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.

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Keywords: protein, structure

MS93.P62

Acta Cryst. (2011) A**67,** C791

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.

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Keywords: thermostable lipase, microgravity

MS93.P63

Acta Cryst. (2011) A**67,** C791

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.

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Keywords: photosynthesis, flavoprotein, reductase

MS93.P64

Acta Cryst. (2011) A**67,** C791-C792

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

at 2.4 Å and 2.25 Å, respectively. This is the first reported structure of a prokaryotic UCK. The overall structure is highly similar to that of human uridine cytidine kinase 2 (UCK2). Structural comparison of the nucleoside-binding site between ttCK and human UCK2 revealed that most amino acid residues around the base moieties are identical between them, except for Tyr59 and Tyr93 in ttCK, corresponding to Phe83 and His117 in human UCK2. These two residues are located near the N4 amino group and the N3 nitrogen atom of cytosine: these atoms differ between cytosine and uracil. Many UCK homologues have a Phe and His residue at the position equivalent to Tyr59 and Tyr93 in ttCK, respectively. As the next step, we prepared ttCK mutants with amino acid substitution at Tyr59 and Tyr93. The substrate specificity of Y59F was the same as that for wild type. In contrast, Y93H had activity for both uridine and cytidine. Whereas the replacement of Tyr93 with Phe or Leu, unaffected the substrate specificity of ttCK, the replacement with Gln, Asn and Glu endowed ttCK with phosphorylation activity toward uridine. These results indicate that a hydrophilic residue at position 93 permits UCK to accept uridine as substrate. A potential hydrogen donor at this position may enable UCK to interact a keto group of uridine specifically. In addition to ttCK, other UCK homologues of 26 species including pathogens have tyrosine at the position equivalent to Tyr93, which predicts that these are cytidine-specific UCKs. This study points to the critical need for experimental studies even of enzymes whose annotation has been accepted.

Keywords: nucleoside, kinase, mutation

MS93.P65

Acta Cryst. (2011) A**67,** C792

Structure-based catalytic optimization of a type III rubisco

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The Calvin-Benson-Bassham cycle is responsible for carbon dioxide fixation in all plants, algae, and cyanobacteria. The enzyme that catalyzes the carbon dioxide-fixing reaction is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco from a hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tk*-Rubisco) belongs to the type III group, and it shows high activity at high temperatures. We have previously determined the crystal structure in the apo-form of this enzyme [1]. We have also found that replacement of the entire α-helix 6 of *Tk*-Rubisco with the corresponding region of the spinach enzyme (SP6 mutant) results in an improvement of catalytic performance at mesophilic temperatures, both *in vivo* and *in vitro*, whereas the former and latter half replacements of the α-helix 6 (SP4 and SP5 mutants) do not yield such improvement [2]. We report here the crystal structures of the wild-type *Tk*-Rubisco and the mutants SP4 and SP6, and discuss the relationships between their structures and enzymatic activities [3].

A comparison among these structures shows the movement and the increase of temperature factors of α-helix 6 induced by four essential factors. We thus supposed that an increase in the flexibility of the α-helix 6 and loop 6 regions was important to increase the catalytic activity of *Tk*-Rubisco at ambient temperatures. Based on this structural information, we constructed a new mutant, SP5-V330T, which was designed to have significantly greater flexibility in the above region, and it proved to exhibit the highest activity among all mutants examined to date. The thermostability of the SP5-V330T mutant was lower than that of wild-type *Tk*-Rubisco, providing further support on the relationship between flexibility and activity at ambient temperatures.

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Keywords: structure, enzyme, thermophile

MS93.P66

Acta Cryst. (2011) A**67,** C792

TTP binding in the active conformation of dCTP deaminase: dUTPase

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The bifunctional dCTP deaminase:dUTPase is a homotrimeric enzyme, which is closely related to the monofuncional dCTPases and dUTPases. The enzyme catalyses the deamination and the phosphate hydrolysis of dCTP to dUMP and it is end-product inhibited by dTTP. Until now the structures of the wild type *Mycobacterium tuberculosis* bifunctional enzyme in its apo form (pdb-entry 2qlp) and with TTP (pdb-entry 2qxx) bound have been solved [1] and the *Methanocaldococcus jannaschii* bifunctional enzyme in the apo form (pdb-entries 1ogh, 2hxb, 1pkh, [2,3]) is also known. Furthermore, two variants of the *M. jannaschii* enzyme have been crystallized: the E145Q variant with diphosphate and magnesium (pdb-entry 3GF0) and the E145A variant of the *M. jannaschii* enzyme with α,β-imido dUTP and magnesium (pdb-entry 2hxd) [4]. It has been concluded that the bifunctional dCTP deaminase:dUTPase exists in two mutually exclusive conformations. An active form, which resembles the apo-form and the structure where α,β-imido dUTP binds and an inactive form that binds dTTP. To investigate the inhibition by dTTP we have made several mutant enzymes, one of which is able to bind dTTP in the active conformation. Furthermore, the wild type enzyme and this mutant enzyme have been enzymatically characterized and interestingly, the mutant enzyme, which binds dTTP in the active conformation, is still inhibited by dTTP.

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Keywords: nucleotide metabolism, bifunctional dCTP deaminase: dUTPase, dTTP inhibition

MS93.P67

Acta Cryst. (2011) A**67,** C792-C793

Structural studies of the aminotransferases LivB and NeoB

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