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Crystallization and preliminary X-ray studies of allophycocyanin from red alga *Porphyra yezoensis*

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Abstract

Allophycocyanin from red alga *Porphyra yezoensis* has been crystallized in three crystal forms. Form 1 crystals with space group $P4_132$ or $P4_332$ and cell parameters $a = 286$ Å, $\alpha = 90^\circ$ were obtained by using isopropanol as precipitant. These crystals did not diffract beyond 5.8 Å and could not be used in structure analysis. Form 2 crystals were obtained when crystallization conditions were slightly changed by adding 0.2 *M* magnesium chloride. The space group of form 2 was not determined because it was difficult to get large single crystals. Form 3 crystals were obtained by using ammonium sulfate as precipitant. The space group of form 3 is *R*32 with cell dimensions $a = b = 105.3$, $c = 189.4$ Å and one $\alpha\beta$ unit in the asymmetric unit. These crystals diffract up to 2.06 Å resolution and are suitable for structure determination by molecular-replacement methods.

1. Introduction

Phycobiliproteins in bacteria, algae and plants are the light-harvesting complexes which absorb and transfer light energy to the photosynthetic reaction centers in the thylakoid membrane. The phycobiliproteins can be divided into three main classes: phycoerythrins (PE), phycocyanins (PC), allophycocyanins (APC) according to their visible light absorption properties. APC is close to the photosynthetic reaction centre, forming a core to which stacks of antenna rods are attached. The rods are composed of PC in the middle and PE on the tip (Glazer, 1984). Energy transfer proceeds successively in the direction of PE→PC→APC→chlorophyll *a* with an overall efficiency of almost 100% (Grabowski & Gantt, 1978).

During the last two decades the crystal structures of several phycobiliproteins have been solved at high resolution such as C-PC from *Mastigocladus laminosus*; C-PC from *Agmenellum quadruplaticum*; C-PC from *Fremyella displosiphon*; phycoerythrocyanin (PEC) from *M. laminosus*; B-PE from *Porphyridium sordidum*; b-PE from *Porphyridium cruentum*; R-PE from *Polysiphonia urceolata* (Schirmer *et al.*, 1985, 1986, 1987; Duerring *et al.*, 1990, 1991; Ficner *et al.*, 1992; Ficner & Huber, 1993; Chang *et al.*, 1996). In 1995, Brejc (Brejc *et al.*, 1995) first reported the crystal structure of APC from the cyanobacterium *Spirulina platensis* at 2.3 Å resolution, but the three-dimensional structure of APC from red alga is still unknown. In this article we report the crystallization and preliminary crystallographic studies of APC from red alga *P. yezoensis*.

2. Methods

APC was purified to homogeneity from red alga *P. yezoensis* as reported previously (Pu *et al.*, 1986). Form 1 crystals were grown at room temperature using the hanging-drop vapor-diffusion method. The 10 µl droplets containing 3 mg ml^{-1} APC, 20 m*M* $(NH_4)_2SO_4$, 5%(*v*/*v*) isopropanol and 2%(*v*/*v*) glycerol in 50 m*M* Hepes buffer (pH = 7.3) were equilibrated with 1 ml reservoir solution 9%(*v*/*v*) isopropanol and 0.45 *M* NaCl in the same buffer. Cubic crystals appeared in 4 d and grew to a size of $0.5 \times 0.5 \times 0.5$ mm in a month (Fig. 1*a*). The addition of glycerol was useful in obtaining large crystals. Unfortunately, these crystals did not diffract beyond 5.8 Å. Diffraction data were collected at room temperature using a Siemens X-200B area detector and a Rigaku rotating-anode X-ray generator at 50 mA and 200 kV. These crystals belong to space group $P4_132$ or $P4_332$ with cell parameters $a = 286$ Å and $\alpha = 90^\circ$. Further investigation of this crystal form was abandoned because of the poor diffraction quality.

When 0.2 *M* $MgCl_2$ was added to the hanging drops of crystal form 1, the cubic crystals changed into hexagonal crystals (form 2), but there were too many small crystals. In addition, the crystals were stacked together, so they were difficult to manipulate. Only on one occasion were we able to produce two fine crystals of about $0.1 \times 0.1 \times 0.3$ mm (Fig. 1*b*).

Form 3 crystals were obtained by using ammonium sulfate and magnesium chloride. The optimized crystallization conditions were: 10 µl droplets containing 4 mg ml^{-1} APC, 0.25 *M* $MgCl_2$ and 0.4 *M* $(NH_4)_2SO_4$ in 50 m*M* Hepes buffer (pH = 7.3) were equilibrated with 1 ml reservoir solution of 0.4 *M* $MgCl_2$ and 1.0 *M* $(NH_4)_2SO_4$ in the same buffer. The crystals were grown at room temperature. Large blue crystals were harvested after 1–2 weeks with a typical size of $0.7 \times 0.7 \times 0.3$ mm (Fig. 1*c*).

