Molecular replacement studies on crystal structure of allophycocyanin from red algae *Porphyra yezoensis*

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Abstract Using the crystal structure of allophycocyanin from cyanobacterium *Spirulina platensis* (APC-SP) as a search model, the crystal structure of allophycocyanin from red algae *Porphyra yezoensis* (APC-PY) has been studied by molecular replacement methods. The APC-PY crystals (Form 3) belong to the space group of R32, cell dimensions $a = b = 10.53$ nm, $c = 18.94$ nm, $\alpha = \beta = 90°$, $\gamma = 120°$; there is one αβ monomer in each crystallographic asymmetric unit in the cell. The translation function search gave a unique peak with a correlation coefficient (Cc) of 67.0% and an *R*-factor of 36.1 % for reflection data from 1.0 to 0.4 nm. Using the results by molecular replacement, the initial model of APC-PY was built, and the coincidence of the chromophore in APC-PY initial model with its $2F_o$-F_c OMIT map further confirms the results by molecular replacement.

Keywords: **allophycocyanin, red algae, molecular replacement method.**

The phycobilisomes in blue-green algae and red algae are supramolecular light-harvesting protein-pigment complexes which can absorb and transfer light energy in the range of 500 nm and 650 nm to the light synthetic reaction centers in the thylikoid membrane, with an overall efficiency of almost 100 % .

Electronic microscopic studies showed that the phycobilisomes are composed of two distinct domains: the core and the rods. The core which is composed of several core cylinders associated by allophycocyanin (APC) discs is in the proximity of the reaction centres, while the rods are attached on the core and are composed of phycocyanin (PC) discs in the middle and phycoerythrin (PE) or phycoerythrocyanin (PEC) discs on the tip. Light energy is transferred from PE or PEC, via PC to APC and finally to the reaction centres[1] .

There are eight three-dimensional structures of phycobiliproteins reported so far [2−10] . APC is the component of phycobiliprotein in the core. Its monomer is composed of two different subunits: α and β subunits, and each subunit covalently attaches a phycocyanobilins (PCB) at the Cys-84 residue. In spite of the important role of APC in the energy transfer process and the complexity of the organization of APC discs, the three-dimensional structure studies of APC are extensively noticed. In 1995, Brejc et al. first reported the 0.23 nm crystal structure of APC from

cyanobacterium *Spirulina platensis* (APC-SP)[10]. But there is no three-dimensional structure of APC from red algae reported. In this paper we report the results of the molecular replacement studies of APC from red algae *Porphyra yezoensis* (APC-PY). The final demonstration of the three-dimensional structure of APC-PY will give us significant information for better understanding of structure-function relationship of APC.

1 Crystal and data

The crystallization and preliminary X-ray studies of APC-PY have been reported[11]. Crystals were grown at room temperature using hanging drop vapor diffusion methods. Three crystal forms were obtained, but only form 3 crystals could give strong diffraction. Form 3 crystals were obtained by using ammonium sulfate as precipitant in Hepes buffer (pH = 7.25). After 1—2 weeks, large crystals suitable for X-ray studies could be obtained. Form 3 crystals belong to space group of R32 with parameters $a = b = 10.53$ nm, $c = 18.94$ nm, $\alpha = \beta = 90°$, $\gamma = 120°$. There is one $\alpha\beta$ pair with molecular weight of about 40 ku in the asymmetric unit ($V_m = 2.51 \times 10^{-3}$ nm$^3 \cdot$ U^{-1}, about 46 % solvent).

Data for form 3 crystals were collected on a Nicolet/Siemens X-200B area detector at National Laboratory of Biomacromolecules, using graphite-monochromated CuKα radiations generated by a Rigaku RU 300 rotating anode operating at 200 kV and 50 mA. A total of 820 omega-scanning oscillation exposure frames in steps of 0.15 ° were measured using a crystal to detector distance of 14 cm with a two-theta angle of 25 °. The data were processed by the XENGEN package. The final collection statistics of form 3 of APC-PY are given in table 1.

