Crystal Structure of Allophycocyanin from Red Algae Porphyra yezoensis at 2.2-Å Resolution*

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‡ To whom correspondence should be addressed: National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Chaoyang District, Beijing 100101, China.

The crystal structure of allophycocyanin from red algae Porphyra yezoensis (APC-PY) at 2.2-Å resolution has been determined by the molecular replacement method. The crystal belongs to space group R32 with cell parameters a = b = 105.3 Å, c = 189.4 Å, α = β = 90°, γ = 120°. After several cycles of refinement using program X-PLOR and model building based on the electron density map, the crystallographic R-factor converged to 19.3% (R-free factor is 26.9%) in the range of 10.0 to 2.2 Å. The r.m.s. deviations of bond length and angles are 0.015 Å and 2.9°, respectively.

In the crystal, two APC-PY trimers associate face to face into a hexamer. The assembly of two trimers within the hexamer is similar to that of C-phycoerycin (C-PC) and R-phycocerythin (R-PE) hexamers, but the assembly tightness of the two trimers to the hexamer is not so high as that in C-PC and R-PE hexamers.

The chromophore-protein interactions and possible pathway of energy transfer were discussed. Phycocyanobilin 1α84 of APC-PY forms 5 hydrogen bonds with 3 residues in subunit 2β of another monomer. In R-PE and C-PC, chromophore 1α84 only forms 1 hydrogen bond with 2β77 residue in subunit 2β. This result may support and explain great spectrum difference exists between APC trimer and monomer.

Phycobilisomes are large supramolecular aggregates attached to the stromal side of the thylakoid membrane in cyanobacteria, red algae, and cryptomonads. These supramolecular aggregates are light-harvesting protein pigment complexes that are composed of phycobiliproteins and linker proteins. Based on the absorption of visible light, the phycobiliproteins can be divided into three main groups: phycocerythin (PE) or phycocerythrocyanin (PEC), phycocyanin (PC), and allophycocyanin (APC). With the help of linker proteins, phycobiliproteins form the two distinct structural domains of phycobilisome, the core and the rods. The core, which is composed of three or more core cylinders associated by APC discs, is in proximity of the reaction centers, whereas the rods are attached on the core and are composed of PC discs in the middle and PE or PEC discs on the tip. Light energy is transferred from PE or PEC via PC to APC and finally to the reaction centers (1).

The crystal structures of several phycobiliproteins have been solved; among them, three are PEs (2–4), three are C-phycoerycins (C-PCs) (5–7), one is PEC:PEC from Mastigocladus laminosus (8), and one is APC:APC from Spirulina platensis (9). All these structures are very similar. The basic building block is an αβ monomer composed of α and β subunits (R-phycocerythin (R-PE) and B-phycocerythin (B-PE) have a third subunit γ in the center of the molecule); three αβ monomers are arranged around a 3-fold symmetry axis to form an (αβ)3 trimer or two (αβ)2 trimers, which are assembled face to face into an (αβ)6 hexamer.

The crystal structure of APC is very special compared with other phycobiliproteins. First, the spectrum difference between APC trimers and its monomer is very large. When APC monomers aggregate to trimer, the absorption spectrum has a 40-nm red shift; the CD spectrum also changes a great deal, and exciton interaction in the trimer of APC was suggested (10), whereas the spectrum difference between C-PC monomer and its trimer is not so large as in APC, although phycocyanin has the same α84PCB and β84PCB as APC.

Second, the functional unit of APC was thought to be a trimer, whereas the function unit of other phycobiliproteins was hexamer (αβ)6 or (αβ)2γ. Brejc and co-workers solved the structure of APC-SP from blue alga S. platensis (9) in the unit cell of APC-SP crystal; two trimers are associated in a “back to back” manner that might represent the assembly state of APC in nature. Red alga is higher than blue alga in evolution, so it would be interesting to know the packing of APC from red alga in the unit cell and in nature.

Third, in PE and PC, the two trimeric discs are superimposed along a 3-fold axis, but in PC and APC the two discs are connected perpendicularly. The pathway of energy transfer between PC and APC is still unknown.

The red algae Porphyra yezoensis is an algae that exists widely in nature. Its phycobilisomes contain R-PE, C-PC, and APC. In this paper we report the crystal structure of APC from P. yezoensis (APC-PY) at 2.2-Å resolution. The organization of APC trimers in the core cylinders of phycobiliproteins and the pathway of energy transfer were discussed.

