Crystal Structure of R-Phycocyanin and Possible Energy Transfer Pathways in the Phycobilisome

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ABSTRACT The crystal structure of R-phycocyanin from *Polysiphonia urceolata* (R-PC-PU) at 2.4 Å is reported. The R-PC-PU crystal belongs to space group $P4_32_12$ with cell parameters $a = 135.1$ Å, $c = 210.0$ Å, and $\alpha = \beta = \gamma = 90°$. The structure was determined by molecular replacement. The crystallographic R-factor of the refined model is 0.189 (R_{free} = 0.239). Comparison of the microenvironment of chromophore β155 in R-PC-PU and in C-PC from *Fremyolla diphosiphon* (C-PC-FD) reveals that their spectral differences may be caused by their different α28 residues. In the R-PC-PU crystal structure, two $(\alpha\beta)_3$ trimers assemble face to face to form a hexamer, and two such hexamers assemble in two novel side-to-side arrangements. Possible models for the energy transfer from phycoerythrin to phycocyanin and from phycocyanin to allophycocyanin are proposed based on several phycobiliprotein crystal structures.

INTRODUCTION

The light-harvesting antenna system in algae is formed by phycobilisomes. Phycobilisomes are mainly composed of phycobiliproteins attached to the thylakoid membrane where the photosynthesis reaction takes place (Glazer, 1985, 1989). Phycobiliproteins have different numbers of chromophores, which are open-chain tetrapyrroles covalently bound to cysteines via thioether bonds. The chromophores can be classified by structure as phycoerythrobilin (PEB), phycocyanobilin (PCB), phycoviolobilin (PVB), or phycourobilin (PUB). Phycobiliproteins can be classified in three main groups as phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) according to the number and species of the chromophores they contain. PE, at the tip of the rod-like phycobilisome, harvests light energy that is transferred to PC, then from PC to APC, and finally to the photosynthesis reaction center. PC, which plays an important role in mediating the energy transfer from PE to APC, can be classified as C-PC and R-PC. C-PC has one absorption peak at 620 nm, because the three chromophores bound to α84, β84, and β155 are all PCB. R-PC can further be divided into three groups according to their spectral characteristics: R-PC(I), R-PC(II), and R-PC(III) (Ong and Glazer, 1987, 1988). They all carry three kinds of chromophores at α84, β84, and β155: R-PC(I) has α84PCB, β84PCB, and β155PEB; R-PC(II) has α84PEB, β84PCB, and β155PEB; and R-PC(III) has α84PUB, β84PCB, and β155PCB.

Several kinds of phycobiliprotein structures have been solved, such as PE (Ficner et al., 1992; Ficner and Huber, 1993; Jiang et al., 1999; Ritter et al., 1999), C-PC(Schirmer et al., 1985, 1986; Duerring et al., 1991; Stec et al., 1999), and APC (Brejc et al., 1995; Liu et al., 1999; Reuter et al., 1999). All these structures are similar. They are composed of two different subunits, α and β, building up an $\alpha\beta$ monomer. These monomers are arranged around a threefold symmetry axis in the trimer $(\alpha\beta)_3$, while two trimers are assembled face to face to form a hexamer (B-PE and R-PE have a third subunit γ, which is in the center of the R-PE and B-PE hexamers). Each $\alpha\beta$ of PE always carries five chromophores at α84, β84, α140a, β155, and β50/61. APC always carries two chromophores at α84 and β84.

Four kinds of C-PC crystal structures have been reported, but the R-PC crystal structure is still unknown. The spectrum of R-PC from *Polysiphonia urceolata* (R-PC-PU) is similar to R-PC(I). R-PC-PU is the first crystal structure of phycocyanin that contains both PEB and PCB. This crystal structure is important for illustrating the relationship between the phycobiliprotein structure and the spectral characteristics.

Although the crystal structures of PE, PC, and APC provide a structural basis for understanding the energy transfer pathways inside each of the proteins, the energy transfer pathways between different phycobiliproteins in phycobilisome are still unknown. Because the crystal structure of R-PE from *P. urceolata* and APC from *Porphyra yezoensis* have been solved in our laboratory, the crystal structure of R-PC-PU is expected to provide information for studying the energy transfer between the different phycobiliproteins in phycobilisome.

MATERIALS AND METHODS

Protein purification and crystallization

R-PC was extracted from the red algae *P. urceolata*, which was harvested in the ocean at Qingdao (Zhang et al., 1995). The R-PC-PU was crystallized by the hanging-drop vapor diffusion method. The hanging-drop contains 6 mg/ml R-PC and 0.05 M Na_2HPO_4/NaH_2PO_4 and $(NH_4)_2SO_4$ (pH 7.0). The diffusion buffer contained 10–20% $(NH_4)_2SO_4$ and 10% NaCl, 0.05 M Na_2HPO_4/NaH_2PO_4. Crystals were grown in the dark at room temperature. Cobalt blue octahedral crystals were often obtained after 2 or 3 weeks with a maximum linear size longer than 1 mm.

