The 6-O-methylglucosyl-containing lipopolysaccharides (MGLPs) and the 3-O-methylmannosylcontaining polysaccharides (MMPs) are two unusual polymethylated polysaccharides (PMPS) produced by mycobacteria. Both PMPS localize to the cytoplasm, where they have been proposed to regulate fatty-acid biosynthesis owing to their ability to form stable 1:1 complexes with long-chain fatty acids and acyl-coenzyme A derivatives. In sequestering the products of fatty-acyl synthase I (FAS I), PMPS are thought to facilitate the release of the neo-synthesized chains from the enzyme, thereby not only reopening active sites essential for enzyme turnover but also terminating their elongation. In addition, PMPS have been proposed to serve as general fatty-acyl carriers, the role of which would be to facilitate the further processing of very long and insoluble fatty-acyl CoAs, including mycolic acids, by increasing the tolerance of mycobacteria to high cytoplasmic concentrations of these products while protecting them from degradation. The glucosyl-3-phosphoglycerate synthase (GpgS), is a retaining a-glucosyltransferase that initiates the biosynthetic pathway of the MGLPs in mycobacteria. The enzyme transfers a Glc*p* moiety from UDP-Glc to the 3 position of the phosphoglycerate to form glucosyl-3-phosphoglycerate. Here we report new crystal structures of the *apo* and UDP complex forms of GpgS from *Mycobacterium tuberculosis* at 2.6 and 3.0 Å resolution respectively. The overall structure shows the two-domain organization typical of GT-A GTs. We propose a plausible model for donor and acceptor substrates recognition and catalysis. The implications of this model for the comprehension of the early steps of MGLPs biosynthesis and the catalytic mechanism of other members of the GT-A family are discussed.

Keywords: Mycobacteria, glucosyl-3-phosphoglycerate synthase (GpgS)

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Crystal structure of cyclophilin-a enzyme from *Azotobacter vinelandii*

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The Peptidyl-Prolyl Isomerases (Cyclophilins, FKBPs and Parvulins) catalyze the cis-trans isomerization of peptide bonds preceding prolyl residues, therefore accelerating protein folding [1]. Cyclophilins (CyPs) have been established as a model system in enzymology in terms of extensive efforts of understanding the mechanism of enzyme catalysis in full depth [2]. *Azotobacter vinelandii* is a well known agricultural, aerobic, soil-dwelling bacterium, which fixes atmospheric nitrogen, converting it to ammonia, which is the most ingestible form of the element for the plants. There are two known cyclophilins in *A. vinelandii*: cytoplasmic AvCyPA and periplasmic AvCyPB.

The crystal structure of the cytoplasmic cyclophilin A was determined by molecular replacement at 1.7 Å resolution. In addition, the crystal structure of the protein complexed with the synthetic tetrapeptide succinyl-Ala-Phe-Pro-Phe-p-nitroanilide (sucAFPFpNA) was determined at 2.0 Å resolution. The tetrapeptide sucAFPFpNA was used as a substrate for an assay that confirmed that *A. vinelandii* AvCyPA possesses PPIase activity. The tetrapeptide is bound as a proline cis-isomer and adopts different conformations from those observed in other related structures. Comparisons between the uncomplexed and complexed structures as well as other CypA structures provides additional insights about structure-function relationships of this enzyme. Also structural studies for PPIAses from a new organism may complement existing studies and help achieve a better understanding of the link between sequence variation and enzymatic function.

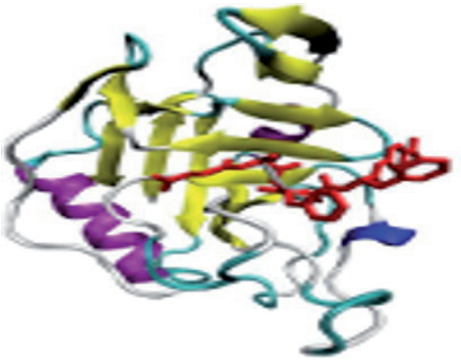

Figure 1: The complex between AvCyPA and synthetic peptide sucAFPFpNA solved at 2.0Å resolution

[1] Wang, J. Heitman, *Genome Biology* **2005**, *6,* 226. [2] E.Z. Eisenmesser, O. Millet, W. Labeikovsky, D.M. Korzhnev, M. Wolf-Watz, D.A. Bosco, J.J. Skalicky, L.E. Kay, D. Kern, *Nature* **2005**, *438, 7064,* 117-121.

