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Crystallization and preliminary X-ray diffraction study of BchU, a methyltransferase from *Chlorobium tepidum* involved in bacteriochlorophyll *c* biosynthesis

Jiro Harada,[a] Kei Wada,[b] Hitomi Yamaguchi,[b] Hirozo Oh-oka,[b] Hitoshi Tamiaki[a] and Keiichi Fukuyama[b]*

[a]Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan, and [b]Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Correspondence e-mail: fukuyama@bio.sci.osaka-u.ac.jp

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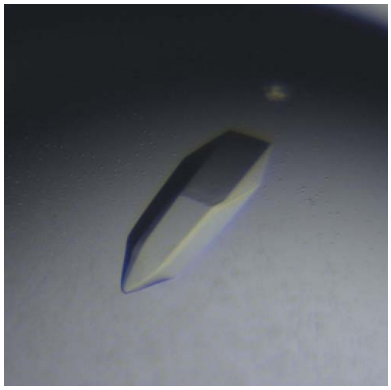

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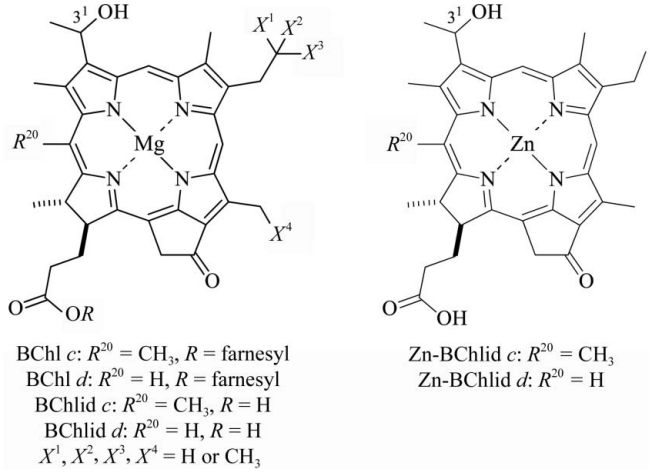
The *S*-adenosylmethionine-dependent methyltransferase BchU is an enzyme involved in the bacteriochlorophyll *c* biosynthetic pathway and catalyzes methylation at the C-20 position of the chlorin moiety. Recombinant *Chlorobium tepidum* BchU overproduced in *Escherichia coli* was purified and crystallized by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. The crystals belonged to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 81.5$, $c = 250.7$ Å. A native data set was collected to 2.27 Å resolution using synchrotron radiation at SPring-8.

1. Introduction

Bacteriochlorophyll (BChl) pigments are widely distributed in bacterial photosynthetic organisms and serve essential roles in energy-converting systems, *i.e.* light-harvesting apparatuses and reaction-centre complexes, where energy from sunlight is effectively captured and converged to initiate a charge-separation process. A variety of chlorophyllous pigments including BChls *a*, *b*, *c*, *d*, *e* and *g* have been found in photosynthetic bacteria. The biosynthetic pathway of BChl *a* has been intensively studied by molecular-genetic and biochemical methods (for a review, see Willows, 2003). Specific pathways from a common intermediate to the other pigments have been proposed but the enzymes have as yet scarcely been identified.

Photosynthetic green bacteria have extramembraneous light-harvesting antenna systems, chlorosomes, which adapt to extremely low-light environments. The main parts of chlorosomes are self-aggregates of BChls *c*, *d* and *e* (see Fig. 1 for BChls *c* and *d*); BChls c and d); *in vitro* reconstitution using only each pigment showed that no protein participates in formation of the chlorosomal aggregates (Blankenship *et al.*, 1995; Tamiaki, 1996; Blankenship & Matsuura, 2003). Recently, the BChl *c* biosynthetic pathway in one of the green sulfur bacteria, *Chlorobium tepidum*, has been proposed from its whole genome analysis (Eisen *et al.*, 2002) and subsequently from molecular-genetic studies (Frigaard *et al.*, 2003; Frigaard & Bryant, 2004). In this pathway, *bchU* was identified as the gene encoding C-20



BChl *c*: $R^{20} = CH_3$, R = farnesyl
BChl *d*: $R^{20} = H$, R = farnesyl
BChlid *c*: $R^{20} = CH_3$, $R = H$
BChlid *d*: $R^{20} = H$, $R = H$
X^1, X^2, X^3, X^4 = H or CH_3

Zn-BChlid *c*: $R^{20} = CH_3$
Zn-BChlid *d*: $R^{20} = H$

Figure 1
Molecular structures of BChls *c* and *d* and BChlids *c* and *d* (left) and Zn-BChlids *c*/*d* (right). The 3^1 stereochemistry is *R* or *S*.

