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Hypothesis

A possible structure for α-crystallin

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α-Crystallin, the major protein of the mammalian eye lens, is found in vivo as a multimeric aggregate composed of two closely related subunits whose molar ratio is widely variable from species to species. Attempts to determine the arrangement of the subunits within the aggregate, or even to determine the size of the aggregate and the number of subunits composing it, have not resulted in general agreement. Because of the variability in α-crystallin particle size, the apparent dependence of this parameter on certain environmental factors (e.g. temperature), the absence of a specific requirement for either α-crystallin isoform in aggregation, and the sharp division in the amino acid sequence between a strong hydrophobic region and a sharply hydrophilic one, it is suggested that the α-crystallin aggregate has the properties of a protein micelle. This hypothesis is consistent with what is known of the α-crystallin molecule and aggregate, and can be tested experimentally. If this hypothesis is shown to be true, then α-crystallin will be the first example of a naturally occurring protein micelle.

α-Crystallin; Protein micelle

The eye lens contains very high concentrations (up to 600 mg/ml) of a unique group of structural proteins – the crystallins. They were first named and described by Berzelius in 1830 [1], and fractionated by Morner in 1894 [2]. Yet, despite considerable efforts by many investigators, our understanding of the structures and functions of these proteins is quite limited. This is particularly true for α-crystallin, the major protein of the mammalian lens.

α-Crystallin is a multimeric protein assembled from two closely related subunits, the A and B chains, each of which has a molecular mass of about 20 kDa. Both are altered by post-translational modification such as deamidation, phosphorylation and partial degradation [3]. Consequently, α-crystallins isolated from old lenses contain a complex mixture of subspecies. Extensive sequence analyses, mainly by De Jong and coworkers [4], have established that the evolution of the proteins has been comparatively slow. This would suggest that there may be considerable constraints on the structure of the protein. These could reflect physiological constraints imposed on the lens for the maintenance of transparency, plasticity and other essential features.

Many attempts have been made to determine the arrangement of the subunits in the α-crystallin aggregates but, to date, there is no general agreement. Despite some claims to the contrary [5–7], it would appear that there is no specific requirement for either subunit in the assembly of the aggregate. Thus, the subunit ratio varies not only with species [4], but also with developmental age [8]. In addition, it is possible to construct α-

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crystallin-like particles using any combination of purified A and B chains from any species [9,10]. In view of these observations and the similarities in their sequences [4], immunochemical properties [11], and the microenvironments of aromatic and other amino acids [12,13], it seems probable that the polypeptides have similar three-dimensional structures and can occupy the same sites in the aggregates. Chemical and immunochemical probing experiments have indicated that these sites are (quasi-) equivalent [14], with all subunits accessible on the surface of the aggregate [12,15].

The protein is generally isolated as a very polydisperse population of macromolecular aggregates, with an average molecular mass of 800 kDa. However, the size varies markedly with the age of the tissue and with the isolation conditions. Values as low as 320 kDa [16] and as high as 15 MDa (HMM-α) [17] have been reported. No evidence has been found for self-association of the protein particles [18]. The molecular mass distribution is independent of protein concentration, indicating that the various forms are not in equilibrium. It is possible that some of the very high values can be attributed to polymerization of the protein as a consequence of the post-translational modifications [19], but variations in the isolation conditions appear to be the main reason for the diversity. Temperature, pH, ionic strength, as well as specific ions, are known to alter the size distribution of the proteins [6,15,16]. It has been demonstrated that the higher molecular mass forms can be converted to smaller particles under relatively mild conditions, e.g. at 37°C [6], with 1 M urea [20], and by exposure to low ionic strength [6]. These observations would suggest that the larger forms are polymers of some simpler form of the protein, perhaps the 240 kDa species obtained when highly purified subunits are allowed to reassemble [9,10]. However, electron microscopy of the different α-crystallins does not support such a conclusion [21,22]. Most preparations consist of approximately spherical particles of very similar appearance, with a range of diameters (6–>20 nm) [21,22] that varies with the molecular mass distribution of the aggregates. Conversion of the 800 kDa particle to smaller species is accompanied by only a slight reduction in particle diameters. Only in the very large high molecular mass proteins isolated from the lens nucleus can polymeric structures, composed of smaller particles, be observed [19].

