

Lens α -crystallin: function and structure

JOSEPH HORWITZ, MICHAEL P. BOVA,
LIN-LIN DING, DANA A. HALEY,
PHOEBE L. STEWART

Abstract

α -Crystallin is a major lens protein, comprising up to 40% of total lens proteins, where its structural function is to assist in maintaining the proper refractive index in the lens. In addition to its structural role, it has been shown to function in a chaperone-like manner. The chaperone-like function of α -crystallin will help prevent the formation of large light-scattering aggregates and possibly cataract. In the lens, α -crystallin is a polydisperse molecule consisting of a 3:1 ratio of α A to α B subunits. In this study, we expressed recombinant α A- and α B-crystallin in *E. coli* and compared the polydispersity, structure and aggregation state between each other and native bovine lens α -crystallin. Using gel permeation chromatography to assay for polydispersity, we found native α -crystallin to be significantly more polydisperse than either recombinant α A- or α B-crystallin, with α B-crystallin having the most homogeneous structure of the three. Reconstructed images of α B-crystallin obtained with cryo-electron microscopy support the concept that α B-crystallin is an extremely dynamic molecule and demonstrated that it has a hollow interior. Interestingly, we present evidence that native α -crystallin is significantly more thermally stable than either α A- or α B-crystallin alone. In fact, our experiments suggest that a 3:1 ratio of α A to α B subunit composition in an α -crystallin molecule is optimal in terms of thermal stability. This fascinating result explains the stoichiometric ratios of α A- and α B-crystallin subunits in the mammalian lens.

Key words α -Crystallin, Cataract, Chaperone, Heat-shock proteins, Lens, Lens proteins

The structural proteins in the eye lens that are responsible for the refractive properties of this tissue are called the crystallins.^{1,2} The crystallins make up more than 90% of the total dry mass of the lens. In the mammalian lens there are, in general, three classes of crystallin: α , β , γ . Each crystallin class constitutes about one-third of the total mass. There are two α -crystallin genes; α A and α B.³ In humans, for example, the α A gene encodes for a 173 amino acid residues polypeptide and the α B gene encodes for a 175

amino acid residues polypeptide. There is a 57% sequence homology between α A and α B. In all vertebrate lenses α -crystallin is found as a heterogeneous multimeric complex with a molecular weight distribution ranging from 300 000 to over 1 million.³ The average molecular weight of α -crystallin in mammalian lenses is estimated at 600 000 to 900 000. Thus each native α -crystallin complex is made up of 30 to 45 subunits. In these native multimeric complexes the ratio of α A to α B is approximately 3:1.

Until the late 1980s it was believed that the crystallins were lens-specific proteins. However, it was found that α B-crystallin is essentially a ubiquitous protein and is a *bona fide* member of the small heat-shock protein family.⁴⁻⁶ α A-crystallin is found mainly in the lens. It has been shown in recent years that α B-crystallin is overexpressed in various neurodegenerative diseases. It is also elevated in the ischaemic heart and in other biological systems where stress is introduced.³

In 1992, it was shown that α -crystallin has chaperone-like properties,⁷ being capable of binding to unfolded or denatured proteins and suppressing non-specific aggregation. The finding that α -crystallin possesses chaperone-like properties suggests that it may have a functional role in the lens, in addition to its structural role as a major refractive element. In the lens there is no protein turnover, repair mechanisms are minimal, post-translational alteration and protein unfolding continue throughout life, and transparency has to be maintained for decades. The chaperone-like properties of α -crystallin seem to be most suitable for a unique tissue such as the eye lens where proteins, especially in the lens centre, are as old as the organism. Some of the properties of α -crystallin in relation to its lenticular function will be discussed here.