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Data collection and structure determination

The R-PC-PU diffraction data were collected at the KEK Photon Factory (Tsukuba, Japan) on Beam Line 6B with a Weissenberg camera (radius 573 mm). Thirty-five frames (size 400 × 800 mm) were collected with a wavelength of 1.0 Å, oscillation angle 2.1° with 0.1° overlap, and an exposure time for each frame of 40 s. The data were processed using the programs DENZO and SCALEPACK (Otwinowski and Minor, 1997). The resolution is 2.4 Å, and the final results are shown in Table 1.

The R-PC-PU structure was solved by molecular replacement with program AMoRe (Navaza, 1994) by using as a model the R-PE structure solved earlier (Jiang et al., 1999). Data from 10–3.5 Å was used for the rotation function calculation; the same solution could be obtained with different integration radii. With the correct rotation angle, the translation function was calculated using data from 10 to 4 Å, for space groups $P4_32_12$ and $P4_12_12$. The translation solution with the highest Cc was obtained using space group $P4_32_12$. Therefore, the space group was determined to be $P4_32_12$. After rigid body refinement, the *R*-value decreased to 39.8%.

The molecular packing in the unit cell was examined with Turbo Frodo (Jones, 1978) on an SGI graphic workstation. Because the R-PC-PU sequence is unknown, a consensus sequence was used to build the initial model. The consensus sequence was from four homologous proteins (Wilson et al., 1991; De Lorimier et al., 1993; Ducret et al., 1994) (Table 2). The X-plor package (Brünger, 1992) was used for structural refinement, with each refinement step followed by manual rebuilding based on sets of omit maps. Residues were adjusted according to the electron density map, and the final sequence determined has 79% homology to the consensus sequence. The resolution was gradually extended and the B-factor refined. A total of 340 water molecules were introduced in batches according to the 2Fo-Fc and Fo-Fc maps in the last stage. The *R*-value of the final model was 18.9% with an *R*-free of 23.9%. The model quality is shown in Table 1. All dihedral angles are in the allowed region except for β77Thr, which in all known phycobiliproteins is in the disallowed region because it forms a hydrogen bond with chromophore α84. The coordinates and reflection data of R-PC-PU have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank as entry 1F99.

TABLE 1 Data collection statistics and parameters of refined R-PC-PU

Parameter	Value
Space group	$P4_32_12$
Cell constants	$a = b = 135.1$ Å, $c = 210.0$ Å, $\alpha = \beta = \gamma = 90°$
Resolution limit (Å)	2.4
Number of observations	266,953
Number of independent reflections	73,674
Completeness (%) ($I > 2a$)	
All data	75.5
Outermost shell (2.5-2.4 Å)	49.0
R_{merge} (on I) of all data (%)	12.2
Number of protein atoms	7482
Number of chromophore atoms	387
Number of active solvent atoms	340
R-factor of final model (8-2.4 Å)	18.9%
Free R-factor	23.9%
Root mean square deviation of bond lengths (Å)	0.010
Root mean square deviation of bond angles (°)	2.4
Temperature factors (Å^2)	
Main-chain atoms	23.3
Side-chain atoms	26.6

RESULTS AND DISCUSSION

Molecular structure

R-PC-PU is composed of α- and β-subunits, with the α-subunit composed of 162 residues and the β-subunit of 172 residues. The α- and β-subunits have similar three-dimensional structures including nine α-helices and loop linkages. Two PCBs are bound to α84 and β84, and one PEB is bound to β155. Three αβ monomers are arranged around a three-dimensional axis to form a disc-shaped $(\alpha\beta)_3$ trimer, which is 30 Å thick and 110 Å in diameter, with a cavity in the center (Fig. 1 *a*).

All known C-PCs crystallized in a trigonal or hexagonal space group. R-PC-PU is the first phycocyanin in space group $P4_32_12$, with one trimer per asymmetry unit. The structural differences between R-PC-PU and C-PC from *Fremyolla diphosiphon* (C-PC-FD) are very small. The root mean square deviation between their main chains is 0.931 Å. The distances between the chromophores in the two proteins are also very similar. The non-crystallographic threefold axis of R-PC-PU is at a 45° angle to the *z* axis of the unit cell. Nevertheless, the two trimers related by a crystallographic twofold axis still assemble face to face to form a hexamer (Fig. 1 *b*). Phycobiliproteins exist in many forms in vitro, mostly as $(\alpha\beta)_3$ and $(\alpha\beta)_6$, depending on their source, concentration, and isolation conditions. The unusual packing function of R-PC-PU in the unit cell of the crystal still forming a hexamer supports the viewpoint that the phycocyanin biological function unit is a hexamer in nature.

The chromophore attached to β155 of R-PC-PU is PEB, which fits the electron density well (Fig. 2). In all crystal structures of C-PC, the chromophores at β155 are PCB. The difference between the chemical structures of PEB and PCB is shown in Fig. 3, where the carbon atom C(16) (in ring D) is a chiral atom in PEB.

