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A phycoerythrin-allophycocyanin complex from the intact phycobilisomes of the marine red alga *Polysiphonia urceolata*

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Abstract

A phycoerythrin-allophycocyanin (PE-AP) complex was obtained from intact phycobilisomes of the marine red alga *Polysiphonia urceolata*. Study of spectral properties and polypeptide components showed that the complex contains PE, phycocyanin, AP, and higher proportional linker proteins of the four groups present in intact phycobilisomes.

Additional key words: absorption spectra; allophycocyanin; fluorescence spectra; light-harvesting complex; phycoerythrin; proteins.

Introduction

Phycobilisomes (PBSs) are predominant light-harvesting complexes found in prokaryotic *Cyanobacteria* and eukaryotic *Rhodophyceae*. PBSs are macromolecular complexes composed of phycobiliproteins of phycoerythrin (PE), phycocyanin (PC), phycoerythrocyanin (PEC), and allophycocyanin (AP). All PBSs comprise two structural domains: the peripheral rows formed by PE, PC, and row linker peptides, and the allphycocyanin core formed by AP, allophycocyanin B (APB), core linker peptides, and core-membrane linker (L_{CM}). PBSs are attached by L_{CM} to thylakoid membranes and structurally coupled with photosystem 2 (Glazer 1984, Zilinskas and Greenwald 1986, Rowan 1989). The radiant energy from 460 to 650 nm that PBSs absorb is efficiently transferred from the peripheral rows to the core. Eventually the energy is transferred to photosystems or other PBSs by two terminal emitters, AP_{LM} and APB complexes (Glazer *et al.* 1985, Glazer 1989, Reuter and Müller 1993, Van Grondelle *et al.* 1994). The directional energy transfer is based on the structural intactness of PBSs. To study energy transfer in PBSs artificial biliprotein complexes are often used as a model (Zhao *et al.* 1999).

The R-phycoerythrin (R-PE), a predominant biliprotein, from *Polysiphonia urceolata* Grev. is an aggregate of $(\alpha_2\beta_2)_3\gamma$. Each ($\alpha\beta$) contains four phycoerythrobilins (PEB) and one phycourobilin (PUB), whose maximum absorption is at 498 nm, and the γ-subunit has one PEB and three PUB (Chang *et al.* 1996). The R-phycocyanin (R-PC) from *P. urceolata* is an $(\alpha\beta)_3$ complex; its α-subunit has one phycocyanobilin (PCB) and its β-subunit has one PCB and one PEB (Zhang *et al.* 1995). Recently we performed some researches on the PBS from *P. urceolata* and its biliprotein components. The PBS was, unlike other reported PBSs, stable at 4 °C in 0.1 M phosphate buffer (pH 7.0) with 0.2 M sucrose. It did not show obvious dissociation until the incubation time was prolonged beyond 10 h. In the meantime, we got from the PBS dissociated for 12 h at 4 °C a dark-brown biliprotein complex. It is poorly soluble in phosphate buffer and fairly stable in solution at 4 °C and room temperature. Moreover, there were no lipid pigments to be extracted from the complex in ethanol and acetone; therefore, it has no lipid pigments. Here we report studies on spectral properties and biliprotein and other polypeptide components of this dark-brown complex from *P. urceolata* PBS.

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Abbreviations: AP - allophycocyanin; APB - allophycocyanin B; AP_{LM} - AP with L_{CM}; L_{CM} - core-membrane linker; PAGE - polyacrylamide gel electrophoresis; PBS - phycobilisome; PC - phycocyanin; PCB - phycocyanobilin; PE - phycoerythrin; PEB - phycoerythrobilin; PUB - phycourobilin; R-PC - R-phycocyanin; R-PE - R-phycoerythrin; SDS - sodium dodecylsulfate.
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Materials and methods

