Acta Crystallographica Section D
**Biological Crystallography**
ISSN 0907-4449

# Crystallization and preliminary X-ray diffraction analysis of the light-harvesting protein phycocyanin from the thermophilic cyanobacterium *Synechococcus elongatus*

**Yoshiharu Toriumi,[a] Sam-Yong Park,[b]† Hideki Hashimoto,[a] Hiroyuki Shimizu,[c] Masahiko Hirano[c] and Nobuo Kamiya[b]***

[a]Toray Research Center Inc., 3-3-7 Sonoyama, Otsu, Shiga 520-8567, Japan, [b]RIKEN Harima Institute, SPring-8, 1-1-1 Kouto, Mikazuki-cho, Sayo, Hyogo 679-5148, Japan, and [c]Toray Research Center Inc., 1111 Kamakura, Kanagawa 248-8555, Japan

† Present address: Division of Protein Design, Graduate School of Integrated Science, Yokohama City University, 1-7-29 Suehiro, Tsurumi-ku, Yokohama 230-0045, Japan.

Correspondence e-mail: nkamiya@sp8sun.spring8.or.jp

Received 17 January 2001
Accepted 12 July 2001

The crystallization and preliminary crystallographic study of phycocyanin from the thermophilic cyanobacterium *Synechococcus elongatus* is reported. Phycocyanin is composed of $\alpha$- and $\beta$-subunits consisting of 162 and 172 amino-acid residues, respectively. These associate to form an $\alpha\beta$ heterodimer, which further associates to give a ring-shaped trimer $(\alpha\beta)_3$. Two trimers bind head-to-head to form a hexamer $(\alpha\beta)_6$. Phycocyanin crystals have been obtained by the sitting-drop vapour-diffusion method with a precipitant solution containing 30%(*w*/*v*) PEG 4000 and 100 m*M* MES pH 7.5–8.0. Using synchrotron radiation, the crystals diffract to 2.0 Å resolution. They belong to the trigonal space group *R*32, with unit-cell parameters $a = b = 186.75$ (3), $c = 59.75$ (4) Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Assuming that the crystallographic triad is identical to the threefold axis of the hexamer and with three $(\alpha\beta)_6$ molecules in a unit cell, the calculated molar volume ($V_M$) is 2.64 Å$^3$ Da$^{-1}$. This value corresponds to a solvent content of approximately 53%, with one $\alpha\beta$ heterodimer occupying the asymmetric unit.

## 1. Introduction

Cyanobacteria have large light-harvesting protein complexes called phycobilisomes, which effectively capture light energy and transfer it to the Photosystem II reaction centre (Sidler, 1994). The phycobilisomes consist of phycobiliproteins and linker polypeptides. Phycobiliproteins are classified into several kinds of homologous proteins, phycoerythrin (PE), phycoerythrocyanin (PEC), phycocyanin (PC) and allophycocyanin (APC) (Betz, 1997). The phycobilisomes isolated from *S. elongatus* are composed of 13 subunits, phycocyanins $\alpha\beta$, allophycocyanins $\alpha\beta$, six linker polypeptides and another three biliproteins including an anchor protein. We have sequenced the *cpc* operon which encodes rod polypeptides of the phycobilisomes (DDBJ Nucleotide Sequence Database accession number D13173; Hirano *et al.*, 1992).

Phycobilisome proteins contain covalently bound open-chain tetrapyrrole chromophores which absorb light energy. Phycobiliproteins form hemidiscoidal supramolecular protein complexes on the surface of the thylakoid membrane on top of Photosystem II. The allophycocyanin complexes form the core units of this structure close to the reaction centre. Other phycobiliproteins stack together to form the rod units. Phycoerythrin and phycoerythrocyanin are on the tips of the antenna rod. There are several linker polypeptides which have structural and regulatory roles in the organization of phycobilisome.