Materials and methods

Bovine α -crystallin, calf α -crystallin, bovine recombinant α A- and human recombinant α B-crystallin were prepared and purified as described previously.⁸ The thermal stability of α -crystallin was assessed by recording the far-ultraviolet circular dichroism spectra on a JASCO model J-600 spectropolarimeter. Cryo-

J. Horwitz
M.P. Bova
L.L. Ding
D.A. Haley
Jules Stein Eye Institute
UCLA School of Medicine
Los Angeles
CA 90095, USA

D.A. Haley
P.L. Stewart
Department of Molecular
and Medical Pharmacology
& Crump Institute for
Biological Imaging
UCLA School of Medicine
Los Angeles
CA 90095, USA

Joseph Horwitz ✉
Jules Stein Eye Institute
100 Stein Plaza
UCLA School of Medicine
Los Angeles
CA 90095, USA
Tel: +1 (310) 825 7521
Fax: +1 (310) 794 2144
e-mail:
horwitz@jsei.ucla.edu

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electron microscopy and three-dimensional image reconstruction of α B-crystallin were performed as described previously.⁹

Results

The insolubilisation of proteins in the lens nuclear region as a function of age

It has been known for over 30 years that with age there is a gradual conversion of lens proteins from a 'water-soluble' native state to a 'water-insoluble' modified and denatured state.¹⁰ Several investigators have found that with increasing age there is a decline in the amount of soluble protein in both the cortex and nucleus of the human lens.^{11,12} Fig. 1 shows a typical gel filtration chromatography elution profile of human lens soluble proteins obtained from the central nuclear area of a 10-year-old and a 50-year-old human lens. The lens is essentially a closed system where proteins cannot diffuse from the centre to the periphery. Thus, comparing the protein profile from the same location allows us to calculate the decrease in the soluble fraction as a function of age. As shown in Fig. 1 there is a 44% loss of the β - and γ -crystallins upon aging from 10 to 50 years; however, there is a 100% loss of the soluble α -crystallin. On the other hand, in the cortical regions there is always some soluble α -crystallin and the decrease in the amount of α -crystallin, as well as of β - and γ -crystallin, is gradual (see fig. 3 in McFall *et al.*¹²). The abrupt disappearance of the soluble α -crystallin from the lens nucleus between the ages of 40 and 50 years suggests that in addition to its refractive role it may have another function as the lens ages.

The heterogeneity of native lens α -crystallin and recombinant α -crystallin

As was stated in the introduction, native lens α -crystallin is not a unique, well-defined complex of the same number of subunits, but rather a heterogeneous population of variable mass. The size and charge

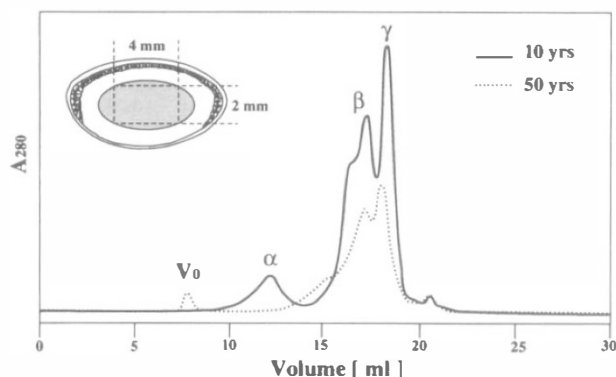


Fig. 1. Gel filtration chromatography of a nuclear lens section 2 mm \times 2 mm \times 4 mm. The soluble lens protein from these lens sections was chromatographed on a Pharmacia HR-6 column using a Pharmacia FPLC system. The flow-rate was 1 ml/min. V_0 denotes the void volume. Continuous line, soluble proteins from a 10-year-old lens; dashed line, soluble proteins from a 50-year-old lens.

