Crystal Structure of C-Phycocyanin from *Cyanidium caldarium* Provides a New Perspective on Phycobilisome Assembly

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ABSTRACT The crystal structure of the light-harvesting protein phycocyanin from the cyanobacterium *Cyanidium caldarium* with novel crystal packing has been solved at 1.65-Å resolution. The structure has been refined to an *R* value of 18.3% with excellent backbone and side-chain stereochemical parameters. In crystals of phycocyanin used in this study, the hexamers are offset rather than aligned as in other phycocyanins that have been crystallized to date. Analysis of this crystal's unique packing leads to a proposal for phycobilisome assembly in vivo and for a more prominent role for chromophore β-155. This new role assigned to chromophore β-155 in phycocyanin sheds light on the numerical relationships among and function of external chromophores found in phycoerythrins and phycoerythrocyanins.

INTRODUCTION

Phycobiliproteins are bile pigment-apoprotein complexes that constitute the major light-harvesting antennae for oxygenic photosynthesis in cyanobacteria and red algae (Gantt, 1990; Glazer, 1989; MacColl and Guard-Friar, 1987). Allophycocyanin, phycocyanin, phycoerythrin, and phycoerythrocyanin, the principal phycobiliproteins, consist of α- and β-subunit polypeptides to which one or more linear tetrapyrrole chromophores are covalently attached (Bryant, 1991; Glazer, 1985; Troxler, 1994). Phycocyanobilin (PCB) is the chromophore of allophycocyanin and phycocyanin. In both proteins the chromophores are attached by cysteinyl thioether linkages at α-84 and β-84 positions; in phycocyanin a second PCB is found attached to cysteine at β-155 in a loop not found in allophycocyanin.

The α- and β-subunits of phycobiliproteins exhibit a high affinity for one another and associate into $\alpha\beta$ monomers, which in turn aggregate into $(\alpha\beta)_3$ trimers and $(\alpha\beta)_6$ hexamers. In vivo, hexameric phycobiliproteins together with linker proteins self-assemble into macromolecular light-harvesting complexes (called *phycobilisomes*) occurring in vivo on the stroma surface of the thylakoid membranes (Bryant, 1991; Gantt, 1990; Glazer, 1989; Glazer and Melis, 1987). Phycobilisomes have two components: 1) the core, usually comprising two or three rods built from two spectroscopically distinct allophycocyanins, which are in contact with the thylakoid membrane, and 2) the rods, usually six, comprising two or more phycocyanin hexamers that radiate out from the core into the stroma space of the thylakoid membrane. In some species, rods also contain two or more hexamers of phycoerythrin or phycoerythrocyanin, which are located distally to phycocyanin hexamers. Core hexamers as well the external rods are joined together by an array of colorless linker proteins.

Phycobilisomes are very effective energy transducers (Porter et al., 1978), transferring the absorbed energy from sunlight to the photosystem II within the thylakoid membrane with more than 90% efficiency (Gantt, 1990). An understanding of the physical processes leading to such effective energy transfer would greatly enhance our knowledge of the energy transfer mechanism in photosynthesis.

Although crystals of phycobiliproteins have been available for some time (Dobler et al., 1972; Fisher et al., 1980; Hackert et al., 1977), the first structure of a phycobilisome protein was that of C-phycocyanin from *Mastigocladus laminosus* (Schirmer et al., 1985). Other hexameric phycobiliprotein structures solved since include C-phycocyanin from *Agmenellum quadruplicatum* (Schirmer et al., 1986), phycoerythrocyanin from *Mastigocladus laminosus* (Duerring et al., 1990), C-phycocyanin from *Fremyella diplosiphon* (Duerring et al., 1991), B-phycoerythrin from *Porphyridium sordidum* (Ficner et al., 1992), and allophycocyanin from *Spirulina platensis* (Brejc et al., 1995). All are remarkably similar.

Generally phycobiliprotein $\alpha\beta$-monomers are organized into crystallographic trimers (Fig. 1), two of which come together to form a face-to-face hexamer with approximate D_3 symmetry. In phycocyanin, two chromophores at positions α-84 and β-84 are found toward the center of trimeric discs in regions that have structural similarity to the heme binding pocket in the globin family of proteins (Pastore and Lesk, 1990; Schirmer et al., 1985), whereas the chromophore β-155 is located in a short loop unique to the β-subunit of phycocyanin and is close to the edge of the trimeric disc.

Crystals of the C-phycocyanin from unicellular rhodophyte, *Cyanidium caldarium,* have an unusual feature: the crystal packing is different from that found in other phycocyanins. The protein molecules are arranged in layers rather than in columns. Because of the unique crystal packing, the spatial orientation of chromophores (particularly β-155) is

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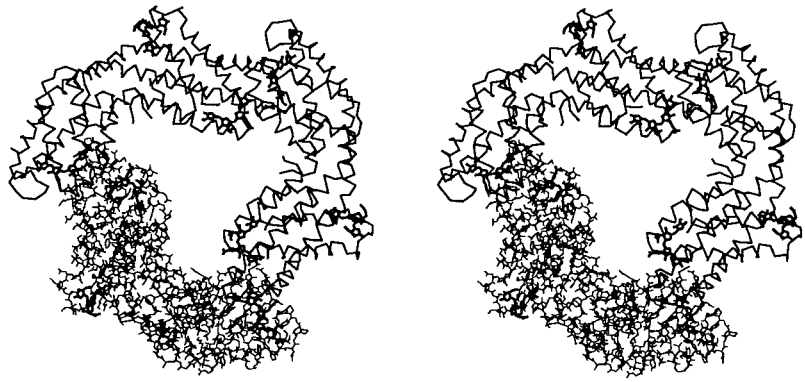

FIGURE 1 Stereoview of the trimer of $\alpha\beta$-monomers. Two of the monomers are in Cα representation, and third monomer is in all-atom bond-stick representation. The chromophores are depicted by bold lines. Note that the β-subunit has two chromophores.

different, and this raises the possibility that β-155 has a more important role in energy transfer between adjacent hexamers than commonly believed. The significance of the possible involvement of the β-155 adduct for modes of energy transfer between the hexamers in other phycobiliproteins and for phycobilisome rods in vivo is discussed below.

