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The crystal structure of R-Phycoerythrin

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Introduction

Phycobilisomes (PBS) 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 which are composed of phycobiliproteins and linker proteins. Phycobiliproteins can be divided into three main groups: phycoerythrin(PE), 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 APC discs which is in proximity of the reaction centers, while the rods are attached on the core and are composed of PC discs in the middle and PE or PEC discs on the tip. There are some kinds of phycobiliproteins had been determined(Schirmer et al., 1985, 1986, 1987; Duerring et al., 1990, 1991 ; Ficner et al., 1992; Ficner & Huber, 1993; Brejc et al., 1995; Chang et al., 1996).

The subunit composition of R-PE is $(\alpha\beta)_6\gamma$ There are five chromophores in each ($\alpha\beta$) unit of R-PE, α84 PEB and 140a PEB link to the α subunit, β84PEB, β155PEB and β50/61 PUB link to the β subunit(Nagy et al.,1985). Recently, the crystal structure of R-phycoerythrin(R-PE) from Polysiphonia urceolata had been refined to a resolution of 1.9 Å (Jiang, 1999), using atomic coordinates of R-PE structure determined at 2.8Å resolution(Chang, 1996).

Materials and results

The crystals of R-PE were gained by vapor diffusion method(Chang et al.,1996). They are belong to R3 space group with cell dimension of a=b=189.8Å, c=60.1Å. The starting model used in the study was the R-PE structure determined at 2.8Å resolution . High quality diffraction data were collected by using Prof. N. Sakabe's Weissenberg camera in KEK, Photon Factory(Tsukuba, Japan) and by using X-200B Area detector at home respectively. The program X-plor(Brunger,1992) was used for refinement and Turbo-frodo(Jones, 1978) was used for model building. After refinement, R-factor of the final mode was 19.5% and R-free was 28.2%. The r.m.s deviations of bond distances
and bond angles are 0.017Å and 2.9°, respectively. The Ramachandran plot of R-PE model is quite reasonable and the fitting of model for electron density map is well.

We used the sequence of R-PE from Polysiphonia boldii for model building of R-PE at 2.8 Å. The increase of resolution to 1.9Å help us to determlne the sequence of R-PE more reliably. Six residues have been changed in each ($\alpha\beta$) unit of R-PE according to the electron density map, they are αl32Ala →Ser, αl36Thr →Met, αl62Phe →Tyr, αl64Thr →Ala, β145Ser →Thr and β151Ile → Ala, the final sequence is shown in Table 2 and the electron density of residue αl36 is shown in figure 1.

