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.

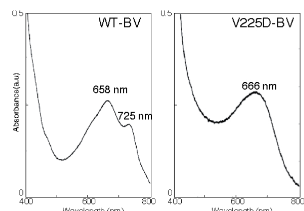

Fig. 1Absorption spectra of BV and BV13 in the presence of the V225D protein or the wild-type PcyA.

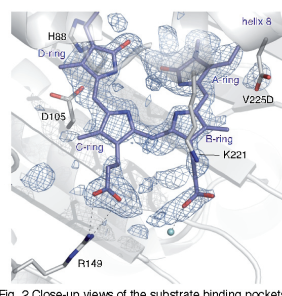

Fig. 2 Close-up views of the substrate binding pockets.

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

Keywords: mutation, pigment, reductase

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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

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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

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pH-dependent substrate recognition in human MTH1

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