka S, Raman B, Ramakrishna T, Rao CM. Unfolding and refolding of a quinone oxidoreductase: alpha-crystallin, a molecular chaperone, assists its reactivation. Biochem J 2001; 359:547-56.
15. Rajaraman K, Raman B, Ramakrishna T, Rao CM. Interaction of human recombinant alphaA- and alphaB-crystallins with early and late unfolding intermediates of citrate synthase on its thermal denaturation. FEBS Lett 2001; 497:118-23.
16. Hook DW, Harding JJ. Molecular chaperones protect catalase against thermal stress. Eur J Biochem 1997; 247:380-5.
17. Marini I, Moschini R, Del Corso A, Mura U. Complete protection by alpha-crystallin of lens sorbitol dehydrogenase undergoing thermal stress. J Biol Chem 2000; 275:32559-65.
18. Santhoshkumar P, Sharma KK. Analysis of alpha-crystallin chaperone function using restriction enzymes and citrate synthase. Mol Vis 2001; 7:172-7 .
19. Rawat U, Rao M. Interactions of chaperone alpha-crystallin with the molten globule state of xylose reductase. Implications for reconstitution of the active enzyme. J Biol Chem 1998; 273:9415-23.
20. Raman B, Ramakrishna T, Rao CM. Temperature dependent chaperone-like activity of alpha-crystallin. FEBS Lett 1995; 365:133-6.
21. Raman B, Rao CM. Chaperone-like activity and temperature-induced structural changes of alpha-crystallin. J Biol Chem 1997; 272:23559-64.
22. Smith JB, Liu Y, Smith DL. Identification of possible regions of chaperone activity in lens alpha-crystallin. Exp Eye Res 1996; 63:125-8.
23. Das KP, Surewicz WK. Temperature-induced exposure of hydrophobic surfaces and its effect on the chaperone activity of alpha-crystallin. FEBS Lett 1995; 369:321-5.
24. Narberhaus F. Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol Mol Biol Rev 2002; 66:64-93.
25. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 1998; 7:471-4.
26. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, Chateau D, Chapon F, Tome F, Dupret JM, Paulin D, Fardeau M. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 1998; 20:92-5.
27. Bova MP, Yaron O, Huang Q, Ding L, Haley DA, Stewart PL, Horwitz J. Mutation R120G in alphaB-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. Proc Natl Acad Sci U S A 1999; 96:6137-42.
28. Kumar LV, Ramakrishna T, Rao CM. Structural and functional consequences of the mutation of a conserved arginine residue in alphaA and alphaB crystallins. J Biol Chem 1999; 274:24137-41.
29. de Jong WW, Caspers GJ, Leunissen JA. Genealogy of the alpha-crystallin—small heat-shock protein superfamily. Int J Biol Macromol 1998 May-Jun; 22:151-62.
30. Merck KB, De Haard-Hoekman WA, Oude Essink BB, Bloemendal H, De Jong WW. Expression and aggregation of recombinant alpha A-crystallin and its two domains. Biochim Biophys Acta 1992; 1130:267-76.
31. Feil IK, Malfois M, Hendle J, van Der Zandt H, Svergun DI. A novel quaternary structure of the dimeric alpha-crystallin domain with chaperone-like activity. J Biol Chem 2001; 276:12024-9.
32. Andley UP, Mathur S, Griest TA, Petrash JM. Cloning, expression, and chaperone-like activity of human alphaA-crystallin. J Biol Chem 1996; 271:31973-80.
33. Pasta SY, Raman B, Ramakrishna T, Rao ChM. Role of the C-terminal extensions of alpha-crystallins. Swapping the C-terminal extension of alpha-crystallin to alphaB-crystallin results in enhanced chaperone activity. J Biol Chem 2002; 277:45821-8.
34. Selcen D, Ohno K, Engel AG. Myofibrillar myopathy: clinical, morphological and genetic studies in 63 patients. Brain 2004; 127:439-51.
35. Selcen D, Engel AG. Myofibrillar myopathy caused by novel dominant negative alpha B-crystallin mutations. Ann Neurol 2003; 54:804-10.
36. Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, Van Impe K, Fedotov V, Dadali E, Auer-Grumbach M, Windpassinger C, Wagner K, Mitrovic Z, Hilton-Jones D, Talbot K, Martin JJ, Vasserman N, Tverskaya S, Polyakov A, Liem RK, Gettemans J, Robberecht W, De Jonghe P, Timmerman V. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet 2004; 36:602-6.
37. Studer S, Obrist M, Lentze N, Narberhaus F. A critical motif for oligomerization and chaperone activity of bacterial alpha-heat shock proteins. Eur J Biochem 2002; 269:3578-86.
38. Kirschner M, Winkelhaus S, Thierfelder JM, Nover L. Transient expression and heat-stress-induced co-aggregation of endogenous and heterologous small heat-stress proteins in tobacco protoplasts. Plant J 2000; 24:397-411.

39. Pace CN, Vajdos F, Fee L, Grimsley G, Gray T. How to measure and predict the molar absorption coefficient of a protein. Protein Sci 1995; 4:2411-23.
40. Bova MP, Ding LL, Horwitz J, Fung BK. Subunit exchange of alphaA-crystallin. J Biol Chem 1997; 272:29511-7.
41. Lambert H, Charette SJ, Bernier AF, Guimond A, Landry J. HSP27 multimerization mediated by phosphorylation-sensitive intermolecular interactions at the amino terminus. J Biol Chem 1999; 274:9378-85.
42. Pasta SY, Raman B, Ramakrishna T, Rao ChM. Role of the conserved SRLFDQFFG region of alpha-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange, and chaperone-like activity. J Biol Chem 2003; 278:51159-66.
43. Kim KK, Kim R, Kim SH. Crystal structure of a small heat-shock protein. Nature 1998; 394:595-9.
44. van Montfort RL, Basha E, Friedrich KL, Slingsby C, Vierling E. Crystal structure and assembly of a eukaryotic small heat shock protein. Nat Struct Biol 2001; 8:1025-30.
45. Van Montfort R, Slingsby C, Vierling E. Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. Adv Protein Chem 2001; 59:105-56.
46. Wotton D, Freeman K, Shore D. Multimerization of Hsp42p, a novel heat shock protein of Saccharomyces cerevisiae, is dependent on a conserved carboxyl-terminal sequence. J Biol Chem 1996; 271:2717-23.
47. van de Klundert FA, Smulders RH, Gijsen ML, Lindner RA, Jaenicke R, Carver JA, de Jong WW. The mammalian small heat-shock protein Hsp20 forms dimers and is a poor chaperone. Eur J Biochem 1998; 258:1014-21.
48. Lindner RA, Carver JA, Ehrnsperger M, Buchner J, Esposito G, Behlke J, Lutsch G, Kotlyarov A, Gaestel M. Mouse Hsp25, a small shock protein. The role of its C-terminal extension in oligomerization and chaperone action. Eur J Biochem 2000; 267:1923-32.
49. Leroux MR, Melki R, Gordon B, Batelier G, Candido EP. Structure-function studies on small heat shock protein oligomeric assembly and interaction with unfolded polypeptides. J Biol Chem 1997; 272:24646-56.
50. Datta SA, Rao CM. Packing-induced conformational and functional changes in the subunits of alpha-crystallin. J Biol Chem 2000; 275:41004-10.

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