heterogeneity of α -crystallin has been studied extensively.³ In these studies on the quaternary structure of bovine α -crystallin, Siezen and co-workers¹³ suggested that there are more than 1000 different hybrids of α -crystallin. Post-translational modifications that constantly take place in the intact lens add to the heterogeneity.³ The recent availability of recombinant α -crystallin rekindled interest in studying the physicochemical properties of this protein. Analytical gel filtration chromatography is a simple way to study the homogeneity or heterogeneity of a protein sample. Fig. 2 shows the molecular weight distribution of native bovine α -crystallin as well as of recombinant α A- and α B-crystallin. As shown, native α -crystallin is the most heterogeneous protein, with a nonsymmetrical distribution. Under the conditions we used, the peak of the native α -crystallin sample corresponds to a molecular weight of 800 000. The molecular weight of the leading edge (between 8 ml and 10 ml) is larger than 1 million. Recombinant α A-crystallin is very similar to native α -crystallin with a significant non-symmetrical molecular weight distribution. On the other hand, recombinant α B-crystallin shows a symmetrical normal distribution, suggesting a more homogeneous population (Fig. 2). However, compared with a well-defined protein oligomer such as GroEL, or a monomeric protein such as ferritin, α B-crystallin is significantly less homogeneous. This is evident from the fact that the elution peak width at its half-height is significantly wider than would be expected for a monomeric protein sample, as shown in Fig. 3. The question to be asked is whether α -crystallin functions better as a polydisperse population.

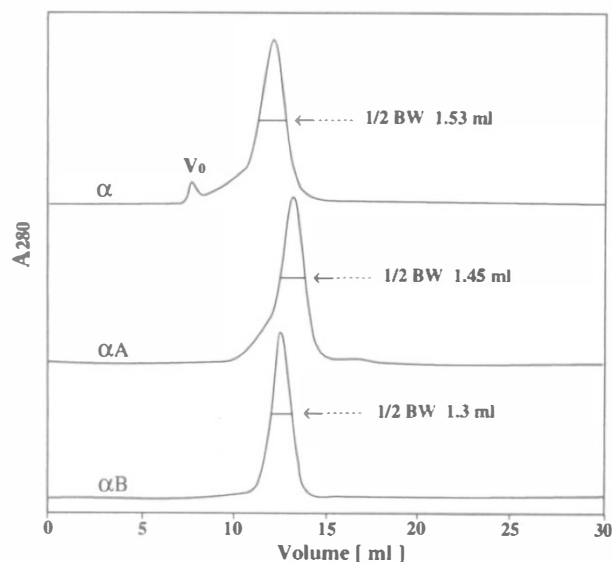


Fig. 2. Elution profile of native bovine α -crystallin, recombinant bovine α A-crystallin and recombinant human α B-crystallin. The system used was a Pharmacia FPLC with HR-6 column. All samples were chromatographed under identical conditions. The sample volume was 100 μ l; the flow rate was 1.0 ml/min. The protein concentration was 0.2 mg/ml.

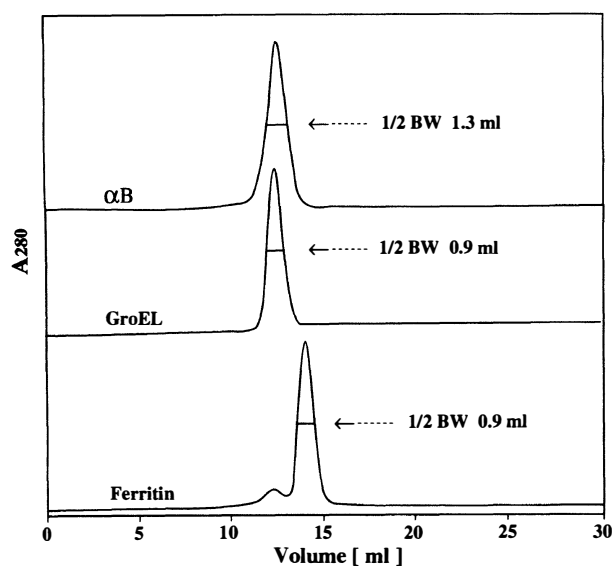


Fig. 3. Elution profile of recombinant human α B-crystallin, GroEL and ferritin. All other conditions were identical to those in Fig. 2.

Is there an advantage in having the ratio of 3 α A to 1 α B that is found in the native α -crystallin complex of the mammalian lens?