Keywords: peptidyl-prolyl isomerase, azotobacter vinelandii, catalysis

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Crystal structure analysis of the genetic encoded photosensi-tizer KillerRed

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Several methods have been developed to elucidate the protein functions and interactions in living cells up to the present. Among them, methods for losing protein functions derived meaningful data from biochemical and cellular biological experiments. Chromophore-assisted light inactivation (CALI) is one of promising techniques to inactivate target proteins in living cells [1]. In CALI, chromophore molecules are used as photosensitizer, which produce highly reactive free radicals including reactive oxygen species (ROS) by irradiation of intense light. ROS have short lifetime, therefore the damage radius is limited to approximately 3-4 nm [2]. This indicates that inactivation of the protein(s) is limited in short timescales and very small regions, where the inactivation light is exposed. So far some fluorescent small molecules such as malachite green and fluorescein were used as photosensitizer for CALI applications. These photosensitizers should exogenously introduce into living specimen, which is the bottleneck of developing versatile application of CALI. KillerRed is the first genetically encoded photosensitizer, which has notable phototoxicity. KillerRed is developed by protein engineering from the hydrozoan chromoprotein anm2CP, a homolog of GFP [3].

For the farther development of KillerRed, we determined the crystal structure of KillerRed to understand the structural basis for its phototoxicity. The crystal structure of KillerRed was solved by S-SAD at 2.8Å resolution. The data sets were collected using the loopless data-collection method [4] with chromium Kα X-rays. The overall structure of KillerRed was 11-stranded β-barrel with an internal α-helix passing through inside of the barrel, which is characteristic of the fluorescent protein family. The chromophore formed by the autocatalytic cyclization

and oxidation of three residues (Gln65-Tyr66-Gly67) located at the center of internal α-helix. The imidazolinone moiety of chromophore was exposed to the outside of the β-barrel through the characteristic water-filled channel. It is considered that oxygen molecules are converted to ROS with light induced energy transfer at chromophore, followed by ROS diffuse to outside of β-barrel through this channel.

[1] F.S. Wang, D.G. Jay, *Trends Cell Biol*. **1996**, *6*, 442-445. [2] S. Beck, T. Sakurai, B.K. Eustace, G. Baste, R. Schier, F. Rudert, D G. Jay, *Proteomics* **2002**, *2*, 247-255. [3] M.E. Bulina, D.M. Chudakov, O.V. Britanova, Y.G. Yanushevich, D.B. Staroverov, T.V. Chepurnykh, E.M. Merzlyak, M.A. Shkrob, S. Lukyanov, K.A. Lukyanov, *Nat. Biotechnol*. **2006**, *24*, 95-99. [4] Y. Kitago, N. Watanabe, I. Tanaka, *Acta Cryst*. **2005**, *D61*, 1013-1021.

Keywords: protein, structure

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Structure improvement and analysis of F16L lipase crystallized under microgravity environment

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Previously we have solved the structure of wild type T1 lipase at 1.5Å which revealed that a unique Na^+-π interaction with Phe16. The 1.5 Å resolution map revealed that a specific atom tightly interacts with the aromatic π-system of Phe16 [1]. To further confirm the positive contribution of the side chain of Phe16 for the present cation-π interaction, we have also solved the crystal structure of mutant F16L T1 lipase at 1.8 Å, which were prepared and crystallized similarly to the wild-type enzyme. Although the resolution was slightly lower (1.8 Å), we found almost zero electron density in mutant F16L enzyme at the position corresponding to the metal ions in the wild-type enzyme. In order to improve the structure of F16L lipase, we send the protein to space under our National Angkasawan Program. Space crystallization was carried out using high-density protein crystal growth apparatus (HDPCG) utilizing vapor diffusion method and X-ray diffraction data was collected at SPring-8 BL41XU, Japan. Slight increase in crystal size and interface was observed. Higher resolution of 1.7 Å was obtained for space crystals with better quality electron-density map distributed over the entire F16L lipase space crystal structure.