MATERIALS AND METHODS

Protein purification and crystallization

Phycobilisomes from *C. caldarium* contain allophycocyanin (plus linker proteins) in the core and phycocyanin as the only colored protein in the rods. The phycocyanin was purified as described earlier (Offner et al., 1981; Troxler et al., 1981) and concentrated to 15 mg/ml. The purity was measured by the ratio of absorbance at 620 nm (chromophore) and 280 nm (protein) and, in protein samples used for crystallization, was more than 8. The crystals were grown by the sitting drop vapor diffusion method from 50-μl droplets of 15 mg/ml protein in 50 mM phosphate buffer (pH 7.0) and 0.45 M ammonium sulfate with 0.02% sodium azide equilibrated against the 1.0 M ammonium sulfate with buffer in the mother liquor. The protein crystallized into the rhombohedral R3 or R32 space group with cell dimensions of $a = b = 106.42$ Å, $c = 176.18$ Å, and $\gamma = 120°$ for the hexagonal cell. The initial precession photograph and Wilson statistics analysis were inconclusive about the space group assignment. Therefore the data were collected in the R3 symmetry.

Data collection and structure solution

A data set to 1.65-Å resolution was collected with two crystals (both $0.5 \times 0.5 \times 0.5$ mm in size) on a Hamlin area detector system (Table 1). About 78,000 unique reflections with overall $R_{merge} = 8.9\%$ gave an 90.3% complete data set to 1.65-Å resolution. The completeness in the last high-resolution shell of data (1.68–1.65 Å) was 65%. The structure was solved by the molecular replacement method in the R3 space group. A dimer of $\alpha\beta$-monomers from *Fremyella diplosiphon* (Duerring et al., 1991) was retrieved from the PDB (Bernstein et al., 1977) and used as a trial structure (PDB code 1cpc). The crystallographic *c* axis constitutes the threefold symmetry axis in each crystal. A rotational search around the crystallographic *c* axis with *F. diplosiphon* coordinates resulted in one clearly correct position with a significantly lower *R* factor (40.5%), which showed a rotation of 25° from the *F. diplosiphon* hexamer position and was chosen as a starting solution for the refinement. This position coincided with the twofold in the R32 space group. Choice of the R32 space group was confirmed by a Patterson self-rotation search (data not shown).

Refinement

The model of phycocyanin from *C. caldarium* was created by graphically "mutating" all nonidentical amino acids of *F. diplosiphon* structure, using the program CHAIN (Sack, 1988). Conformations of the "mutated" residues were adjusted to avoid interpenetrations. The refinement as well as molecular replacement were carried out using program X-PLOR 3.1. Several slow-cool cycles of refinement combined with the manual modeling after each cycle were necessary to obtain convergence. Each slow-cool cycle contained 50 cycles of Powell minimization, molecular dynamics run at 2000 K for 1 ps, and a slow cooling cycle (30 K every 50 steps), followed by 100 cycles of Powell minimization and individual temperature factor refinement. Refinement was initiated in the R3 space group, using the program X-PLOR (Brunger, 1992) as well as in the R32 space group. After three heat-cool cycles of refinement in the resolution range 10.0–1.65 Å followed by manual rebuilding, the *R* factor converged to 19.5% in the R3 space group and with $R = 18.3\%$ in the R32 space group. The model refined in the R32 space group had very good stereochemistry (RMS on bonds 0.009 Å and on angles 1.8°). Differences between the models were negligible; therefore, only the model refined in the R32 space group is described. The refined model has been deposited to the PDB (code 1phn).

Energy transfer calculations

Theory by Förster (1967) provides the background for calculating the energy transfer rates in a weakly coupled system of chromophores by an isolated dipole approximation. Assuming the validity of the theory and

TABLE 1 Data collection and the refinement statistics

Refinement statistics	
R_{merge}	8.9%
Resolution range of refinement	10.0–1.65Å
Number of reflections: data collection	44677
(after resolution and F > 2σ(F) cutoff)	43637
Completeness, data col. (refinement)	90.3% (88.4%)
Redundancy	3.3
Final *R* factor (R_{free})	18.4% (27.2%)
RMS deviations	
Bond lengths (Å)	0.009
Bond angles	1.81
Dihedral angles	24.3
Improper angles	1.79
B factor (Å^2)	
Overall	12.3
α-subunit	8.9
β-subunit	15.6

neglecting the spectral differences between the chromophores, we have calculated the energy transfer rates for the *C. caldarium* phycocyanin crystals according to the simplified Förster formula:

$$k_{et} = \kappa^2/\tau_o(R_o/r)^6$$

where k_{et} is the energy transfer rate, κ^2 ($\kappa = e_i e_j - 3(e_i r_{ij})(e_j r_{ij})$) is the orientation factor of the chromophore with reference to its transition dipole moment, τ_o is the excitation lifetime of the phycocyanobilin (~1.9 ns), R_o is the Förster radius (50 Å), and r is the distance between the chromophores.

RESULTS AND DISCUSSION

Description and quality of the structure

The sequence similarity between the phycocyanins from *C. caldarium* and from *F. diplosiphon* is high: 72% identity for the α-subunit and 76% identity for the β-subunit. The changes are observed mostly on the surface of the molecules, and most of them are highly conservative (Asp-Glu, Ser-Thr, Arg-Lys). There is one exception, however. There is a patch of hydrophobic residues at the interface between the subunits comprising residues 9, 5, 24, and 27 from the α-subunit and residues 38, 100, and 106 from the β-subunit. These residues are homologous but different in size between these two phycocyanins. These substitutions may be responsible for slightly different packing of $\alpha\beta$-subunits and the different crystal packing.

The high sequence homology suggests that the corresponding three-dimensional structures should be very similar. Indeed, the RMS difference between Cαs of the starting model and of the refined structure is only 0.76 Å compared to the positional errors estimated by the Luzzati method (Luzzati, 1952) of 0.2 Å. This shows the strong structural homology to the *F. diplosiphon* phycocyanin. Nevertheless, the different crystal packing suggests that there are crucial differences between the structures. Below, we first describe the fold of the protein, then elucidate the common features of the models, and finally focus on the differences.