We compared the crystal structures of R-PC-PU and C-PC-FD. Superposition of their β155 and its associated microenvironment (Fig. 4) shows that the D ring of β155PEB in R-PC-PU is much further away from the conjugate plane formed by the B and C rings of R-PC-PU than that of β155PCB in C-PC-FD. The reason is that the OB atom in ring D of β155PEB forms a hydrogen bond with α28Asn in R-PC-PU, which pulls ring D out of the BC conjugate plane. In C-PC-FD, α28 is Phe, which modifies the conformation of β155PCB by hydrophobic interaction, preventing the D ring from deviating from the BC conjugate plane. We know that C-PC and APC have the same kind of chromophores α84PCB and β84PCB but quite different spectral characteristics, which indicates that the chromophore microenvironments play a very important role in influencing the spectral characteristics of phycobiliproteins. The conclusion can be made that the different α28 residues create a different

TABLE 2 Alignment of R-PC amino acid sequences

	Sequence
α-Subunit	
A1	**MKTP**I**TEA**IAT**AD**N**QGRFLS**N**TE**L**QA**VN**GR**YQ**RAT****ASL**T**AAK**AL**T**GS**A**QR
A2	**MKTP**L**TEA**VSA**AD**S**QGRFLS**S**TE**V**QA**AS**GR**FN**RAA****ASL**E**AAK**AL**S**AK**A**DA
A3	**MKTP**L**TEA**VAA**AD**S**QGRFLS**N**TE**V**QA**AS**GR**FN**RA**K**ASL**E**AAK**AL**T**SK**A**DS
A4	**MKTP**L**TEA**VAA**AD**S**QGRFLS**N**TE**V**QA**AS**GR**FN**RA**K**ASL**E**AAK**G**L**TAK**A**DS
CON	**MKTP**L**TEA**VAA**AD**S**QGRFLS**N**TE**V**QA**AS**GR**FN**RA**K**ASL**E**AAK**AL**T**AK**A**DS
SEQ	**MKTPLTEAIAAADSQGRFLSNTELQVVNGRYNRATSSLEAAKALTANADR**
A1	**L**ITG**AA****QAVY**N**KFPYTTQM**PG**PA**Y**ASSAI**GKAKC**A**RD**I**GYYLRM**V**TY**TLV**
A2	**L**VNG**AA****QAVY**N**KFPYTTQME**G**SN**Y**STTPE**GKAKC**S**RD**V**GYYLRM**I**TY**CLV**
A3	**L**VNG**AA****QAVY**S**KFPYTTQME**G**SN**Y**SATPE**GKAKC**S**RD**V**GYYLRM**I**TY**CLV**
A4	**L**VSS**AT****QAVY**T**KFPYTTQME**G**PN**Y**SATSE**GKAKC**S**RD**I**GYYLRM**I**TY**CLV**
CON	**L**VNG**AA****QAVY**N**KFPYTTQME**G**.N**Y**SATPE**GKAKC**S**RD.GYYLRM**I**TY**CLV**
SEQ	**LISGAAQAVYSKFPYTTQMPGPNYSSTAIGKAKCARDIGYYLRMVTYCLV**
A1	V**GGTGPMDEYLVAGLEEINR**S**FDLSPSWY**I**EAL**QY**I**KNS**HGLSG**QV**AN****EA**
A2	A**GGTGPMDDYLIAGLDEINR**T**FELSPSWY**V**EAL**KY**I**KSN**HGLSG**DA**AT****EA**
