Structure of Phytobilin Synthesis Enzyme

Photosynthetic organisms utilize phytobilins, which are linear tetrapyrrole pigments, for photosynthesis and light sensing. Such organisms develop light-harvesting systems to accomplish efficient photosynthesis in their living environments. Red algae and cyanobacteria have giant protein-pigment complexes called phycobilisomes as a light-harvesting system, in which phycobilins (one of phytobilins) are utilized for light-harvesting pigments, whereas higher plants and green algae have light-harvesting complexes, in which chlorophylls are mainly utilized as the light-harvesting pigments [1]. Phycobilins absorb lights in the range from red to green, which are not well absorbed by chlorophylls. This characteristic of these pigments is reflected in the habitats of photosynthetic organisms; cyanobacteria and red algae can grow in deeper water rather than green algae. In addition, phytochromes, red-light sensitive photoreceptors in plants, covalently bind phytochromobilin (one of phytobilins). Phytochromes are involved in various physiological reactions such as the photoperiodic induction of flowering, the induction of germination, and leaf senescence; these reactions are initiated by the photoisomerization of phytochromobilin in phytochromes.

Phytobilins are biosynthesized from heme by heme oxygenase and ferredoxin dependent bilin reductases (FDBRs) (Fig. 1). First, the porphyrin ring of heme is oxidatively cleaved by heme oxygenase to produce BV. Then, BV is reduced by FDBRs to produce phytobilins [2]. This pathway has attracted much attention not only from the viewpoint of biological importance but also from the biotechnological aspect [3]. Phycocyanobilin:ferredoxin oxidoreductase (PcyA), one of such FDBRs, is unique in reducing BV to phycocyanobilin by two sequential steps [4]. The first step is the reduction of the vinyl group of the BV D-ring to produce 18^1, 18^2-dihydrobiliverdin IXα, and the second step is the reduction of the A-ring of 18^1, 18^2-dihydrobiliverdin IXα to produce phycocyanobilin. Each reduction step uses two electrons supplied by ferredoxin. To achieve these sequential reductions, PcyA must possess a molecular structure that allows discrimination between the A- and D-rings of BV and control of the reaction sequence.

The crystal structure of PcyA from cyanobacterium *Synechocystis* sp. PCC 6803 in complex with BV has been determined from data obtained at 1.51 Å resolution using synchrotron radiation at beamlines **BL41XU** and **BL44B2** [5]. This structure, the first determined tertiary structure of an FDBR family member, reveals how an FDBR recognizes its bilin

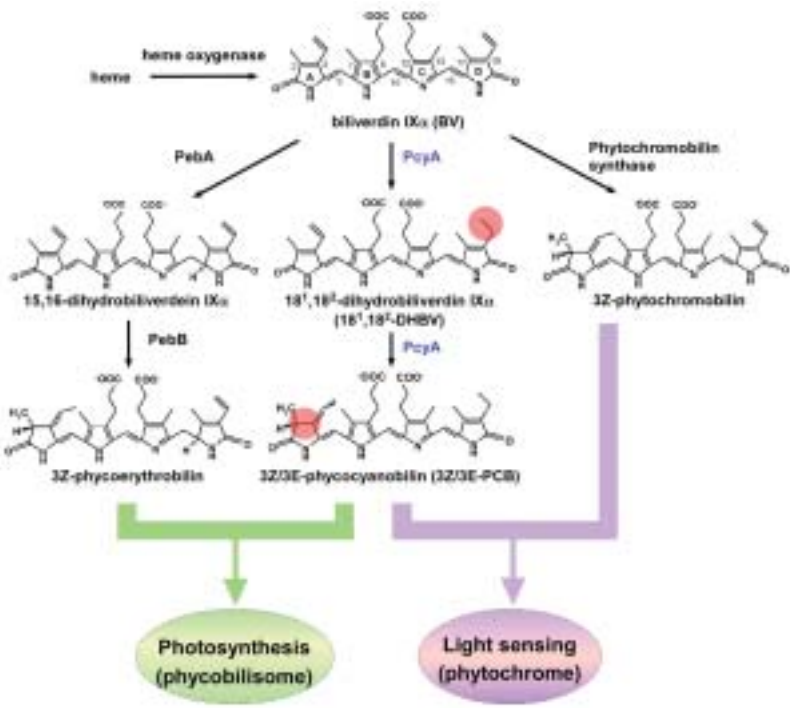

Fig. 1. Biosynthetic pathway of phytobilins by FDBRs.