[1] H. Matsumura, T. Yamamoto, T.C. Leow, T. Mori, A.B. Salleh, M. Basri, T. Inoue, Y. Kai, *R.N.Z.R.A. Rahman* **2008**, *70(2),* 592-598.

Keywords: thermostable lipase, microgravity

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Crystal structure of the engineered cross-linked complex between Fd and FNR

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In photosynthetic organisms, Ferredoxin (Fd) and Fd-$NADP^+$ reductase (FNR) are redox partner proteins responsible for the conversion between $NADP^+$ and NADPH. The plant-type Fd is an electron carrier protein containing one [2Fe-2S] cluster, and FNR catalyzes the reduction of $NADP^+$ using two electrons donated from reduced Fds. Electron transfer between Fd and FNR requires the formation of transient Fd-FNR complex. Three X-ray crystal structures of Fd-FNR complexes from maize leaf and root tissues and the cyanobacterium *Anabaena* have been reported so far [1-3]. However, the specific interaction mode of Fd and FNR is largely different among the three known X-ray structures of Fd-FNR pairs, which exhibit distinct physicochemical and physiological properties. Understanding the mechanism of electron transfer between redox partners is a complex problem involving specific recognition between redox partners, subsequent conformational changes, and redox potentials of redox centers.

To investigate the relative contribution of these factors to the electron transfer between Fd and FNR, we introduced the specific disulfide bonds between maize leaf Fd and FNR by engineering cysteines into the two proteins resulting in 13 different Fd-FNR cross-linked complexes[4]. These variants display a broad range of activity to catalyze the NADPH-dependent cytochrome *c* reduction, categorized into three groups with more than 70% activity in comparison to wild-type Fd/FNR, with intermediate activity (7-46%), and with less than 3% activity. These distinctive electron transfer rate of Fd-FNR must be closely related to the distance and orientation of two redox centers, and environment around them. We have crystallized the two types of Fd-FNR cross-linked complexes, with high and intermediate activities. Furthermore, the X-ray diffraction data were collected at 2.7 Å, and 4.5 Å resolution, respectively, on the synchrotron-radiation beam line BL44XU at the SPring-8. The crystal structures of the two types of engineered cross-linked Fd:FNR complexes were solved by molecular replacement. We will describe the possible determinant to tune the electron transfer rate between the plant-type Fd and FNR based on two complex structures.

[1] R. Morales, G. Kachalova, F. Vellieux, M. Charon, M. Frey, *Acta Crystallography* **2011**, *D56*, 1408-1412 [2] G. Kurisu, M. Kusunoki, E. Katoh, T. Yamazaki, Y. Onda, Y. Kimata-Ariga, T. Hase, *Nature Structural biology* **2001**, *8*, 117-121. [3] G.T. Hanke, G. Kurisu, M. Kusunoki, T. Hase, [4] Y. Kimata-Ariga, Y. Sakakibara, T. Ikegami, T. Hase, *Biochemistry* **2010**, *23*, 10013-10023.

Keywords: photosynthesis, flavoprotein, reductase

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A single amino acid limits the substrate specificity of uridine-cytidine kinase

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Nucleoside metabolism is one of important metabolic systems. Many anti-cancer and anti-viral drugs have been developed to act on nucleoside metabolic enzymes. Insight into the structure and mechanism of the enzymes can aid in the development of potent inhibitors. Uridine-cytidine kinase (UCK) catalyzes the phosphorylation of cytidine and uridine to CMP and UMP, respectively. However, mechanism of substrate specificity in UCK has not been investigated in detail. We found that the UCK homologue from Thermus thermophilus HB8 (ttCK) has the substrate specificity toward only cytidine. Activity of ttCK was inhibited by CTP, but not uridine, UMP, and UTP, could suggesting that uridine cannot bind to ttCK. In order to elucidate the reason for the exclusion of uridine by ttCK, we determined the X-ray crystal structures of ttCK in ligand-free form and in complex with CMP