α-Crystallin quaternary structure: molecular basis for its chaperone activity

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Abstract α-Crystallin, the major protein in all vertebrate lenses, functions as a chaperone. In the present analysis, an 'open' micellar structure composed of αA subunits is used to simulate chaperoning of partially heat denatured soluble γ-crystallin. The interaction is both electrostatic and hydrophobic and satisfies experimental evidence for a 1:1 α/γ molar ratio, a doubling of molecular mass and a minimal increase in the dimensions of the complex [J. Biol. Chem. (1994) 269, 13601–13608; Invest. Opthalmol. Vis. Sci. (1995) 36, 311–21]. These data are also in accord with Farahbaksh et al. [Biochemistry (1995) 34, 509–16]; i.e. the bound γ-crystallin monomers are not in a central cavity, but are separated by αA subunits.

Key words: α-Crystallin; Chaperone; γ-Crystallin; Lens

1. Introduction

α-Crystallin, the major protein in all vertebrate lenses, is one of a number of globular proteins recruited for maintaining lens transparency and establishing refractive properties. α-Crystallin exists in the lens as heteropolymers of αA and αB subunits, each ~20 kDa. Originally, the most prevalent α-, β- and γ-crystallins were considered very stable proteins unique to the lens. However, the sequence homology and functional overlap with small heat shock proteins (sHsp) [1] plus the identification of the two subunits of α-crystallin in many normal and abnormal cell types [2–5] suggest multiple functions for α-crystallin. The more recent discovery that α-crystallin has chaperone-like activity [6] provides an opportunity to probe the function of these very important polypeptides.

The missing link in examining structure/function relationships at the molecular level is the 3D structure of α-crystallin subunits and their aggregate. α-Crystallin has not been crystallized and its molecular mass (~800 kDa) is too large for high resolution NMR. Consequently, the tertiary structure of the two subunits of α-crystallin [7] and the quaternary structure of its aggregate [8] have been proposed by our group using computer assisted molecular modeling in conjunction with experimental data on the secondary structure. The validity of these working models can be tested by how well they correlate with experimental data accumulated over the past several decades.

In the present study, our 'open' micellar quaternary structure of αA subunits (Fig. 1) [8] and the crystal structure of bovine γ-B crystallin (formerly γ-II) [9] are used to explain, on the molecular level, recent experimental observations [10,11]. The data show that α-crystallin binds to partially heat denatured soluble γ-crystallin in a 1:1 monomer stoichiometry. This binding protects γ-crystallin from further heat induced thermal denaturation [10] as well as thiol oxidation [11]. Wang and Spector [10] suggest that each monomer in α-crystallin binds a soluble, partially denatured γ-crystallin molecule at a single binding site. The 1:1 ratio has also been reported by Borkman et al. [12] for the α/γ complex following UV radiation. Using our quaternary model for simulation of these observations, the results show that γ-crystallin N-terminal domain is intercalated in the space between the adjacent C-terminal domains of α-crystallin subunits.

2. Materials and methods

To simulate 1:1 α/γ complex formation, the crystal structure of γB (PDB entry 4GCR) and the computer-generated bovine αA subunit and homopolymer (Fig. 1) were used along with molecular modeling SYBYL6.1 software. In order to simulate the experimental protocol, our working models of the αA subunit and the γB crystal structure were subjected to annealing. The structural changes at 65°C were analyzed with respect to the original geometries. In both molecules, minimal changes in the Cα backbone indicated the absence of protein unfolding that characteristically exposes buried hydrophobic residues. The observed minimal changes were the orientation of sidechains of charged amino acids. These data are in agreement with the observation of Wang and Spector [10] that γ-crystallin remains soluble and only 'partially' denatures at 62–65 °C.

Following annealing the electrostatic patterns and potential at all atomic sites of αA- and γ-crystallin alone and in the complex were calculated from DelPhi2.2.0 (InsightII2.2.0). This information was used to locate a putative binding site. γ-Crystallin was successfully docked in the intersubunit space of the αA homoaggregate (Fig. 2).