EXPERIMENTAL PROCEDURES

Crystallization and data collection of APC-PY was reported earlier (11). The crystals of APC-PY belong to space group R32 with parameters a = b = 105.3 Å, c = 189.4 Å, α = β = 90°, and γ = 120°.

Molecular replacement using program AMoRe (12) was carried out using the 2.3-Å structure of APC-SP as a model. Model cell parameters were a = b = c = 150.0 Å, α = β = γ = 90°, integrate radius was 30 Å, and rotation function calculation gave a rather high coefficient solution,
The consensus sequence (CONS) was derived from the alignment. The model sequence (SEQ) is based on the electron density. Conserved amino acids are marked in bold letters.

### $\alpha$ subunit

| ANCY | SIVTKAIV NADAEARYLS PGLDKIKSF VAGGASRLRI AQLTEKNR |
| CALO | SIVTKSIV NADAEARYLS PGLDKIKSF VSSGGERLRI AQLTEKNR |
| SYNO | MSIVKSIV NADAEARYLS PGLERIKSF VVGGDRRLRI AQTAEER |
| MALA | SIVTKSIV NADAEARYLS PGLERIKSF VSSGGERLRI AQLTDNKR |
| CYCA | MSIVTKSIV NADAEARYLS PGLERIKSF VLSQQRLNZ AQLTDNKR |
| AGNE | MSIVKSIY NADAEARYLS PGLERIKSF VLLSQQRLNZ AQLTDNKR |
| CONS | SIVTKSIV NADAEARYLS PGLERIKSF VLSQQRLNZ AQLTDNKR |

### $\beta$ subunit

| ANCY | MQDAITSVIN SSDVQKYL D TAAEKLGY FATGELRVR AATTASANAA |
| CALO | AQDAITSVIN SSDVQKYL D SAAEKLGY FATGELRVR AATTASANAA |
| SYNO | MQDAITSVIN SSDVQKYL D SAAEKLGY FATGELRVR AATTASANAA |
| MALA | MQDAITSVIN SSDVQKYL D TAAEKLGY FATGELRVR AATTASANAA |
| CYCA | MQDAITSVIN SSDVQKYL D SAAEKLGY FATGELRVR AATTASANAA |
| AGNE | MQDAITSVIN SSDVQKYL D SAAEKLGY FATGELRVR AATTASANAA |

### $\gamma$ subunit

| ANCY | SIVQAGQ1QLF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |
| CALO | SIVQAGQQ1LF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |
| SYNO | SIVQAGQQ1LF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |
| MALA | SIVQAGQQ1LF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |
| CYCA | SIVQAGQQ1LF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |
| AGNE | SIVQAGQ1QLF QRQGQVSPG GNAQDEEM TATCRDLDY YLRLYTYIG |

### $\delta$ subunit

| ANCY | SQDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| CALO | SQDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| SYNO | SQDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| MALA | AGDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| CYCA | AGDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| AGNE | AGDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |
| CONS | GSDVTCIERI GIVGMTREM K SLGPTIYAV GVVAAKMKV AATLQAEDSS |

### $\epsilon$ subunit

| ANCY | EAGSIFVDYV GMQ |
| CALO | EAGSIFVDYV GALS |
| SYNO | EAGSIFVDYV GALS |
| MALA | EAGSIFVDYV GALS |
| CYCA | EAGSIFVDYV GALS |
| AGNE | EAGSIFVDYV GALS |
| CONS | EAGSIFVDYV GALS |

### $\zeta$ subunit

| ANCY | EMGSIYID1S SGLS |
| CALO | EMGSIYID1S SGLS |
| SYNO | EMGSIYID1S SGLS |
| MALA | EMGSIYID1S SGLS |
| CYCA | EMGSIYID1S SGLS |
| AGNE | EMGSIYID1S SGLS |
| CONS | EMGSIYID1S SGLS |

### $\eta$ subunit

| ANCY | ...F........V.M. |
| CALO | ...F........V.M. |
| SYNO | ...F........V.M. |
| MALA | ...F........V.M. |
| CYCA | ...F........V.M. |
| AGNE | ...F........V.M. |
| CONS | ...F........V.M. |
$\alpha = 60.07$, $\beta = 3.06$, $\gamma = 88.03$, $Cc = 20.0$. The orientations and positions of one $\alpha$ in the asymmetric unit were determined by the translation function with a high correlation coefficient of 66.9%. The R-factor in the range from 10 to 4 Å was 36.1%. After rigid-body refinement, R-factor dropped to 33.1%, and the correlation coefficient increased to 71.2%. The packing of molecules in the unit cell was reasonable.

