Evolution of a light-harvesting protein by addition of new subunits and rearrangement of conserved elements: Crystal structure of a cryptophyte phycoerythrin at 1.63-Å resolution

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ABSTRACT Cryptophytes are unicellular photosynthetic algae that use a lumennally located light-harvesting system, which is distinct from the phycobilisome structure found in cyanobacteria and red algae. One of the key components of this system is water-soluble phycoerythrin (PE) 545 whose expression is enhanced by low light levels. The crystal structure of the heterodimeric α2β2 PE 545 from the marine cryptophyte Rhodomonas CS24 has been determined at 1.63-Å resolution. Although the β-chain structure is similar to the α and β chains of other known phycobiliproteins, the overall structure of PE 545 is novel with the α chains forming a simple extended fold with an antiparallel β-ribbon followed by an α-helix. The two doubly linked β50/β61 chromophores (one on each β subunit) are in van der Waals contact, suggesting that exciton-coupling mechanisms may alter their spectral properties. Each α subunit carries a covalently linked 15,16-dihydrobiliverdin chromophore that is likely to be the final energy acceptor. The architecture of the heterodimer suggests that PE 545 may dock to an acceptor protein via a deep cleft that may be transferred via this intermediary protein to the reaction center.

Light-harvesting proteins increase the efficiency of photosynthetic organisms growing in low-light regimes. They act as antennae, capturing photons over a broad frequency spectrum and transferring energy to membrane-bound reaction centers (1). In cyanobacteria and red algae, the light-harvesting phycobiliproteins (PBPs) are water soluble and organized into phycobilisomes (large, multiprotein complexes bound to the stromal face of the thylakoids). Individual PBPs and phycobilisomes have been studied by X-ray crystallography (2-11) and electron microscopy (12, 13) as well as biochemically (14). The individual proteins are structurally conserved with a basic αβ unit (referred to by convention as monomer) arranged around a 3-fold axis forming an (αβ)3 trimer. Cryptophyte algae also use PBPs to harvest light but these differ from those of the cyanobacteria and red algae in several significant ways (15). In any one species, there is only one type of PBP, either phycocyanin (PC) or phycoerythrin (PE); allophycocyanin is never present. The PC or PE is not organized into a phyco- chlorophyll; DBV, 15,16-dihydrobiliverdin.

Abbreviations: PE, phycoerythrin; PBP, phycobiliprotein; PEB, phycoerythroblin; DBV, 15,16-dihydrobiliverdin.

Data deposition: The coordinates have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID code 1qgw).

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RESULTS

Structure. The PE 545 \( \alpha_2\alpha_2\beta\beta \) dimer forms a boat-shaped molecule (approximately 75 Å \( \times \) 60 Å \( \times \) 40 Å). A \( C_6 \) tracing of the dimer, which forms the asymmetric unit of our crystal form, is shown in Fig. 1A. The \( \beta \) subunits are similar to each other and both \( \alpha \) and \( \beta \) subunits of the PBPs (2–10), forming part of the globin family of protein structures (Fig. 2A). The major differences between the two \( \beta \) subunits, and among the members of PBP family, are in the N-terminal regions, including helices X and Y that are separate from the main body, and in the GH loop (2–10). Least-squares alignment of the two PE 545 \( \beta \) subunits gives a rms deviation D of 0.6 Å (over 161 C\(_{\alpha}\) atoms) compared with 0.7 and 0.8 Å for the two \( \beta \) subunits (C over 144 and D over 140 C\(_{\alpha}\) atoms, respectively) aligned to the phycobilisome PE \( \beta \) subunit (10).

The \( \alpha \) subunits consist of an antiparallel \( \beta \)-ribbon followed by an \( \alpha \)-helix (Figs. 1 and 2). Each \( \alpha \) subunit is extended along its respective \( \beta \) subunit. The \( \alpha \) subunits are not identical as expected from previously determined sequence differences (22, 31, 32). We observe two \( \alpha \) subunits, \( \alpha_1 \) and \( \alpha_2 \), respectively (Figs. 1 and 2) with the major sequence and structural differences residing in the C-terminal regions; \( \alpha_1 \) is longer than \( \alpha_2 \) (76 versus 67 residues) and the C-terminal extension of \( \alpha_1 \) is important in chromophore binding (Fig. 3A). The C-terminal helix in the \( \alpha_2 \) subunit is longer than that in \( \alpha_1 \) (Fig. 2D). Both \( \alpha \) subunit C termini are approximately in the same position with respect to their \( \beta \) subunits. Our electron density maps

### Table 1. Data collection and refinement statistics

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<td>Disallowed</td>
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*From PROCHECK (43).