Preparation of intact phycobilisomes: Intact PBSs from *Polysiphonia urceolata,* which is luxuriant from February to April when the temperature of the sea water where it grows is about 5 to 15 °C, were prepared by centrifugation of step-sucrose gradients in 0.75 M phosphate buffer, pH 7.0 (Gantt 1980). 25 g of fresh algae was washed twice in 0.9 M phosphate buffer (pH 7.0) and ground in 100 cm^3 of 0.9 M phosphate buffer containing 1 mM NaN_3. The ground sample was passed through a French press cell twice at 7.04×10^6 kg m^{-2}. Then *Triton X-100* was added in the suspension of the ruptured algae up to the final concentration of 2 % (v/v). The suspension was centrifuged with the rotor *RPR20-3-1286* of the *20PR-520* centrifuge (*Hitachi*) at 30 000×*g* for 20 min at 20 °C. Intact PBSs were obtained from the PBS extracts of the red middle part of the centrifuged tubes by the step-sucrose-gradient centrifugation. The gradients were composed of 1.0 cm^3 of 1.00, 0.85, 0.50, and 0.25 M sucrose in 0.75 M phosphate buffer (pH 7.0). On the top of the gradients in 12 cm^3 tubes, about 8 cm^3 of the PBS extract was loaded. The gradients were spun at 250 000×*g* in an angle rotor *RP557* of the *55P-72* centrifuge (*Hitachi*) at 22 °C for 2.5 h. Intact PBSs were collected from the purple precipitate in the gradient tubes.

Preparation of PE-AP complexes: The intact PBSs suspended in 0.1 M phosphate buffer (pH 7.0) with 0.2 M sucrose were dissociated at 4 °C for 12 h. Then the dissociated PBS, loaded on the step-sucrose gradient tubes with 1.0 cm^3 of 1.00, 0.75, 0.50, and 0.30 M sucrose in 0.6 M phosphate buffer (pH 7.0), were centrifuged in an angle rotor (*RP557*) of the *55P-72* centrifuge (*Hitachi*) at 250 000×*g* for 2 h at 22 °C. Dark-brown precipitate under the gradients was collected, suspended in 0.6 M phosphate buffer (pH 7.0), and centrifuged at 1 200×*g* for 20 min at 4 °C in the rotor *RPR20-3-1286* of the *20PR-520* centrifuge (*Hitachi*) to remove insoluble substances. This precipitate is named PE-AP complex.

Spectrum measurements: Absorption and fluorescence spectra of the intact PBSs suspended in 0.75 M phosphate buffer (pH 7.0) and of the PE-AP complex suspended in 0.6 M phosphate buffer (pH 7.0) were measured at room temperature using the *UV-3400* spectrophotometer (*Hitachi*) and the *850* spectrophotometer (*Hitachi*), respectively.

SDS-polyacrylamide gel electrophoresis: The PBS and the PE-PA complex were incubated for 4 h in 17.6 mM Tris, pH 8.2, 16.4 mM H_3BO_3 with 2 % (m/v) sodium dodecyl sulfate (SDS), 2 % β/2-mercaptoethanol (v/v), and 5 % glycerin (v/v) at 37 °C. The incubated samples were centrifuged at 15 000×*g* for 10 min to remove insoluble substances. Subunit constituents of the PE-PA complex were analysed by the SDS-PAGE using a gradient gel (1.5 mm) of 9-28 % (m/v) in the pH 8.2 buffer containing 88 mM Tris, 82 mM H_3BO_3, and 0.4 % SDS (m/v). The SDS-PAGE was performed with a constant current of 16-20 mA for 8-10 h. As the electrode buffer (pH 8.2), 44 mM Tris-42 mM H_3BO_3 with 0.3 % (m/v) SDS was employed. After the SDS-PAGE, the slab gel was stained in Coomassie Blue G-250 solution.

Results

Spectral properties of the PE-AP complexes: The spectral properties of intact PBS from *P. urceolata* are shown in Fig. 1. No distinctive absorption from 660 to 680 nm and the relatively weak absorption from 400 to 450 nm in its absorption spectrum (Fig.1, *solid curve*) demonstrate that the obtained intact PBSs did not contain chlorophyll (Gantt 1980). Absorption spectrum of the PE-AP complex from the intact PBS is in Fig. 2. When excited at 497 nm, the complex had maximum fluorescence emission at 578 nm (Fig. 3*A*); when excited at 600 nm, it had a peak at 664 nm and a weak shoulder at 638 nm (Fig. 3*B*). When excited at 620 and 640 nm, the complex had an emission maximum at 665 nm (Fig. 3*C,D*). Fluorescence excitation spectra of the PE-AP complex showed that the 578 nm emission comes from a biliprotein of the R-PE kind (Fig. 4*A*). The 640 nm emission (typical PC emission) was from an R-PC component to which the PE component contributes (Fig. 4*B*), and the 665 nm emission, the typical AP emission, was from an AP component and the PE and PC components contributed to it only a little (Fig. 4*C*) (Glazer 1984, Zilinskas 1986). Furthermore, the absorption of the complex from 350 to 450 nm (Fig. 2) plays only a little role in generation of the three emissions. Therefore, this absorption may be attributed to some polypeptides that hardly have a structural relationship to chromophores of the biliprotein components. The spectral properties of the complex confirm that it contains PE, PC, and AP biliproteins and some other polypeptides.