The structure was refined using X-PLOR (13). The consensus sequence was used for the initial model building. Fourier transform and electron density were first calculated in the resolution range of 10 Å to 3.5 Å. Residues that could not be fitted into the electron density map were omitted from the phase calculation in the next refinement cycle. After several cycles of rigid body, positional refinement, and manual model adjustment, the R-factor dropped to 25.4%, and a 2Fo-Fc Fourier map looked quite good. Then the resolution was extended to 2.2 Å. After the chromophores were fitted in the map and followed by several cycles of positional refinement and model adjustment, the electron density improved further. At this stage almost all side chains were well defined except those on the surface. Residue exchanges were carried out at this stage according to the omit map. After several cycles of positional refinement and model adjustment, the R-factor was converged to 24.0%, the individual B-factors were then refined, and the R-factor dropped to 21.5%. 169 water molecules were added to the model according to the Fo-Fc and 2Fo-Fc maps, and the final R-factor of the model was 19.3% (R-free factor was 26.9%) in the range of 10 Å to 2.2 Å.

RESULTS AND DISCUSSION

Amino Acid Sequence—Because the amino acid sequence of APC from $P$. yezeoensis is still unknown, the following six APC amino acid sequences were used to get a consensus sequence for model building. Among these sequences, four are from cyanobacteria, $Anabaena$ cylindrica (14), $Calotrix$ PCC7601 (15), $Fischerella$ PCC7603 (16), and $Synechococcus$ PCC6301 (17), and two are from red algae, $Aglaothamnion$ neglectum (18) and $Cyanidium$ caldarium (19). The alignment of these six sequences is shown in Table I.

Quality of the Model—The final crystallographic R-factor for APC-PY model is 19.3%, in the range of 10.0 Å to 2.2 Å. The Luzzati plot gives a mean positional error of 0.26 Å (20). The r.m.s. deviations of bond lengths and bond angles are 0.015 Å and 2.9°, respectively. The quality of the final model is summarized in Table II. The Ramachandran plot shows that all dihedral angles fall into most favored or allowed regions with the only exception of $\beta77$Thr (Fig. 1) (21), which has a conserved unusual dihedral angle in all known phycobiliprotein structures. In APC-PY, the N atom of $\beta77$Thr forms a hydrogen bond with OD (the oxygen atom in the ring D of chromophore).

![Fig. 1. Ramachandran plot of the APC-PY residues. Glycine residues are marked as squares. Nonglycine residues are marked as crosses. $\beta77$ (277) is in an unusual region. PSI and PHI are dihedral angles $\psi$ and $\phi$.](image)

![Fig. 2. Omit electron density map of residues $\beta35$Glu and $\beta36$Leu in APC-PY.](image)
oxygen atom of α84PCB. The electron density of this residue is well defined in APC-PY. The consensus amino acid sequence (Table I) was used to build the initial model and later modified according to the electron density map. In the 2.3-Å-resolution crystal structure of APC-SP (9), 28 residues were not well defined with 102 atoms of zero occupancy; these residues are α25 Asp, α35Glu, α36Arg, α49Glu, α50Arg, α53Lys, α54 Gln, α76 Tyr, α79 Asp, α120Lys, α127Glu, β2 Gln, β10Asn, β17Lys, β20 Asp, β25 Gln, β35Glu, β36Leu, β39Arg, β50Asn, β58Lys, β65 Asp, β68Arg, β116Lys, β117Glu, β131 Gln, β138Glu, β150Lys. The fit of these residues to our electron density map is better in APC-PY; most of them behave well at 1σ density level (Fig. 2); others behave well at 0.7σ density level except α76 in the loop, which has a density at 0.5σ density level.

Comparing the crystallographic sequences of the final model of APC-PY and APC-SP, there are 37 nonidentical residues, 25 in the α subunit and 12 in the β subunit (Table III). In comparison with other APC sequences, the 37 residues of APC-PY are more conserved than those of APC-SP. For example, α52Val and α61Gln of APC-PY are identical to other known sequences. The electron density of these two residues in APC-PY are well defined.

**Molecular Structure**—The asymmetric unit of APC-PY contains α and β subunits. The α subunit is composed of 160 residues, and the β subunit contains 161 residues. Three αβ monomers are arranged around a 3-fold axis to form a disc shaped (αβ)3 trimer of 30 Å in thickness and 110 Å in diameter with a cave in the center. The α and β subunits in the αβ monomer have similar structures, with nine α-helices (X, Y, A, B, E, F, F', G, H) separated by irregular loops (Fig. 3).