methyltransferase (Maresca *et al.*, 2004) which was proposed to convert bacteriochlorophyllide (BChlid) *d* into BChlid *c* (Fig. 1) using *S*-adenosylmethionine (SAM) as a methyl-group source (Harada, Saga *et al.*, 2005). In the species *C. vibrioforme* NCIB 8327, this gene was inactivated by a frame-shift mutation owing to a single-base-pair insertion, which results in exclusive production of BChl *d* (Maresca *et al.*, 2004; Harada, Oh-oka *et al.*, 2005). Such BChl *d*-producing species would be natural mutants deficient in C-20 methyltransferase activity. We have reported that recombinant His-tagged BchU catalyzed the C-20 methylation of Zn-BChlid *d* (Fig. 1) as a slightly modified substrate in the presence of SAM to produce Zn-BChlid *c* (Harada, Saga *et al.* 2005).

In order to clarify the molecular mechanism of the specific methylation by BchU, we initiated its three-dimensional structure determination by the X-ray diffraction method. In this paper, we describe the overproduction, purification and crystallization of BchU and its preliminary X-ray diffraction studies.

2. Materials and methods

2.1. Purification of BchU

The expression and purification of His-tagged BchU have been reported previously (Harada, Saga *et. al.*, 2005) and are briefly described here. The full-length *bchU* gene from *C. tepidum* strain WT2321 (Wahlund & Madigan, 1995) was cloned into expression vector pET-15b (Novagen). The plasmid was transformed into *Escherichia coli* BL21(DE3) cells. The transformant was grown in 5 l liquid Terrific broth containing ampicillin (100 µg ml^{-1}) at 310 K to an optical density of 0.6 at 600 nm. After addition of 0.5 m*M* isopropyl-*β*-D-thiogalactopyranoside (final concentration) to induce the transcription of the *bchU* gene, the culture was further incubated overnight. The harvested cells were suspended in lysis buffer containing 20 m*M* Tris–HCl pH 7.8, 300 m*M* NaCl and 20 m*M* imidazole. After disruption by sonication followed by centrifugation at 22 200*g* for 30 min, the His-tagged protein His$_6$-BchU was purified from the supernatant using Ni^{2+}-affinity resin (Qiagen Inc.) according to standard protocols. Eluted His$_6$-BchU was further purified by gel filtration using a Hi-Prep 16/60 Sephacryl S-200 HR column (Amersham Biosciences) equilibrated against buffer containing 20 m*M* Tris–HCl pH 7.8, 150 m*M* NaCl. All purification steps were performed at 277 K. The purity of the pooled fractions was checked by SDS–PAGE according to Laemmli (1970).

2.2. Crystallization

The purified His$_6$-BchU solution was concentrated for crystallization with a Vivaspin filter (Vivascience). All crystallization trials were carried out using the hanging-drop vapour-diffusion method at 293 K. Crystallization drops containing 1 µl protein solution in 20 m*M* Tris–HCl pH 7.8, 150 m*M* NaCl and 1 µl precipitant solution were equilibrated against 500 µl precipitant solution. Initial trials were performed using the commercially available sparse-matrix screening kits Crystal Screens I, II and Lite, PEG/Ion Screen (Hampton Research), Wizard I and II (Emerald Biostructures) and JB Screen (Jena Bioscience GmbH). The initial conditions that produced crystals were optimized by varying the concentrations of protein, precipitants and buffer systems and the pH.

2.3. Data collection

Crystals were soaked for a few seconds in paraffin oil, a cryoprotectant. The crystal was mounted in a cryoloop and flash-cooled in a cryostream at 100 K. A complete data set was collected on an ADSC Quantum 4R detector using synchrotron radiation (λ = 1.000 Å) at the BL38B1 station of SPring-8 (Hyogo, Japan). The crystal-to-detector distance was 200 mm and 120 images were recorded at 1° intervals with an exposure time of 10 s per image. The intensity data were processed and scaled with the program *HKL*2000 (Otwinowski & Minor, 1997).