Table 1 Data collection of form 3

Parameters	
Total observations	44677
Unique reflections	18059
Resolution/nm	0.22
Completeness (%)[a)]	87/65
Completeness (2σ cutoff) (%)[a)]	75/45
Average I/σ[a)]	15.3/2.1
R_{merge}[b)]	0.082

a) For 2-0.22/0.223-0.220 nm. b) $R_{merge} = \sum_h \sum_i | I(h)_i - \langle I(h) \rangle | / \sum_h \sum_i I(h)$.

2 Structure determination

2.1 The model selection

By now, the only one known structure of APC is the crystal structure of APC from cyanobacterium *Spirulina platensis*. Though the amino acid sequence of APC-PY is still unknown, two sequences of APC from red algae have been reported, one from *Aglaothamnion neglectum*[12] and the other from *Cyanidium caldarium*[13]. The sequence similarity between these two sequences is 89%, and the sequence similarities between each of these two sequences and the consensus sequence (the sequence from alignment) of the known structure APC-SP are both 84%. Thus it is

reasonable to attempt to determine the crystal structure of APC-PY by molecular replacement method, using the crystal structure of APC-SP as a search model.

2.2 The cross rotation function

The program AMoRe[14] was applied to the cross rotation function search, for which the data between 1.0 and 0.4 nm were used, the patterson maps of the model were calculated by placing the probe model in a cubic box with 15.0 nm cell edges, and the low limit of the integration radius of 3 nm was used according to the distance from the centroid of mass of the model to the furthest atom. Table 2 lists the five solutions with higher correlation coefficient (Cc). The number one solution (RF1) with three rotating angles at $\alpha = 60.07$, $\beta = 3.06$, $\gamma = 88.03$ has an apparently higher Cc than other solutions. Thus this solution is the correct rotation function solution of the model.

Table 2 Results of cross rotation function calculation

No.	α	β	γ	T_x	T_y	T_z	Cc	R
RF1	60.07	3.06	88.03	0.00	0.00	0.00	20.0	0.0
RF2	58.86	26.58	151.83	0.00	0.00	0.00	12.3	0.0
RF3	106.02	88.08	232.03	0.00	0.00	0.00	10.7	0.0
RF4	55.95	47.45	28.65	0.00	0.00	0.00	10.5	0.0
RF5	14.46	90.00	51.87	0.00	0.00	0.00	10.2	0.0

2.3 The translation function

After the model was rotated by the angles corresponding to the best cross function solution RF1, the translation function search was performed by using the program AMoRe. In translation function search, the data from 1.0 to 0.4 nm were used and the searching range was the whole unit cell. The results for translation function search are listed in table 3 and the schematic diagram of translational function search is shown in fig. 1. According to the symmetry of the space group R32 of APC-PY, the three translation function solutions in table 3 are the symmetry-related solutions. The high correlation coefficient of 67 % and the low R-factor of 36.1 % of these solutions clearly indicated that these solutions are the correct translation function solutions of the model.

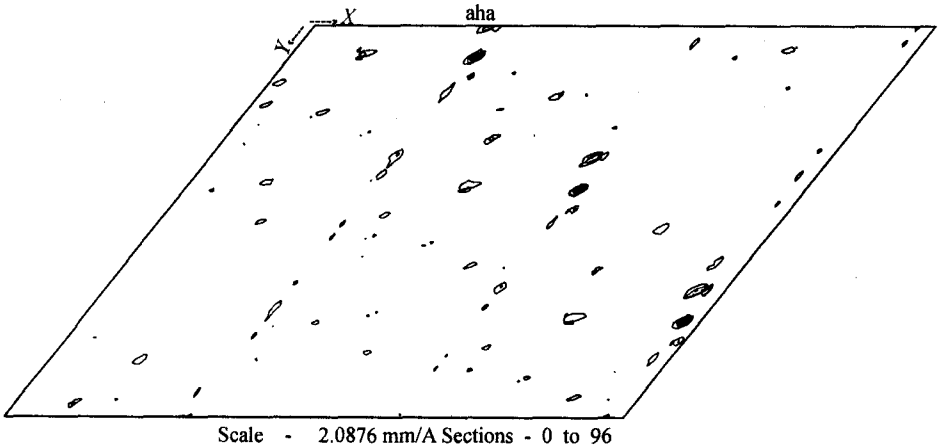

Fig. 1. Schematic diagram of the translation function search of APC-PY.

