A Novel Quaternary Structure of the Dimeric α-Crystallin Domain with Chaperone-like Activity*

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αB-crystallin, a member of the small heat-shock protein family and a major eye lens protein, is a high molecular mass assembly and can act as a molecular chaperone. We report a synchrotron radiation x-ray solution scattering study of a truncation mutant from the human αB-crystallin (αB57–157), a dimeric protein that comprises the α-crystallin domain of the αB-crystallin and retains a significant chaperone-like activity. According to the sequence analysis (more than 23% identity), the monomeric fold of the α-crystallin domain should be close to that of the small heat-shock protein from Methanococcus jannaschii (MJHSP16.5). The theoretical scattering pattern computed from the crystallographic model of the dimeric MJHSP16.5 deviates significantly from the experimental scattering by the α-crystallin domain, pointing to different quaternary structures of the two proteins. A rigid body modeling against the solution scattering data yields a model of the α-crystallin domain revealing a new dimerization interface. The latter consists of a strand-turn-strand motif contributed by each of the monomers, which form a four-stranded, antiparallel, intersubunit composite β-sheet. This model agrees with the recent spin labeling results and suggests that the αB-crystallin is composed by flexible building units with an extended surface area. This flexibility may be important for biological activity and for the formation of αB-crystallin complexes of variable sizes and compositions.

αA- and αB-crystallin, which share 54% amino acid sequence identity, build the subunits of α-crystallin, a major eye lens protein, comprising up to 40% of the total lens proteins. The structural function of the α-crystallin is to assist in maintaining transparency in the lens (1). The chaperone-like function of αB-crystallin helps to avoid formation of large light-scattering aggregates and possibly helps to prevent cataract in the lens. Moreover, neurodegenerative diseases, ischemia, or multiple sclerosis lead to an overexpression of this protein, which makes it an object of special medical interest (2).

The α-crystallin as well as other mammalian small heat-shock proteins (sHSPs)1 form large globular complexes with a diameter of about 10–25 nm. Cryoelectron microscopy and image analysis revealed that αB-crystallin is a hollow spherical shell with variable quaternary structure (3), and a frequent exchange of subunits between the particles was observed. The chaperone activity of αB-crystallin is associated with partial perturbation of the substrate protein tertiary structure, leading to a multimeric molten globule-like state with increased hydrophobicity (4, 5). The exposed hydrophobic regions of α-crystallin interact with substrate proteins possessing an increased surface hydrophobicity but a low degree of unfolding (6).

The molecular structure and subunit interactions in α-crystallin have long been under investigation. The stretches of residues promoting formation of lower or higher molecular weight α-crystallin oligomers have been identified. Upon addition of 1% deoxycholic acid, the α-crystallin oligomer dissociates into tetramers (7). The latter can also be formed by deletion of amino acids 1–63 in αA-crystallin (8). Site-directed spin-labeling demonstrated that the αA-crystallin dimers are formed by subunit interactions along a highly conserved β-strand in the region of residues 109–121 (9). This stretch of residues forms a sheet of two antiparallel β-strands that extends across the dimer interface.

The oligomers of bacterial sHSPs are monodisperse and more rigid. Their gel filtration profile shows a single narrow, symmetric peak, whereas α-crystallin, αB-crystallin, or HSP27 yield broader peaks (10). A 2.9 Å resolution crystal structure of MJHSP16.5, a sHSP isolated from a hyperthermophilic archaean, has been determined (11). The topology of the MJHSP16.5 monomer resembles an immunoglobulin fold with a central β-sandwich of two sheets, consisting of four β-strands each. Upon dimerization, one of the sheets is extended by an additional strand contributed by the neighboring subunit (Fig. 1, left panel). This fifth strand labeled β6 (11), a small stretch of five residues from 93 to 97, is part of a long loop of 19 residues (84–102) protruding into the structural core of the monomer. This strand is therefore crucial for the stabilization of the MJHSP16.5 dimer.

A dimeric model of the α-crystallin domain has recently been generated (12) by homology modeling based on the crystal structure of MJHSP16.5. The quaternary structure of the homology model and thus the dimeric interface are virtually identical to those of MJHSP16.5 displayed in Fig. 1, left panel. The loop-strand-loop motif (residues 84–102 of subunit A) in-...
teracting with strand β2 (residues 45–49 of subunit B) in MjHSP16.5 has been modeled in human αB-crystallin as a large loop lacking secondary structure (residues 104–115 of subunit A) that is adjacent to strand β2 (residues 66–70 of subunit B). The homology model (12) does not show contacts between residues 114–118 in subunits A and B, and this contradicts the spin-labeling results (9).

