Cryo-EM Structures of a Single Ring Chaperonin from Bacteriophage OBP P.fluorescence in Nucleotide-free and ADP-bound States

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Chaperonins are the Hsp60 (heat-shock protein 60) family of molecular chaperones, which ensure the correct folding of denatured or newly synthesized proteins. They consist of several subunits organized in barrel-like structures with an inner cavity where the folding occurs. The common feature of all chaperonins is the ATP-dependent transition between open and closed conformations. Recently, chaperonins have been found in bacteriophage genomes (Kurochkina et al, 2012; Semenyuk et al, 2016). The object of the present study is a single-ring chaperonin of bacteriophage OBP *P. fluorescence*.

Using cryo-electron microscopy we studied 3D structures of OBP chaperonin in nucleotide-free and ADP-bound states. The protein was isolated from *E. coli* lysate and purified using Q sepharose chromatography. Micrographs were collected on an FEI Titan Krios microscope equipped with a Falcon 2 direct detector; image processing was performed in Relion-2.1 (Kimanius et al, 2016). In total 43098 particles were used to build 3D reconstructions of nucleotide-free state (final resolution 7.3 Å) and 75955 for ADP-bound state (final resolution 6.5 Å) without imposing symmetry.

In contrast to the vast majority of chaperonins which are double-ring complexes, the OBP chaperonin is a single-ring structure. Despite being assembled from seven chemically identical subunits, it demonstrates a surprising degree of asymmetry in both nucleotide-free and nucleotide-bound states (Fig. 1A, B). In nucleotide-free state C7 symmetry is only displayed at the level of equatorial domains (Fig. 1C), while apical domains are arranged as three pairs and one unpaired domain (Fig. 1D). The unpaired subunit is less resolved in the reconstruction, which implies its higher mobility compared to subunits within pairs. In ADP-bound conformation subunit mobility is increased presumably due to the loss of contacts between subunits within pairs.

Fitting of a homology-based atomic structure into the density map of an OBP chaperonin indicated a novel subunit conformation. Compared to GroEL the apical domains of OBP chaperonin are rotated relative to the intermediate domains resulting in a different set of intra-ring contacts (Fig. 2). Structural differences between the OBP chaperonin and chaperonins of group I and group II indicate the existence of a new group of chaperonins and a yet unknown mechanism of chaperonin activity.

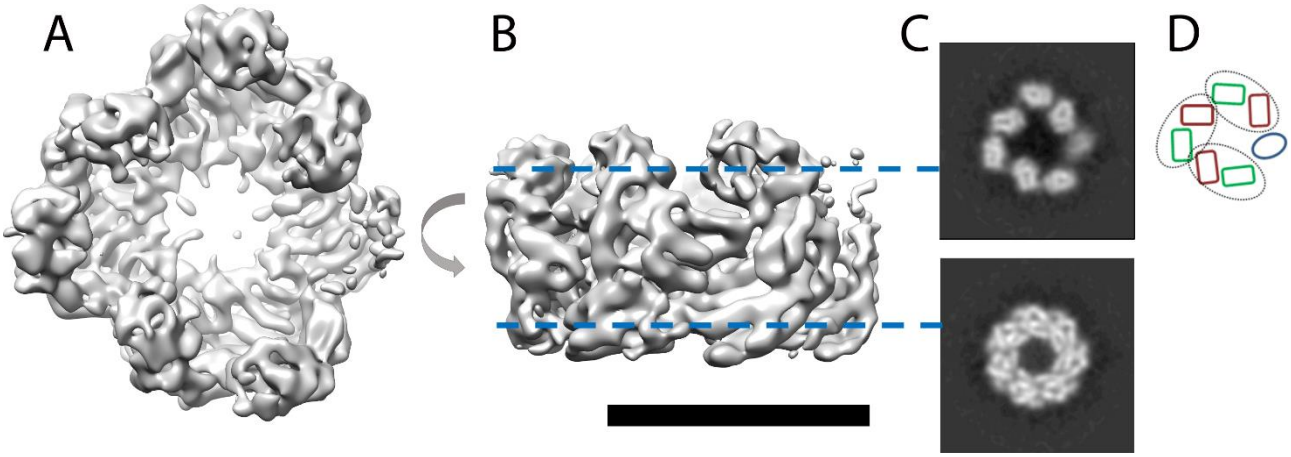

Figure 1. Cryo-EM structure of a single-ring OBP chaperonin in its nucleotide-free state. (A) Symmetry-free 3D reconstruction (top view); (B) side view. Scale bar is 10 nm. (C) sections through reconstructions at the level of apical domains (upper image) and equatorial domains (bottom image); (B) the hypothetical arrangement of the apical domains.

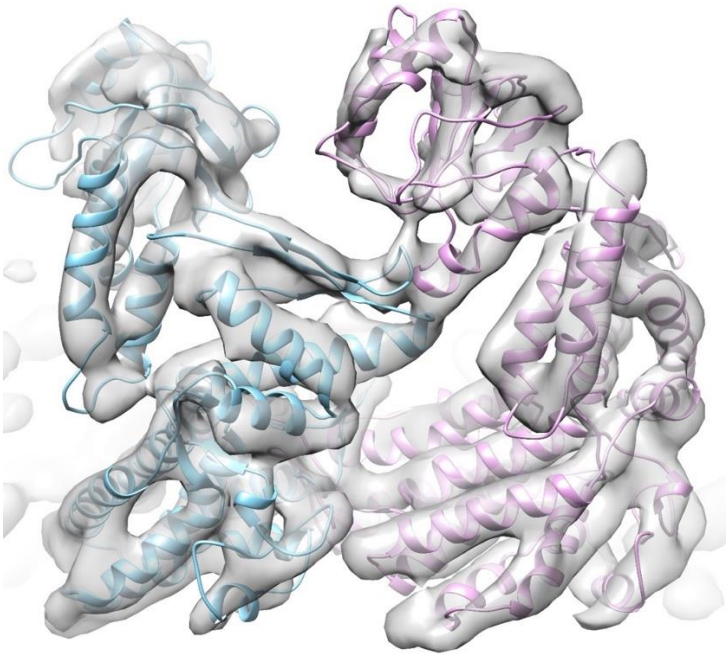

Figure 2. Fitting of the homology-based model of OBP chaperonin into EM density of a subunit pair.

The authors acknowledge funding from the Russian Foundation for Basic Research (grants #16-04-01587 to O.S. and #18-04-01281 to L.K.)