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.

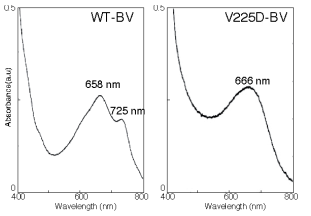

Fig. 1Absorption spectra of BV and BV13 in the presence of the V225D protein or the wild-type PcyA.

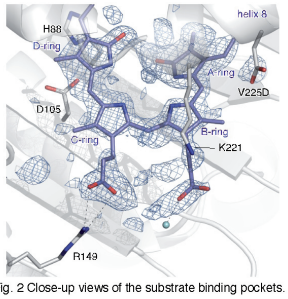

Fig. 2 Close-up views of the substrate binding pockets.

[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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Human MutT homolog-1 (hMTH1) hydrolyzes a variety of oxidized nucleoside triphosphates such as 8-oxo-dGTP, 2-oxo-dATP and 2-oxo-ATP to their corresponding monophosphates and prevents replicational and transcriptional errors caused by their misincorporations into DNA and RNA. We have determined crystal structures of hMTH1 complexed with 8-oxo-dGTP and 2-oxo-dATP and found that hMTH1 recognizes the two different oxidized nucleotides, 8-oxo-dGTP and 2-oxo-dATP, by the exchange of the protonation site between the neighboring Asp residues (Asp119 and Asp120) in the active site pocket. To our knowledge, this is a very novel mechanism for expression of broad substrate specificity by enzymes. In this study, we investigated the pH dependence of substrate-active site interactions in the crystal structures of the hMTH1-8-oxo-dGTP and hMTH1-2-oxo-dATP complexes. Under the crystallization condition with ammonium sulfate as precipitate, the hMTH1-8-oxo-dGTP crystals over pH 7.0 reveal that the substrate binding mode is altered toward an inactive form, in which the triphosphate group is located far from the catalytic residues in the Nudix motif. In the hMTH1-2-oxo-dATP crystals, electron densities for 2-oxo-dATP disappear at pH8.0. These are suggested that the deprotonation of Asp119 in the hMTH1-8-oxo-dGTP crystals and Asp120 in the hMTH1-2-oxo-dATP crystals occurs at these pHs.

Keywords: genome stability, broad substrate specifity, pH-dependence

MS94.P01

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Inclusion of the insecticide endosulfan in cyclodextrins

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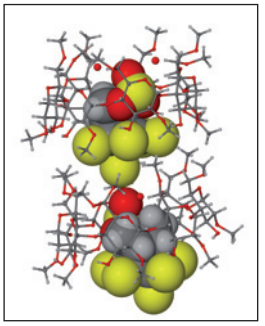
Most agrochemicals are highly insoluble in water, highly toxic and have reduced stability against chemical and photolytic degradation which makes them environmentally hazardous [1]. Endosulfan is an organochlorine insecticide and acaricide with a combination of these poor physical properties. Improving these physical properties has been attempted by complexing endosulfan with native and derivatised cyclodextrins (CDs).

Solid state inclusion complexes have been formed with β-CD, γ-CD and a derivatised CD known as DIMEB (heptakis(2,6-di-O-methyl)-β-CD). The single crystal X-ray structures of both the β-CD complex and the DIMEB complex of the symmetrical β-endosulfan isomer have been elucidated. The asymmetric unit of the DIMEB-β-endosulfan complex contains two DIMEB molecules with a disordered guest molecule situated in each (Figure). This complex shows a novel packing arrangement as the DIMEB molecules pack head-to-tail in infinite columns with adjacent columns parallel to one another rather than anti-parallel.



The asymmetric unit showing only the major disordered guest component

An amorphous CD, randomly methylated β-CD (RAMEB), was also investigated for inclusion with endosulfan using PXRD. Kneading experiments between RAMEB and endosulfan resulted in a semi-crystalline material with distinct peaks at low 2θ values. The peaks do not match those of the pure crystalline guest material but do show some similarity to the DIMEB-β-endosulfan complex PXRD pattern.

An understanding of the host-guest interactions forms an essential part of complex characterisation needed for developing new agrochemical formulations.

[1] E. Morillo, in *Cyclodextrins and their Complexes* ed. H. Dodziuk. John Wiley & Sons, Weinheim, **2006**, *16.3*, 459-467

Keywords: cyclodextrin, inclusion, crystal structure

MS94.P02

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180° Domain detection by surface phase sensitive second harmonic generation microscopy of polar materials

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Phase Sensitive Second Harmonic Generation Microscopy (PS-SHM) technique [1], [2] was developed to map 180° domains of polarization. Domain contrast is achieved by using the interference effect between SHG responses of a sample and a homogeneous reference material. Previously it was shown [1] that interpretation of domain mapping is simple if the crystal thickness is homogeneous and in the range of or below the coherence length l_c. Our objective is to demonstrate experimentally, that PS-SHM technique can be performed using a sufficiently flat surface of samples irrespective of their thickness. This is offering most feasible conditions to investigate various as grown materials, and especially those which could not be obtained in μm thick layers.

Using the PS-SHM technique in transmission mode, we have demonstrated [3] either a mono- or bi-polar state for different nonlinear optical crystals such as *N,N*-dimethyl-2-acetamido-4-nitroaniline (DAN), 2-cyclo-octylamino-5-nitropyridine (COANP), a channel-type inclusion compound of perhydrotriphenylene (PHTP) / *N,N*-dimethyl-3-nitroaniline (DMNA), potassium dihydrogen phosphate (KH_2PO_4) crystals stained by dyes and the zeolite $AlPO_4$-5 loaded by 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran (DCM). For all these cases a phase contrast was found irrespective of thickness and surface quality.

In this work, we have demonstrated that the application of PS-SHM can be realized under non phase matching conditions of samples much thicker than the coherence length. Further development is exploring the possibility to perform PS-SHM under reflection.

[1] P. Rechsteiner, J. Hulliger, M. Flörsheimer, *Chem. Mater.* **2000**, *12*, 3296-3300. [2] S. Kluge, F. Budde, I. Dohnke, *Appl. Phys. Lett.* **2002**, *81*, 247-249. [3] H. Aboulfadl, J. Hulliger, *Cryst. Growth Des. to be published* **2011**.

Keywords: microscopy, phase contrast, polarity

MS94.P03

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Molecular recognition and cluster size in supersaturated solutions of $NaClO_3$

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