required to clarify the role of Rpn14 in 26S proteasome assembly.

Keywords: proteasome, chaperones, assembly

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3D structure of small dense LDL; Application of low-resolution diffraction and *ab initio* methods

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Until now, no three-dimensional structural data of the 'especially bad' small dense (sd-LDL) particles existed. Our previously published X-ray structure [Lunin et al. 2001] and the one obtained by Orlova et al. [1999] (cryo EM) correspond to the larger LDL-2 particles. Both papers showed that LDL particles have a cylindrical respectively ellipsoidal shape and are not spherical particles as commonly assumed. In the particle core, a system of flat layers was observed. At present, we are able to grow crystals of native, human LDL particles from all LDL subfractions, except LDL-4. Whereas LDL-1 to LDL-3 (large LDL) crystallize in space group C2, LDL-5 and LDL-6 (sd-LDL) crystallize in space group P2(1). By special methods we were able to collect 100% complete datasets of LDL crystals in a resolution range of 300-27A. A simple analysis of the parameters of the reduced cells shows that large-LDL and sd-LDL differ predominantly in the short axis of the crystallographic unit cell (large-LDL: a=180 Å, sd-LDL: a=143 Å). These results lead to the hypothesis that sd-LDL particles, if approximated as cylinders, have a height reduced by 37 Å compared to large-LDL. As the repeating distance of smectic layers of LDL cholesterol esters is also 37 Å, the observed reduction of one cell axis could correspond well to the disappearance of one layer of cholesterol esters in the particle core. This is perfectly supported by the now available electron density maps calculated from LDL-5 and LDL-6 datasets by ab initio methods were in fact the disappearance of one layer inside the particle core is visible. While differences in core structure are obvious, the analysis of the particle surface (ApoB fold) is more complicated due to the dense packing of the crystals, and still in progress.

Keywords: lipoproteins, *ab-initio structure* determination, low resolution

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The hexameric structures of the archeal secretion ATPase revealed by X-ray crystallography and SAXS

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Secretion ATPase superfamily is a widely conserved ATPase family that play the central role in many bacterial and archaeal macromolecular transport and filamentous subunit assembly mechanisms; such as type II secretion, type IV secretion, natural competence, type IV pilin, and archaeal flagellar systems. They commonly form hexameric ring structure, by which they would function as a motor via the conformational transition upon ATP hydrolysis. Here, we report the crystal structure of the archaeal secretion ATPase, GspE from Archaeoglobus fulgidus, complexed with the non-hydrolysis ATP analog, AMP-PNP. It represents the alternating open and closed subunit conformations within a hexameric ring. The active site conformation bound with AMP-PNP and Mg^{2+} in the closed form reveals the catalytically active conformation. Solution structures analyzed by small angle x-ray scattering (SAXS) showed that the gamma-phosphate of AMP-PNP can lock the closed conformation to form the symmetric all-closed hexamer. In contrast, SAXS ADP structure revealed the asymmetric hexameric ring similar to crystal structure with AMP-PNP, suggesting the mixture of the active closed conformation and inactive open conformations. Together with these results, we propose the conformational transition mechanism upon ATP hydrolysis and its catalytic cycle, which would be common for all secretion ATPase superfamily.

Keywords: ATPase, hexameric ring, SAXS

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Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes

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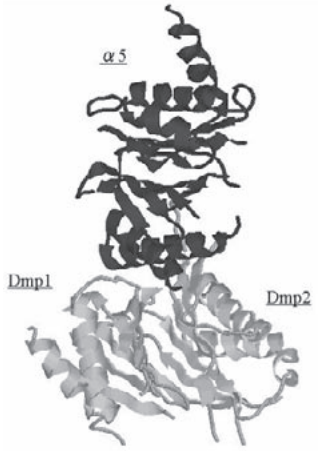
Eukaryotic 20S proteasomes are composed of two α-rings and two β-rings, which form an $\alpha\beta\beta\alpha$ stacked structure. Here we describe a proteasome-specific chaperone complex, designated Dmp1-Dmp2, in budding yeast. Dmp1-Dmp2 directly bound to the α5 subunit to facilitate α-ring formation. In delta-dmp1 cells, α-rings lacking α 4 and decreased formation of 20S proteasomes were observed. We also determined the crystal structure of Dmp1-Dmp2 delta loop-α5 complex and illustrated an intermediate state of proteasome assembly. A model of Dmp1-Dmp2 interacting with the αring was generated by superimposed the α5 subunit from Dmp1-Dmp2 delta loop-α5 on the structure of the 20S proteasome (PDB ID code 1RYP).Since the β2, β3 and β4 subunits of the proteasome are thought to attach to the α-ring during the primary stage of β-ring assembly, the steric hindrance caused by β4 and Dmp1 must trigger the release of Dmp1-Dmp2 from the α-ring during the attachment of the β subunit onto the α-ring.



Keywords: proteasome, chaperones, macromolecular X-ray crystallography