Phycocyanin is located in the middle of the rod complex and has an absorption maximum ($\lambda_{max}$) of 615–640 nm. It is composed of an $\alpha$-subunit (162 amino-acid residues) and a $\beta$-subunit (172 amino-acid residues), with three phycocyanobilins as the chromophore. One phycocyanobilin is attached to the polypeptide chain of the $\alpha$-subunit by a thioether bond to Cys84. Two phycocyanobilins are also attached to the $\beta$-subunit (at Cys84 and Cys155). The two subunits form a heterodimer ($\alpha\beta$) and three dimers form a cyclic trimer $(\alpha\beta)_3$, the minimal functional unit. Two trimers aggregate head-to-head to form a hexamer structure $(\alpha\beta)_6$. The hexameric phycocyanin is believed to play an important role in phycobilisomes. Crystal structures of some phycocyanins have already been reported: C-PC from *Mastigocladus laminosus* (Schirmer *et al.*, 1985, 1987), C-PC from *Agmenellum quadruplicatum* (Schirmer *et al.*, 1986, 1987), C-PC from *Fremyella diplosiphon* (Duerring *et al.*, 1991), C-PC from *Cyanidium caldarium* (Stec *et al.*, 1999) and C-PC from *Spirulina platensis* (Moreno *et al.*, 1997; Padyana *et al.*, 2001). Crystal structures of other phycobiliproteins are also available: PEC from *M. laminosus* (Duerring *et al.*, 1990), B-PE from *Porphyridium sordidum* (Ficner *et al.*, 1992), APC from *Spirulina platensis* (Brejc *et al.*, 1995) and R-PE from *Polysiphonia urceolata* (Chang *et al.*, 1996). We describe here the crystallization and preliminary X-ray diffraction analysis of the C-phycocyanin from the thermophilic cyanobacterium *S. elongatus*. The phycocyanin from

*S. elongatus* is stable to about 333 K. This temperature is about 20 K higher than the optimum temperature of phycocyanin from mesophilic cyanobacteria. It is hoped that the crystal structure analysis of thermostable phycocyanin will indicate the mechanism of thermostability.

Of the cyanobacteria mentioned above, *M. laminosus* is also thermophilic. *S. elongatus* and *M. laminosus* both grow well at 328 K. Primary structures show 79.0 and 82.6% homology for the $\alpha$- and $\beta$-subunits, respectively, which is about the same as the C-PC homology between *S. elongatus* and mesophilic cyanobacteria. This shows that the overall similarity of primary structures does not correlate with thermostability. The three-dimensional structures of these proteins are required in order to understand their thermostability.

## 2. Materials and methods

*S. elongatus* was grown at 328 K for 3 d (Hirano *et al.*, 1980) and the cells were collected by centrifugation. After cell lysis in a French press, crude extracts were fractionated with 50% ammonium sulfate followed by hydroxyapatite chromatography. Phycocyanin and allophyocyanin were completely separated by the purification procedures and the purity of the phycocyanin obtained was over 95% by SDS–PAGE. For crystallization, the protein was concentrated to 2.3 mg ml$^{-1}$ (0.02 m*M*) in 10 m*M* MES buffer pH 6.5.

## 3. Results and discussion

### 3.1. Crystallization of phycocyanin

A single crystal form of phycocyanin was obtained by the sitting-drop vapour-diffusion method. Crystals were grown at 293 K in 100 m*M* MES solution pH 7.5–8.0 using 30%(*w*/*v*) PEG 4000 as a precipitant. The initial droplets contained 5 µl protein solution (2.3 mg ml$^{-1}$) and 5 µl precipitant solution and were equilibrated against 1 ml precipitant solution in the reservoir. Crystals grown under these conditions reached their maximum size within 5 d at 293 K; their typical dimensions are approximately 0.15 × 0.15 × 0.10 mm (Fig. 1).

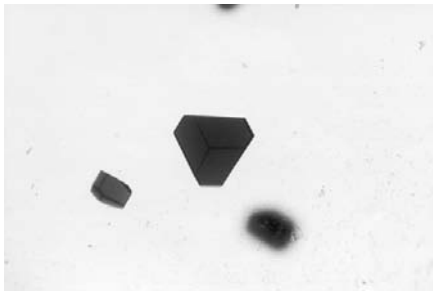

**Figure 1**
A single crystal of phycocyanin grown from 30% PEG 4000, 100 m*M* MES pH 7.5. Approximate dimensions of the crystal are 0.15 × 0.15 × 0.10 mm.