The chain fold for *C. caldarium* phycocyanin (Fig. 1) is mostly α-helical and, as indicated above, is similar to that of *F. diplosiphon* and other phycobiliproteins (Duerring et al., 1990; Ficner et al., 1992). Both α- and β-subunits form well-defined, helical globin-like domains with six helices folded into an arrangement similar to that of myoglobin, with PCB chromophores α- and β-84 (tetrapyrroles) located at sites corresponding to the heme group in myoglobin. The globin domains are complemented with the two additional helices (X, Y) (Schirmer et al., 1985), which are responsible for the formation and stability of $\alpha\beta$-monomers. Helices Y, A, and B can be considered as one bent helix. In the *C. caldarium* structure this helix has a slightly different bending angle. As a result, α- and β-subunits are pinched closer to each other, and the tips of the most distant loops are closer by ~1.5 Å (Cα-121α–Cα-121β).

In the *C. caldarium* phycocyanin, the globin domains are also distorted slightly and show a roll away from the threefold axis by ~1.5°. This causes an increased ruggedness of the surface of the hexamer. The hexamer, as refined in R32 space group, has a lower and upper trimer of $\alpha\beta$-monomers related by the crystallographic twofold axis. We can speculate that the above-mentioned hydrophobic patch at the trimer-trimer interface may cause these global conformational changes.

The backbone conformation is well within allowed regions on the Ramachandran plot (Ramachandran et al., 1963), with the exception of β-Thr^{77}. The unusual conformation of this residue, which is located on the loop enveloping chromophore β-84, is caused by the close contact with the chromophore α-84 of the neighboring monomer. The backbone conformation of this residue in the *C. caldarium* phycocyanin structure ($\Phi = 86.7°$, $\Psi = 153.3°$) agrees well with previous findings (Duerring et al., 1991).

Methylation of β-Asn^{72} was also detected as before (Duerring et al., 1991; Klotz et al., 1986). Asn^{72} was initially sequenced as Thr (Offner et al., 1981); later this misassignment was corrected when the DNA sequence became available (RFT, unpublished data). The side chain of Asn^{72} provides a direct link of the chromophore β-84 to solvent. It has been proposed that this conserved methylation serves a functional role (Swanson and Glazer, 1990) by providing a better separation of the chromophore from the solvent. One can easily speculate that the electronic properties of the β-84 chromophore would have been different without the

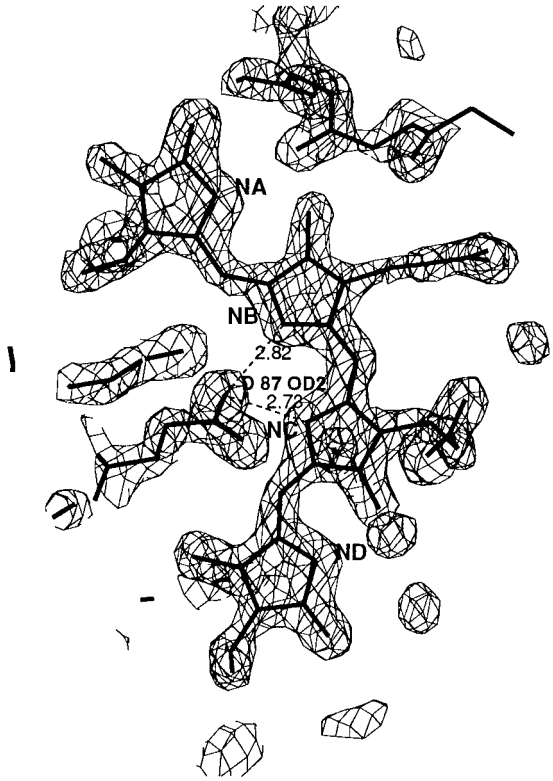

FIGURE 2 The chromophore-phycocyanobilin (PCB) α-84 covered with 2Fo-Fc electron density contoured on the 2.5σ level. In the figure, the nitrogen atoms NA, NB, NC, ND are labeled. Crucial hydrogen bonds from the central nitrogens of the B and C pyrrole rings to Asp^{87} are also shown.

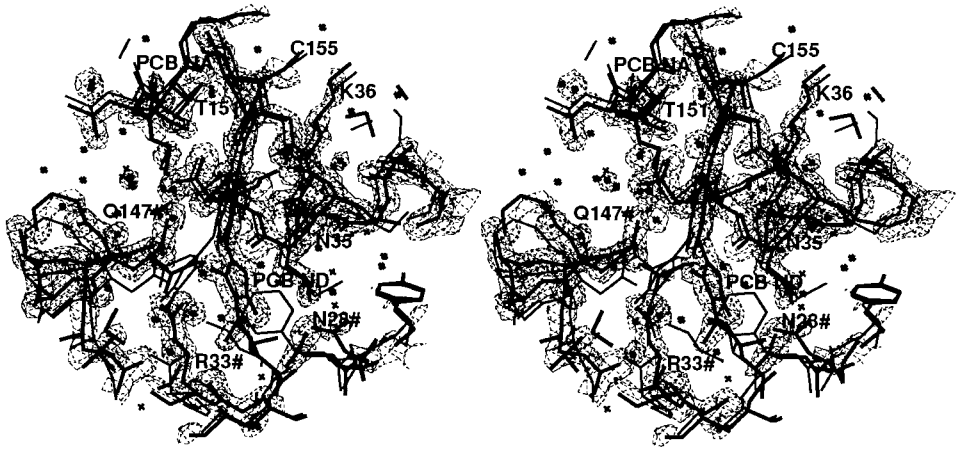

FIGURE 3 The comparison of the chromophores β-155 of *F. diplosiphon* and *C. caldarium.* The 2Fo-Fc electron density contoured on the 2σ level covers the chromophore and its immediate surroundings. Please note the change in conformation of the ring D caused by amino acid substitutions at the lower trimer. The symbol # denotes the symmetry-related trimer.

methylation, which consequently would lead to a less efficient chromophore.