Subunit and polypeptide components of the PE-AP complex: Fig. 5 shows the subunit and linker polypeptide components of the PE-AP complex revealed by SDS-PAGE. The PE-AP complex (*lane c*) contains all the subunits and the linker polypeptides as the intact PBS (*lane b*) does, for example the four groups of linker protein bands, the bands of the α- and β-subunits and that of the γ-subunit (Zilinskas 1986, Rowan 1989). However,

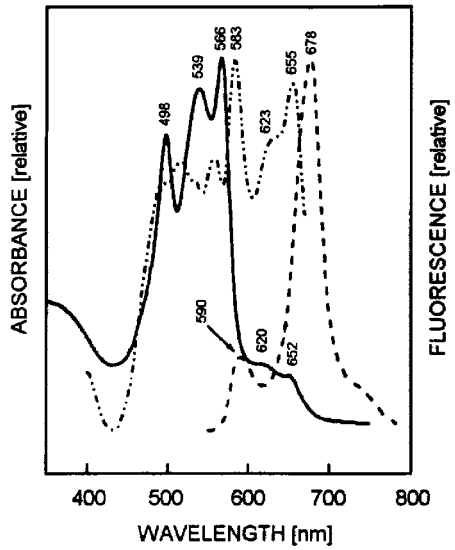

Fig. 1. Absorption (*solid curve*), emission excited at 498 nm (*dashed curve*), and excitation examined at 680 nm (*dot-dash curve*) spectra of intact phycobilisomes from *Polysiphonia urceolata* Grev. in 0.75 M phosphate buffer (pH 7.0).

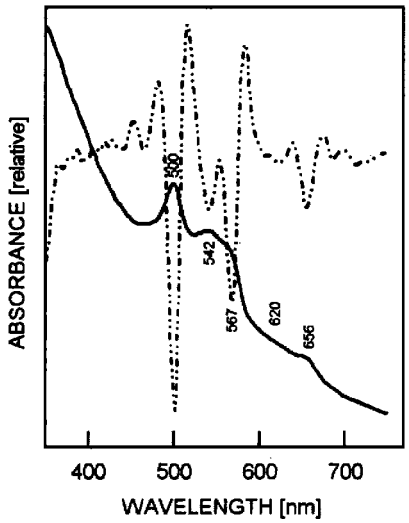

Fig. 2. Absorption (*solid curve*) and second derivative (*dot-dash curve*) spectra of PE-AP complex in 0.6 M phosphate buffer (pH 7.0).