The three-dimensional structure of APC-PY α and β subunits are very similar to the known structure of APC-SP. The inter-subunit interactions within the (αβ) monomer and the (αβ)3 trimer are also very similar to these of APC-SP and other phycobiliproteins. In the (αβ) monomer of APC-PY, the ionic- and polar-interacting residues between the two subunits are α3 Ser-β3 Asp, α13 Asp-β94 Tyr, α13 Asp-β110Arg, α17Arg-β97 Tyr, α18 Tyr-β93Arg, β13 Asp-α93Arg, β13 Asp-α97 Tyr, β18 Tyr-α89 Asp.

In the APC-PY crystal, two trimers associate face to face into the (αβ)6 hexamer through a crystallographic dyad perpendicular to the triad. There are three (αβ)6 hexamers in a unit cell, located at (0,0,0), (2/3, 1/3, 1/3), and (1/3, 2/3, 2/3) (Fig. 4a). The assembly of two trimers in this hexamer is completely different from that of APC-SP. In APC-SP crystal, the two trimers are associated loosely through β subunits in a “back to back” manner (Fig. 4b) in the hexamer (9), but in APC-PY crystal, the two trimers in the hexamer contact through α subunits, and the assembly of the two trimers is much tighter than that in APC-SP.
The assembly of the hexamer in the APC-PY crystal is similar to that of C-PC from *Fremylla diplosiphon* (C-PC-FR) and R-PE from *Polysiphonia urceolata* (R-PE-PU) hexamers; two \((\alpha\beta)_3\) trimers associate face to face in the hexamer. The \(\alpha\) subunits provide the contacting surface, and the two trimers fit complementarily in the hexamer.

Despite the similarity in assembly in APC-PY, C-PC-FR, and R-PE-PU hexamers, the superposition of the \(C_\alpha\) atoms of APC-PY and C-PC-FR hexamers shows that the assembly of the two trimers in APC-PY hexamer is obviously looser than that in C-PC-FR and R-PE-PU hexamers. The calculated accessible areas between the trimers in C-PC-FR and R-PE-PU hexamers are about 5900 and 6900 Å\(^2\). In APC-PY hexamer, this value is about 3200 Å\(^2\), which is much bigger than that in APC-SP (600 Å\(^2\)); thus, the APC-PY hexamer can be described as a "loose hexamer." The interactions between the trimers in APC-PY hexamer are different from those in C-PC-FR and R-PE-PU hexamers.

First, the number of the residues involved in the interactions between the two trimers in APC-PY hexamer is smaller than that in C-PC-FR and R-PE-PU hexamers, indicating a weaker association. This is consistent with the calculated accessible areas between the two trimers in C-PC-FR, R-PE-PU, and APC-PY hexamers. The special polar network present in C-PC of *Agmenellum quadruplicatum* (C-PC-AQ), formed by residues 1\(\beta\)46 Asn-6\(\alpha\)164 Asn-1\(\alpha\)21 Asn-6\(\alpha\)161 Glu-6\(\alpha\)33 Glu-6\(\alpha\)30 Arg (6) is not conserved in APC-PY hexamer. In addition, the electrostatic interactions between 1\(\alpha\)2Lys-6\(\alpha\)23Glu, 1\(\alpha\)17Arg-6\(\alpha\)108 Asp, and 1\(\alpha\)120Arg-4\(\alpha\)174 C-terminal carboxyl group, which were suggested to be involved in the hexamer formation in C-PC-FR (22), are also not present in APC-PY hexamer. Furthermore, the comparison of APC-PY with C-PC-FR and R-PE-PU reveals that all the conserved polar and ionic inter-
actions between the two trimers in C-PC-FR and R-PE-PU hexamers are not present in APC-PY hexamer.

Despite the above difference, the interactions that maintain APC-PY as a loose hexamer seem still to be the polar and charged interactions between the two trimers. In APC-PY hexamer, the polar and charged interactions are 1α25 Asp-6α37Arg, 1α22 Gly-6α26Arg, 1α25 Asp-6α161Glu, 1α25 Asp-6α165 Tyr, and 1α28Lys-6α147 Asp. In APC-SP, only a few polar and charged interactions (<4 Å) exist between the two trimers, such as 1β65 Asp-6β131 Gln and 1β120Asn–6β120Asn, indicating a very loose packing.