3. Results and discussion

The purification of His$_6$-BchU yielded approximately 8 mg protein, the purity of which was evaluated to be over 95% by Coomassie blue-

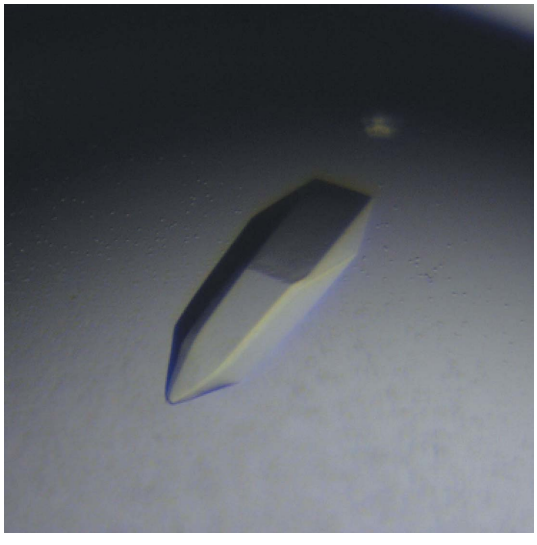


Figure 2
A typical crystal of the His$_6$-BchU from *C. tepidum*. The dimensions are approximately 0.1 × 0.1 × 0.4 mm.

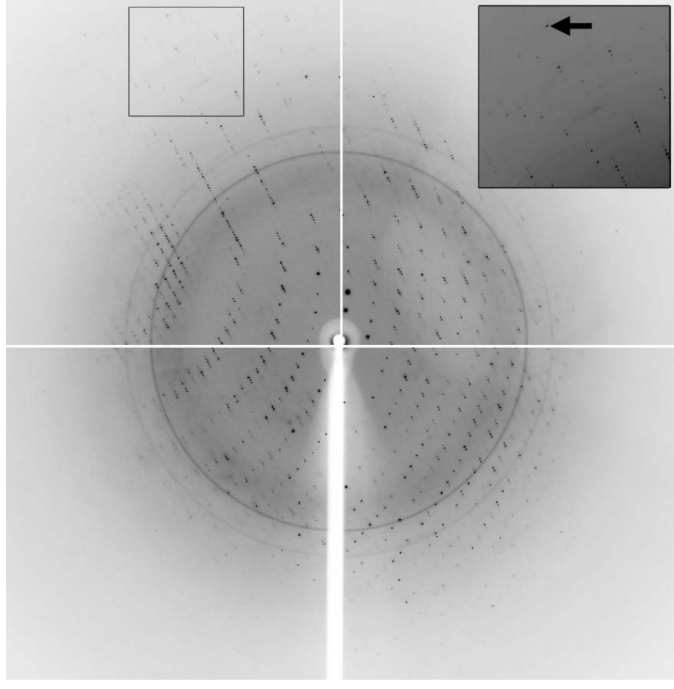


Figure 3
Diffraction pattern of the His$_6$-BchU crystal. The resolution of the spot indicated by the arrow is 2.10 Å.

Table 1
Summary of crystallographic statistics.

Values in parentheses are for the outermost shell (2.35–2.27 Å).

Crystal system	Hexagonal
Space group	$P6_122$ or $P6_522$
Unit-cell parameters (Å)	$a = b = 81.5$, $c = 250.7$
No. of observations	267219
No. of unique reflections	23709
Resolution range (Å)	50.0–2.27
R_{sym}† (%)	5.0 (22.0)
Redundancy	11.3 (10.9)
Completeness (%)	99.1 (100)
Mean $I/\sigma(I)$	16.4

† $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity for multiple recorded reflections.

stained gels. In the initial crystallization trials, small crystals were obtained from several drops containing ammonium sulfate as a precipitant (*e.g.* Crystal Screen I condition Nos. 4 and 39; Crystal Screen II condition Nos. 32 and 42; JB Screen 6 conditions B1 and C4). The crystallization conditions finally established consisted of a protein solution of 10 mg ml^{-1} in 50 m*M* Tris–HCl pH 7.8 containing 150 m*M* NaCl and a reservoir solution consisting of 1.5 *M* ammonium sulfate, 100 m*M* Tris–HCl pH 8.5 and 12%(*v*/*v*) glycerol. Crystals grew to maximum dimensions of 0.1 × 0.1 × 0.4 mm in a week (Fig. 2).

The crystals belong to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 81.5$, $c = 250.7$ Å. A complete data set was collected to a resolution of 2.27 Å using synchrotron radiation at 100 K. A typical X-ray diffraction pattern of BchU is shown in Fig. 3. Diffraction spots along the c^* axis were so close together that synchrotron radiation was required to resolve individual spots. It is noted that the crystal has anisotropy in its X-ray diffraction power; it diffracts X-rays to better than 2.1 Å resolution along the c^* axis but to at most 2.3 Å along the a^* axis. Detailed crystal parameters and data-collection statistics are shown in Table 1. When the asymmetric unit contains one His_6-BchU subunit, the respective V_M and V_{sol} are 3.0 Å^3 Da^{-1} and 59% (Matthews, 1968).

We now aim to solve the structure by the MAD technique with selenium as the anomalous scattering atom. Preparation of the SeMet derivative of the BchU and its crystallization are in progress.

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