3. Results and discussion

A sector of the aggregate illustrated in Fig. 2 represents the N-terminal domain of γ-crystallin in a pocket provided by four sequences in two adjacent αA subunits. The sequences lining the right side of the pocket comprise two highly conserved regions, HCR1 and HCR2 shared with other sHsps; the left side of the pocket contains the sequences for Ser 122 phosphorylation and the C-terminal extension (Lys^{166}-Ser^{173}). In Fig. 3 the sequence comparison of the C-terminal domain of bovine α-crystallin and five sHsps shows the two highly conserved homologous regions, HCR1 and HCR2. HCR1 in αA includes residues 96–103 and HCR2 includes residues 131–140. The latter, HCR2, contains a putative ATP binding site delineated by our molecular modeling studies [13]; subsquently it was

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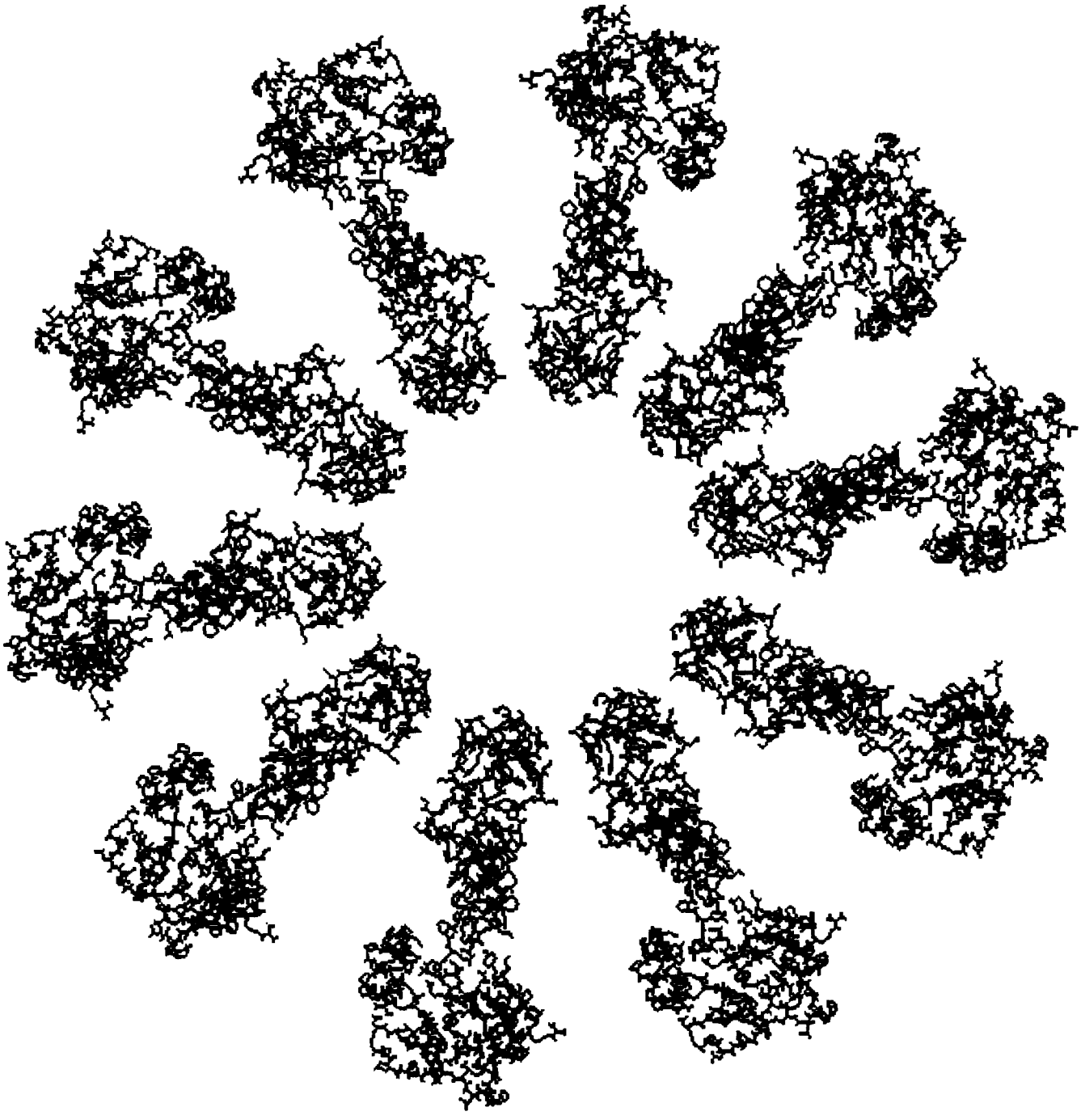

Fig. 1. Cross-sectional view (through diameter plane) of spherical quaternary structure of α-crystallin consisting of 42 αA subunits with molecular mass 840 kDa; radius 101 Å and radius of the cavity 24 Å. The hydrophobic N-terminal domain provides the driving force for subunit aggregation. The outer surface of the hydrophilic C-terminal domain of each subunit is exposed to the solvent and imparts aggregate solubility.

recognized by Kantorow et al. [14] that the sequence contains an autophosphorylation site. The conservation of these amino acids in the C-terminal domain of α-crystallin and sHsp suggests functional importance. The sequences in the binding pocket share the same structural base consisting of two sequences, Thr^{86}-Val^{94} and Asp^{151}-Glu^{156}. The latter sequence is subject to proteolysis ([15] and refs. therein).