Second, in C-PC-FR and R-PE-PU hexamers, the trimer-trimer association is mediated almost exclusively by polar and charged residues (6), but in the APC-PY hexamer, some hydrophobic residues are also involved, such as α21 Pro, α22 Gly, α104 Val, α164 Phe, β42 Ala, and β46 Ala.

Chromophores α84PCB and β84PCB—In APC, two phycocyanobilins are covalently bound to cysteine residues at position α84 and β84 (Fig. 5). Both chromophores are well defined in APC-PY (Fig. 6). The geometry and protein environment of these two chromophores resemble those of APC-SP. The α84 PCB chromophores have a protein environment similar to that of β84PCB. The polar and ionic protein-chromophore interactions in α84PCB and β84PCB are shown in Table IV.

Chromophores α84PCB and β84PCB have similar hydrophobic environment; there are three aromatic residues close to α84, such as α90 Tyr, α91 Tyr, and α119 Tyr, and three close to β84, such as β90 Tyr, β91 Tyr, and β119 Tyr. In C-PC-FR, α90 and α91 are all Tyr, and β90 and β91 are all Ile. In R-PE-PU, α90 and α91 are His and Tyr, respectively, and β90 and β91 are all Ile. But in all known APCs, α90, α91, β90, and β91 are all Tyr. So the microenvironment of α84 and β84 in APC-PY is similar to that in C-PC-FR and R-PE-PU, indicating that α84PCB and β84PCB have similar conformation and spectrum character.

Table IV

| Chromophore | α84 PCB for APC-PY | β84 PCB for APC-PY |
|-------------|-------------------|-------------------|
| OA          | 3.8 OD1 1α72 Asn  | 3.8 OD1 1β72 Asn  |
| NA          | 3.3 OD1 1α72 Asn  | 3.3 OD1 1β72 Asn  |
| NB          | 2.9 OD2 1α87 Asp  | 2.9 OD2 1β87 Asp  |
| NC          | 3.0 OD1 1α87 Asp  | 3.0 OD1 1β87 Asp  |
| NC          | 2.8 OD2 1α87 Asp  | 2.8 OD2 1β87 Asp  |
| O2B         | 3.4 NH2 1α86 Arg  | 3.4 NH2 1β86 Arg  |
| O1C         | 3.2 O2 2β87 Tyr   | 3.2 O2 2β87 Thr   |
| O2C         | 3.3 O1G 2β87 Thr  | 3.3 O1G 2β87 Thr  |
| OD          | 3.2 N 2β87 Thr    | 3.3 NH2 1β86 Arg  |

β90 Tyr and β91 Tyr stabilize the β84PCB ring D conformation, which may make PCB have different spectrum characteristics in APC-PY and C-PC.

Energy Transfer—There are 12 PCBs in APC-PY hexamer; the arrangement of these chromophores is shown in Fig. 7. The theory of shot-distance exciton interaction (23) and long distance dipole-dipole resonance mechanism (24) has been used to explain the energy transfer rate between chromophores.

Inside trimer of APC-PY, the distance between 1β84PCB and 2β84PCB in APC-PY is about 34 Å and that between 1α84PCB and 2β84PCB is about 21 Å. These values are similar to those in C-PCs. The chromophores are too far away to have exciton interaction. It is also difficult to explain why exciton interaction exists in APC but not in C-PC. Our study of chromophore-protein interactions and comparison of microenvironments in R-PE-PU, C-PC-FR, and APC-PY show that almost all the chromophore-protein interactions exist within the same monomer (αβ), the only exception being α84PEB in R-PE-PU, which
forms a hydrogen bond with \( \beta 77 \) Thr in another monomer. In C-PC-FR, the situation is the same as in R-PE-PU. However, it is different in APC-PY; its \( \alpha 84\)PCB forms five hydrogen bonds with the residues in other monomer, such as \( \alpha 84\)PCB 2β62 Tyr OH, O1C-2β62 Tyr N, O1C-2β67 Thr OG1, O2C-2β67 Thr OG1, and OD-2β77 Thr N (see Table IV). We believe this difference may explain why the spectrum of APC changes greatly when its monomers associate to trimer. In APC-SP, distances of \( \alpha 84\)PCB 2β62 Tyr OH, O1C-2β62 Tyr N, O1C-2β67 Thr OG1, O2C-2β67 Thr OG1, and OD-2β77 Thr N are all within the distance of hydrogen bond formation. As we know, \( \beta 62 \) Tyr and \( \beta 67 \) Thr are close to chromophore \( \beta 84\)PCB and may control the conformation of chromophore and bridge between \( \alpha 84 \) PC and \( \beta 84 \) PCB to make the exciton interaction occur.