The electron density is of sufficiently high quality to locate every nonhydrogen atom (Figs. 2 and 3). This high quality electron density allowed us to model more than 500 water molecules, some deep in the protein interior, using the difference electron density maps. The criterion adopted was that the water molecule was incorporated into the model if the electron difference density was above 2.4σ. Electron density is weak and disconnected only for residues 115–123 in the β-subunit. This sequence contains the single free cysteine in the structure. The backbone temperature factors in this region were above 40 Å^2, whereas elsewhere they were below 20 Å^2.

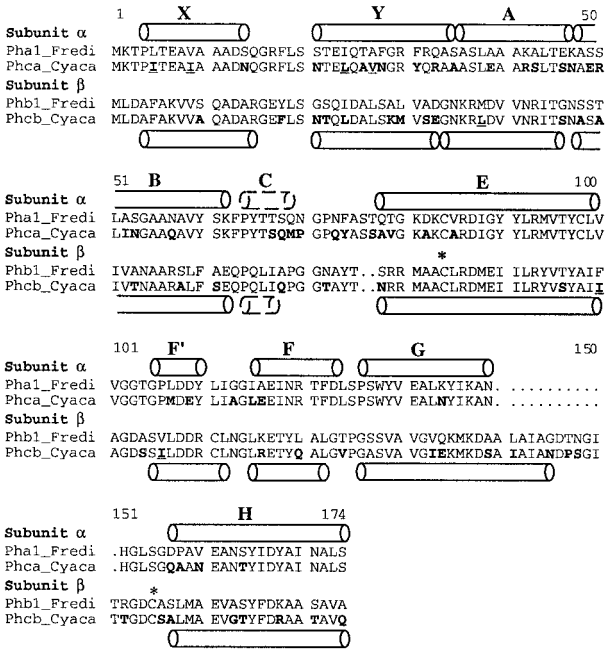

FIGURE 4 The alignment of the amino acid sequences for the *F. diplosiphon* and *C. caldarium* cyanobacteria. The identities of the sequences are 72% for the α-subunit and 76% for the β-subunit. The nonidentical residues are presented in bold. Above and below the sequences the secondary structure elements are marked by cylinders and the cysteinyl attachments for the chromophores by an asterix. Hydrophobic residue differences in X and Y helices are underlined.

Chromophores

The chromophores α- and β-84 (tetrapyrroles) are located in positions similar to that of the heme group in myoglobin. The additional chromophore β-155 is attached to the short loop that is not present in the α-subunit. All chromophores are covalently attached to the protein by the thioether bonds. These bonds have the same stereochemistry as those described for *F. diplosiphon*, i.e., the stereoisomers for α-84 and β-84 are R, whereas for β-155 the stereoisomer is S. The chromophores α-84 and β-155 in *C. caldarium* phycocyanin structure are very well defined and have temperature factors below 10 Å^2 (Figs. 2 and 3). Although the electron density of chromophore β-84 gets weaker as it approaches the ill-defined region of β-115, every single atom can be placed unequivocally. All three chromophores α-84, β-155, and β-84 display the Z configuration on the D ring, as is also found in the phycocyanin from *F. diplosiphon* (Duerring et al., 1991).

The α-84 chromophores from *C. caldarium* and *F. diplosiphon* superimpose exactly. This result might be attributed to the high homology of the immediate protein surroundings (Fig. 4). Most of the neighborhood of chromophore β-84 is also highly conserved, but the location of the chromophore reflects the change in conformation of the unwound helical region 105–115 of the β-subunit. The D ring of the chromophore is pushed ~1.5 Å deeper into the protein's cavity as compared to *F. diplosiphon*. The surrounding of chromophore β-155 is much less conserved and exhibits notable structural changes. The residues Asn^{28}, Arg^{33}, Gln^{147}, and Asn^{150} in *C. caldarium* are replaced by Phe, Gln, Asp, and Val, correspondingly, in *F. diplosiphon*. This causes a different packing arrangement at the interface between the trimers and the significant rotation of the D ring. Two dihedral angles between the C and D rings change by ~75° and ~20° (Fig. 3).