The present study is aimed at the analysis of the properties and structure of the α-crystallin domain of the human αB-crystallin. A dimeric α-crystallin domain is expressed, purified, and characterized using different biochemical and physical methods. Its quaternary structure is studied by synchrotron radiation x-ray scattering. The latter method allows us to determine overall structures of native biological macromolecules under nearly physiological conditions (13). Comparisons between experimental x-ray solution scattering curves and those evaluated from crystallographic structures are widely used to verify structural similarity between macromolecules in crystals and in solution (14, 15). Moreover, the use of crystallographic models of individual subunits permits the building of structural models of complex particles in solution by rigid body refinement against the scattering data (16–18). Below, solution scattering is used to demonstrate that the quaternary structure of the α-crystallin domain dimer differs from that of sHSP from Methanococcus jannaschii (9.1). A model of the former, constructed by rigid body refinement, displays a new dimerization interface, suggesting that the α-crystallin domain may be flexible in solution.

**EXPERIMENTAL PROCEDURES**

**Cloning**—The amino acid sequence alignment between human αB-crystallin and MjHSP16.5 was used as a basis (12). The residue Ala-57 in αB-crystallin was aligned with Gln-36 in MJHSP16.5, the N-terminal residue of the first β-strand β1. The Arg-157 in αB-crystallin was aligned with Ile-144 in MJHSP16.5, located in the last β-strand, β10. The sequence Ala-57 through Arg-157 covered the core α-crystallin domain plus an additional nine residues from the N-terminal domain. The αB-crystallin deletion mutant gene was excised from plasmid pET16b at the restriction sites NcoI and XhoI. The gene fragment was ligated into expression vector pETM-11 at the NcoI and XhoI sites. The resulting plasmid was named pB57–157.

**Expression and Purification of the α-Crystallin Domain**—Plasmid pB57–157 was transformed into BL21(DE3), and 1-liter expression cultures were grown in Luria Broth medium supplemented with kanamycin to a final concentration of 50 μg/ml. The expression was induced at A600 = 0.8 by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM. After 4–6 h of induction, cells were collected by centrifugation, and the cell pellets were resuspended in 50 mM of starting buffer (20 mM sodium phosphate, 0.5 mM NaCl, pH 7.4). 400 μl of lysozyme (10 mg/ml; Sigma) were added to the cell suspension, and the suspension was stirred in an ice-water bath for 20 min. After the addition of 1 μl of benzazepine (250 units/μl; Merck), the suspension was incubated at 23 °C for 10 min with constant stirring. Insoluble cellular debris was removed by sedimentation at 36,000 × g for 30 min at 4 °C. The supernatant was filtered through a 0.22-μm filter (Millipore). At a flow rate of 1 ml/min, 25 ml of cell extract were applied on a 1-ml Hi-Trap metal-chelating column (Pharmacia), charged with 100 mM NiCl2 solution, and equilibrated with starting buffer. The column was washed with 10 column volumes of starting buffer. For 10 column volumes, a linear gradient from 0 to 500 mM imidazole was applied. The His-tagged αB57–157 fusion protein eluted at 400 mM imidazole concentration. The positive fractions were pooled, and a His-tagged TEV-protease was added in a 1:100 ratio (w/w). The cleavage reaction was dialyzed against starting buffer at 20 °C for 24 h. The reaction mixture was passed through a mini-column (Bio-Rad) filled with 1 ml of Ni-NTA resin (Qiagen), which had been equilibrated with starting buffer. The flow-through was collected and analyzed for purity on a 10% SDS-polyacrylamide gel electrophoresis (Novex) stained with Coomassie Blue or silver staining methods. Protein samples were concentrated using Centricon (Millipore) filter units with a molecular mass cutoff of 3 kDa. Protein concentrations for all measurements were determined by the Bradford method, using the Bio-Rad protein assay reagent with bovine serum albumin as a standard.

**Chaperone Activity Assay**—The effect of the α-crystallin domain on protein aggregation was measured as described previously (19). Aggregation of alcohol dehydrogenase (ADH) at 37 °C was measured as an apparent optical density at A415 using a Kontron Uvikon 922 Spectrophotometer equipped with a thermostated cuvette holder. In a total reaction volume of 400 μl, 5 μM yeast ADH (Sigma) was incubated with varying amounts of purified α-crystallin domain or 1 μM human αB-crystallin. The reaction buffer was a 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM NaCl, and 2 mM EDTA. The optical density in the cell was recorded every 2 min. For all molar ratios, three independent experiments were conducted.