The distances of chromophores between the two trimers in APC-PY hexamer are similar to those in C-PC-FR and R-PE-PU hexamers. Based on the 1.9-Å resolution crystal structure, the possible pathway of energy transfer within and between the two trimers of R-PE-PU were discussed (25). There are three pairs of short distance interactions between two trimers. Similar energy pathways (1a84PCB → \( \alpha 84\)PCB) also exists in C-PC-FR hexamers (26). In C-PC-FR, R-PE-PU, and APC-PY hexamers, the distances between 1β64 (C10 atom) and 4β64 (C10 atom) are 27.5, 28.7, and 30.3 Å, respectively; which are comparable. Therefore, the distance between the two chromophores of APC-PY seems adequate for effective energy transfer.

In addition to the energy pathway composed of chromophores, the aromatic pathway formed by aromatic residues may play an important role in energy transfer. The energy transfer from chromophores to aromatic residues vice versa can be explained by exciton interaction mechanism, because the distances between some chromophores and aromatic residues, such as \( \alpha 84\)PCB-\( \alpha 90 \) Tyr, \( \alpha 84\)PCB-\( \beta 91 \) Tyr, \( \beta 84\)PCB-\( \beta 90 \) Tyr, \( \beta 84\)PCB-\( \beta 91 \) Tyr are very short (~4 Å). Förster's dipole-dipole resonance transfer can occur between different aromatic residues rather than between chromophores and aromatic residues, because the overlap integral between chromophore absorption (\( \lambda _{\text{max}} \approx 650 \) nm for fluorescence spectrum) and aromatic residue emission (\( \lambda _{\text{max}} \approx 300 \) nm for fluorescence spectrum) is quite small. In APC-PY there are two areas abundant in aromatic residues as shown in Fig. 8. One is close to chromophore \( \alpha 84\)PCB and composed of \( \alpha 164 \) Phe, \( \alpha 165 \) Tyr, \( \alpha 166 \) Phe, \( \alpha 168 \) Tyr, \( \alpha 90 \) Tyr, \( \alpha 91 \) Tyr, \( \alpha 97 \) Tyr, \( \alpha 119 \) Tyr, and \( \beta 18 \) Tyr. Another is close to chromophore \( \beta 84 \) and composed of \( \beta 165 \) Tyr, \( \beta 166 \) Phe, \( \beta 168 \) Tyr, \( \beta 90 \) Tyr, \( \beta 91 \) Tyr, \( \beta 94 \) Tyr, \( \beta 97 \) Tyr, \( \beta 119 \) Tyr, and \( \alpha 18 \) Tyr. \( \alpha 164 \) Phe is involved in the hydrophobic interactions between the two trimers. The aromatic residues in the \( \alpha \) subunit and the \( \beta \) subunit have high homology and similar locations. Other aromatic residues are on the periphery of the disc, such as \( \alpha 76 \) Tyr, \( \alpha 60 \) Phe, \( \beta 76 \) Tyr, \( \beta 62 \) Tyr, \( \beta 30 \) Tyr, \( \beta 31 \) Phe, \( \beta 30 \) Phe, and \( \beta 81 \) Tyr; among them \( \alpha 76 \) Tyr and \( \beta 62 \) Tyr may mediate the energy transfer between \( \alpha 84\)PCB and \( \beta 84\)PCB. Because PC and APC are connected perpendicularly in vivo, aromatic residues on the periphery may mediate the energy transfer from the chromophores of PC to those of APC.

**Functional Unit**—In the core cylinders of phycobilisomes, several APC trimers are close together, but the association manner of these APC trimers is still unknown. Based on dissociation experiments, it was suggested that allophycocyanin does not form hexamers (27), because almost all the residues involved in the trimer-trimer aggregation in C-PC-AQ and C-PC-FR hexamers are not conserved in APC. Similar conclusions were reported later (6, 9). In the APC-PY hexamer, all the interactions involved in the formation of C-PC-AQ and C-PC-FR hexamers and all the conserved polar and charged interactions in C-PC-FR and R-PE-PU hexamers are not present, but APC-PY can still associate face to face to form a hexamer, which is maintained by some polar and charged in-
interactions, different from those in C-PC-FR and R-PE-PU. Because the distances of chromophores between the two trimers in this hexamer are also adequate for effective energy transfer, we assume that the loose hexamer may represent the basic unit of APC in physiological conditions. It is possible that linker proteins may help to stabilize the loose hexamers.

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