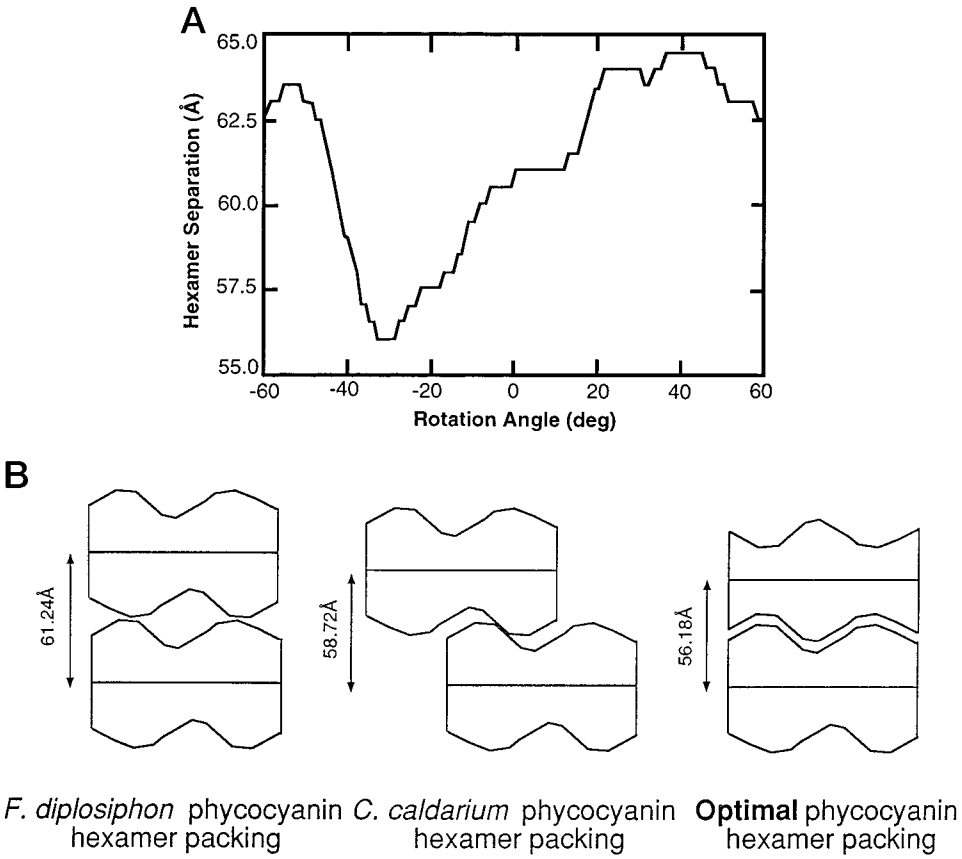

FIGURE 5 Rotation of hexamers from modeling and from crystal packing. (*A*) Plot of the distances between optimally packed hexamers versus rotation angle around the axis of the phycobilisome rod. The minimum (d = 56.18 Å) is located 30° from the position in crystals of *F. diplosiphon* phycocyanin, where hexamers are on top of each other. (*B*) Comparison of hexamer packing in *F. diplosiphon* crystals, in *C. caldarium* crystals, and in optimally packed rods.

Crystal contacts

Although *F. diplosiphon* and *C. caldarium* crystallized in similar rhombohedral space groups (R3 and R32, respectively), there is a profound difference in the lattice dimensions and cell organization. In crystals of phycocyanin from *C. caldarium* the hexamers are offset, whereas in *F. diplosiphon* crystals they are organized in columns with a crystallographic translational repeat of 61.24 Å along the *c* axis. The hexamers in *C. caldarium* crystals have unique stacking contacts along the body diagonal. This offset stacking along the cell diagonal allows the hexamers to pack over one another more tightly than in *F. diplosiphon* because the hexamers are effectively rotated relative to one another. The tighter packing is apparent from the smaller average hexamer-hexamer distance along the *c* axis in *C. caldarium* crystals of 58.72 Å (176.18/3), as compared to 61.24 Å for *F. diplosiphon*. The tight packing of hexamers in *C. caldarium* phycocyanin crystals results in crystals that are very

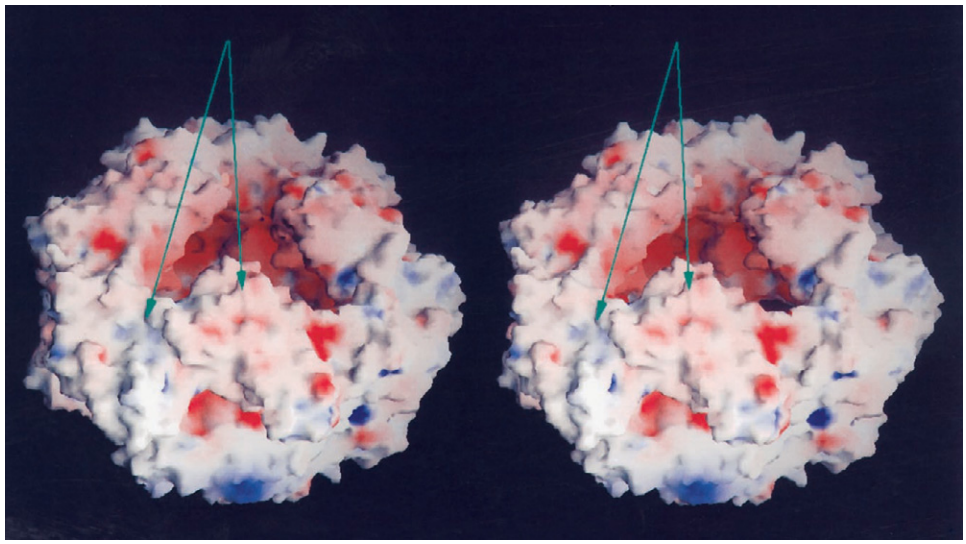

FIGURE 6 A stereo representation of the tilted hexamer with the electrostatic field projected onto the molecular surface. The picture was prepared with the program GRASP. Note a charge complementarity of two rotated hexamers, as indicated by the green arrows.

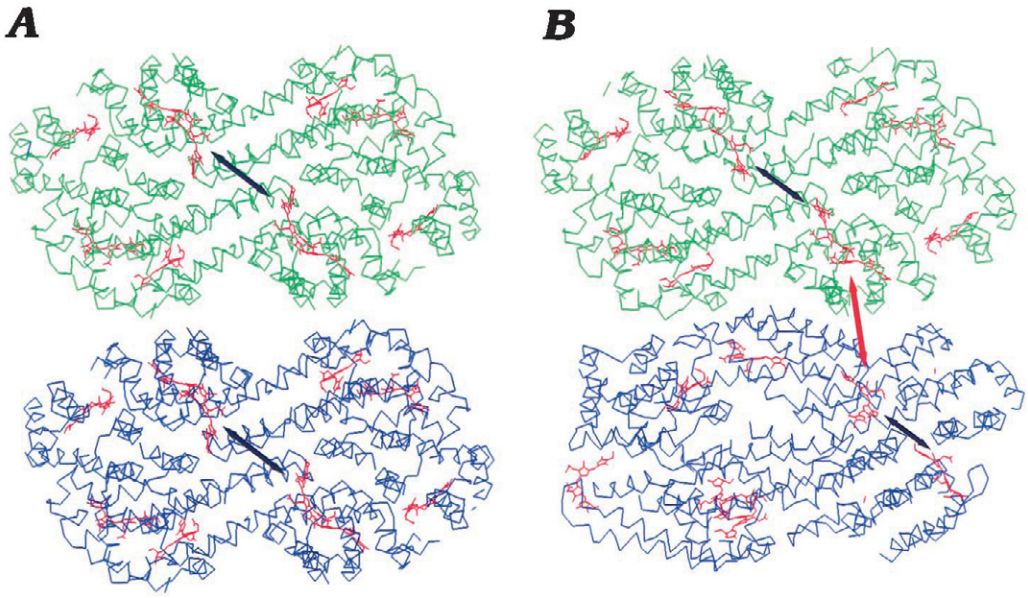

FIGURE 7 Color representation of the two proposed phycobilisome rod packings. The β-155 contacts within hexamers are shown by parallel black lines. In optimally packed hexamers (*B*), β-155 chromophores approach closely and are well aligned for energy transfer, indicated by the red arrow. The lower hexamer (in *blue*) is rotated between *A* and *B*. (*A*) Two hexamers of *F. diplosiphon* phycocyanin arranged into a rod with no rotation between them. This stacking is based on *F. diplosiphon* crystal packing of hexamers. (*B*) Two hexamers of *C. caldarium* phycocyanin arranged with hexamers rotated 30° from one another. Note the knobs-into-holes arrangement and the closer approach of the hexamers.

stable mechanically, which diffract well, even when heated above 50°C.

