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Structural characterization of pneumococcal surface protein PGK by X-ray diffraction

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Streptococcus pneumoniae is the major cause of pneumonia, meningitis and septicemia [1], [2]. Pneumococcal infections are a major global health problem worldwide. The search of novel combat strategies is urgently needed. Phosphoglycerate kinase (PGK) from S. pneumoniae is a major enzyme in the gycolytic pathway, and also important as surface protein. We have solved the structure of PGK from *S. pneumoniae*. Further investigations will reveal the role of PGK as virulence factor. This information could be relevant in the fight against infection diseases caused by pneumococcus

[1] S. Bergmann, S. Hammerschmidt. *Microbiology* **2006,** *152*, 295-303. [2] S. Hammerschmidt. *Curr Opin Microbiol* **2006** *9*, 12-20.

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Structure of ubiquitin-like small archaeal modifier protein 1 (SAMP1) from *Haloferax volcanii*

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The ubiquitin-like (Ubl) system has been shown to be ubiquitous in all three kingdoms of life following the very recent characterization of ubiquitin-like small archaeal modifier proteins (SAMP1 and 2) from *Haloferax volcanii*. The ubiquitin (Ub) and Ubl molecules in eukaryotes have been studied extensively and their cellular functions are well established. Biochemical and structural data pertaining to prokaryotic Ubl protein (Pup) continue to be reported. In contrast to eukaryotes and prokaryotes, no structural information on the archaeal Ubl molecule is available. Here we determined the crystal structure of SAMP1 at 1.55 A resolution and generated a model of SAMP2. These were then compared with other Ubl molecules from eukaryotes as well as prokaryotes. The structure of SAMP1 shows a β-grasp fold of Ub, suggesting that the archaeal Ubl molecule is more closely related to eukaryotic Ub and Ubls than to its prokaryotic counterpart. The current structure identifies the location of critical elements such a single lysine residue (Lys4), C-terminal di-glycine motif, hydrophobic patches near leucine 60, and uniquely inserted α-helical segments (a1 and a3) in SAMP1. Based on the structure of SAMP1, several Ub-like features of SAMPs such as poly-SAMPylation and non-covalent interactions have been proposed, which should provide the basis for further investigations concerning the molecular function of archaeal Ubls and the large super-family of β-grasp fold proteins in the archaeal kingdom.

Keywords : X-ray, structure, ubiquitin

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Structural Insights of the S1 Pocket in the Yeast 20S Proteasome

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Proteasomes are multicatalytic protein complex of large molecular weight. That is known that the ubiquitin proteasome system plays an important role in regulated proteolysis. The 26S proteasome is composed of two 19S regulatory components and a 20S proteasome. 20S proteasome forms barrel shape and consist of 4 protein rings arranged in parallel. Upper two rings are arranged symmetrically with lower two rings and they are called α- and β-rings. Each ring consists of 7 heterogeneous subunits. There is a little homology between 7 subunits within the ring, and those between α- and β-ring. N-terminal tails of α-ring subunit form an entry gate to facilitate passage of substrate. Each of 1, 2 and 5 βsubunit has different enzyme activities; 1 has caspase-, 2 tryptic- and 5 chymotryptic-like activities. At was found recently that inhibition of 20S proteasome activity resulted in decrease and disappearance of cancer cells. Therefore the inhibitor of 20S proteasome serves as a new anticancer reagent. It is necessary to disclose the structural details of S1 active site of 20S proteasome and its complex with the inhibitor. We have investigated inhibitor compounds and their reaction mechanisms by an auto-dock simulation against the S1-pocket of the bovine 20S proteasome, and found structural characteristics in such region, and have checked inhibitory reaction with yeast 20S proteasome. Therefore we have analyzed the 20S proteasome from yeast, and are going to study the mechanism or S1-pocket specificities with atomic resolution. Here we report on the preparation of the yeast 20S proteasome and the enzymatic activities with inhibitor, and also on the crystallization and initial structure determination.

Yeast 20S proteasome was prepared by over expression in yeast (*Saccharomyces cerevisiae*). Cells were homogenized by glass beads and the crude protein solution was purified by M2 affinity chromatography and Mono-Q anion exchange one. Isolated 20S proteasome was concentrated by ultrafiltration, and was co-crystallized with small molecule ligand. Sitting drop vapor diffusion method was applied using 0.1M MES-NaOH (pH 6.5), 15% MPD, 50 mM magnesium acetate as a reservoir solution and 10 mg/ml proteasome as a sample solution. Crystals are isomorphous as described in the previous report [1] and belong to the space group $P2_1$. Diffraction images were recorded at 100 K by using Rayonix MX-225HE CCD detector installed in BL44XU, SPring8, Japan. These images were processed by using HKL2000, intensity data up to 2.5 Å become available. Initial phases were determined by molecular replacement method and the structure model without a ligand was refined by *Refmac*.

Three-dimensional direction and localization of amino acid residue around S1 pocket are available for one inhibitor; TYR(135) and GLY(128) interact with -OH and -C=O groups, but VAL(129) interferes with such region. Results show possible inhibition with yeast proteasome according to bovine structural information and biological assay of the yeast one. We are preparing crystals by means of a co-crystallization or inhibitor-soaking crystal method. Their structural analyses are now in progress.

[1] M. Groll, L. Ditzel, J. Lowe, D. Stock, M. Bochtler, H.D. Bartunik, R. Huber, *Nature*, **1997**, *386***,** 463-471.

Keywords: proteasome, structure, X-ray