A3	A**GGTGPMDDYLIAGLDEINR**T**FELSPSWY**V**EAL**KH**I**KAN**HGLSG**DA**AT****EA**
A4	A**GGTGPMDDYLIAGLDEINR**T**FELSPSWY**V**EAL**KH**I**QSN**HGLSG**DA**AT****EA**
CON	A**GGTGPMDDYLIAGLDEINR**T**FELSPSWY**V**EAL**K.**I**KSN**HGLSG**DA**AT****EA**
SEQ	**VGGTGPMDDYLVAGLEEINRTFELSPSWYIEALKYIKNNHGLSGDVANEA**
A1	**NAYIDYAIN**TSL
A2	**NSYIDYAIN**ALI
A3	**NSYIDYAIN**ALI
A4	**NSYIN****YAIN**ALT
CON	**NSYIDYAIN**ALI
SEQ	**NTYIDYAINTLS**
β-Subunit	
A1	**ML****DAFA****KVVAQADARG**EFL**S**NTQ**IDAL**SK**MV**KEG**N**Q**RLDIV**NKVTS**NAS**A
A2	**MF****DAFT****KVVAQADARG**Q**FIS**ASE**IDAL**AA**MV**SDS**N**K**RLDAV**NRISS**NAS**T
A3	**MF****DAFT****KVVAQADARG**Q**FIS**TSE**IDAL**AA**MV**SDS**N**K**RLDAV**NRISS**NAS**T
A4	**MF****DAFT****KVVAQADARG**Q**FIS**TSE**IDAL**AA**MV**SGR**N**K**RLDAV**SRISN**NAS**T
CON	**MF****DAFT****KVVAQADARG**Q**FIS**TSE**IDAL**AA**MV**SDS**N**K**RLDAV**NRISS**NAS**T
SEQ	**MLDAFAKVVAQADARGEFLSNTQIDALLAAVSEGNKRLDVVNKITNNASA**
A1	**IV**TNS**AR**A**LFA**E**QP**Q**LI**Q**PGGNAYTSR**N**MAACLRDMEI**V**LRYV**S**Y**AMLA**G**
A2	**IV**ASA**AR**Q**LFA**Q**QP**S**LI**A**PGGNAYTSR**R**MAACLRDMEI**I**LRYV**T**Y**ASFA**G**
A3	**IV**ASA**AR**Q**LFA**Q**QP**A**LI**A**PGGNAYTSR**R**MAACLRDMEI**I**LRYV**T**Y**SAFT**G**
A4	**IV**ASA**AR**E**LFA**Q**QP**A**LI**S**PGGNAYTSR**R**MAACLRDMEI**I**LRYV**T**Y**SAFT**G**
CON	**IV**ASA**AR**Q**LFA**Q**QP**A**LI**A**PGGNAYTSR**R**MAACLRDMEI**I**LRYV**T**Y.AF.G**
SEQ	**IVTNAARALFAEQPQLISPGGNAYTSRRMAACLRDMEIVLRYVSYAMIAG**
A1	**D****S****SV**LD**DRCLNGLRETY**Q**ALGTPG**S**SVA**VAIQK**MK**DASVALA**ND**TT**G**TPI
A2	**DA****SV**LE**DRCLNGLRETY**L**ALGTPG**A**SVA**AGVNL**MK**ESALAIV**ND**RA**G**ISA
A3	**DA****SV**ME**DRCLNGLRETY**L**ALGTPG**A**SVA**AGVNL**MK**DAALAII**ND**KA**G**ISA
A4	**DA****SV**LE**DRCLNGLRETY**L**ALGTPG**T**SVA**AGVNL**MK**DAALSIV**ND**RA**G**ISN
CON	**DA****SV**LE**DRCLNGLRETY**L**ALGTPG**T**SVA**AGVNL**MK**DAALAIV**ND**RA**G**ISA
SEQ	**DASVLDDRCLNGLRETYQALGTPGASVAVAIQKMKDAALALVNDTTGTPA**
A1	**GDC****S****SL**VA**E**LAG**YFDRA**AVS**V**V
A2	**GDCA****SL**SS**E**IGT**YFDRAA**AA**V**A
A3	**GDCA****SL**SS**E**IGT**YFDRAA**AS**V**A
A4	**GDCA****SL**SS**E**IGT**YFDRAA**AS**V**A
CON	**GDCA****SL**SS**E**IGT**YFDRAA**AS**V**A
SEQ	**GDCASLVAEIATYFDRAAAAVA**