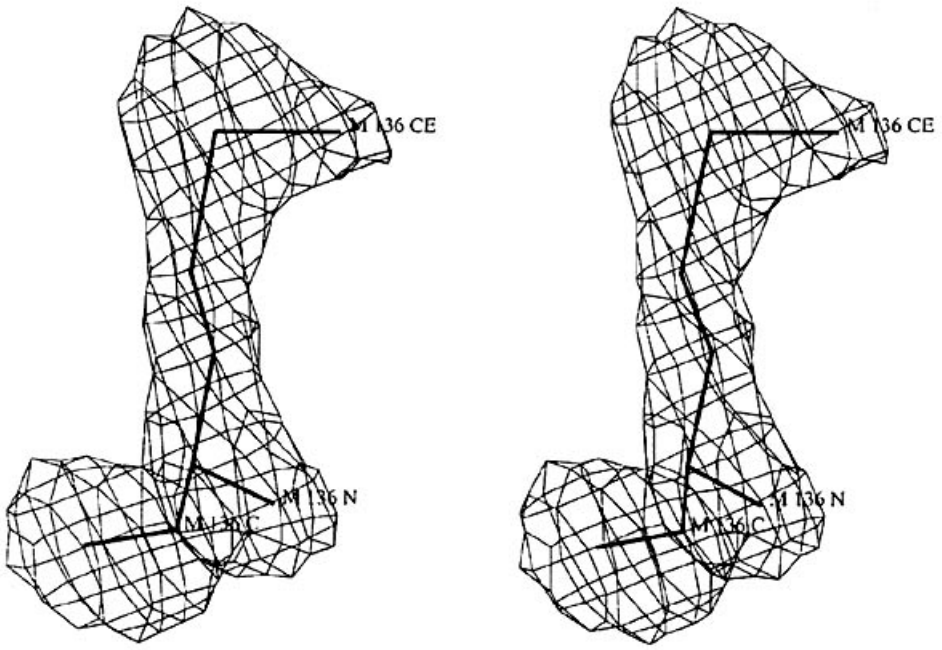

Figure 1 The electron density map of α136 Met

The microenvironment and conformation of chromophores

The mechanism of energy transfer was mainly explained by Forster's dipole-dipole resonance and exciton interaction theory. The approximate formula was usually adopted for calculating the

$$K_{et}=k^2/\tau_0(R_0/\gamma)^6$$

energy transfer rate, where: K_{et} is the energy transfer rate; k^2 is the orientation factor; R_0=50 Å, is the forster residue; To is the florescence life time; and γ is the distance between two chromophores.

Chromophores with same chemical formula may have different spectrum characteristic in phycobiliprotein since that the microenvironment influence the conformation of chromophores, so the analysis of the structure of chromophores and their environment in detail is very important, all the chromophores have the same 4 nitrogen and 6 oxygen atoms can form hydrogen bond with protein residues(Fig.2), which influence the spectrum characteristics and energy transfer of phycobiliproteins.

Almost all the nitrogen atom in ring B and ring C form hydrogen bonds with Asp or Arg and all the oxygen atom in ring C forms hydrogen bond with NH1 and NH2 of Arg. Since all the ring B and ring C connected with double bond and from a conjugate plane with rigidity, so the strong interactions between Arg and atoms in ring B and ring C of chromophore imply that Arg plays an important role in keeping the orientation of chromophore, in other words, the orientation factor k^2 of chromophore may be greatly influenced by Arg.

Since ring A of PEB and the ring A, D of PUB combine with the remains of chromophore through single bond, we assumed the flexibility of chromophore was influenced by the interactions between

protein residues and oxygen atoms in ring D and ring A, so the flexibility changes the fluorescence lifetime τ_0.

Almost all the chromophores form hydrogen bonds with residues which are in the same ($\alpha\beta$)unit, the only one exception is the oxygen atom in ring D of α84PEB forms hydrogen bond with N atom of β77 which is in another ($\alpha\beta$)subunit, in all phycobiliproteins the dihedral angle of β77 is the only one which is in the unreasonable area of Ramachandran plot. It indicates that the free energy of residue β77 is quite high, so this hydrogen bond is not stable and makes the ring D of chromophore α84 has some flexibility, since the conformation of ring D influences the absorption characteristic of α84 PEB, so the flexibility fits the need of energy absorbing, storing and releasing of chromophore.