Modeling hexamers in the phycobilisome rods

Because in other phycocyanins that crystallized in rhombohedral space groups the hexamers are organized in columns due to the crystal translational repeat, it has been suggested (e.g., Schirmer et al., 1986) that the organization of hexamers in vivo is similar to what occurs in crystals. The exact translational repeat precludes the possibility that hexamers in columns could be rotated by a small angle (Fig. 5) to adjust the contacts. However, efficient packing of hexamers would be required in the native antenna to keep the exposed phycobilisome rods stable in the surrounding solvent.

To determine the optimal van der Waals packing of phycocyanin hexamers in columns (rods), a hexamer contact search was performed by rotating one hexamer on top of another in one degree steps over a 120° range, starting with exactly aligned hexamers as in the *F. diplosiphon*. The contacts were optimized at every step by shifting the top hexamer up and down so that the closest approach of the Cα in rotated hexamers would be within 5–5.5 Å. The result of this optimization is presented in Fig. 5 *A*. At the minimum, which was located ~30° from the initial position, the hexamers were separated by 56.18 Å.

The hexamers in *C. caldarium* have slightly more rugged surfaces than those of the phycocyanin from *F. diplosiphon*. This ruggedness is associated with the $\alpha\beta$ packing differences (see above). To evaluate the contribution of electrostatic energy to the stability of the rods composed of optimally packed, rotated hexamers, we calculated the electrostatic field and projected it onto the molecular surface (Fig. 6). The regions in contact in the rotated adjacent hexamers are marked by green bars. Visual inspection shows that these red (negative) and blue (positive) charged patches at the knobs and holes, respectively, on the hexamer surface would interact favorably (Fig. 6). This observation agrees also with the tentative conclusions of the electrostatic calculations carried out on the *F. diplosiphon* protein (Karshikov et al., 1991). Karshikov et al. find a shallow minimum. Although their global minimum in hexamer-hexamer rotation is 0°, the 30° rotated position is less than 1 kcal higher in energy. Note that for phycocyanin the dominant electrostatic field is negative (red), particularly in the center of the hexamers. This charge distribution may be important for efficient energy transfer and for strong binding of the linker proteins (Lundell et al., 1981).

We propose that the same structural feature of rotated hexamers that is used in the crystal lattice of *C. caldarium* phycocyanin is important for the native phycobilisome rods. The difference between the arrangement of hexamers packed into rods proposed by others (e.g., Schirmer et al., 1986) and that proposed here can clearly be seen in Fig. 7. Considerably more space is found between the hexamers in Fig. 7 *A*, where the hexamers are not rotated, as compared to Fig 7 *B*, where rotation to the optimal angle of 30° is used. The tighter packing of the rotated rods would be stabilized not only by van der Waals contacts, but by electrostatic interactions, as just noted. Furthermore, this rotated hexamer packing has implications for the impor-

tance of each chromophore in energy transfer along the rods.

Energy pathways

In phycobilisomes, energy is transferred along the rods to the core by the chromophores. Chromophores are divided into two spectroscopic classes (Teale and Dale, 1970). The s (sensitizing) class absorbs at the blue edge of the absorption band and transfers the excitation energy in a radiationless (resonant) manner to the f (fluorescing) chromophores. Excitation at the red absorption edge by the f-chromophores results in depolarization, which suggests that the energy is transferred in the rods along the line of f-chromophores.

Using Förster theory (Förster, 1967), Schirmer et al. (1986), as well as others (Sauer and Scheer, 1988), have calculated the energy transfer rates for the different systems of chromophores in different crystals and concluded that chromophore β-84 is a type f chromophore. Analysis of the chromophore distances in *C. caldarium* lattice suggests that chromophore β-155 should play a more significant role than that ascribed to it before.

The calculations discussed here are not intended to compete with more sophisticated methods such as those presented by Sauer and Scheer, (1988) but were meant to provide the background for the structural as well as functional comparisons. The levels of these calculations are nevertheless fully compatible with those presented by Schirmer et al. (1986) and Duerring et al. (1990, 1991). In fact, the orientation factors and transmission rates are very similar in all of those species, despite small structural differences. The values presented in Table 2 should be compared with those presented in figure 11 and table 9 of Duerring et al. (1991).

In Fig. 8, we compare the chromophore packing schemes in the crystal lattices of *F. diplosiphon* and *C. caldarium*. Table 2 gives the distances, orientation factors, and transition rates calculated from the Förster theory for the *C. caldarium* lattice. The β-84 chromophore distances are similar in the two lattices. A comparison indicates that the only significant difference is the closeness of chromophores α-84 and β-155 between translationally related molecules in the lattice (26.7 Å, 30.4 Å) (Fig. 8). These contacts are absent in the *F. diplosiphon* lattice.

