

Poster Presentation

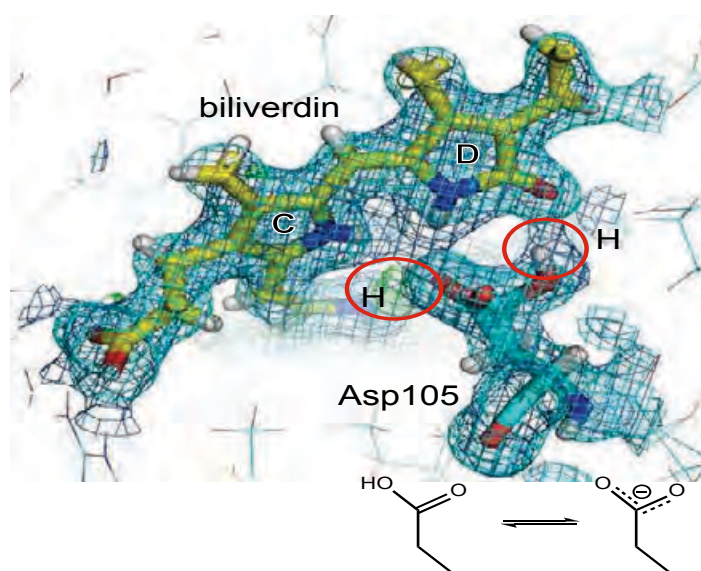
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Neutron Structure of Phycocyanobilin:oxidoreductase Complexed with Biliverdin

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Phytobilins are linear tetrapyrrole compounds used as chromophore for light harvesting and photoreceptor proteins in higher plants, algae, and cyanobacteria. Phytobilins are synthesized from biliverdin IX(alpha) (BV). Phycocyanobilin:oxidoreductase (PcyA) is an enzyme to produce phycocyanobilin (PCB) used as chromophore for light harvesting and photoreceptor proteins. PcyA is unique because it catalyzes the reduction of BV by two sequential steps; the first step is the reduction of the vinyl of the BV D-ring to produce 18(1)-18(2)-dihydrobiliverdin (18EtBV), and the second step is the reduction of the A-ring. In these reduction steps, four hydrogen atoms are delivered to BV. The earlier studies showed that the carboxyl group of Asp105 showed dual conformations. This has been attributed to the difference of its protonation states. The catalytically essential His88 was suggested to be protonated (i. e. His88 is a proton donor) to donate the proton to BV. BVH⁺ (N-protonated) state, in which four pyrrole N atoms of BV were fully protonated, was proposed to be partially formed when BV was bound to PcyA. Further, another tautomeric BVH⁺ state in which three of four pyrrole N atoms of BV were protonated and the lactam (C=O) group of BV D-ring was protonated as lactim (C-OH; O-protonated) was proposed. Additionally, newly identified water molecule near BV has been suggested to be a proton donor. To elucidate the H atom positions of these molecules, we determined the neutron crystal structure of the PcyA-BV complex at 1.95 Å resolution. Crystal with approximately 2.2 X 1.8 X 0.8 mm³ size, which was soaked into the deuterium-exchanged crystallization solution, was used in the diffraction experiment. The neutron diffraction intensity data was collected using IBARAKI Biological Crystal Diffractometer (iBIX) in J-PARC. In this conference, we report the protonation states of catalytically important residues and BV as well as orientations of water molecules in the PcyA-BV complex.



Keywords: Neutron Crystallography, protonation, Redox enzyme