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Fig. 1. \( C_6 \) tracing of the PE 545 dimer plus two views of the polar slot. (A) A stereo \( C_6 \) diagram of the PE 545 dimer showing all of the chromophores (\( \alpha_1 \) cyan, \( \alpha_2 \) green, \( \beta \) chains gold and magenta, and chromophores blue). The view is down the pseudo 2-fold axis. The pair of \( \beta \)0/\( \beta \)1 chromophores is toward the viewer, just covered by loops from the \( \alpha_1 \) and \( \beta \) chains. \( A \) was produced with the program SETOR (42). (B and C) The solvent-filled slot that separates the two \( \alpha \beta \) monomers is shown as a molecular surface (blue). (B) The view is down the pseudo 2-fold axis from the face opposite the one containing the two \( \beta \)0/\( \beta \)1 chromophores. The backbone is shown as a red tube. The \( \alpha \)19 subunit helices can be seen entering the edges of the slot. The monomer on the left is \( \alpha \beta \) while \( \alpha \beta \) is on the right. Note that the slot is wider in the upper half because of the flat face of the C-terminal helix of the \( \alpha_2 \) subunit. The chromophores are shown in pink. The \( \alpha \)19 chromophores are at the interface between the two monomers, bounding the ends of the slot. (C) An orthogonal view where the \( \beta \)0/\( \beta \)1chromophores are on the top surface near the center. The slot enters from the bottom and is not symmetric about the pseudo 2-fold axis (running up the page). The \( \alpha \)19 chromophores are at the bottom left and right demarcating the ends of the slot. \( B \) and \( C \) were produced by using the program GRASP (35).
show no evidence for the reported heterogeneity in cryptophyte dimers caused by the α subunits (22, 32, 33).

A unique feature of our PE 545 structure is the presence of a β-sheet, as all other structures of PBPs are devoid of β-sheet structure (2–10) with the exception of the linker polypeptide (11). In our dimer, there are two nearly identical β-sheets, each composed of two strands from an α subunit plus one strand from its partner β subunit. The portion of the β subunit that forms the third β-strand is part of the N-terminal extension of the standard globin fold.

Two ions were clearly identified in the structure. The electron density for a Mg$^{2+}$ ion octahedrally coordinated by six water molecules was observed at the C terminus of the α-helix in the α subunit. The electron density, coordination geometry, and correlation between the B factors of the ion and its oxygen ligands support our assignment. A second ion was identified as a Cl$^{-}$ ion (Fig. 3A), which coordinated one of the pyrrole nitrogens in the β50/61 chromophore in β subunit D. The coordination shell of the Cl$^{-}$ is completed by the OH of Ser-147 and the side chain NH of Gln-148 from the GH loop of β subunit D, as well as a water ligand (Fig. 3A). This Cl$^{-}$ ion was identified by the fact that it showed extra electron density in difference maps and its B factor dropped to 2 Å$^2$ during each refinement cycle (compared to > 5 Å$^2$ for typical water molecules). In addition, it gave a 7-σ peak in an anomalous Fourier map. We note that both of these ions could result simply from the 0.1 M MgCl$_2$ present in the crystallization solution.

Two posttranslationally modified residues per monomer were identified in the electron density map. As expected, residue 72 in the β subunit is a γ-N-methylasparagine, a modification conserved in most PBPs (34). Lys-4 in both α subunits also appears to be modified, with the electron density and chemical environment being consistent with 5-hydroxylysine. Both modified residues are in the vicinity of the β82 chromophore (Fig. 3B).