TABLE 2 Relative distances r (Å), orientation factors κ, and Förster transfer rates k_{et} (1/ns) between the *C. caldarium* chromophores

A. Chromophores in a hexamer*

	$\alpha\beta$-Monomer		Upper trimer						Lower trimer								
	1β 84	1β 155	2α 84	2β 84	2β 155	3α 84	3β 84	3β 155	4α 84	4β 84	4β 155	5α 84	5β 84	5β 155	6α 84	6β 84	6β 155
1α -84																	
r	50.9	47.3	69.4	20.9	39.5	69.4	57.9	86.1	68.1	35.4	36.3	25.2	62.1	59.2	77.3	62.3	87.3
κ^b	1.87	0.56	1.31	−1.38	0.35	1.31	0.93	0.65	0.67	0.92	1.03	1.17	0.74	0.43	0.68	0.56	1.26
k_{et}	1.56	0.23	0.13	185.5	0.26	0.13	0.19	0.01	0.04	3.56	3.80	43.9	0.79	0.04	0.02	0.04	0.03
1β-84																	
r		33.8	57.9	37.7	67.6	20.9	37.7	48.6	35.4	50.9	50.2	62.1	54.9	73.2	62.3	37.7	46.7
κ^b		0.78	0.93	0.48	0.60	−1.38	0.48	−0.02	0.92	1.42	1.38	0.74	1.29	1.34	0.56	0.41	−0.44
k_{et}		3.37	0.19	0.67	0.03	185.5	0.67	0.00	3.56	0.95	0.98	0.08	0.50	0.09	0.04	0.48	0.14
1β-155																	
r			86.1	48.6	79.8	39.5	67.6	79.8	36.3	50.2	27.3	59.2	73.2	89.9	87.3	46.8	69.7
κ^b			0.65	−0.02	0.83	0.35	0.60	0.83	1.03	1.38	1.65	0.43	1.34	0.79	1.25	−0.43	0.09
k_{et}			0.01	0.00	0.02	0.26	0.03	0.02	3.81	0.98	54.4	0.04	0.09	0.01	0.03	0.15	0.001

B. Other selected chromophores (different hexamers) with close contacts (<40 Å) or substantial transfer rates (>1.0)

1α 84	15β 155	18β 155	35β 84	36α 84	1β 84	28β 84	29α 84	1β 155	8β 155	20α 84	24α 84	36β 155	41β 155
r	26.7	30.4	29.4	37.5	r	34.3	29.5	r	38.1	26.7	30.4	41.4	53.7
κ	−0.20	−0.99	−0.95	0.79	κ	1.73	−0.95	κ	1.21	−0.20	−0.99	1.45	1.94
k_{et}	0.91	10.40	11.35	1.83	k_{et}	15.01	11.35	k_{et}	4.00	0.91	10.4	3.41	1.29

*The symmetry related molecules were created by applying the symmetry operators according to the convention $1 = (x, y, z)$, $2 = (-y, x - y, z)$, $3 = (y - x, -x, z)$, $4 = (y, x, -z)$, $5 = (-x, y - x, -z)$, $6 = (x - y, -y, -z)$, $8 = -y - 1, x - y, z)$, $15 = (y - x, -x - 1, z)$, $18 = (x - y, -y - 1, -z)$, $20 = (-y - 1, x - y - 1, z)$, $24 = (x - y - 1, -y - 1, -z)$, $28 = (y - 1/3, x + 1/3, -z + 1/3)$, $29 = (-x - 1/3, y - x + 1/3, -z + 1/3)$, $35 = (-x - 1/3, y - x - 2/3, -z + 1/3)$, $36 = (x - y - 1/3, -y - 2/3, -z + 1/3)$, $41 = (-x - 4/3, y - x - 2/3, -z + 1/3)$.

#Orientation factor $\kappa = \mathbf{e}_i\mathbf{e}_j - 3(\mathbf{e}_i\mathbf{r}_{ij})(\mathbf{e}_j\mathbf{r}_{ij})$, where $\mathbf{e}_i$ is the direction of chromophore i, $\mathbf{r}_{ij}$ is a vector joining the chromophores $r = |\mathbf{r}_{ij}|$.

The distances were calculated between the geometric centers of the conjugated parts of the tetrapyrroles, and orientation factors were calculated from the fitted lines to the directions of the conjugated part of the chromophores.

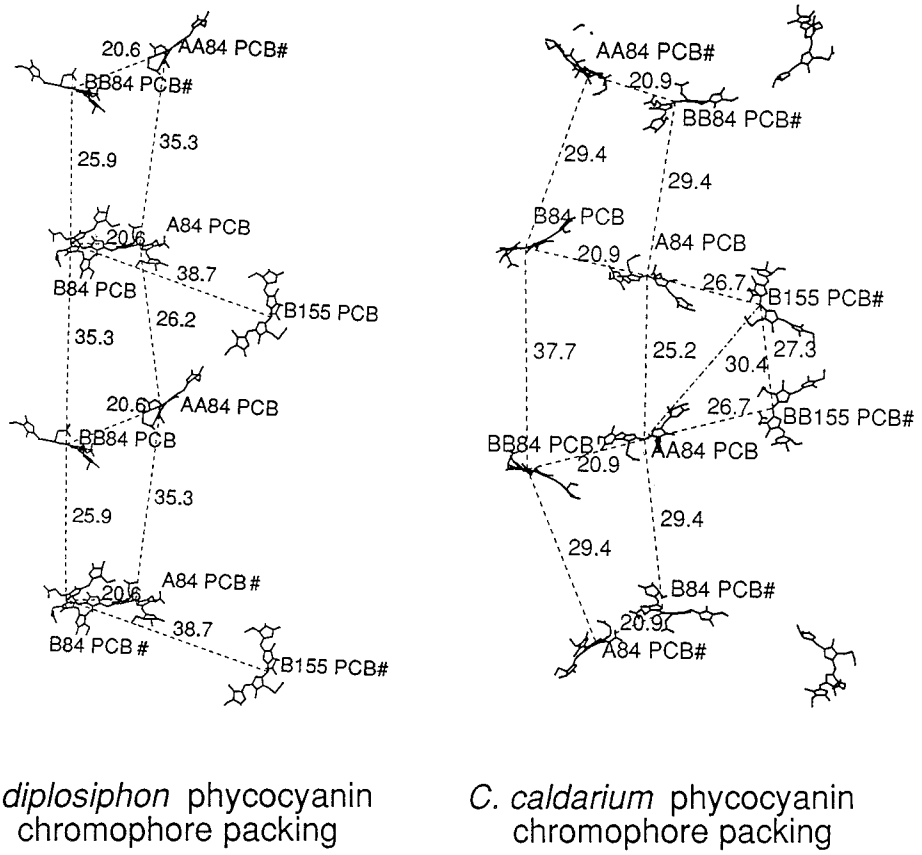

FIGURE 8 The chromophore packing with distances in *F. diplosiphon* and *C. caldarium* showing the possible energy transfer pathways. Both lattices have the β-84 pathway preserved. In *F. diplosiphon* lattice the shortest distance to the β-155 is depicted. Note the short distances in *C. caldarium* lattice between β-155 and α-84. The α-subunit is represented by A (AA), and the β-subunit by B (BB). A and B denote subunits of an upper trimer, and AA and BB denote subunits of a lower trimer in a hexamer. # denotes translationally related molecules.