The α/γ complex simulation satisfies the requirement of a 1 : 1 molar ratio, a doubling of molecular mass and a minimal increase in the dimensions of the complex [10]. Both experimental data [16] and our model of the quaternary structure [8] show that the radius of α-crystallin aggregate (40–42 αA subunits) is ~100 Å and volume occupied by the subunits is only ~30%. This provides sufficient space to accommodate binding of γ-crystallin and other proteins. In Fig. 2, the N-terminal domain of γ-crystallin is bound between the αA subunits while its C-terminal domain extends into the solvent. Our calculations show that the effective radius of the α/γ complex is ~125 Å since

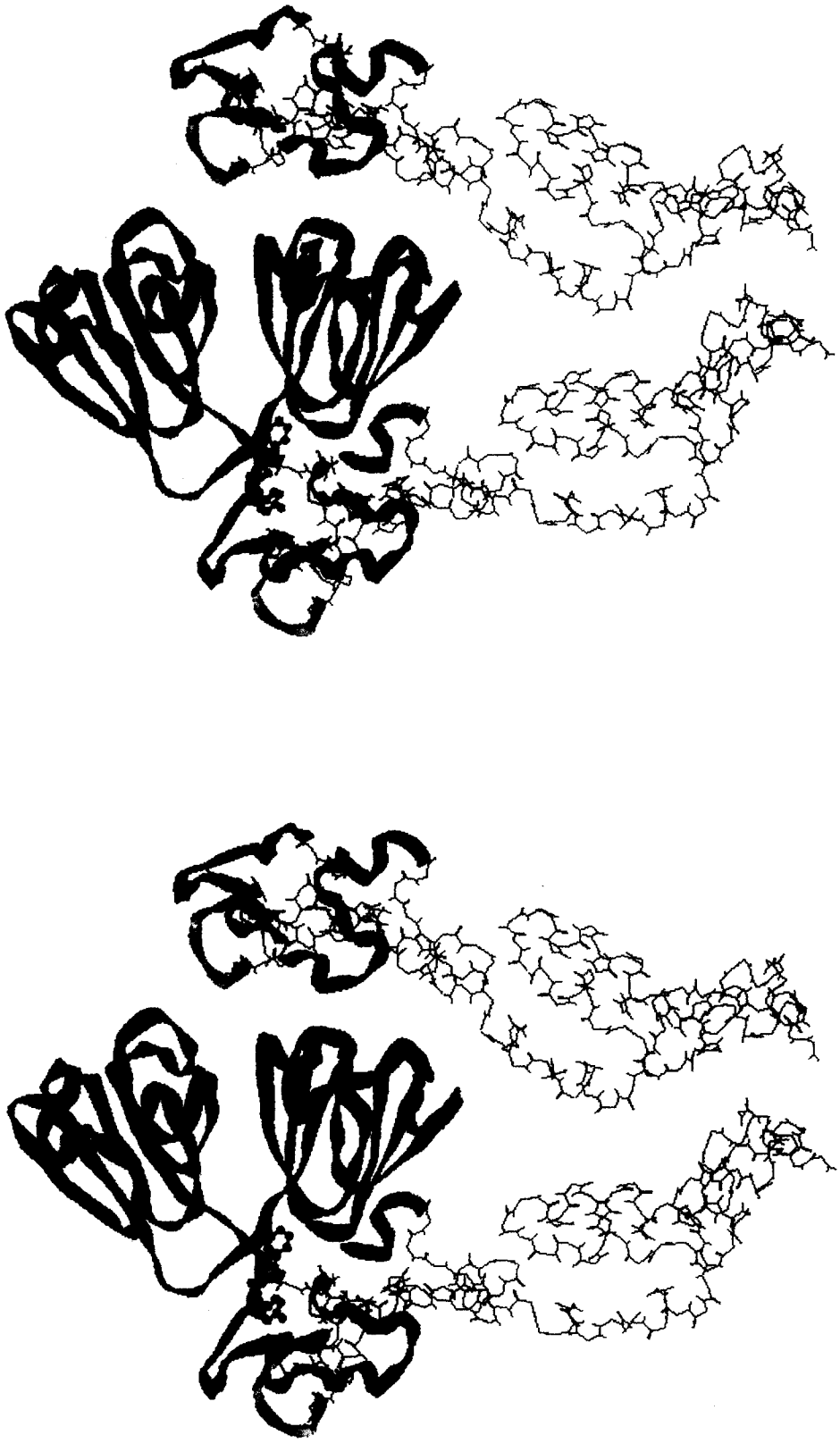

Fig. 2. Stereoview of the interaction of γ-crystallin (red ribbon) with αA-crystallin subunits (peptide backbone). Two of the ten αA subunits in the cross-section as described in Fig.1 are illustrated. Color code in αA: yellow, green, purple and magenta represent HCR1 (yellow ribbon); HCR2 (green ribbon); phosphorylation site (purple ribbon) and C-terminal extension (magenta ribbon). Phe^{175} and Tyr^{174} of γ-crystallin are represented in ball-and-stick which participate in the interaction.

the calculated width of the C-terminal domain of γ-crystallin is ~25 Å. This theoretical dimension of the α-γ complex agrees with the minimal increase observed in the radius of the complex by Wang and Spector [10] and our earlier high voltage electron microscopic observations of structures within the lens nuclear region [17].

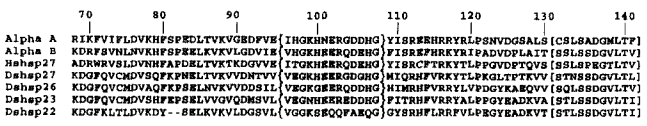

Fig. 3. Sequence comparison of bovine αA, bovine αB, *Drosophila* shsp27, human sHsp27, *Drosophila* sHsp26, sHsp23 and sHsp22. The highly conserved residues (HCR1 and HCR2) are shown in curly and square brackets, respectively.

The α/γ interaction is predominately electrostatic with some hydrophobic contribution. Table 1 contains the electrostatic potential at various atomic sites in unbound αA subunit, γ-monomer and the α/γ complex. The amino acids included in the table are from the C-terminal domain and interconnecting segment of αA and the N-terminal domain of γ-crystallin. Residues 95–136 of αA are within the region of homology with other sHsps (Fig. 3). Electrostatic complementarity