The eye lens chaperone αB-crystallin forms defined 24meric globular assemblies

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α-crystallins are molecular chaperones that belong to the small heat-shock protein (sHsp) family. sHsps constitute an important component of the stress response. Their cellular function is to bind partially unfolded polypeptides and maintain them in a refolding competent state [1]. α-crystallins form large oligomers that were reported to display great heterogeneity. The current opinion is that α-crystallins cannot be obtained in homogeneous form. Thus neither the structure of monomeric α crystallin nor the topology of the subunit assembly within the oligomer are yet known [2].

To re-evaluate the degree of heterogeneity and the quaternary structure of acrystallins, we analyzed the structures of recombinant human α-A- and αB-crystallin.

Human αB-crystallin was recombinantly expressed in E. coli and purified to homogeneity. For both proteins, mass spectrometry (MS) revealed the correct monomer masses. Aggregation assays with the model substrate lysozyme confirmed the chaperone activity of the purified proteins.

For α-crystallins, biochemical and structural data in the literature suggest heterogeneous assemblies with a continuum of different oligomeric states. In our preparations, however, both recombinant α-crystallins eluted in analytical SEC columns in single narrow and symmetric peaks. According to linear calibration, the elution time of αBcrystallin corresponded to a molecular mass of ~475 kDa consistent with a complex of 24 subunits (20.2 kDa monomeric mass). In addition sedimentation velocity (SV) analysis of αB-crystallin generated a single sedimentation boundary, implying the absence of significant fractions of differently sized particles.

In line with the results from analytical SEC and SV, transmission electron microscopic (TEM) analysis also confirmed the presence of rather homogeneous αB-crystallin preparations. For three-dimensional reconstruction, single particle TEM-images of αB-crystallin from negative stain and cryo preparations were subjected to multivariate statistical analysis (MSA) and classified. Class averages clearly indicated the presence of well-defined oligomeric structures some of them with obvious 3-fold symmetry (Fig. 1). As in addition αB-crystallin oligomers were determined to be 24mers by SEC and sedimentation equilibrium ultracentrifugation, tetrahedral symmetry was imposed for the calculation of the three-dimensional model. By means of angular reconstitution based on selected class averages, a three-dimensional model of the αB-crystallin oligomer could be established which was then iteratively refined, in analogy to a prevesiously reported 3D-TEM-analysis [3].

The three-dimensional reconstruction shows that human αB-crystallin oligomers are roughly spherical (Fig. 2A) with a diameter of 13.5 nm. The oligomer accomodates a large central cavity with 8.5 nm in diameter, which is surrounded by a protein shell. Its mean thickness is about 2.5 nm, reaching up to 4 nm (Fig. 2B). The protein shell contains openings

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at the positions of the 3- and 2-fold axes, with diameters of about 3.5 nm for the 2-fold axes and 3 nm and 2 nm for the two non-equivalent 3-fold axes, equivalently.

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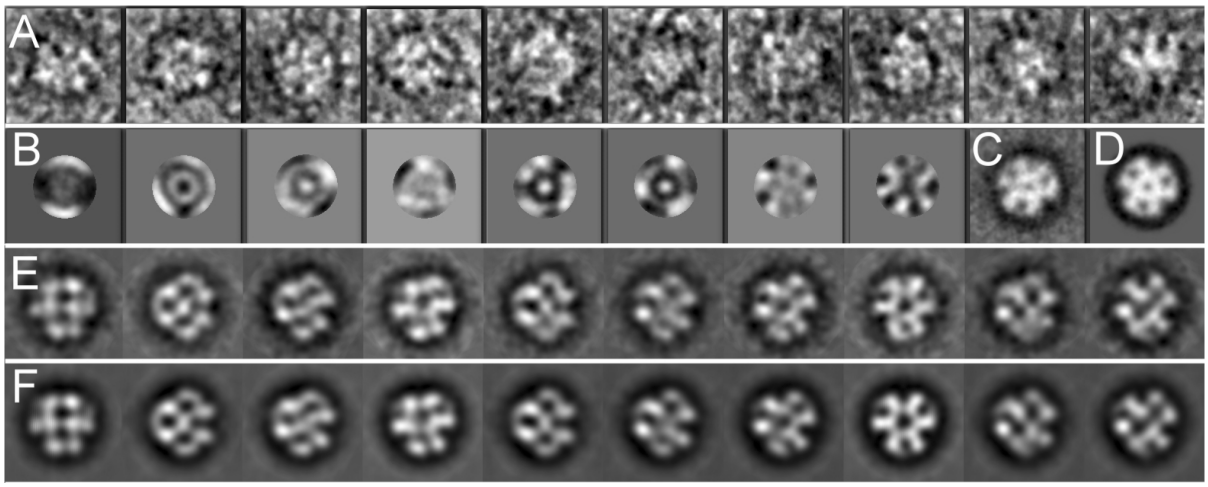

Figure 1. (A) Gallery of single, negatively stained αB-crystallin oligomers (stained with 1.5%[w/vol] ammonium molybdate, pH 5.5). (B) First eigenimages obtained after translational alignment of the original dataset (C) Characteristic class average with 3-fold symmetry. (D) Class average from C with imposed 3-fold symmetry. (E) Representative final class averages. (F) Two-dimensional reprojections of the three-dimensional model of αB-crystallin into directions corresponding to the orientations of the above class averages. Scale bar: 10 nm.

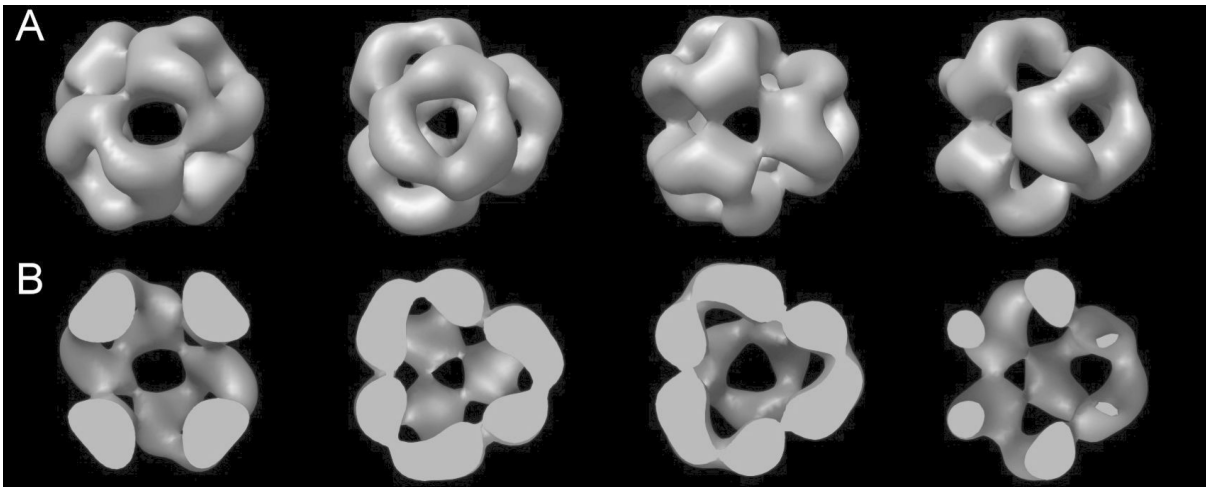

Figure 2. (A) Surface representations of the 3D model of human recombinant αB-crystallin, viewed along the 2-fold axis,the 3-fold axes and perpendicular to the dimeric α-crystallin domain. (B) Density cross sections through the 3D model of αB-crystallin.

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