Alignment of the α- and β-subunits of R-PC from *Porphyridium cruentum* (A1), *Synechococcus* sp. (strain WH7803) (A2), *Synechococcus* sp. (strain WH8103) (A3), and *Synechococcus* sp. (strain WH8020) (A4). The consensus sequence (CON) was derived from the alignment, and conserved amino acids are marked in bold.

chromophore microenvironment, which influences the conformational changes of the chromophores leading to different spectral characteristics. Thus C-PC-FD has a single absorption peak (620 nm) whereas R-PC has two absorption peaks (one is at 617 nm caused by α84PCB and β84PCB and the other at 560 nm caused by β155PEB).

Energy transfer inside the phycobilisome

A main goal of the crystal structure determination of phycobiliprotein is to study the energy transfer process between different phycobiliproteins. The first objective is to identify the relative locations of the chromophores in the different

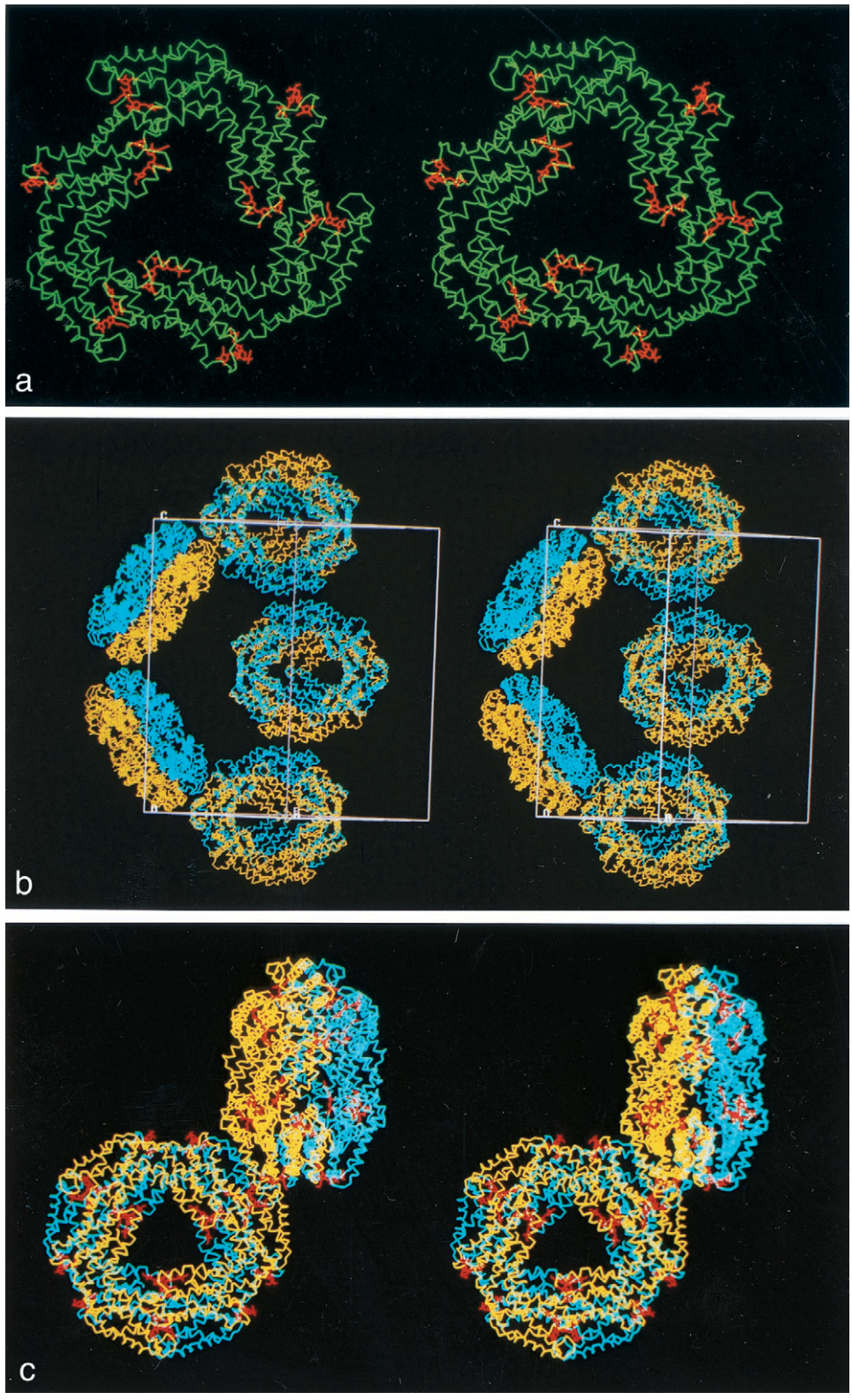

FIGURE 1 (*a*) Stereo view of the Cα chain of the R-PC-PU trimer (*green*) and the chromophores (*red*); (*b*) Stereo view of R-PC-PU packing in the unit cell; (*c*) Assembly of two perpendicular side-to-side hexamers in the unit cell.

phycobiliproteins. Although the crystal structures of several kinds of phycobiliproteins have been solved and electron microscope studies have revealed the approximate assembly functions of several kinds of phycobilisomes (Bryant et al., 1990; Glauser et al., 1992; Wehrmeyer, 1983), the energy transfer processes are not clear because the distances between chromophores in different phycobiliproteins are unknown. Trimeric phycobiliproteins are disc-like with a face

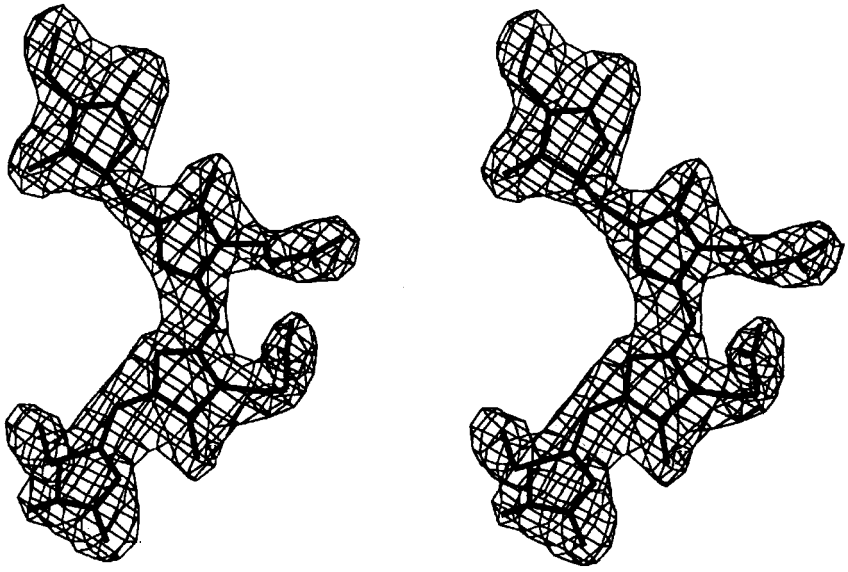

FIGURE 2 Stereo view of β155 PEB in the omit 2Fo-Fc electron density map.

