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Ruffling of metalloporphyrins bound to IsdG and IsdI, two heme degrading enzymes

Woo Cheol Lee[1], Georgia Ukpabi[1], Michelle L. Reniere[2], Eric P. Skaar[2], Michael E. P. Murphy[1]
[1]University of British Columbia, Microbiology and Immunology, 2350 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3, Canada, [2]Vanderbilt University Medical Center, Nashville, Tennessee, 37232, USA, E-mail：cheol@interchange.ubc.ca

IsdG and IsdI are paralogous proteins from *Staphylococcus aureus* that reductively degrade hemin. Final heme degradation products of IsdG and IsdI are yet to be elucidated, nor has it been determined how oxygen binds to initiate the reaction. The crystal structures of an inactive N7A variant of IsdG in complex with Fe^{3+}-protoporphyrin IX (IsdG-hemin) and of IsdI in complex with cobalt protoporphyrin IX (IsdI-CoPPIX) were solved to 1.8 Å or better resolution. These structures show that the metalloporphyrins are buried into similar deep clefts and the propionic acids form salt bridges to two Arg residues. His77 (IsdG) or His76 (IsdI), a residue required for activity, is coordinated to Fe^{3+} or Co^{3+} atoms, respectively. The bound porphyrin rings form extensive steric interactions in the binding cleft such that the porphyrin rings are highly distorted from the planar. This distortion can be described as ruffled and places the b- and d-meso carbons close to the distal oxygen-binding site. In the structure of IsdI-CoPPIX, the distal side of the CoPPIX accommodates a chloride ion in a cavity formed through a conformational change in Ile55. The chloride ion participates in a hydrogen bond to the side chain amide of Asn6. We also have determined the crystal structures of IsdG-hemin and IsdI-hemin bound to cyanide to resolutions of ~1.8 Å. Structural information from these complexes is valuable in understanding the regiospecificity of ring cleavage. We propose a reaction mechanism in which reactive peroxide intermediate proceeds with nucleophilic oxidation at the b- or d-meso carbon of the hemin.

Keywords: iron acquisition, heme degradation, staphylococcus aureus

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Crystal structural analysis of photosystem II with the novel method to reduce X-ray radiation damage

Yasufumi Umena[1], Keisuke Kawakami[2], Akio Ohkuma[2], Shinya Saijyou[3], Hisashi Naitow[3], Jian-Ren Shen[2], Nobuo Kamiya[1]
[1]graduate school of science, Osaka city university, laboratory for biological structural chemistry, 3-3-138, Sugimoto, Sumiyoshi-ku, Osaka-city, Osaka, 558-8585, Japan, [2]Graduate School of Science and Technology, Okayama University, 1-1-1, Tsushimanaka, Okayama-city, Okayama, 700-8530, Japan, [3]RIKEN Harima Institute, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, E-mail：umena@sci.osaka-cu.ac.jp

Photosystem II (PSII) is a multi-subunit membrane protein complex functioning in photosynthesis. It performs a series of light-induced electron transfer reactions leading to the splitting of water and generation of molecular oxygen. The catalytic center is composed of four manganese atoms and one calcium atom. The detailed locations of these metal atoms are not fully understood. Recent X-ray absorption fine structure analysis has shown that the Mn_4Ca-cluster is seriously damaged by X-ray irradiation onto PSII crystals resulting in changes in the coordination structure among these metal atoms. In order to reveal the intact structure of the Mn_4Ca-cluster, we adopted a slide-oscillation method to collect the low-dose X-ray data for the crystal structure analysis. In this method, the irradiation point was slid to an adjacent point on the crystal along the oscillation axis after recording of every image and after some slides the irradiation point was brought back to the initial position. These slides were repeated through the required oscillation range. This method reduced the overlap of the irradiated regions in each cross section of the crystal 20 times lower than the normal oscillation method. Using X-ray beam (0.035 x 0.035 mm) at BL41XU of SPring-8, we collected 300 frames of diffraction images from the crystal (1.0 x 0.3 mm) of PSII from *Thermosynechococcus vulcanus* and processed them to 3.3 Å resolution. Comparing the electron-density map obtained by the slide-oscillation method with that obtained by the normal method, we found remarkable differences at the region of the Mn4Ca-cluster in the difference Fourier map. We will discuss the structure of the Mn4Ca-cluster from the low X-ray dose data and speculate the intact structure of the Mn4Ca-cluster.

Keywords: measuring techniques, membrane protein complexes, metal clusters

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Biosynthesis of Fe-S clusters by SUF system: implications from crystal structure of SufCD complex

Kei Wada[1], Takayuki Sato[1], Norika Sumi[1], Yasuhiro Takahashi[2], Keiichi Fukuyama[1]
[1]Osaka University, Department of Biological Sciences, Graduate School of Science, 1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan, [2]Saitama University, Saitama, Saitama, 338-8570, Japan, E-mail：keiwada@bio.sci.osaka-u.ac.jp

Iron-sulfur (Fe-S) clusters act as cofactors of various Fe-S proteins that are widely distributed in nature. Recent studies revealed that maturation of Fe-S proteins in several Eubacteria as well as Archaea and eukaryotic chloroplast is achieved by the SUF system. This system is encoded in *E. coli* by the *sufABCDSE* operon, and consists of six Suf components. SufS is a cysteine desulfurase and, together with SufE, delivers sulfur to SufBCD. The SufBCD forms a tertiary complex, in which Fe-S cluster is synthesized prior to its delivery to target Fe-S proteins. Here we report the crystal structure of the $SufC_2D_2$ complex and the mutational analysis of SufD, aiming at exploring the mechanistic outline of the SufBCD complex. Coexpression of SufC and SufD in *E. coli* resulted in formation of a binary complex, of which the crystal structure was determined. In this complex, SufD forms a homodimer, where each subunit has a unique β-helix central domain that is flanked by N- and C-terminal α-helical domains. Two molecules of SufC, the ATPase component sharing similarity with the ABC ATPase, were bound to the C-terminal helical domains of dimeric SufD. Notably, conformational changes of SufC were observed upon complex formation with SufD; SufC was changed to have the structure suitable for ATP binding/hydrolysis. In vivo complementation analysis of SufD has located residues and domains that are essential for the SufD function. Furthermore, SufB bears similarity with SufD, and biochemical studies have indicated that the SufBCD complex is composed of 1xSufB, 1xSufD, and 2xSufC. On the basis of these findings, we would like to propose that the Fe-S cluster is transiently assembled at the interface between SufB and SufD during the biosynthetic reaction.

Keywords: iron sulfur clusters, ATPases, conformational change