a precipitant solution containing $CaCl_2$, PEG 4000 and a pH 7.5. We have collected X-ray datasets up to 2.1 Å resolution, revealing an orthorhombic crystal system with the unit cell parameters: a= 46.18 Å, b= 80.82 Å, c= 104.5 Å, α=β=γ=90º. Space group is $P2_12_12_1$ and there is one molecule per asymmetric unit. The phasing method used to solve this structure was molecular replacement. The three-dimensional structure shows the general fold for this protein and presents valuable information about its function.

Keywords: macromolecules, mechanism, structure

MS93.P71

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The structure of a novel bifunctional dehydratase/isomerase from fungal secondary metabolism

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Several fungi contain a dehydratase/isomerase that converts metabolites from lignin degradation into small compounds that show anti-tumor and antimicrobial effects against both Gram-positive and Gram-negative bacteria. The reaction occurs in two steps, a dehydratase reaction resulting in a relatively stable reaction intermediate followed by a unique isomerase step that leads to the final product. The enzyme is a dimer of 200 kDa, and the enzyme subunit (900 amino acids) does not show significant overall sequence homology to any protein of known structure. The X-ray structure of this enzyme has been solved using SAD to 2.0 Å resolution. The enzyme belongs to the all beta class of proteins and consists of three domains. Anomalous diffraction experiments revealed the presence of three Zn2+ ions and removal of zinc resulted in the loss of catalytic activity. Complexes with substrate and a reaction intermediate allowed the identification of the two active sites and suggest that two of the zinc ions participate in catalysis. The two active sites are about 20 Å apart, which requires that the reaction product of the first step has to diffuse from the enzyme in order to reach the second active site. This observation is consistent with NMR data that show release of this intermediate into the bulk solution. The crystal structures of the enzyme-ligand complexes allow some mechanistic conclusions that can be probed by other biochemical methods.

Keywords: macromolecular crystallography, bifunctional enzyme, dehydratase/isomerase

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Structures of exo-β-D-glucosaminidase, a chitosan degrading enzyme from *Trichoderma reesei*

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The 93-kDa extracellular protein composed of 892 amino acids from *Trichoderma reesei*, named Gls93, is an *exo-β*-D-glucosaminidase (GlcNase), belonging to glycoside hydrolase family 2. Gls93 catalyzes the degradation of chitosan into *β*-D-glucosamine (GlcN) monomers. For crystallographic studies, Gls93 was overexpressed in *Pichia pastoris* cells. The recombinant Gls93 had two molecular forms of approximately 105 kDa (Gls93-F1) and approximately 100 kDa (Gls93-F2), with the difference between them being caused by N-glycosylation pattern.

Initial crystal screenings for Gls93-F1 and Gls93-F2 produced several microcrystals in a week. Trials to improve the crystallization conditions were performed by varying the pH, the buffer system and the precipitant concentration. As a result, rectangular parallelepiped-shaped crystals were obtained from different conditions. Both crystal forms of gls93-F1 and gls93-F2 were suitable for X-ray structure analysis[1]. Initial phase determination for the Gls93-F1 crystal was performed by the molecular-replacement (MR) technique using the coordinate set of a bacterial GHF2 GlcNase, *A. orientalis* CsxA (PDB ID 2VZS; van Bueren *et al*., 2009), which has approximately 37% amino-acid sequence identity to Gls93, as a search model. Initial phase determination for the Gls93-F2 crystal was performed by the MR method with coordinate set of Gls93-F1. We have also successfully determined the crystal structure of Gls93 complexed with glucosamine. The crystal structures of Gls93-F1, Gls93-F2, and Gls93-F1/glucosamine were refined at 1.8, 2.4, and 2.6 Å resolutions, respectively. In addition, affinities of oligo sugars to the Gls93 enzyme were examined by surface plasmon resonance (SPR). The present crystal structure analyses and SPR measurements provide useful information for the substrate recognition mechanism of Gls93.