**Sedimentation Velocity Analysis and Equilibrium Centrifugation**—A Beckman XLA analytical ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) equipped with ultraviolet absorption optics was used for the sedimentation velocity and the sedimentation equilibrium studies at 4 °C and at 10 °C, respectively. Protein in 50 mM phosphate buffer, pH 7.0, was loaded into 12-mm double-sector aluminum cells placed in an An60 Ti rotor. For the sedimentation velocity analysis, sample volumes of 350 μl were centrifuged at 60,000 rpm. Radial scans of absorbance at 290 nm were taken at 2-min intervals. Data were analyzed to provide the apparent distribution of sedimentation coefficients by means of the programs DCDT (20) and Svedberg (21). Hydrodynamic modeling was done with ULTRASCAN (22).

For the sedimentation equilibrium analysis, a sample volume of 180 μl at 3 mg/ml protein in 50 mM phosphate buffer, pH 7.0, was centrifuged at 26,000 rpm. Each data point measured at 297 nm was an average of 50 measurements. The solution density (1.0029 g/ml) and the partial volume of the protein (0.7217 ml/g) were calculated with ULTRASCAN, using the known buffer composition and amino acid composition of the protein. The degree of hydration was estimated based on the amino acid composition by the method of Kuntz and Brassfield (23).

**Scattering Experiments and Data Analysis**—The synchrotron radiation x-ray scattering data were collected using standard procedures on the X33 camera (24–26) of the European Molecular Biology Laboratory (EMBL) on storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) and the multicrystal prediffracted chambers with delay line readout (27). The solutions were measured at protein concentrations of 5, 10, and 25 mg/ml. The scattering curves were recorded at a wavelength λ = 0.15 nm for sample detector distance 1.4 m covering the momentum transfer range 0.40 < s < 5.1 nm⁻¹ (s = 4π sinθ/λ, where 2θ is the scattering angle). The data were normalized to the intensity of the incident beam and corrected for the detector response, the scattering of
FIG. 2. Sequence alignment between human aB-crystallin and M. jannaschii HSP16.5. Only the region relevant for the modeling of the dimer interface is presented, beginning with the N terminus of strand β5 and ending with the C terminus of strand β6. The secondary structure was assigned using PROMOTIF (46). The β-strands of MJHSP16.5 are labeled as reported previously (11), and a similar labeling scheme was used for aB-crystallin.

The coordinates of the crystallographic model of the HSP16.5 from M. jannaschii were taken from the Protein Data Bank, entry 1shs (11). The scattering curves from the x-ray structure and the homology models were calculated using the program CRYDAM, a modified version of the program CRYSOL (32). The program takes into account the scattering from the solution shell at the temperature of the model protein. The latter is covered by a 0.3-nm-thick hydration layer with an adjustable density, which may differ from that of the bulk solvent. The scattering intensity is

\[ I(s) = \langle A_{s}(s) - \rho_{0}sA_{s}(0) \rangle^2 \]

(Eq. 1)

where \( A_{s}(s) \) is the scattering amplitude from the particle in vacuo, \( A_{s}(0) \) and \( \rho_{0} \) are, respectively, the scattering amplitudes from the excluded volume and the hydration layer, both with unitary density, \( \delta p_{0} = \rho_{0} - \rho_{f} \); and \( s \) stands for the average over all particle orientations (Ω is the solid angle in reciprocal space, \( s = (s, \Omega) \)). Given the atomic coordinates, the program fits the experimental data using the surface-to-volume ratio of the particle, the excluded volume of the particle and the contrast of the hydration layer \( \delta p_{0} \), to minimize the discrepancy between the experimental and calculated curve

\[ \chi^2 = \frac{1}{N-1} \sum_{j=1}^{N} \left( \frac{I_{\text{calc}}(s_j) - I_{\text{exp}}(s_j)}{\sigma(s_j)} \right)^2 \]

(Eq. 2)

where \( N \) is a number of the experimental points, and \( I_{\text{calc}}(s_j) \) and \( \sigma(s_j) \) denote the experimental intensity and its S.D., respectively.