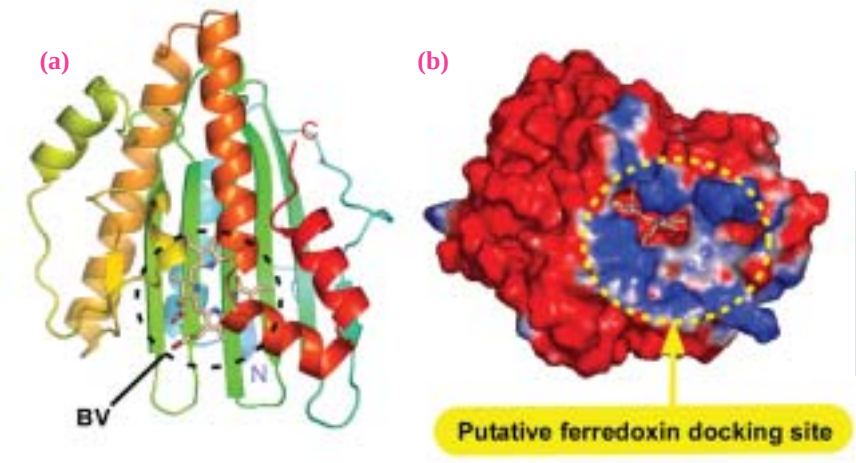

Fig. 2. Overall structure and electrostatic potential of PcyA-BV complex. **(a)** The ribbon model is displayed with the N-terminal side in blue and the C-terminal side in red. **(b)** Positive and negative surfaces are shown in blue and red, respectively. BV is shown as a stick model.

substrate and ferredoxin. PcyA is folded in a three-layer α/β/α sandwich structure, in which BV in a cyclic conformation is positioned between the β-sheet and C-terminal α-helices (Fig. 2(a)). The basic patch on the PcyA surface near the BV molecule may provide a binding site for acidic ferredoxin, allowing the direct transfer of electrons to the propionate groups of BV (Fig. 2(b)).

The electron density of BV was clearly visible, indicating that PcyA strictly recognizes the orientation of BV to reduce the BV site specifically (Fig. 3, left). On the basis of the active site structure, the conservation of amino acids in PcyAs, and the absolute configuration of the product, we propose the mechanism by which the sequential reduction of the D- and A-rings is controlled; Asp 105 located between the two reduction sites would play the central role by changing its conformation during the reaction (Fig. 3). Homology modeling on the basis of PcyA structure yielded the putative structures of other FDBRs. The model of phytochromobilin synthase fits well with the previous genetic data. Thus, the PcyA structure may provide a structural basis for understanding the common reaction mechanism of FDBRs and the specificity of the substrate-reducing site in each FDBR.

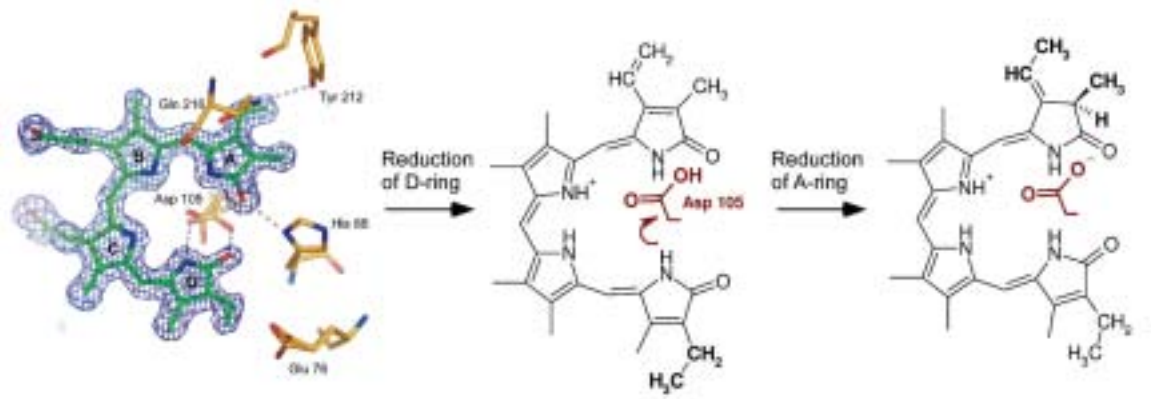

Fig. 3. Possible scheme of sequential reduction of BV catalyzed by PcyA. Electron density for BV is superimposed on the stick models of BV and candidate active residues (left). The side chains of Glu 76 and Asp 105 show multiple conformers. The essence of the sequential reduction of BV is that the side chain of Asp 105 changes its conformation after D-ring reduction allowing the stereospecific reduction of the A-ring.

Keiichi Fukuyama[a,*] and Masakazu Sugishima[b]

[a] Department of Biological Sciences, Osaka University
[b] Department of Medical Biochemistry, Kurume University School of Medicine

*E-mail: fukuyama@bio.sci.osaka-u.ac.jp

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