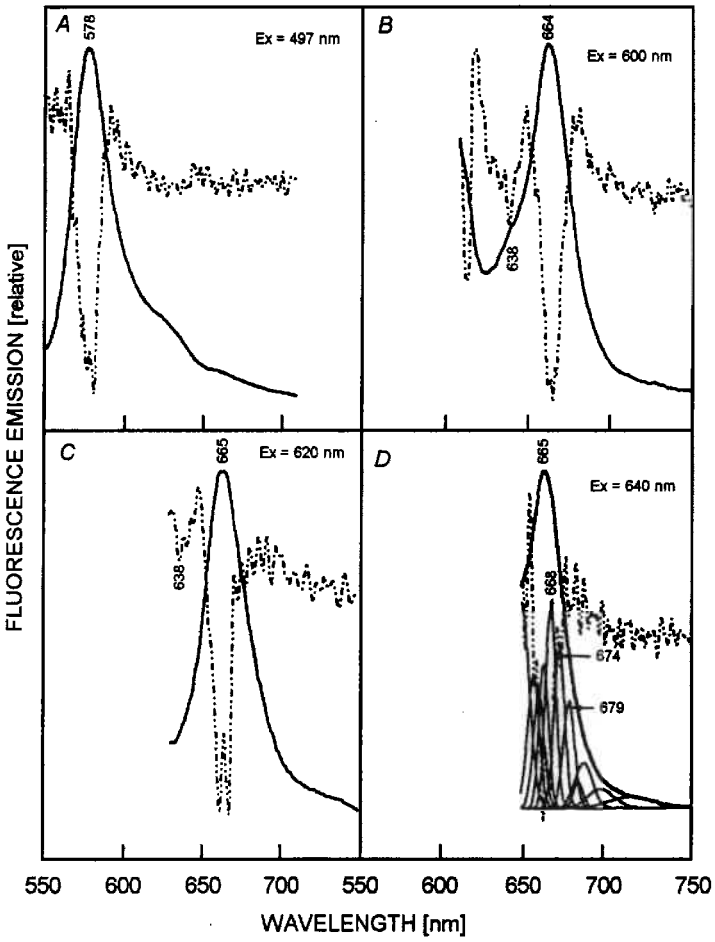

Fig. 3. Fluorescence emission (*solid curves*) and second derivative (*dot-dash curves*) spectra of PE-AP complex in 0.6 M phosphate buffer (pH 7.0). The four spectra of *A*, *B*, *C*, and *D* were recorded when PE-AP complex was excited at 497, 600, 620, and 640 nm. *D* also shows the Gaussian deconvolution pattern.

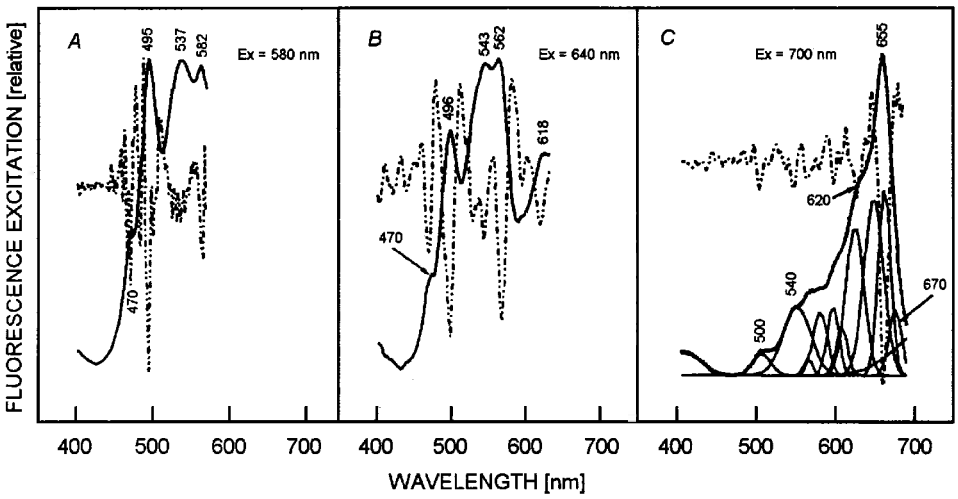

Fig. 4. Fluorescence excitation (*solid curves*) and second derivative (*dot-dash curves*) spectra of PE-AP complex in 0.6 M phosphate buffer. The three spectra of *A*, *B*, and *C* were recorded when the emissions of 580, 640, and 700 nm, respectively, were examined. *C* also shows the Gaussian deconvolution pattern.

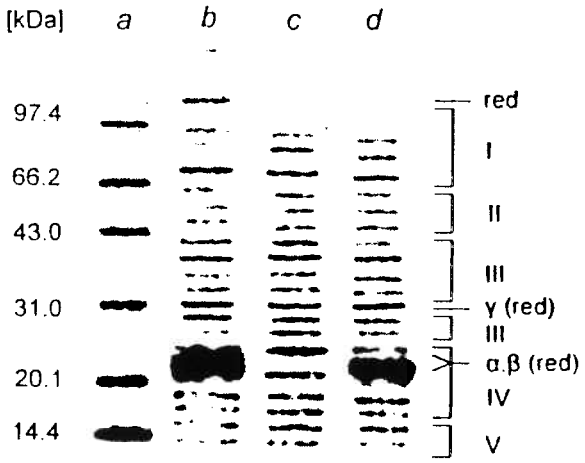

Fig. 5. SDS-PAGE pattern of PE-AP complex polypeptides: *a* - markers; *b* - intact phycobilisomes incubated at 37 °C; *c* - PE-AP complex; *d* - intact phycobilisomes incubated at 100 °C. IV contains subunits; I, II, III, and V are linker proteins.