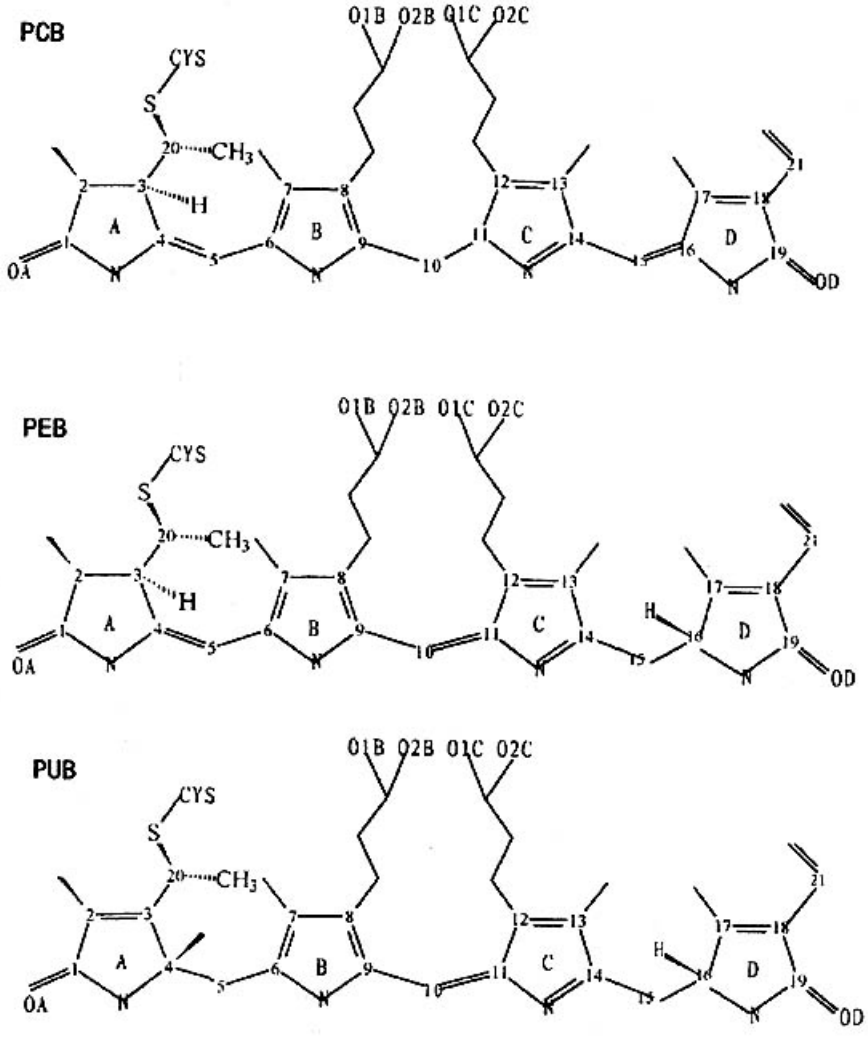

Figure2 Chemical structure of PCB, PEB and PUB

As we know, the chromophore linked to β50 and β61 is PUB in R-PE but is PEB in B-PE, the chemical structure of PEB and PUB is shown in Fig.2, carbon atom C(4) (in ring A) is chiral atom in PUB. We compared the microenvironment of them, and found that PUB form an additional hydrogen bond

between oxygen atom in ring A of β50/61 PUB and N atom(in β148b Gln), the bond length is 2.84 Å. This hydrogen bond is a main factor in controlling the conformation of ring A of PUB, which may be a main factor cursing the spectrum difference between PEB and PUB.

Energy transfer

As we know, in each (α β) unit of phycobiliproteins , α84 and β84 are on the inter surface and β155, β140a, β50 on the outside surface. The distance among chromophores is the most important factor in calculating the K_{et}, so we calculated the distances among chromophores in an special (α β) unit and 30 chromophores in $(\alpha\beta)_6$.

From the calculation, we found that the shortest distance is between α84PEB and β50/61 PUB (19Å) , the distance between β84PEB and β50/61 PUB is also quite short (23.9Å), which indicates that β50/61 PUB play a very important role in transferring energy from the outside surface of molecule disc to the inside surface of molecule disc since it absorbs higher energy than others. α84PEB play a role in passing the outside energy from β50/61 PUB and α140a to β84PEB, which is the terminal energy acceptor in R-PE molecule.

Between two $(\alpha\beta)_3$ of R-PE, there are only three coupling distances shorter than 30Å, such as 1α84 to 4β84, α140a to β155 and β155 to β155. These are regarded as possible pathways of energy transferring between two $(\alpha\beta)_3$ trimmers.

When we calculated the distances between the chromophores which are belong to different $(\alpha\beta)_6$, and found some of these distances are very short, the distance between the nearest two atoms is only 3.4Å, which is between chromophore β155 and α140a(shown in Fig. 3), such short a distance makes it possible that the two chromophores form a conjugate system, which means the energy transfer between them will be very quickly. Although we assume this may be only cursed by molecular packing in crystal, but from the characteristic of the molecule shape of phycobiliproteins, we believe that the permutation of phycobiliproteins in the real phycobilisomes are similar to their packing function in the crystal. This was also proved by the electron microscopy result (Glazer, 1984, Ducret et al. 1996). According to this, we are trying to explain the function of chromophore β155 in the phycocyanin, that is maybe the same as α140aPEB of R-PE can transfer the energy rapidly to the β155PCB of C-PC which is located in the adjacent rod, since chromophore 155 has the same position in R-PE and C-PC.