A complex model of the quaternary structure of the α-crystallin aggregates has been proposed by Hoenders and co-workers [6]. This suggests that the subunits are arranged in three concentric layers, with only A chains in the core and different proportions of A and B subunits in the other two layers. The outer layer was suggested to be incomplete to allow for the addition of more subunits with the ageing of the lens. A slightly modified version of this model was recently proposed by Tardieu et al. [23]. The models are consistent with the changes in molecular mass mentioned above, but not with the observations indicating that the subunits occupy equivalent sites in the aggregate or with the range of particle diameters. Furthermore, it is hard to accept that the A subunits could be located in three extremely different environments and the B subunits in two.

An alternative model suggests that α-crystallin is a dodecamer displaying T (tetrahedral), or 23 point group, symmetry [10,24]. This is consistent with the equivalence of the subunits, but also fails to explain the variations in size of the particles. It is possible that the smallest species which can be isolated from the lens, and the aggregates constructed from purified subunits, exhibit this structure. However, the populations with higher molecular masses do not appear to consist of simple multiples of a 12-subunit particle.

It is clear that neither model offers a satisfactory description of the structure of the aggregate. This raises the question of whether it will be possible, at all, to explain the many apparently conflicting observations in the literature in terms of a unique structure or set of structures in which the position of any subunit can be described with a closed set of simple rules for translation and rotation.

Most multimeric proteins have a unique quaternary structure in which the subunit orientation is determined by the distribution of polar and apolar regions on the surfaces of the subunits. However, in a number of well-characterized cases [e.g. tobacco mosaic virus (TMV) and tomato bushy stunt virus (TBSV) coat proteins], there is more than one way in which subunits can bind to each other. The tobacco mosaic virus subunit can bind to its identical neighbors in the capsid in one of two ways, depending on whether RNA has been incorporated

into the virus [25]. X-ray crystallography has shown that both sets of structural determinants coexist on the subunit surface and that the transition from one to the other is a function of the conformational state of that portion of the subunit which interacts with the RNA. In tomato bushy stunt virus, as in many other pseudo-icosahedral particles, the geometric problem of creating a closed surface from a large number of identical small subunits is solved by quasi-equivalent protein interactions [26]. Thus, the subunits can exist in one of two related conformations, with a flexible interdomain region allowing the interconversion. It should be noted that, unlike the TMV subunit, the two conformations of the TBSV subunit coexist within a single coat protein assembly.

Similar arrangements in α-crystallin could explain some of the apparent flexibility of the aggregate. However, there is no evidence to suggest that the protein interacts with any other lens constituent which could induce a conformational change, thereby favoring an alternative set of subunit interactions. It is possible that the B subunits have different conformations, and that their presence in the aggregates would disrupt the preferred dodecameric packing of the A chains sufficiently for the insertion of extra subunits. However, the B subunit contents of the different sized populations are apparently identical [16]. Similar arguments lead to the conclusion that post-translational modification of the subunits does not alter the packing either.

If α-crystallin has a uniquely determined structure or set of structures in the sense of other closed-surface protein aggregates, then it should exhibit certain characteristics. Completely reconstituted particles should show narrow size distributions, and these should be independent of protein concentration during the reconstitution above the critical concentration. Variations in pH, ionic strength, and temperature might affect the ability of the subunits to interact, but should not alter the size of any aggregates which may still be present. Furthermore, interactions between particles should be specific and geometrically orientated; this could result in the formation of ordered polymers of the particles themselves, as in the case of sickle cell hemoglobin, and, in situ, would lead to a loss of lens transparency.

None of these characteristics seems to apply to α-crystallin. It would appear that its quaternary structure is flexible and not constrained by the symmetry requirements which apply to most other aggregates. In fact, many aspects of the protein's behavior more closely resemble those of a population of micelles.

Micelles are self-assembled aggregates of amphiphilic molecules orientated such that the non-polar regions of the molecules are segregated from the solvent. This leads to a significant reduction in free energy, with a strongly positive entropy term; i.e. entropy is the driving force for the formation of micelles [27,28]. In general, initial formation of micelles occurs within a narrow concentration range for a given set of environmental conditions (the critical micelle concentration), and the aggregates formed near this boundary are spherical. At higher concentrations, well above the range necessary for initial micelle formation, there is often a second transition from spherical or ellipsoidal micelles to extended cylinders and/or bilayers. Thus, the sizes and shapes that can be formed are quite variable. This is due to the limitations of the spherical shape: in a polar solvent, the micelle core of a spherical particle could not be much greater than the length of two non-polar regions, since the solvent is excluded. Once formed, the micelle is stabilized by a combination of the entropic effects and interactions of the hydrophilic regions of the molecules with the solvent. There are no specific interactions between the molecules in the hydrophobic core. Hence, if the hydrophilic regions do not interact specifically with each other either, the molecules would be free to rotate or even to move over the micelle surface.