**Table 1**
Crystal parameters and data reduction of phycocyanin.

Values in parentheses are for the outermost resolution shell (2.11–2.00 Å).

| | |
|---|---|
| Space group | *R*32 |
| Unit-cell parameters (Å, °) | $a = b = 186.75$ (3), $c = 59.75$ (4), $\alpha = \beta = 90$, $\gamma = 120$ |
| Wavelength (Å) | 0.70 |
| Resolution range (Å) | 28.0–2.00 |
| Measured reflections | 318257 |
| Unique reflections | 26889 |
| Completeness (%) | 99.7 (99.4) |
| $R_{\rm merge}$† (%) | 6.6 (28.5) |
| Multiplicity | 11.8 (11.1) |
| Mean $\langle I/\sigma(I)\rangle$ | 8 (2.7) |

† $R_{\rm merge} = \sum |I_i - \langle I\rangle| / \sum |I_i|$, where $I_i$ is the intensity of an observation and $\langle I\rangle$ is the mean value for that reflection; summations are over all reflections.

### 3.2. Diffraction data collection

Diffraction data of the crystals of phycocyanin were obtained using the synchrotron-radiation source at the RIKEN beamline 2 (BL44B2) station of SPring-8, Harima, Japan (Adachi *et al.*, 2001). Intensity data were collected with a MAR CCD detector mounted on a Huber alignment table. The cryogenic head of the Rigaku cryostream was mounted close to the goniometer head. The crystal was mounted with the *c* axis as the axis of rotation; its distance from the CCD detector was 220 mm. Measurements were performed at 100 K and the wavelength of the incident X-ray was 0.7 Å. Diffraction data were integrated and scaled with the *MOSFLM* (Leslie, 1994) and *SCALA* programs (Collaborative Computational Project, Number 4, 1994).

The crystal of phycocyanin diffracted to a resolution of 2.0 Å, as shown in Fig. 2, and was found to belong to space group *R*32, with unit-cell parameters $a = b = 186.75$ (3), $c = 59.75$ (4) Å, $\alpha = \beta = 90$, $\gamma = 120°$. Assuming that the crystallographic triad is identical to the threefold axis of the hexamer and that there are three $(\alpha\beta)_6$ molecules in a unit cell, the calculated molar volume ($V_{\rm M}$) is 2.64 Å$^3$ Da$^{-1}$ (Matthews, 1968). One $\alpha\beta$ heterodimer occupies the asymmetric unit of the cell. This $V_{\rm M}$ value corresponds to a solvent content of approximately 53%. The reflection data have an $R_{\rm merge}$ value of 6.6% for 26 889 in-

**Table 2**
Space groups and unit-cell parameters of phycocyanin crystals.

| Species | Space group | Unit-cell parameters (Å, °) |
|---|---|---|
| *Agmenellum quadruplicatum* | *P*321 | $a = b = 184.5$, $c = 60.5$, $\alpha = \beta = 90$, $\gamma = 120$ |
| *Cyanidium caldarium* | *R*32 | $a = b = 106.42$, $c = 176.18$, $\alpha = \beta = 90$, $\gamma = 120$ |
| *Fremyella diplosiphon* | *R*3 | $a = b = 180.26$, $c = 61.24$, $\alpha = \beta = 90$, $\gamma = 120$ |
| *Mastigocladus laminosus* | $P6_3$ | $a = b = 154.6$, $c = 40.5$, $\alpha = \beta = 90$, $\gamma = 120°$ |
| *Spirulina platensis*† | *P*6 or $P6_3$ | $a = b = 182.38$, $c = 60.87$, $\alpha = \beta = 90$, $\gamma = 120°$ |
| *Spirulina platensis*‡ | $P2_1$ | $a = 107.2$, $b = 115.4$, $c = 183.04$, $\alpha = 90$, $\beta = 90.2$, $\gamma = 90$ |
| *Synechococcus elongatus* | *R*32 | $a = b = 186.75$, $c = 59.75$, $\alpha = \beta = 90$, $\gamma = 120$ |

† Moreno *et al.* (1997). ‡ Padyana *et al.* (2001).