A puzzling question is why the eye lens contains a mixture of α A and α B subunits. A second question is why there is a specific ratio of a 3 α A subunits to 1 α B subunit. Since they have 57% sequence homology, α A and α B have many similar physicochemical properties. There are, however, significant differences between them, such as isoelectric point, hydrophobicity, thermal stability and the ability to bind unfolded proteins.^{3,8} The thermal stability can be monitored by measuring the circular dichroism spectra of the protein in question. Fig. 4 shows the far-ultraviolet circular dichroism spectra of recombinant α B-crystallin at various temperatures. There is a significant change in the secondary structure conformation of α B-crystallin as the temperature is

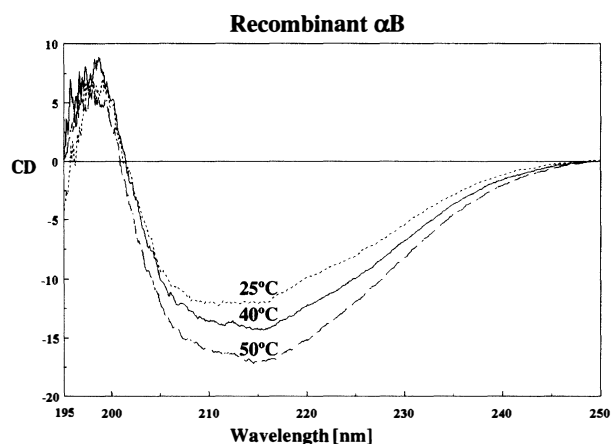


Fig. 4. Far-ultraviolet circular dichroism spectra of human recombinant α B-crystallin. Each spectrum represents the average of 16 scans. The protein concentration was 1.8 mg/ml and the pathlength was 0.2 mm.

Table 1. Change in circular dichroism intensity of native bovine α -crystallin, recombinant α A-crystallin, recombinant α B-crystallin and various mixtures of recombinant α A- and α B-crystallin upon heating from 25 °C to 50 °C

25 °C → 50 °C	α_{native}	α A	α B	α B + α A (1:1)	α B + α A (1:3)
% change	10%	19%	47%	18%	9%

raised from 25 °C to 50 °C. To a first approximation, the larger the changes, the less stable the protein. In Table 1 we compare the percentage change in the circular dichroism intensity at 217 nm of recombinant α B-, α A- and native α -crystallin, as the temperature changes from 25 °C to 50 °C. For native α -crystallin, there is only a 10% change whereas for α A- and α B-crystallin the changes are 19% and 47% respectively. Mixing different ratios of α A- and α B-crystallin reveals an interesting finding. A ratio of 3:1 α A- to α B-crystallin is the most stable, and is significantly more stable than either α A alone or α B alone.

The structure of α -crystallin

The three-dimensional structure of α -crystallin has been a subject of intense investigation for many years. Many hypothetical models have been proposed.^{3,14} These include various 'three-layers' models in which α -crystallin subunits are depicted as hard balls and layered to produce the desired complex.^{15,16} Other models include a 'protein micelle'-like model,¹⁷ a rigid cubic and rhombic dodecahedron model,¹⁸ a GroEL-like model,¹⁹ a micellar model with elongated subunits,²⁰ and a 'pitted-flexiball'²¹ model. It should be emphasised that, to date, in spite of intensive efforts by several laboratories, no one has succeeded in crystallising α -crystallin. It is conceivable that the heterogeneous nature of the α -crystallin complex prevents it from being crystallised. Recently, using cryo-electron microscopy, we have succeeded in gaining some insight into the quaternary structure of α -crystallin.⁹ We have chosen recombinant α B-crystallin for our studies, because we have found that it is less heterogeneous than α A-crystallin or native α -crystallin (Fig. 2). The cryo-electron microscopy results suggest that α B-crystallin has a variable quaternary structure. Our results also show that the α B-crystallin assemblies are approximately 11 nm in diameter, having a thick protein shell, approximately 3 nm thick, surrounding a central cavity. While the resolution of our work is relatively low (approx. 4 nm), this is the first time that an average structure of α -crystallin based on hard data has been constructed. A three-dimensional image reconstruction of α B-crystallin is shown in Fig. 5.