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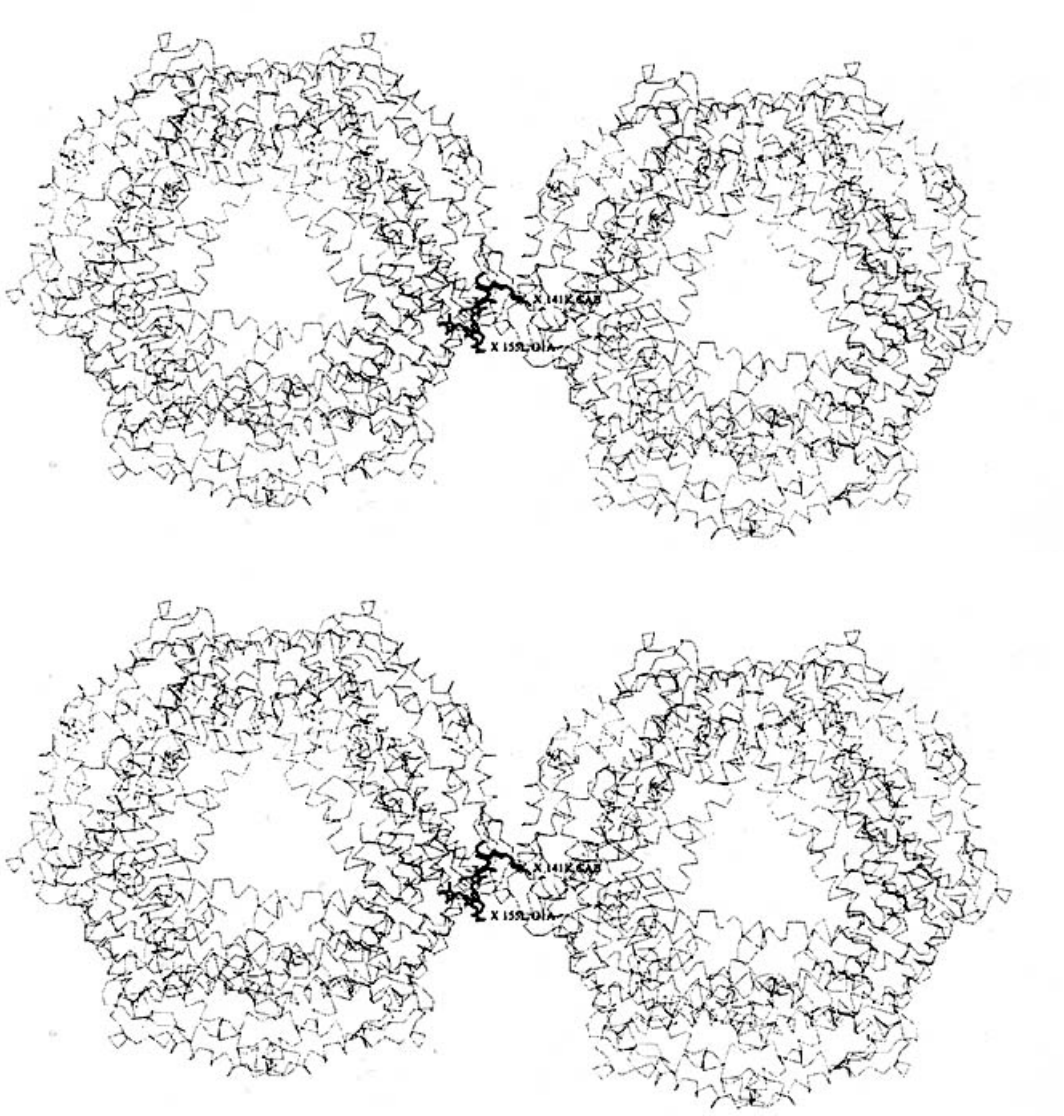

Figure3 Two adjacent R-PE hexamers and chromohores α140a and β155

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