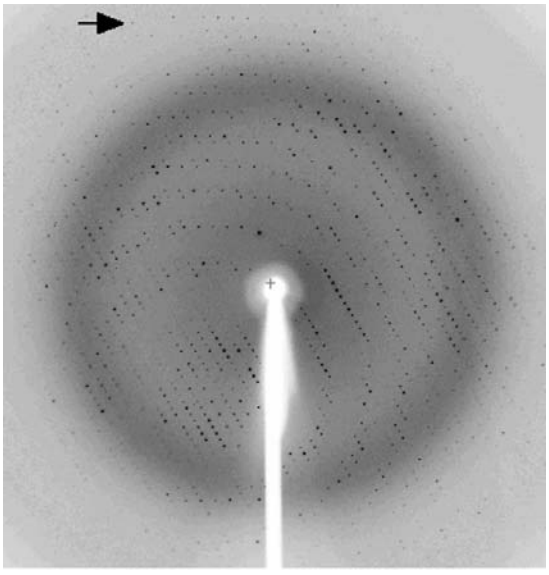

**Figure 2**
X-ray diffraction photograph of the phycocyanin crystal. A 1° oscillation image of a crystal having dimensions 0.15 × 0.15 × 0.10 mm was exposed for 20 s. The arrow at the edge of the image indicates the diffraction at 2.0 Å resolution.

dependent reflections derived from 318 257 observations. The completeness of the data set is 99.7% (28.0–2.0 Å resolution). Data-collection statistics are summarized in Table 1.

The space groups and unit-cell parameters of crystals of C-PC from other organisms are shown in Table 2 for comparison. At least five different space groups have been reported. Although the space group of C-PC from *S. elongatus* is the same as that of C-PC from *Cyanidium caldarium*, the unit-cell parameters differ considerably, indicating that the crystal packing is different. Molecular replacement is now in progress using the *Fremyella diplosiphon* C-PC structure as a starting model.

This research was supported in part by a grant from the New Energy and Industrial Technology Development Organization (to HS and MH).

## References

Adachi, S., Oguchi, T., Tanida, H., Park, S.-Y., Shimizu, H., Miyatake, H., Kamiya, N., Shiro, Y., Inoue, Y., Ueki, T. & Iizuka, T. (2001). In the press.
Betz, M. (1997). *Biol. Chem.* **378**, 167–176.
Brejc, K., Ficner, R., Huber, R. & Steinbacher, S. (1995). *J. Mol. Biol.* **249**, 424–440.
Chang, W.-R., Jiang, T., Wan, Z.-L., Zhang, J.-P., Yang, Z.-X. & Liang, D.-C. (1996). *J. Mol. Biol.* **262**, 721–731.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Duerring, M., Huber, R., Bode, W., Ruembeli, R. & Zuber, H. (1990). *J. Mol. Biol.* **211**, 633–644.
Duerring, M., Schmidt, G. B. & Huber, R. (1991). *J. Mol. Biol.* **217**, 577–592.
Ficner, R., Lobeck, K., Schmidt, G. & Huber, R. (1992). *J. Mol. Biol.* **228**, 935–950.
Hirano, M., Satoh, K. & Katoh, S. (1980). *Photosynthesis Res.* **1**, 149–162.
Hirano, M., Soga, M., Shimazu, T. & Katoh, S. (1992). *Research in Photosynthesis*, Vol. 1, pp. 73–76. Dordrecht: Kluwer Academic Publishers.
Leslie, A. G. W. (1994). *MOSFLM Users Guide*, MRC-LMB, Cambridge.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Moreno, A., Bermejo, R., Talavera, E., Alvarez-Pez, J. M., Sanz-Aparicio, J. & Romero-Garrido, A. (1997). *Acta Cryst.* D**53**, 321–326.
Padyana, A. K., Bhat, V. B., Madyastha, K. M., Rajashankar, K. R. & Ramakumar, S. (2001). *Biochem. Biophys. Res. Commun.* **282**, 893–898.
Schirmer, T., Bode, W., Huber, R., Sidler, W. & Zuber, H. (1985). *J. Mol. Biol.* **184**, 257–277.
Schirmer, T., Huber, R., Schneider, M., Bode, W., Miller, M. & Hackert, M. L. (1986). *J. Mol. Biol.* **188**, 651–676.
Schirmer, T., Bode, W. & Huber, R. (1987). *J. Mol. Biol.* **196**, 677–695.
Sidler, A. (1994). *The Molecular Biology of Cyanobacteria*, pp. 139–216. Dordrecht: Kluwer Academic Publishers.
Stec, M., Troxler, R. F. & Teeter, M. M. (1999). *Biophys. J.* **76**, 2912–2921.