Table 3 Results of translation function calculation

No.	α	β	γ	T_x	T_y	T_z	Cc	R
TF1	60.07	3.06	88.03	0.583 89	0.955 95	0.236 87	67.0	36.1
TF2	60.07	3.06	88.03	0.250 40	0.288 92	0.070 45	66.9	36.1
TF3	60.07	3.06	88.03	0.917 13	0.622 23	0.403 49	66.8	36.1

2.4 The initial model building

The αβ monomer of the model APC-SP was operated into the unit cell of APC-PY by using the rotation and translation parameters obtained by the rotation and translation function search, thus the initial model of APC-PY was built.

In order to further refine the orientation and the position of the molecules in the unit cell of APC-PY, the initial refinement was performed in the resolution range of 1.0 – 0.4 nm with the αβ monomer as a rigid body first, then the α and β subunits as a rigid body respectively, by using the rigid body refinement technique of XPLOR[15]. After 10 cycles of refinement, the *R*-factor dropped to 32.3 %. By placing the APC-PY model after rigid body refinement into its unit cell, it can be seen that the APC-PY molecules show reasonable packing in crystal cell (fig. 2). Table 4 lists the Van der Walls' contacts between symmetry-related APC-PY αβ monomer. There is no obviously close contact found. The coincidence of the chromophore α84 in APC-PY model with its $2F_o$-F_c OMIT maps further confirms the correctness of the results by molecular replacement (fig. 3).

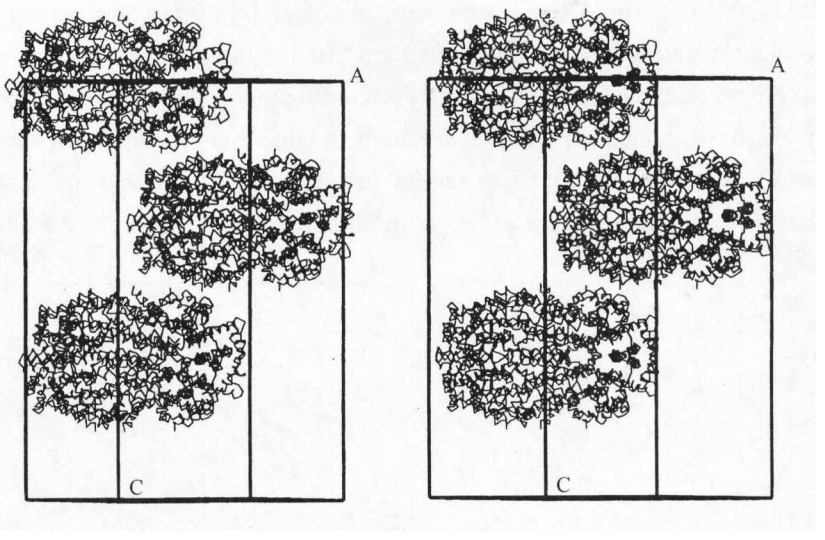

Fig. 2. Packing diagram of APC-PY molecules in the cell.

3 Discussion

Using the crystal structure of allophycocyanin from cyanobacterium *Spirulina platensis* as a search model, the crystal structure of allophycocyanin from red algae *Porphyra yezoensis* has been studied by molecular replacement method. Both cross rotation function search and transla-

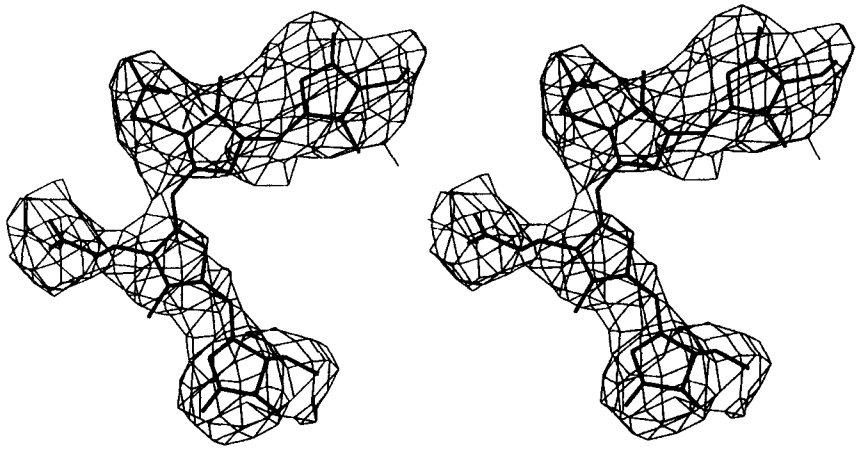

Fig. 3. The chromophore α84 and its $2F_o$-F_c OMIT map (σ =1).