Recent results have suggested that chromophores α-84 and β-84 are the ones most involved in interhexamer energy transfer and that β-84 is of type f (Schirmer et al., 1987; Siebzehrubl et al., 1987). In fact, the distance between these chromophores (~20 Å) would indicate that the excitonic coupling should occur, and both chromophores should be electronically coupled. However, those results were obtained on isolated dimers and trimers and do not put strict constrains on the hexamer interactions. In the optimally packed hexamers chromophores β-84 of the neighboring hexamers are properly oriented and at appropriate distances (~30 Å), so they can efficiently exchange energy by the Förster mechanism. Energy transfer rates calculated for chromophores β-155 in optimally packed hexamers (Fig. 7) have interhexamer energy transfer along the β-155 chromophore path only slightly less efficient than along the line of the β-84–α-84 path (Table 3). This would create an additional pathway on the periphery of the hexamers, which would involve the chromophore β-155.

The proposed role of chromophore β-155 would lead to a modified picture of an auxiliary energy transfer pathway along the outer surface of phycobilisome rods (Fig. 9) in addition to the dominant one along the linker proteins filling the inner space of the rods (Glazer, 1989; MacColl and Guard-Friar, 1987). However, because this auxiliary pathway is more exposed to solvent, it must be less efficient than the internal fully isolated pathway along chromophores β-84. Nevertheless, this proposal would offer a tentative answer to the question of why additional external chromophores are found more frequently in phycobilisomal proteins located closer to the tip of the rods (phycoerythrins and phycoerythrocyanins). Such external chromophores would collect light efficiently and then partially transfer the energy to the core and partially conduct it along the surface of the rods through an array of other external chromophores. The spectroscopic ladder formed by the different types of chromophores with descending order of absorbed and emitted wavelengths would promote an efficient transfer to the core on both internal and external pathways. Decreasing the number of external chromophores while forming an exten-

TABLE 3 Data similar to those in Table 2 calculated from the optimally packed hexamers

Chromophore pair	r	κ	k_{et}
α84-β84(10)*	35.9	−1.28	6.21
-α84(11)	35.5	1.22	6.08
β84-α84(10)	35.9	−1.30	6.44
-β84(10)	31.6	0.21	0.36
β155-β155(10)	38.9	1.52	5.45

Note substantial transfer rates and optimal orientation of chromophores β-155.

*Where numbers in parentheses stand for: (10), first trimer in translated hexamer; (11), second trimer in an optimally packed translated hexamer.

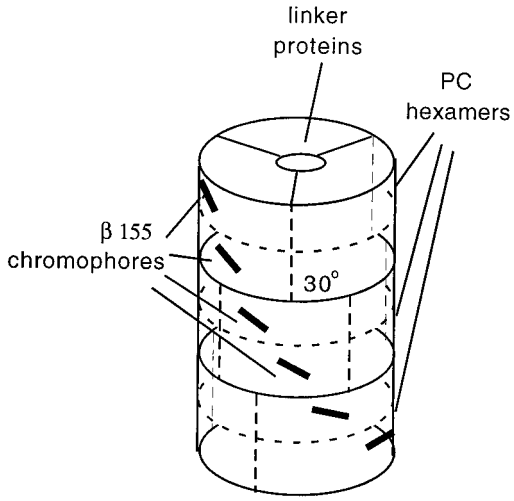

FIGURE 9 A schematic diagram of an additional energy transfer pathway that is proposed to function in native phycobilisome rods. Three hexamers of phycocyanin are depicted with 30° offset angles between them. The chromophores β-155 are represented by short thick lines, which when connected would form a helical path around the periphery of the rod, leading toward a core of a phycobilisome. For simplicity, only one of three helical paths of chromophores is shown.

sive antenna system would also form an "energy funnel" that would eventually lead to an efficient energy transfer to the core.

In assessing the importance of the new pathways for energy transfer at physiological conditions, the role of the linker proteins on the energy transfer capabilities of isolated hexamers versus intact rods must also be considered. Relatively little is known about the structural features of the linker proteins, except for the fact that they do not have chromophores and they have to modify the structure of colored proteins to achieve a high quantum efficiency of energy transfer not seen in isolated dimers, trimers, or hexamers. To address this question we had purified phycocyanin with at least one linker protein (27 kDa), as testified by absorption spectroscopy and by the sodium dodecyl sulfate gel chromatography of the initial material and the dissolved crystals, and crystallized it. Unfortunately this 27-kDa linker protein did not influence the crystal packing arrangement, and the crystals showed R3 (R32) space group. Therefore, it meant that the linker protein did not have any external contacts and was three-way disordered in the crystal and hence invisible. In those crystals the positions of chromophores were identical to the pure α-β dimer crystal structure, which argued that these particular linker proteins had no influence on the geometric characteristics of the hexamers. The spectrum of the complex (red-shifted with a shorter wavelength shoulder, as observed before; Lundell et al., 1981) did not change much in the isolated hexamers. This would argue that the transmission pathways in isolated hexamers are not greatly influenced much by linker proteins. Therefore, we would expect that a complete three-dimensional assembly of the phycobilisomal rod has a much greater influence on energy transfer and spectroscopic characteristics.

The hypothesized auxiliary pathways and the importance of external chromophores could be tested experimentally by mutagenesis of β-155 cysteine, the external chromophore attachment site, and spectroscopic measurement of the energy transfer through the reconstituted rods. Such an experiment would answer an important question about energy transduction by phycobilisome rods. The first step had already been taken by Debreczeny et al. (1993), who measured absorption and emission spectra of individual chromophores in $\alpha\beta$-monomers of the wild type and in the Cys^{155}-to-Ser mutant of cyanobacterium *Synechocus* sp.

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