(the surface always participating in hexamer formation), a back (the surface opposite to the face and not participating in hexamer formation), and a side (edge of the disc). Fig. 5 shows a phycobilisome model based on several electron microscope observations of phycobilisomes(Glazer, 1989; Yamanaka et al., 1980), which summarizes five types of

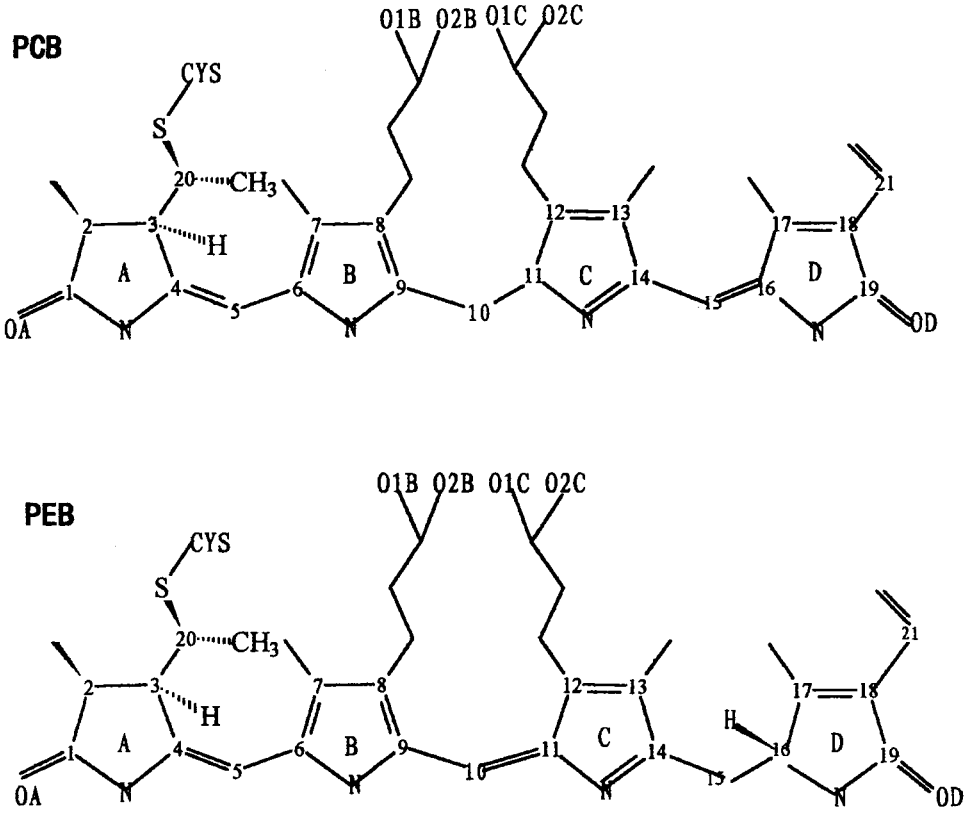

FIGURE 3 Chemical structures of phycocyanobilin (PCB) and phycoerythrobilin (PEB).

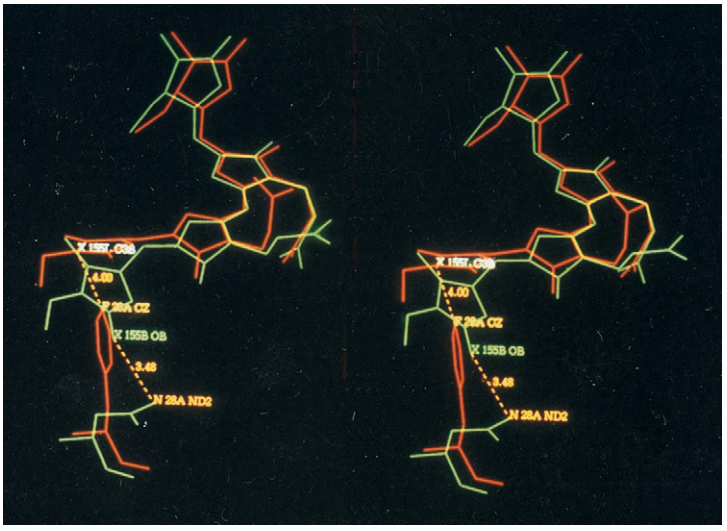

FIGURE 4 Stereo view of chromophore β155 and residue α28 in R-PC-PU (*green*) and C-PC-FD (*red*).

assembly functions for phycobiliproteins. The first type is the back-to-back assembly between PE and PC. The second is the parallel side-to-side assembly between PE and PC. The third is the back-to-side assembly between PC and APC. The fourth is the perpendicular side-to-side type I between PC and APC. The fifth is the perpendicular side-to-side type II between two PCs. The crystal structures of three different phycobiliproteins in phycobilisome (R-PE, R-PC, and APC) have been solved in our laboratory, and possible energy transfer pathways in all five types are discussed based on these structures.