Table 4 Van der Waals' contacts between symmetry-related APC-PY αβ monomers

Atoms	80-MET-CE	and	262-TYR-OH	(XSYM # 2)	0.308 nm
Atoms	93-ARG-NH2	and	276-TYR-OH	(XSYM # 2)	0.309 nm
Atoms	110-ILE-O	and	278-THR-OG1	(XSYM # 2)	0.307 nm
Atoms	111-GLY-CA	and	278-THR-OG1	(XSYM # 2)	0.348 nm
Atoms	113-VAL-O	and	278-THR-OG1	(XSYM # 2)	0.346 nm
Atoms	118-MET-SD	and	278-THR-CG2	(XSYM # 2)	0.340 nm
Atoms	118-MET-CE	and	278-THR-CG2	(XSYM # 2)	0.334 nm
Atoms	121-SER-CB	and	285-ILE-CD1	(XSYM # 2)	0.345 nm
Atoms	121-SER-OG	and	285-ILE-CD1	(XSYM # 2)	0.347 nm
Atoms	121-SER-O	and	253-LYS-CD	(XSYM # 2)	0.347 nm
Atoms	122-LEU-CD2	and	281-TYR-OH	(XSYM # 2)	0.337 nm
Atoms	213-ASP-OD1	and	276-TYR-OH	(XSYM # 2)	0.347 nm
Atoms	262-TYR-OH	and	84A-CYC-O2D	(XSYM # 3)	0.313 nm
Atoms	267-THR-OG1	and	84A-CYC-O1A	(XSYM # 3)	0.315 nm
Atoms	277-THR-N	and	84A-CYC-OB	(XSYM # 3)	0.323 nm
Atoms	277-THR-CA	and	84A-CYC-OB	(XSYM # 3)	0.310 nm
Atoms	277-THR-C	and	84A-CYC-OB	(XSYM # 3)	0.313 nm
Atoms	278-THR-N	and	84A-CYC-OB	(XSYM # 3)	0.305 nm
Atoms	278-THR-CG2	and	84A-CYC-C4B	(XSYM # 3)	0.331 nm
Atoms	278-THR-CG2	and	84A-CYC-C3B	(XSYM # 3)	0.306 nm
Atoms	278-THR-CG2	and	84A-CYC-CAB	(XSYM # 3)	0.328 nm
Atoms	21-PRO-CG	and	164-SER-O	(XSYM # 4)	0.334 nm
Atoms	22-GLY-O	and	26-ARG-NH2	(XSYM # 4)	0.319 nm
Atoms	164-SER-OG	and	242-THR-CG2	(XSYM # 4)	0.346 nm
Atoms	167-ASP-OD2	and	246-ALA-CB	(XSYM # 4)	0.345 nm
Atoms	120-LYS-CD	and	174-SER-O	(XSYM # 6)	0.312 nm
Atoms	331-GLN-CG	and	331-GLN-CD	(XSYM # 12)	0.341 nm

tion function search show apparent peaks and thus the orientation and position of the αβ monomer in asymmetric unit of APC-PY form 3 crystal are determined, respectively. The coincidence of the chromophore in APC-PY initial model with its $2F_o$-F_c OMIT map further confirms the correctness of the results by molecular replacement.

Electron microscopic studies showed that the core of phycobilisome is composed of several core cylinders which are built up by the association of several APC trimers. It is still unknown in which manner the APC trimers are associated into the core cylinders. It was suggested that the

APC trimers may associate into a hexamer in a standard way (by face to face) or form the core cylinders only by translations of APC trimers. In APC-PY crystal, it is clear that two APC-PY trimers associate into a hexamer by face to face through a crystallographic dyad perpendicular to the triad. The deep-going studies of the association conduct of this hexamer will probably have important significance in illuminating the organization manner of the APC trimers in the core cylinder in phycobilisome.

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