Discussion

There are two unique features to the vertebrate eye lens. First, there is a very high concentration of structural proteins (crystallins), reaching values in excess of

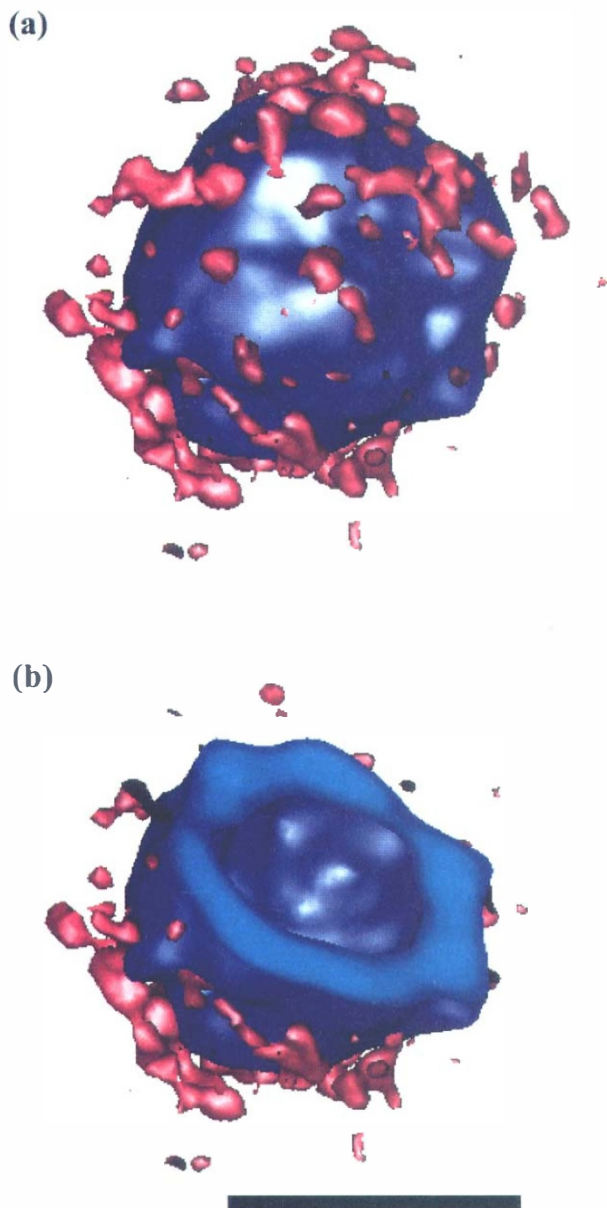


Fig. 5. A three-dimensional reconstruction (blue) of an approximately 32-subunit α B-crystallin assembly surrounded by three-dimensional variance (red) that demonstrates significant structural variability in the assembly. Refer to Haley et al.⁹ for more details. The bar represents 10 nm.

500 mg/ml.^{10,22} The high protein concentration is needed to obtain the necessary refractive index for the lens. Second, in the lens there is continued growth throughout life and lack of protein turnover in the terminally differentiated lens fibre cells.^{10,23} These features are the consequence of the 'design' and the programmed development and growth of the eye lens. It is well established that many of the lens crystallins in the animal kingdom were 'recruited' from existing enzymes to serve an additional role as a structural protein.^{23,24} The dual function of a protein that can serve both as a structural protein and as an enzyme led to the recent concept of 'gene-sharing'.^{24,25} This concept simply means that the same gene acquires an additional function without

duplication. α -Crystallin is a clear example of gene sharing. α B-crystallin, which is a *bona fide* small heat-shock protein, can be found, for example, in the embryonic mouse heart before the eye is formed.²⁶ α A-crystallin, on the other hand, is thought to arise by gene duplication with a more specific role as a lens crystallin.