Energy transfer from PE to PC

Back to back is the main type of assembly function between PE and PC hexamers, but the energy transfer pathway in such an assembly function is not very clear. We investigated the packing of hexamers in known phycobiliproteins and found that the assemblies in R-PE from *P. urceolata* and in C-PC-FD are almost the same: if one of the two adjacent hexamers were translated ~60 Å along the threefold axis, the structure would then match the structure of the other hexamer. The electron microscope observations of the assemblies of PE and PC in phycobilisome were very similar.

When two such back-to-back hexamers were R-PE and R-PC, respectively, six pairs of chromophore-chromophore distances between R-PE and R-PC shorter than 40 Å were observed (Fig. 6). Previous research has considered four energy transfer pathways: PEβ84→PCα84 (35 Å), PEβ84→PCβ84 (27 Å), PEα84→PCβ84 (35 Å), and PEα84→PCα84 (32 Å). In our analysis, the neglected pathways PEβ50→PCα84 (27 Å) and PEβ50→PCβ84 (35 Å) are also short enough for energy transfer, but they may be relatively subsidiary pathways for energy transfer because their relative orientations are not as conducive to energy transfer as the other four.

In the back-to-back assemblies, β155PEB and α140a PEB of PE and β155 of PC does not seem to participate in the energy transfer from PE to PC. However, earlier research work at 1.9-Å resolution on the R-PE structure shows that the distance between α140a PEB and β155, which belong to two adjacent hexamers, is only ~3.4 Å. Therefore, we suppose that in the parallel side-to-side assembly

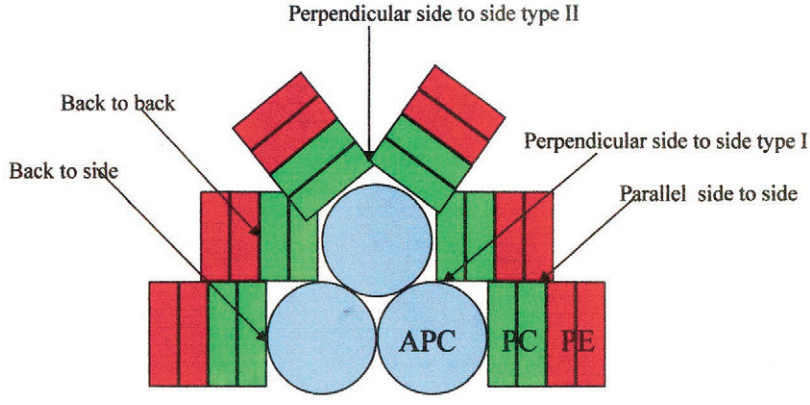

FIGURE 5 Phycobilisome model summarizing the different types of phycobiliprotein assemblies.

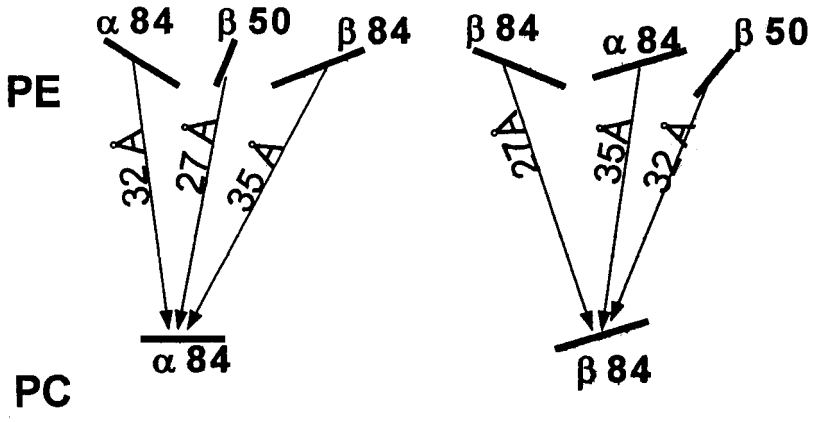

FIGURE 6 Possible energy transfer pathways from PE to PC (in back-to-back assembly).

between PE and PC, the α140a PEB of R-PE may efficiently transfer energy to the β155 of PC (Jiang et al., 1999).