Diffraction data were collected on form 3 on an area detector at room temperature. A total of 820 ω scanning oscillation exposure frames in steps of 0.15° were measured using a crystal-to-detector distance of 140 mm. The exposure time per frame was 180 s with a 2θ angle of 25°. The data were processed by using the *XENGEN* 2.0 package (Howard *et al.*, 1987). The cell dimensions were given by *XENGEN* automatically. The crystals belong to the trigonal system with parameters $a = b = 105.3$, $c = 189.4$ Å, $\alpha = \beta = 90$ and $\gamma = 120^\circ$. Processing with *P*3 showed systematic absences of the $h + k + l \neq 3n$ reflections, indicating an *H* cell of lattice *R*. Data reduction according to space group *R*32 and *R*3 gave $R_{\rm merge}$ values of 8.2 and 6.9%, respectively, and data reduction from space group *R*3 (with 28 933 unique reflections) to *R*32 gave a lower $R_{\rm merge}$ value of 4.9%. Thus, the correct space group of form 3 is *R*32. On the basis of the molecular weight and cell dimensions, there is one $\alpha\beta$ pair with a molecular weight of about 40 kDa in the asymmetric unit [$V_m = 2.51$ Å^3 Da^{-1}; about 46% solvent (Matthews, 1985)]. These crystals can diffract up to a resolution of 2.06 Å, but data-collection statistics indicated a clear fall-off in intensity beyond 2.2 Å

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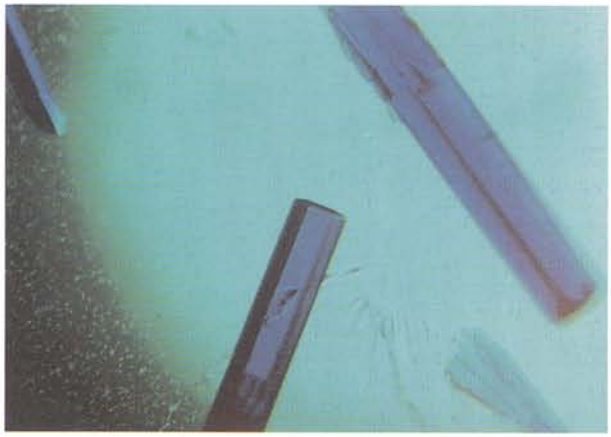
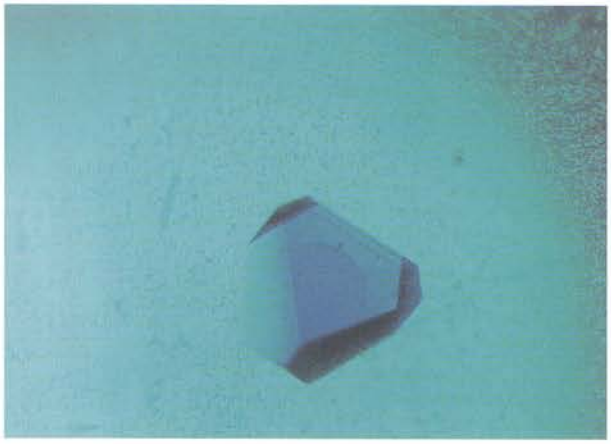
resolution. So the realistic resolution limit is defined as 2.2 Å. The data-collection statistics (to 2.2 Å) of form 3 of APC are given in Table 1.

(*a*)



(*b*)



(*c*)

Fig. 1. Crystals of APC from *P. yezoensis* (*a*) form 1; (*b*) form 2; (*c*) form 3.

Table 1. *Data collection of form 3*

Total No. of observations	44677
No. of unique reflections	18059
Resolution (Å)	2.2
Completeness (%)†	87/65
Completeness (2σ cutoff) (%)†	75/45
Average $I/\sigma(I)$†	15.3/2.1
$R_{\rm merge}$‡	0.082

† For 20–2.2/2.23–2.2 Å. ‡ $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$.

3. Results and discussion

Magnesium chloride is probably important for the crystallization of APC. No crystal of form 3 was obtained using ammonium sulfate as precipitant until a definitive concentration of magnesium chloride was used, and crystal form changed from form 1 to form 2 only when magnesium chloride was added. Magnesium chloride is also present in the crystallization conditions of APC from *S. platensis*, in which the precipitant is PEG 4000. Why APC is susceptible to magnesium chloride in crystallization is still unknown.

The sequence of PAC from *P. yezoensis* is still unknown, but two sequences of PAC from red algae are available (Apt & Grossman, 1993; Offner & Troxler, 1983). The sequence similarity between these two sequences is 89%, and the sequence similarities between each of these two sequences and the consensus sequence of APC from *S. platensis* (Brejc *et al.*, 1995) are both 84%.

Structure of the trigonal crystals of APC from red alga *P. yezoensis* by molecular replacement, using the crystal structure of APC from cyanobacterium *S. platensis* as a search model, is currently under way.

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