FEBS Letters 580 (2006) 3823–3828

Induced-fitting and electrostatic potential change of PcyA upon substrate binding demonstrated by the crystal structure of the substrate-free form

Yoshinori Hagiwara[a], Masakazu Sugishima[b,1], Yasuhiro Takahashi[a], Keiichi Fukuyama[a,*]

[a] *Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan*
[b] *Department of Medical Biochemistry, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*

Received 24 April 2006; revised 30 May 2006; accepted 30 May 2006

Available online 12 June 2006

Edited by Hans Eklund

Abstract Phycocyanobilin:ferredoxin oxidoreductase (PcyA) catalyzes the sequential reduction of the vinyl group of the D-ring and the A-ring of biliverdin IXα (BV) using ferredoxin to produce phycocyanobilin, a pigment used for light-harvesting and light-sensing in red algae and cyanobacteria. We have determined the crystal structure of the substrate-free form of PcyA from *Synechocystis* sp. PCC 6803 at 2.5 Å resolution. Structural comparison of the substrate-free form and the PcyA–BV complex shows major changes around the entrance of the BV binding pocket; upon BV binding, two α-helices and nearby side-chains move to produce tight BV binding. Unexpectedly, these movements localize the positive charges around the BV binding site, which may contribute to the proper binding of ferredoxin to PcyA. In the substrate-free form, the side-chain of Asp105 was located at a site that would be underneath the BV A-ring in the PcyA–BV complex and hydrogen-bonded with His88. We propose that BV is protonated by a mechanism involving conformational changes of these two residues before reduction.

Keywords: Redox; Induced-fit; Light-harvesting pigment; FDBR family; Phytobilin; Phycobilisome

1. Introduction

Phycobilins are members of the phytobilin chromophores, linear tetrapyrrole pigments that are used for light harvesting and/or photoreception in plants, algae, and cyanobacteria [1,2]. Phycobiliproteins, in which phycobilins are covalently bonded to the polypeptide, are assembled on the outer surface of the thylakoid membrane to form the phycobilisome, a large light-harvesting antenna complex for Photosystem II in red algae and cyanobacteria [3]. In higher plants, phytochromobilin, a phytobilin, is covalently bonded to phytochrome, a red-sensitive photoreceptor that is involved in photomorphogenesis, photoperiodic induction of flowering, chloroplast development, leaf senescence, and leaf abscission [4]. The photoisomerization of phytochromobilin is the trigger for these physiological reactions. Recently, phytochrome homologs have been found in photosynthetic and non-photosynthetic bacteria. These phytochrome homologs are covalently bonded to phycobilins or biliverdin IXα (BV), a precursor of phytobilins [2,5].

Biosynthesis of phytobilins begins with the cleavage of the porphyrin ring of heme catalyzed by heme oxygenase [6,7] to produce BV. BV is further reduced by ferredoxin-dependent bilin reductases (FDBRs) to phytobilins [1]. Because FDBRs show weak sequence homology to each other and prefer ferredoxin (Fd) as an electron donor, FDBRs have been considered to constitute a gene family [8]. Although NADPH-dependent biliverdin reductases found in mammals [9] and cyanobacteria [10] also catalyze BV reduction, these enzymes are distinct from FDBRs not only in the preference of Fd as an electron donor but also in their amino acid sequences. Phycocyanobilin:Fd oxidoreductase (PcyA), a member of the FDBR family, is unique in that it catalyzes the reduction of BV in two sequential steps to produce 3Z/3E-phycocyanobilin, one of the major pigments in the phycobilisome (Fig. 1) [11]. Upon BV binding to PcyA, neutral BV is converted into a fully N-protonated cationic form, BVH^+ [2] [12]. Then, PcyA reduces the vinyl group of the BVH^+ D-ring to generate 18^1,18^2-dihydrobiliverdin. In the next step, PcyA reduces the A-ring of 18^1,18^2-dihydrobiliverdin to generate 3Z/3E-phycocyanobilin. In each reduction step, two electrons are supplied by Fd. Recently, we determined the crystal structure of the PcyA–BV complex from *Synechocystis* sp. PCC 6803 at 1.51 Å resolution [13]. PcyA is folded into a three-layer α/β/α sandwich structure: four N-terminal α-helices (H1/H2/H4/H6), a seven stranded (S1–S7) antiparallel β-sheet, and five C-terminal α-helices (H3/H5/H7/H8/H9). BV has a cyclic 'porphyrin-like' conformation and is positioned between the β-sheet and C-terminal α-helices. The electrostatic potential on the molecular surface of the complex is clearly separated into acidic and basic regions, suggesting that Fd likely binds the basic patch near the BV binding pocket. Based on the structure of the BV binding pocket and the stereochemical configuration of the product, we proposed that Asp105 is critically involved in the sequential reductions by PcyA.