the ratio of band densities of the PE-AP complex subunits to that of its linkers was smaller than those of the intact PBS. This demonstrates that the PE-AP complex is composed of AP and PC components as well as of PE, and that the proportion of the red α- and β-subunits from the PE component except the γ-subunit is much smaller than in intact PBS. In other words, the PE-AP complex contains proportionally more of AP, PC, and linker components with respect to the PE. The PBS that was dissociated by the incubation at 37 °C for 4 h had a red PE band in the front of the gel lane (Fig. 5, *lane b*). This band was absent in the PBS that was incubated at 100 °C for 2 to 3 min (*lane d*) which indicates that the PE of the red band is thermostable.

Discussion

The obtained PBSs only showed a rather faint coloured bands in the upper layers of the sucrose gradients except the precipitated PBSs after they was dissociated at 4 °C for 4 h in the 0.1 M phosphate buffer with 0.2 M sucrose. The coloured bands occurred obviously during the dissociation reaching 8 h. The thick coloured bands were present only if the incubation of PBSs lasted beyond 10 h. Therefore we incubated the intact PBSs at 4 °C for 12 h to ensure that they were adequately dissociated. We believe that the stability of PBS at 4 °C is closely linked with the living habit of *P. urceolata* at 5 to 15 °C. Because of this stability, the prolonged incubation hardly gave the dissociated PBS components a chance to create artificial biliprotein complexes by re-association. More recently, we got a PE-component fairly different from R-PE in the biliprotein extract of *P. urceolata* in 0.05 M phosphate buffer (pH 7.0) using a *Sephadex G-150* column. Absorption spectrum (not shown) of the PE-component was fairly similar to that of the PE-AP complex, but without distinct absorption at 620 and 650 nm. Moreover, the PE-component gave no emissions of R-PC and AP that the PE-AP complex presented. In *P. urceolata,* hence, the PE component is another PE biliprotein that takes part in the formation of the PE-AP complex. Accordingly, the obtained PE-AP complex is a natural complex from the dissociation of the *P. urceolata* PBS.

The spectral characteristics and the polypeptide components of the PE-AP complex evidence that it is composed of AP, PC, and various linker proteins as well as a three-peak PE of the R-PE kind. The AP and PC components of the complex account for the absorption at 655 and about 620 nm that is distinctive in the second derivative spectrum (Fig. 2). The highest absorption peak of 500 nm is ascribed to the higher proportion of the γ-subunit in the complex (Glazer 1984, Zilinskas 1986, Rowan 1989). The emission and excitation spectra of PE-AP complex (Figs. 3 and 4) allow a conclusion that there is poor energy coupling among its biliprotein components AP, PC, and PE.

The larger proportional linker components, to which the strong absorption from 350 to 450 nm is attributed, may account for the poor solubility of the PE-AP complex in the phosphate buffer. Its various linkers are adequately consistent with its biliproteins, the PE, PC, and AP. Moreover, possessing the linkers of 120-70 kDa suggests that the PE-PA complex may be adjacent or attached to the membranes. Although its emission at 665 nm is several nanometer shorter than that of the terminal emitters of the intact PBS, the fact that the 670 nm band (Fig. 4*C*) attributes to α^{APB} (Füglistaller *et al.* 1987, Reuter *et al.* 1990) and the emission bands from 668 to 680 nm (Fig. 3*D*) shown distinctively in the second derivative spectra and the Gaussian deconvolution imply that the obtained PE-PA complex may have some terminal emitters of APB or AP_{CM} (Reuter and Wehrmeyer 1990, Capuano *et al.* 1991). In conclusion, the obtained PE-AP complex is in PBS a mediator of radiant energy transfer between the R-PE of the row and the terminal emitters of the core.

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