[1] Y. Sakamoto, M. Ike, N. Tanaka, Y. Suzuki, W. Ogasawara, H. Okada, Y. Morikawa, KT. Nakamura, *Acta Crystallogr F* **2010***, 66,* 309-312.

Keywords: crystallization, glucosaminidase

MS93.P73

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One residue substitution in PcyA leads to unexpected changes in tetrapyrrole substrate binding

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Phycocyanobilin:ferredoxin oxidoreductase (PcyA) catalyzes the sequential reduction of the vinyl group of the D-ring and A-ring of biliverdin IXα (BV), using reducing equivalents provided by ferredoxin. This reaction produces phycocyanobilin, a pigment used for light-harvesting and light-sensing in red algae and cyanobacteria. The crystal structure of PcyA-BV reveals that BV is specifically bound in the PcyA active pocket through extensive hydrophobic and hydrophilic interactions. During the course of a mutational study of PcyA, we observed that mutation of the V225 position, apart from the processing sites, conferred an unusual property on PcyA; V225D mutant protein could bind BV and its analog BV13, but these complexes showed a distinct UV-vis absorption spectrum from that of the wild-type PcyA-BV complex (Fig. 1). In order to gain a better understanding of this non-canonical spectrum, we have determined the crystal structures of V225D mutant protein in complex with BV and with BV13.

The crystal structures of BV- and BV13-bound forms of V225D protein revealed that gross structural changes occurred near the substrate-binding pocket, and that the BV/BV13 binding manner in the pocket was dramatically altered (Fig. 2). That is, the orientation of the bound BV in the V225D protein was inverted relative to the PcyA-BV complex along the axis connecting the γ-position and the mid-point of the A- and D-rings. Furthermore, the BV in the V225D protein was translated by approximately 3 Å from the corresponding position in PcyA-BV. Protein folding in V225D-BV/BV13 was more similar to that of substrate-free PcyA than that in PcyA-BV; the "induced-fit" did not occur when BV/BV13 was bound to the V225D protein. The

unexpected structural change presented here provides a cautionary note about interpreting functional data derived from a mutated protein in the absence of its exact structure.

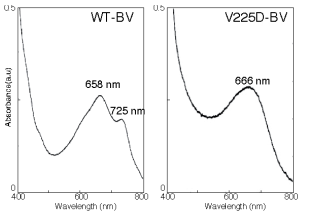

Fig. 1Absorption spectra of BV and BV13 in the presence of the V225D protein or the wild-type PcyA.

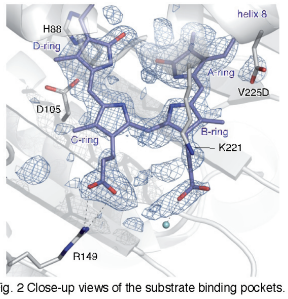

Fig. 2 Close-up views of the substrate binding pockets.

[1] K.Wada, Y.Hagiwara, Y.Yutani, K.Fukuyama, *Biochem. Biophys. Res. Commun.***2010**, *402*, 373-377 .

Keywords: mutation, pigment, reductase

MS93.P74

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Mapping of protein-protein interaction sites in the plant-type [2Fe-2S] ferredoxin

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Ferredoxins (Fds) are small iron-sulfur proteins with low redox potentials that are involved in diverse electron-transfer systems. The plant-type [2Fe-2S] Fds function not only in photosynthesis, where they transfer electron from photosystem I to ferredoxin-NADP$^+$ reductase, but also in electron transfer to such Fd-dependent enzymes as sulfite reductase, nitrite reductase, and Fd-thioredoxin reductase. It was well known that Fd is a hub redox protein, and therefore extensive studies on the structure and function have been made to solve the puzzle of how this small protein partitions electron to a variety of Fd-dependent enzymes.