is represented in terms of the electrostatic field. During recognition of ligand by receptor, one expects a decrease in the electrostatic field at various points. This is represented here by a decrease in electrostatic potential. From this table, it is evident that the absolute value of electrostatic potential at various atomic sites is decreased in the complex. This shows electrostatic complementarity between α- and γ-crystallin and signifies that an electrostatic interaction has occurred. In addition there is possibility of hydrogen bond formation due to close proximity of Ser^{173}, Lys^{166}, Ser^{168}, Glu^{113} in the C-terminal domain of αA and the respective Gly^{1} and Glu^{17}, Ser^{19}/Ser^{20}, Arg^{59} in the N-terminal domain of γ-crystallin. The participation of γ-Ser^{19} and γ-Ser^{20} in α/γ binding and proximity to γ-Cys^{18} and γ-Cys^{22} may be important in modifying their tendency to oxidize. This may explain, in part, the inhibitory effects of α-crystallin on γ-crystallin oxidation [11]. Also, His^{97} (in HCR1), in the αA interconnecting segment, interacts with the C-terminal residue, Tyr^{174} in γ-crystallin. This agrees with ^{1}H NMR spectroscopic data of Carver et al. [18] that shows Tyr^{174} participates in α-γ binding. In addition, data of Takemoto et al. [19] and Takemoto [20,21] have shown that truncation of the C-terminal extension in α-crystallin diminishes its capacity to chaperone. In another study, Cooper et al. compared α-crystallin both in solution and in lens homogenates using ^{1}H NMR spectroscopy and concluded that the resonance of the C-terminal extension remains intact on binding [22]. However, close inspection of TOCSY spectra of both preparations [22,23] revealed at least two missing cross-peaks corresponding to residues 168–170 which suggests partial involvement of this extension in binding. Also the proximity of γ-crystallin residues, Phe^{173} and Tyr^{174}, to αA-Ile^{96}, His^{97} and Gly^{98} suggests that they may participate in hydrophobic interactions in the α/γ complex. Representative distances between atoms Gly^{98}-H and Tyr^{174}-OH, His^{97}-HD and Tyr^{174}-CE2, Ile^{96}-CB and Tyr^{174}-CB and Ile^{96}-CD1 and Phe^{173}-C are 4.816, 3.705, 4.128 and 5.337 Å, respectively.