**Quaternary Structure.** The PE 545 dimer is formed by the association of two tightly linked αβ monomers (Fig. 1A). Within the monomer, the α chain makes intimate contacts with the β chain via the formation of the β-sheet and via the packing of the α chain helix against helices B and E of the β subunit. The formation of the α1β and α2β monomers results in the burial of 4,900 Å$^2$ and 4,300 Å$^2$ of accessible surface area respectively. The assembly of the two monomers into the dimer results in the burial of an additional 4,000 Å$^2$ of accessible surface area and is commensurate with the stability of the dimer (36). We note that the computed buried accessible surface area on dimer formation is an overestimate because of the difficulties posed by the presence of a deep polar slot.

The interface between the two αβ monomers is unusual in that it is very polar and highly solvated (hence the high proportion of ordered water molecules, Table 1). Intimate contacts between the monomers primarily are formed in the vicinity of the α19 chromophores and the β50/61 chromophores, the latter being in van der Waals contact at the interface between monomers (Fig. 3A). Almost all protein–protein contacts between the two monomers are mediated through the α subunits, and the only contact between the two β subunits is in the vicinity of the two β50/61 chromophores.

**Polar Slot.** A striking feature of the dimer is the cavity filled with ordered water molecules that forms a slot between the two monomers (Fig. 1B and C). The slot enters the dimer across the pseudo 2-fold axis relating the two monomers. The slot is approximately 15 Å deep and 20 Å long while its width varies from 5 to 10 Å. Its shape is asymmetric because of the sequence differences between the C-terminal helices of the α subunits. In particular, the C-terminal helix of the α2 subunit presents a flat face to the slot because of the presence of Gly-52, Gly-56, Ala-59, and Ala-63. There are continuous chains of hydrogen-bonded water molecules that pass all the way through the dimer (Fig. 1C). The slot is reminiscent of a docking site for a peptide or other medium-sized molecule that may interact with PE 545.
Comparison to Phycobilisome Proteins. We have overlayed the PE 545 α1α2ββ dimer on the (αβ)6 hexamer structure that forms the basis of the phycobilisome (3). The overlay of the PE 545 β subunit with the phycobilisome β subunit results in overwhelming steric clashes between the PE 545 dimer and the hexamer. Overlaying the PE 545 β subunit with the phycobilisome α subunit results in minimal clashes between the PE 545 dimer and the phycobilisome hexamer. We conclude that the subunit interactions within the PE 545 dimer is unlikely to participate in a higher-order aggregate that resembles the phycobilisome structure.

Recently, the structure of a PBP has been determined that includes the linker polypeptide Lc 7–8, which is one of the proteins responsible for the strict sequential assembly of the phycobilisomes (11). Like the α subunit of PE 545, the linker polypeptide is small (67 residues) and unlikely to have a...
the aspartate side chain. This need for a second negatively charged ligand on the two β50/β61 chromophores is likely to have implications for their physical state and spectroscopic properties.

The chromophores linked to the α subunit are chemically distinct from the β subunit PEB chromophores (Fig. 3 D and E). Our structure is consistent with the presence of a singly linked 15,16-dihydrobiliverdin (DBV) chromophore as determined previously by NMR and MS (21). This chromophore differs from PEB in that pyrrole A is oxidized so that all atoms within the ring are in an sp2 state (Fig. 3 D and E) with the result that all atoms attached to pyrrole A are coplanar with the pyrrole ring.

The ligands attached to the nitrogen atoms in the α19 chromophore also differ from those of the β subunit PEB chromophores, as nitrogen atoms in the two central pyroles are coordinated by a water molecule (Fig. 3D), which, in turn, is coordinated by His-16 of the respective α subunit and another water molecule. Consequently, there is no negatively charged ligand at this central coordination site, distinguishing the α19 chromophore from all other phycobilin structures (2–10). Also, in contrast to the β subunit PEB chromophores, the nitrogen atom of pyrrole D is coordinated to the side chain of Glu-25 of its respective α subunit (Fig. 3D).

The additional conjugation of the α19 chromophore compared with that of the PEBs alters its spectral properties, moving the absorption maximum of DBV to 562 nm (compared with 550 nm for PEB; ref. 21). Thus, it is a likely candidate for the final photon energy acceptor in this form of PE 545 (i.e., the point at which all photon energy arrives before transfer to a chromophore of an acceptor protein). Its location near the polar slot may be of functional significance.