We have refined the crystal structure of a recombinant plant-type [2Fe-2S] Fd I from the blue green alga *Aphanothece sacrum* (*As*Fd-I) at 1.46 Å resolution on the basis of the synchrotron radiation data. Incorporating the revised amino-acid sequence, our analysis corrects the 3D structure previously reported; we identified the short a-helix (67-71) near the active center, which is conserved in other plant-type [2Fe-2S] Fds. Although the 3D structures of the four molecules in the asymmetric unit are similar to each other, detailed comparison of the four structures revealed the segments whose conformations are variable. Structural comparison between the Fds from different sources showed that the distribution of the variable segments in *As*Fd-I is highly conserved in other Fds, suggesting the presence of intrinsically flexible regions in the plant-type [2Fe-2S] Fd. A few structures of the complexes with Fd-dependent enzymes clearly demonstrate that the protein-protein interactions are achieved through these variable regions in Fd. The results described here will provide a guide for interpreting the biochemical and mutational studies that aim at the manner of interactions with Fd-dependent enzymes.

Keywords: ferredoxin, iron-sulfur cluster protein, protein-protein interactions

MS93.P75

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Crystallographic analyses of the ISC proteins involved in *de novo* Fe-S cluster biogenesis

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Iron-sulfur (Fe-S) clusters act as cofactors of various Fe-S proteins that are widely distributed in nature and required to maintain fundamental life processes. Recent studies revealed that the assembly of Fe-S clusters in several bacteria as well as eukaryotic mitochondria is achieved by a multicomponent system, called ISC machinery. This machinery is generally encoded by the *iscSUA-hscBA-fdx* operon, and consists of six ISC proteins. Among them, the components playing central roles in *de novo* Fe-S cluster biogenesis are IscS and IscU. IscS is a cysteine desulfurase that catalyses the sulfur atom abstraction from cysteine substrate and provides the sulfur atom for the biosynthesis. IscU serves as a scaffold for assembly of a nascent Fe-S cluster, prior to its delivery to target Fe-S protein, in which directly accepts the sulfur atoms from the IscS. We have so far determined the unique trimeric structure of the [2Fe-2S] cluster-bound IscU from the hyperthermophilic bacterium *Aquifex aeolicus* (*Aa*) [1]. This structural information provided mechanistic implications that the dynamic association/dissociation of IscU must be the critical events to interact with the other components in the assembly for the nascent Fe-S clusters. Here we focus on the transient complex between IscS and IscU, to clarify the detail manners of protein-protein interactions underlying the sulfur transfer.

Coexpression of *Aa* IscS and *Aa* IscU in *E. coli* resulted in formation of a binary complex, of which the crystal was obtained. A data set was collected to 2.0Å resolution on beamline BL41XU at SPring-8. The structure of IscS moiety was solved with the molecular replacement method using a model of the dimeric *Aa* IscS as the search prove. Although electron densities derived from IscU were mostly invisible, some secondary structures could be assigned. We modeled the structure of IscU moiety in IscS-IscU complex by fitting the previously determined *Aa* IscU into the assigned fragments. The resultant model revealed that two IscU monomers bound near each C-terminus of dimeric IscS where the catalytic pocket of IscS and the Fe-S cluster binding site of IscU were located away from each other. We assume that the flexible loop conserved among IscS orthologues probably mediates the transfer of the sulfur source by invariant cysteine residue at the tip of the loop. In addition, our model indicated the possibility that the positively charged area found in the IscS surface might act as the binding site of the other ISC proteins having a biased negative charge such as IscA and Fdx. These findings imply that a novel role of IscS as a scaffold for the protein-protein interaction in ISC machinery.

[1] Y. Shimomura, K. Wada, K. Fukuyama, Y. Takahashi, *Journal of Molecular Biology* **2008**, *383*, 133-143.

Keywords: iron-sulfur cluster, cysteine desulfurase, protein-protein interactions

MS93.P76

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pH-dependent substrate recognition in human MTH1

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