In this study, we have determined the crystal structure of the substrate-free form of PcyA from *Synechocystis* sp. PCC

*Corresponding author. Fax: +81 6 6850 5425.
E-mail address: fukuyama@bio.sci.osaka-u.ac.jp (K. Fukuyama).

[1] Research fellow of the Japan Society for the Promotion of Science, Japan.

Abbreviations: BV, biliverdin IXα; Fd, ferredoxin; FDBR, Fd-dependent bilin reductase; PcyA, phycocyanobilin:Fd oxidoreductase; r.m.s., root-mean-square

[2] We ignored the contribution of the propionates to the net charge on BV.

0014-5793/$32.00
doi:10.1016/j.febslet.2006.05.075

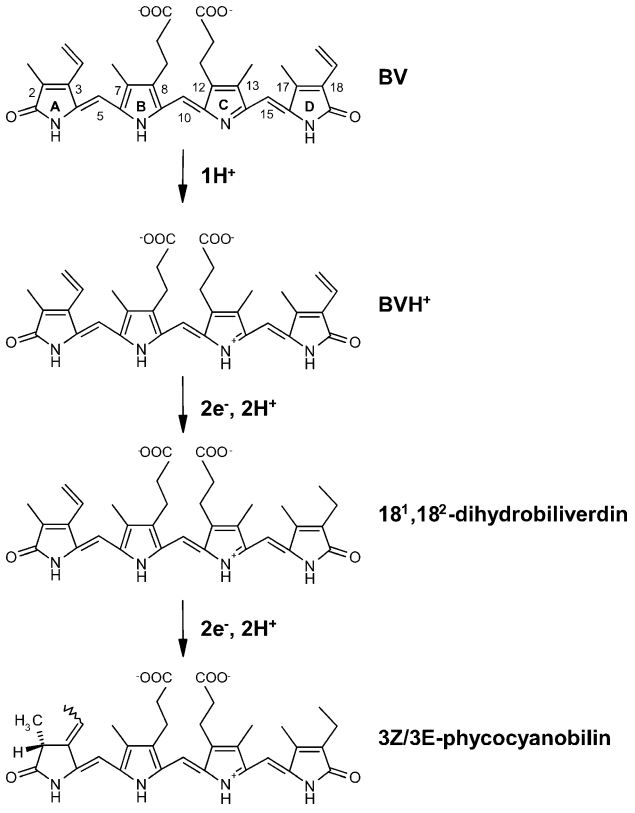

Fig. 1. Enzymatic reaction of PcyA. Upon BV binding to PcyA, it is protonated to BVH^+. BVH^+ is then reduced to 3Z/3E-phycocyanobilin via 18^1,18^2-dihydrobiliverdin using electrons supplied by ferredoxin.

6803 at 2.5 Å resolution. Structural comparison of the substrate-free form of PcyA with the PcyA–BV complex shows the interesting changes in electrostatic potential on the molecular surface; BV binding to PcyA induces the structural changes that increase the positive charges around the BV binding site. Furthermore, upon BV binding, the side-chain conformation of Asp105 markedly changes. Here, we discuss these results from the viewpoint of the characteristic PcyA reaction.