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**Fig. 3. SDS-polyacrylamide gel electrophoresis analysis of a-crystallin domain purification. Lane 1, marker; Lane 2, His-tagged TEV-protease, and a-crystallin domain (αB57–157) cleavage mixture, which was applied to the Ni-chelating column; Lanes 3–5, a-crystallin domain (αB57–157) elution fractions.**

**Fig. 4. Far-ultraviolet circular dichroism spectra of the a-crystallin domain.** Measurement was made at a protein concentration of 0.15 mg/ml in 50 mM sodium phosphate buffer, pH 7.0, using a 1-mm path length cuvette. The spectrum is the average of eight scans.

**RESULTS**

Characterization and Chaperone Activity—The a-crystallin domain corresponds to the peptide 57–157 from aB-crystallin, which was isolated after a tryptic digest of aB-crystallin (34). It was the smallest peptide identified after trypsinization, which comprises the a-crystallin domain. With the expression of αB57–157 and after proteolytic cleavage of the His-tagged fusion protein, the a-crystallin domain had an additional three N-terminal, vector-derived amino acids (Gly, Ala, and Met). The untagged form of the a-crystallin domain eluted with a >98% purity in the flow-through of the Ni-NTA column (Fig. 3). Proper folding of the protein was confirmed by far-ultraviolet circular dichroism spectroscopy (Fig. 4). The spectrum showed negative ellipticity with a minimum at 215 nm, typical for a β-sheet structure and in agreement with secondary structure predictions made for the a-crystallin domain of aB-crystallin (35–37) and with far-ultraviolet CD spectra observed for wild type αB-crystallin (12) and other sHSPs (38).

The functional groups of the a-crystallin domain was also confirmed by the chaperone activity assay (Fig. 5). The ADH aggregation was effectively suppressed with a 5-fold molar excess of a-crystallin domain over ADH and was comparable to the protection by wild type aB-crystallin in a ratio of ADH (5 μM):aB-crystallin (1 μM). When comparing the aggregation values of the ADH control and ADH (5 μM):a-crystallin domain (5...
at the 32 min time point, the control showed 100% ADH aggregation, whereas the ADH in the presence of α-crystallin domain aggregated only to 45%. At a 10-fold molar excess of ADH over α-crystallin domain, no protection of ADH aggregation was observed. The decrease of absorption for ADH (5 μM) and ADH (50 μM):α-crystallin domain (5 μM) beyond the 34 min time point was caused by precipitation of large protein aggregates in the cuvette. With an average of 32 subunits/wild type αB-crystallin molecule, the protection from aggregation per dimer was 100% at a 3.2-fold molar excess of αB-crystallin over ADH. With the α-crystallin domain dimer, a 5-fold molar excess was effective in protecting the same amount of ADH. Although the chaperone activity of the wild type αB-crystallin dimer exceeded that of the α-crystallin domain dimer by about 1.5-fold, the effectiveness of the α-crystallin domain in preventing ADH aggregation remains high.

Solution Structure of the α-Crystallin Domain—The molecular mass of the α-crystallin domain estimated from the analytical ultracentrifugation (22 ± 2 kDa) indicated that the protein is dimeric in solution. The velocity sedimentation data confirmed that the solution was monodisperse. The sedimentation coefficient 1.82 ± 0.06 (s20,W) and the hydrodynamic modeling suggested that the dimer is rather elongated with an estimated axial ratio of 1.10.

The synchrotron x-ray scattering curve from the α-crystallin domain is presented in Fig. 6a. The maximum dimension of the particle was 7.0 ± 0.2 nm, and the radius of gyration was 2.27 ± 0.03 nm. The Porod volume (i.e. the excluded volume estimate) of 42 ± 3 nm3 was compatible with the expected excluded volume of a hydrated dimer and incompatible with a monomeric protein. It can thus be concluded that the α-crystallin domain is dimeric in solution, in agreement with the abovementioned results of the analytical ultracentrifugation. The dimeric structure has been found at all protein concentrations.

The comparison between the scattering curve computed from the dimeric x-ray model of the sHSP (11) (Fig. 1, left panel) and the experimental scattering curve is presented in Fig. 6a, fit (1). The two curves display noticeable systematic deviations (the discrepancy between the experimental and calculated data is χ = 1.89). To check whether the data can be fitted by minor modifications of the crystallographic structure, several tentative models were generated by rigid body movements (up to ± 0.2 nm) and rotations (up to ± 10 degrees) of the second monomer. All these models yielded poor fits with χ between 1.8 and 2.5. This suggests that the quaternary structure of the α-crystallin domain of the human αB-crystallin differs from that of HSP16.5 from M. jannaschii.