Although the characteristics described have been derived both experimentally and theoretically for small model compounds such as phospholipids and extended linear hydrocarbons, it would be expected that a protein with the same three-dimensional distribution of polar and non-polar surfaces could behave in a similar manner. Amphiphilic subunits in a polar solvent would arrange themselves so that the non-polar regions would be segregated from the solvent and the polar regions exposed. The final particle shape would depend on the concentration of the subunits, but the 'critical micelle concentration' would be much lower than that observed for small model compounds, since

the protein amphiphile is much larger than the model compounds and fewer molecules would be required to form a closed surface. The particles would contain a variable number of subunits within limits determined by the subunit size and shape, the absolute minimum for a closed surface being dependent on both factors, and the subunits would arrange themselves on the surface only insofar as necessary for close packing. With these constraints satisfied, there would be no unique structural organization of the subunits on the surface and internally. Since there are no specific interactions within the hydrophobic core, it would be expected that each subunit would freely rotate in position and/or move relative to other subunits over the surface; these degrees of freedom could be affected, of course, by the nature of the hydrophilic region of the subunits and the possible specificity of their interactions with each other.

The major requirement of this model is that the subunits contain clearly distinct hydrophobic and hydrophilic surfaces. Examination of the amino acid sequences of the A and B chains suggests that this could be so. It would appear that each subunit consists of three domains corresponding to the three exons found in the α-crystallin genes [29]. Hydrophilicity prediction profiles [30] reveal that the N-terminal domain (residues 1–63 in the A chain) is strongly hydrophobic, whereas the other domains are hydrophilic. Fluorescence quenching and probing experiments indicate that amino acids in this domain are located close to the center of the aggregates [13,31]. This suggests that the N-terminal domain represents the hydrophobic end of the amphiphilic subunit.

Many of the experimental observations on α-crystallin are consistent with this micellar model. Particle size is variable, as would be expected at the high concentrations found in vivo [19], and increases with age as the concentration of the protein increases, due to the compression of the fiber cells in the center of the lens. The particle size can be altered in vitro, being dependent on factors such as temperature [6,8], ionic strength [6], and specific ion effects [6,15]. Reaggregation of the protein is concentration dependent [7], and increased concentrations result in increased particle size [22]. Any combination of subunits from any species will form a viable α-crystallin particle [9,10]. All subunits, regardless of the size of the particle, are in equivalent locations, all accessible on the surface [9,10,15].

In some respects, α-crystallins appear to resemble the casein aggregates which are generally referred to as micelles [32]. These aggregates are isolated as approximately spherical particles with variable diameters which are sensitive to changes in their environment, e.g. in pH, temperature, ionic strength and composition. However, the casein particles are much larger (40–280 nm) and appear to contain an ordered arrangement of α-s and β-casein subunits in the core, covered by an outer coat of $\varkappa$-casein. Thus, the different subunit types are not in equivalent locations as they are in α-crystallin. Furthermore, calcium is required for casein micelle formation, while no specific ion requirements have been found for the lens protein. It would therefore appear that the two structures are not related.

The non-specific structure of micellar aggregates could be an important factor in the transparency of the lens. If the particles are variable in size and shape, their close proximity in situ cannot lead to local order and, thus, diffraction of the incoming light. Even if all the α-crystallin were present in similarly sized spherical particles, as it might be in the very young lens, long-range order could still not be attained, due to the lack of uniqueness of the organization of the subunits on their surfaces and the post-translational modification of the subunits, which begins early in life. The former militates against geometrically specific interparticle orientation, while the latter specifically creates geometrically randomized interparticle interactions. With time, however, and the innate liquidity of the micellar interior, it is possible that specific interactions could eventually occur through movements of the subunits on the particle surfaces. This possibility, combined with solvent and solute exclusion from the nascent close-packed α-crystallin particles, may be an important factor in senile cataract development.

In summary, we propose that the structure and properties of α-crystallin can best be explained by a micellar model for the subunit organization. This concept is consistent with the amino acid sequence of the subunits, a variety of experimental observations obtained with different techniques and under different conditions, and direct observations of the particles in the electron microscope. Furthermore,

it can be used to explain the maintenance of lens transparency and the reasons for post-translational modifications, and offers a possible etiology for cataractogenesis. This hypothesis is currently being tested in our laboratories.

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