Molecular simulation of the binding of the heat denatured soluble form of γ- to α-crystallin correlates well with experimental observations of a 1 : 1 ratio and a doubling of the molecular mass without substantial increase in molecular dimensions. The data of Wang and Spector [10] also show that α-crystallin does not bind to insoluble, heat denatured γ-crystallin. Under their experimental conditions, the results suggest that, in the aggregate form, α-crystallin does not have significant surface exposure of hydrophobic sidechains. These observations and the relative instability of the association between γ- and α-crystallin suggest that electrostatic and limited hydrophobic interactions constitute reasonable binding sites. It also suggests that α-crystallin may detect the early stages of protein conformational changes that occur under more subtle, physiological levels of stress. Functionally, the ready reversibility of the interaction would favor the release of γ-crystallin following stress.

'How does α-crystallin in its role as a chaperone recognize

Table 1
Electrostatic potential at various atomic sites in αA and γ-crystallin alone and in complex[a]

αA				γ-crystallin			
Amino Acid	Atom	Electrostatic Potential (kT/e)		Amino Acid	Atom	Electrostanic Potential (kT/e)	
		Alone	In Complex			Alone	In Complex
Glu95	OE1	−132	−65	Arg9	NH1	121	59
	OE2	−139	−92		NH2	120	8
Glu102	OE1	−123	−90	Lys2	NZ	167	40
	OE2	−187	−127	Arg31	NH1	142	52
					NH2	98	63
Asp105	OD1	−143	−97	Arg36	NH1	172	71
	OD2	−105	−50		NH2	166	66
Asp106	OD1	−96	−54	Arg47	NH1	160	66
	OD2	−89	−24		NH2	100	81
Glu113	OE1	−153	−35	Arg58	NH1	141	102
	OE2	−60	−56		NH2	158	147
Asp125	OD1	−150	−97	Arg76	NH1	177	106
	OD2	−111	−76		NH2	101	52
Asp136	OD1	−60	−47	Arg89	NH1	143	49
	OD2	−109	−96		NH2	101	89
Asp151	OD1	−151	−139	Arg91	NH1	126	101
	OD2	−176	−157		NH2	103	65
Glu156	OE1	−113	−109	Arg95	NH1	159	114
	OE2	−229	−132		NH2	99	55
Ser173	OXT	−188	−135				

[a] The amino acid residues selected in αA are from connecting segment and C-terminal domain those for γ-crystallin are from N-terminal domain.

dysfunctional protein?' Although the present study does not answer this question, it does provide some insight. In the α/γ complex, the C-terminal 'tail' region of both proteins is involved in the interaction. The C-terminal extension of αA-crystallin [23] is flexible and lacks a preferred secondary structure; therefore it is capable of a variety of configurations to meet the binding requirements of a number of proteins. In vitro, trypsin treated αA crystallin results in the cleavage of its C-terminal extension and reduces chaperone activity [19]. Age-dependent truncation is also observed in vivo [21]. The involvement of the γ-crystallin C-terminal amino acids (Phe^{173} and Tyr^{174}) is supported by ^1H NMR studies which show that these resonances are absent following interaction with α-crystallin. This C-terminal region of γ-crystallin is also subject to modification in vivo. The observation of Garland et al. [24] suggests that such post-translational changes are consistent and exquisitely controlled during lens fiber cell maturation. Since both α- and γ-crystallin C-termini are modified, their interactive behavior would be significantly altered with age.

In a recent study by Farahbaksh et al. [25], spin-labeled derivatives of insulin B chain and melettin were used to investigate the mode of the protein binding to α-crystallin in its capacity as a chaperone protein. The absence of spin–spin interactions of the spin labeled proteins show that neither melettin nor insulin B chain are present in a central cavity or on the α-crystallin oligomer surface. The data suggest that within the complex the bound proteins are separated by 25 Å or more. The results parallel our α/γ complex simulation. In our model, the chaperoned proteins are intercalated in the space between the C-terminal domains of α-crystallin and the proteins are separated by a distance of at least 25 Å. Similar to γ-crystallin, the melettin binding was electrostatic in nature. These data are well represented by the conformation and dimensions of both subunits and quaternary structure of our open 'micellar' model. It has been shown that our working models correlate well with other attributes of α-crystallin determined experimentally: i.e. heat stability [26], polydispersity [15], average aggregate size [16], dynamic quaternary micellar structure [27], flexible C-terminal extensions subject to proteases [23], independence of adjacent C-terminal domains in the quaternary structure [28], electrostatic parameters [16], solvent accessibility of each subunit [28], and a sizable space (70%) for binding proteins [16]. Further simulations using our working model may be useful in determining strategies for crystallization of α-crystallin and X-ray diffraction structure determination.

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