2. Materials and methods

PcyA was prepared as described [13]. Crystallization conditions for the substrate-free form of PcyA were screened by the hanging-drop vapor-diffusion method using Crystal Screen and Crystal Screen 2 kits (Hampton Research) at 293 K. PcyA (20 mg/ml) was mixed with equal volumes of each reservoir solution and equilibrated. Octahedral crystals were obtained after a few months using a reservoir solution containing 2.0 M ammonium sulfate, 0.1 M HEPES–NaOH at pH 7.5, and 2% (v/v) polyethylene glycol 400.

The crystals were soaked in crystallization solution containing 15% (v/v) glycerol as a cryo-protectant and flash-cooled under a nitrogen gas stream at 100 K. Diffraction data were collected at 100 K using synchrotron radiation ($\lambda = 1.0000$ Å) from the BL38B1 beamline at SPring-8 and a Jupiter 210 CCD detector (Rigaku). The data were processed and scaled with HKL2000 [14]. The crystal belonged to the space group $P4_32_12$ with unit-cell dimensions $a = b = 75.98$ Å and $c = 84.97$ Å. The structure of the substrate-free form was solved by the molecular replacement method using a model of the PcyA-BV complex [13] as the search probe, where BV and solvent molecules were excluded. Rotational and translational searches with MOLREP [15] in the CCP4 package [16] using the diffraction data (15.0–4.0 Å resolution) located a molecule in an asymmetric unit. The coordinates of the model and individual temperature factors were refined using CNS [17] and manually revised with Xfit [18]. After several cycles of refinement and model adjustments, water molecules and ions were included in the model. Diffraction data and refinement statistics are summarized in Table 1. Coordinates of the substrate-free form of PcyA have been deposited at the RCSB Protein Data Bank under the accession code 2DKE.

Table 1
Diffraction and refinement statistics

Diffraction statistics[a]	
Resolution (Å)	2.5
No. of observations	50826
No. of unique reflections	9064
Completeness (%)	97.6 (98.8)
Mean $I_0/\sigma(I)$	11.5 (3.6)
R_{sym}[b] (%)	7.9 (23.7)
Refinement statistics	
R[c]/R_{free}[d](%)	24.3/29.9
No. of Cl^- ion/water molecules	1/47
R.m.s. deviations from ideal values	
Bond length (Å)	0.008
Bond angle (°)	1.51
Ramachandran plot	
Most favored (%)	90.4
Additionally allowed (%)	8.7
Generously allowed (%)	1.0

[a]Values in parentheses correspond to the highest-resolution shell (2.59–2.50 Å).
[b]$R_{sym} = \sum_{hkl}\sum_i|I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$.
[c]R-factor $= \sum_{hkl}||F_o(hkl)| - |F_c(hkl)||/\sum_{hkl}|F_o(hkl)|$.
[d]R_{free} value calculated for 5% of the data set not included in refinements.

3. Results and discussion

3.1. Induced-fitting of PcyA upon BV binding

We have determined the structure of the substrate-free form of PcyA from *Synechocystis* sp. PCC 6803 at 2.5 Å resolution. The model contains 241 of the 248 residues of PcyA, together with a chloride ion and 47 water molecules. Residues 1–4 and 246–248 could not be built due to poor electron density. The overall structure of the substrate-free form was similar to that of the complex [13] (root-mean-square [r.m.s.] distance between the corresponding Cαs was 1.19 Å for 240 residues), but notable structural differences were observed in the A-loop, H2′, the B-loop, S4, H5, and H8 (Fig. 2a and supplementary Fig. 1a). In the substrate-free form, the α-helical structures from Leu13 to Pro15 (H1) and from Pro153 to Ile157 (H5) were disrupted, and a new α-helical structure was formed between Glu44 and Gly47 (H2′). H5 and H8, which constitute the entrance of the substrate binding pocket, moved so that the entrance was narrowed upon BV binding (Fig. 2b). This movement increases the hydrophobic interaction between PcyA and the BV tetrapyrrole ring to stabilize the PcyA–BV complex. In addition, several hydrophilic interactions formed upon the conformational changes associated with BV binding. Trp154 in H5 and Lys221 in H8 formed a hydrogen-bond and a salt-bridge, respectively, to the propionate groups of BV when these helices moved upon BV binding. Moreover, the