**Implications for Light Harvesting.** All the chromophores in this PE 545 structure are in close proximity (<45 Å between centers of mass of the conjugated atoms). Calculation of energy transfer parameters for all possible pairs of chromophores in the α1α2ββ dimer, assuming Förster resonance dipole–dipole coupling, indicates that 11 of the 28 chromophore pairs have calculated transfer rates, k_{et}, exceeding 10 ns⁻¹ (Table 2), which is rapid compared with the lifetime of the PEB excited state, τo = 2.7 ns. Thus, energy will rapidly migrate through the network of chromophores to the terminal acceptors.

The central β50/β61 chromophores have a separation of 12.4 Å with favorable stacking of their A pyrrole rings. It is possible that they possess a strong exciton interaction, which may alter their chromophoric properties. In contrast, the two β158 chromophores are the most isolated in terms of energy transfer. They only participate in one pairing with k_{et} > 10 ns⁻¹, which is with their respective β50/β61 chromophores. Each α19 chromophore appears to be coupled with the β82...
and β50/β61 chromophores from its opposite β subunit, with the two β-chromophores coupled to each other.

**DISCUSSION**

The structure of PE 545 demonstrates the evolution of a functional protein complex based on the rearrangement of related elements (from phycobilisome PBPs) and the addition of new subunits that are unlikely to fold in isolation. As such, it is important to examine how the new arrangement alters the properties of the light-harvesting unit. PE 545 has several new features that include the contacting β50/β61 chromophore pair, the α subunits with their DBV chromophores, and the inter-monomer slot. These may hold clues as to how the cryptophyte light-harvesting system operates.

The PE 545 dimer has been studied by biochemical and spectroscopic techniques (36). The dimeric form was shown to be stable, requiring nonbiological conditions to produce the monomer. Dissociation into the monomer resulted in the reduction of the absorbance at 550 nm by approximately 20% and a drop in the positive and negative CD bands at 540 and 580 nm by about 1/3. Two photon fluorescence lifetime measurements showed that monomerization resulted in the loss of the fastest lifetime measured (2.4 ps increasing to 40 ps in the monomer). The structure indicates that these changes are likely to be caused by the separation of the pair of β50/β61 chromophores, which is consistent with the proposal that the change in CD is the result of the disruption of a pair of chromophores, which is consistent with the proposal that the change in CD is the result of the disruption of a pair of chromophores that are exciton-coupled in the dimer (36).

However, the persistence of positive and negative CD bands in the monomer indicates that the chromophores within the monomer are also exciton-coupled, but this coupling is not immediately obvious from the structure. Combining these findings, it appears that the most significant advantage of the dimeric form is the presence of extremely rapid energy transfer between the two monomers, which is mediated by the pair of β50/β61 chromophores.

The α19 chromophore (DBV) is the most likely candidate for the final acceptor on PE 545. This arrangement is true for the most cryptophyte PBPs (21) with some exceptions (37). The question remains as to how this energy then is transferred to the chlorophylls of the reaction center. Based on steady-state fluorescence measurements, it has been suggested that energy transfer is primarily to photosystem II (PSII) as in the thylakoid lumen. Possible candidates include the inner antenna chlorophylls of the reaction center. Based on steady-state fluorescence measurements, it has been suggested that energy transfer is primarily to photosystem II (PSII) as in the cryptophyte light-harvesting system operates.

In the PE 545 structure reported here the large cavity containing bound water could be a site for interaction with a component of photosystem II (PSII) as in the phycobilisome (38, 39). However, attempts to isolate and characterize a PE-PSII particle have not shown clear coupling between these components.

In the PE 545 structure reported here the large cavity containing bound water could be a site for interaction with a component of photosystem II (PSII) as in the thylakoid lumen. Possible candidates include the inner antennas of PSII, Cp43, and Cp47. However, the sequences of Cp43 (psbC) and Cp47 (psbB) for the cryptophyte, *Guillardia theta*, show no evidence of new features that might implicate unique binding sites for PE (40). Time-resolved fluorescence emission demonstrated that PE 565 in *Cryptomonas CR1* transferred its energy, possibly via a 655-nm component, to chlorophyll 682, which was assigned to the intrinsic integral membrane light-harvesting complex (LHC) and subsequently to Cp43/Cp47 (41). Thus, cryptophyte PE may interact directly with LHC or possibly the 655-nm component protein. It is possible that these putative interactions are mediated by the polar slot.

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