Energy transfer from PC to APC

Back to side was regarded as the main way of assembly between PC (back) and APC (side), and a large amount of spectral data of phycobilisome was collected (Debreczeny and Sauer, 1995; Gillbro et al., 1983; Sandstrom et al., 1988; Suter et al., 1984; Suter and Holzwarth, 1987). Nevertheless, different explanations have been given for the PC→APC energy transfer procedure because of unknown distances between the chromophores. In an early spectral study of rod-core complexes, Bittersmann and Vermaas (1991) observed a 20-ps component, and Sandstrom et al. (1988) observed a 17-ps component, which was regarded as from PC→APC. Sandstrom et al. (1988) also detected an additional 55-ps component. The 17-ps or 20-ps component was assigned to PC α84 or PC β84→APC whereas the 55-ps component was assigned to PC β155→APC by the mediation of PC α84 or PC β84.

Recently, Zhang and his co-workers synthesized a complex of APC and PC with 18-ps and 55-ps spectral components (Zhang et al., 1997). They assumed that the 18-ps (17–20-ps) component is PC→APC, the 55-ps component representing the energy transfer from PC β155 to PC β84 but not PC β155→APC. They also proposed a possible model of energy transfer from the PC trimer to the APC trimer to explain why only one spectral component (18 ps) from PC to APC was detected: PC and APC assemble in a face (PC)-to-side (APC) way with APC α84PCB located on the threefold axis of PC, so that the three PC β84s transfer energy equally to APC α84PCB.

In previous research, the APC unit was assumed to be a trimer. Zhang's model is also based on this prerequisite, but from our recent work we suggested that APC in red algae may exist as a loose hexamer (Liu et al., 1999). Therefore, it appears that the APC loose hexamer assembling to a PC hexamer may be closer to the natural state.

When one loose hexamer of APC was closely packed with a trimer of R-PC-PU in a perpendicular way to form a back (PC)-to-side (APC) assembly, the measured distances between chromophores suggest a possible model for energy transfer, shown in Fig. 7.

Fig. 7 shows four isometric pathways numbered 1, 2, 3, and 4 (~32 Å long). They are possible pathways for energy transfer from PC β84 to APC α84. This can explain the 17–20-ps component. Pathway 5 (47 Å) is a possible pathway from β155 of PC to α84 of APC, pathway 6 (46 Å) is

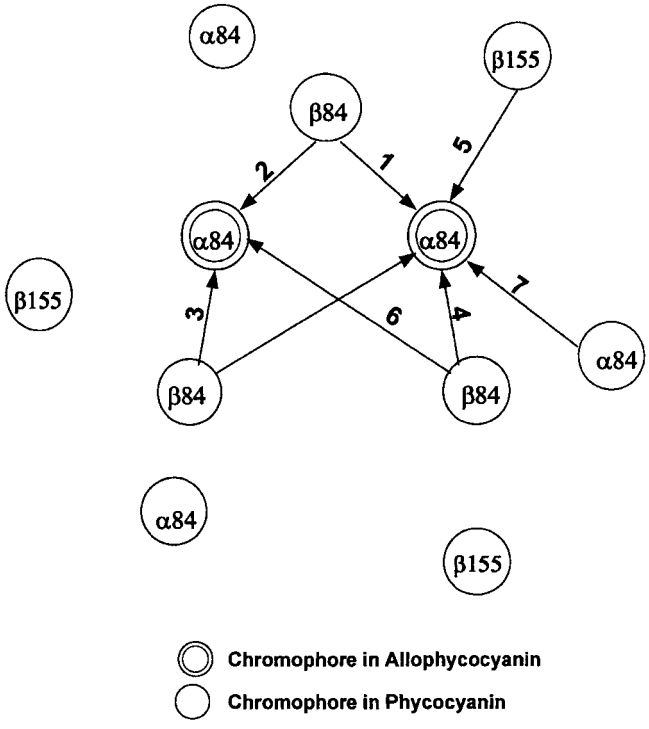

FIGURE 7 Possible energy transfer pathways from PC to APC (in back-to-side assembly).

a possible pathway from β84 of PC to α84 of APC, and pathway 7 (46 Å) is a possible pathway from α84 of PC to α84 of APC. These pathways may explain the 55-ps component. So it appears that β155 of PC can transfer energy to α84 of APC without the mediation of α84 or β84, which is different from Sandstrom's assumption.

The R-PC-PU structure reported here is the first phycocyanin crystal structure belonging to a tetragonal system. The packing of R-PC-PU in the unit cell is quite unique, and two molecular packing functions never before found in C-PC crystal structures were observed. The two functions are similar to the two assembly ways identified as perpendicular side-to-side type I and perpendicular side-to-side type II (Fig. 5).

The perpendicular side-to-side type I (Fig. 1 *c*) assembly has two energy pathways shorter than 40 Å, $\beta155 \rightarrow \alpha84$ (25 Å) and $\beta155 \rightarrow \beta84$ (34 Å), implying that energy transfer from β155 of PC to APC α84 and β84 is possible in this assembly.

In the perpendicular side-to-side type II assembly, the distances between chromophores belonging to different trimers are quite large. All distances between chromophores are greater than 40 Å except for the distance between two α84 chromophores in different trimers, which are 35 Å apart. Such an assembly does not seem to facilitate highly efficient energy transfer and does not seem to be an important way of assembling chromophores.

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