The chaperone-like properties of the α -crystallins may be the reason why these particular proteins were 'recruited' to serve the lens. If one considers, for example, a 60-year-old normal person, the proteins in the centre of this person's lens are 60 years old! During the six decades these proteins were subjected to a variety of insults, stresses, and major post-translational modifications, causing them to unfold and denature. Denatured or unfolded proteins tend to aggregate, and protein aggregation in the lens will lead to light scattering and cataract. Since the old proteins in the centre of the lens cannot be repaired, and nor can they be catabolised and resynthesised, having a protein with chaperone-like properties in large amounts can alleviate the problems. α -Crystallin, which recognises unfolded or denatured proteins, can selectively bind to them and arrest non-specific aggregation. The evidence suggests that α -crystallin serves as a one-way 'sink' in the lens, binding and controlling the unavoidable denaturation of proteins taking place during normal ageing. Unlike chaperone systems in other cells, where the ultimate goal is to *refold* an unfolded protein, α -crystallin in the lens serves only as a part of a typical complex chaperone pathway. In the lens there is no way to refold the damaged old protein, so α -crystallin, by binding to them, controls and avoids non-specific aggregation.²⁷ Fig. 1 suggests that on ageing it is only β - and γ -crystallin that are denaturing. It should be emphasised, however, that in these fractions there are relatively small but significant amounts of housekeeping enzymes such as glyceraldehyde-3-phosphate dehydrogenase, enolase, leucine aminopeptidase and aldehyde dehydrogenase. These enzymes are much more susceptible to denaturation than the other crystallins.²⁸ It was shown recently that without the protein of α -crystallins, aggregation of minor lens components can contribute to the process of opacification.²⁸

The experimental data available suggest that the heterogeneity of the α -crystallins is beneficial to the system. Our circular dichroism experiments show that an α -crystallin mixture of 3 α -crystallins to 1 α B-crystallin is the most stable. Chaperone assay experiments also show that, in general, the hybrid α -crystallin complexes are more stable than either α A or α B alone (data not shown). α B-crystallin tends to aggregate by itself at temperatures around 62 °C. It was shown recently that the addition of α A to α B stabilised the system.²⁹ While the above-mentioned experiment was conducted at non-physiological temperatures where the α -crystallin undergoes irreversible changes, it still lends support to the idea that the hybrid 3:1 α A-crystallin to α B-crystallin mixture is more stable.

More direct evidence for the idea that hybrid α -crystallin molecules are beneficial to the system comes from a recent transgenic mouse experiment with a targeted disruption of the mouse α A-crystallin gene.³⁰ This mouse developed cataracts and inclusion bodies containing α B-crystallin that were found throughout the lens fibre cells. These findings suggest that high concentration of pure α B-crystallin may lead to aggregation.

While the heterogeneity of the α -crystallin complex is beneficial to its function, it certainly hampered progress in elucidating its three-dimensional quaternary structure. Our recent results using cryo-electron microscopy show that, indeed, α -crystallin does not possess a single conformation but has a dynamic variable structure.⁹ The variable and dynamic property of the α -crystallin molecule is consistent with studies showing that there is an intermolecular exchange of the subunits. This was first observed by Van der Oetelaar *et al.*³¹ More recent work by Bova *et al.*³² showed that this subunit exchange strongly depends on the temperature, with extremely high activation energy of 60 kcal/mol and rate constant of 0.075 min^{-1} at 37 °C. The exchange rate increases significantly upon increasing the temperature from 37 °C to 42 °C. All these results are consistent with the findings that the chaperone-like properties of α -crystallin are much better at physiological temperature than at 20 °C, for example. At the higher temperature the capacity of α -crystallin for binding unfolded protein increases significantly.³³

A high-resolution three-dimensional structure of the α -crystallin complex is still not available. Recently, the crystal structure of a small heat-shock protein from *Methanococcus jannaschii* (a hyperthermophilic archaeon) was obtained.³⁴ This 16.5 kDa protein is distantly related to α -crystallin. It should be emphasised, however, that this small heat-shock protein has a unique structure that is always composed of 24 subunits. Similar to what we have found for α -crystallin, this rigid and well-defined 16.5 kDa small heat-shock protein also has a central cavity but, in addition, it has a high degree of symmetry, unlike the variable shapes and sizes of α B-crystallin.^{9,35} The similarity between some of the domains of the 16.5 kDa small heat-shock protein and α -crystallin should help us to solve the quaternary structure of α -crystallin and understand its mode of action in the lens and in other tissues throughout the body.

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