The focus of the molecular modeling of the αB-crystallin mutant was the generation of an interface between two subunits, which is in agreement with (i) the sequence alignment, (ii) the spin-labeling study (9), and (iii) the general molecular interactions involved in protein stabilization and fits the experimental scattering data. In keeping with the low resolution of solution scattering, the modeling of the dimer of αB-crystallin was performed in terms of rigid body movements and rotations of the monomeric subunit derived by homology modeling from the crystal structure of MjHSP16.5. The amino acid sequence of the α-crystallin domain of human αB-crystallin and the fits calculated from the atomic models. (1), the crystallographic dimer of MjHSP16.5; (2–6), scattering from the dimeric homology models in B. The curves (1–6) are displaced down by one logarithmic unit for clarity, and the discrepancies with the experimental data are presented. B, the five α-crystallin domain models (3–6) tested against scattering data with increasing compactness from top to bottom. The left-hand monomer is in the same orientation for each model. The models are numbered to match the fits in A.
β6.2, contributed by the two subunits A and B, which built a four-stranded, antiparallel, intersubunit composite β-sheet with the following strand order: Aβ6.1-Aβ6.2-Bβ6.2-Bβ6.1. The modeled strands β6.1 and β6.2 are separated from β5 and β7 by one residue only, Glu-105 and Glu-117, respectively. Both residues are in a nonextended main chain dihedral conformation, resulting in a kink between the sheet made up by β5 and β7 and the β6.1-β6.2 sheet. The angle between the core β-sandwich and the intersubunit composite sheet varies depending on the actual main chain dihedrals of Glu-105, Glu-117, and their flanking residues. Accordingly, the dimer may have a more closed, compact or a more open, elongated shape. Five different dimer models have been tested (Fig. 6b), ranging from a dimer with an open L-shape (top; (2)) to more compact structures and, finally, a V-shaped dimer (bottom; (6)). The fits to the experimental data computed by CRYDAM are presented in Fig. 6a, curves (2–6). All these models yield better fits than that provided by the crystallographic model of the MjHSP16.5 dimer (curve (1)). The most compact, V-shaped model providing the best agreement with the experimental data is also displayed in Fig. 1 (right panel) for comparison with the crystallographic model of MjHSP16.5.


discussion

Probably the most intriguing functional finding in our study of the dimeric α-crystallin domain from human αB-crystallin is that the chaperone activity does not require a multimeric αB-crystallin complex. The existence of two sites of interactions between sHSP monomers has been proposed previously (39). Our results confirm that after deletion of the N-terminal domain and, moreover, does not require multimeric assembly to be active (40). The chaperone activity at different levels of oligomerization has also been observed for murine HSP25, a member of the mammalian sHSP family (41).

The final model of the α-crystallin domain dimer in Fig. 1 (right panel) provides an excellent fit to the solution scattering data in a wide angular range. A nominal resolution of the data is 1.2 nm, and details of the tertiary structure can obviously not be validated experimentally at this level of resolution, which is why we restricted ourselves to rigid body modeling. Solution scattering is rather sensitive to rigid body movements of structural domains and has successfully been used to model the quaternary structure of proteins (15–17). The use of rigid body modeling is justified by a high sequence homology between the actual main chain dihedrals of Glu-105, Glu-117, and their counterparts of the dimeric α-crystallin domain (Fig. 1). These two residues are identical in HSP16.2 from D. melanogaster, B-crystallin, and tetramers (42). The flexibility might also be an essential property enabling variable sizes and compositions of the multimeric complex of αB-crystallin and yielding mixed sHSP species (i.e., αA-crystallin, αB-crystallin, and HSP27) in a single hetero-oligomeric complex (44). It should be stressed that the potential flexibility of the protein is problematic for crystallographic analysis but not for solution scattering modeling (the experimental scattering pattern corresponds to an average position of the monomers).

The dimer interface in the proposed model contains the residue Arg-116 that causes autosomal dominant congenital cataactract in humans when mutated to Cys. Circular dichroism and 1H-1H-bi(4-anilino)naphthalene-5,5′-disulfonic acid fluorescence spectra indicate that R116C αA-crystallin is structurally different from the wild type protein (45). Arg-116 is an identically conserved residue between αA- and αB-crystallin. According to the present model, this residue plays an essential role in dimerization, and detrimental effects on the quaternary structure and biological activity can be expected after mutation.

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