Phycobilisome Structure of *Porphyridium cruentum*¹

**POLYPEPTIDE COMPOSITION**

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**ABSTRACT**

Purified phycobilisomes of *Porphyridium cruentum* were solubilized in sodium dodecyl sulfate and resolved by sodium dodecyl sulfate-acrylamide gel electrophoresis into nine colored and nine colorless polypeptides. The colored polypeptides accounted for about 84% of the total stainable protein, and the colorless polypeptides accounted for the remaining 16%. Five of the colored polypeptides ranging in molecular weight from 13,300 to 19,500 were identified as the α and β subunits of allophycocyanin, R-phycocyanin, and phycocerythrin. Three others (29,000–30,500) were orange and are probably related to the γ subunit of phycocerythrin. Another colored polypeptide had a molecular weight of 95,000 and the characteristics of long wavelength-emitting aBophycocyanin. In addition, the core fraction was enriched in a colored 95,000 dalton polypeptide. Inasmuch as a polypeptide with the same molecular weight is found in thylakoid membranes (free of phycobilisomes), it is suggested that this polypeptide is involved in anchoring phycobilisomes to thylakoid membranes.

PBS,² of the red alga *Porphyridium cruentum*, are known to consist largely of B-PE and b-PE, some R-PC, and a small amount of APC (4, 6, 15). In the initial investigation on the pigment composition of the PBS of this alga, it was reported that the phycobiliproteins could account for the total protein composition (4). In light of the findings by Tandeau de Marsac and Cohen-Bazire (19) and others (20) that in PBS of cyanobacteria, up to 15% of Coomassie stainable protein is due to colorless polypeptides, the PBS of *P. cruentum* have been reexamined. Colorless polypeptides have been suggested to be involved in PBS structure by functioning as possible linker proteins between phycobiliproteins, or in attaching PBS to the thylakoid (19).

With the exception of two preliminary reports (1, 18), this investigation represents the first detailed results on the polypeptide patterns of PBS of a red alga. It shows that the polypeptide pattern of these PBS is the most complex of any thus far studied. Evidence is also presented here indicating that most of the colorless polypeptides are associated with the PBS core. In addition, one of the high mol wt core components is proposed as a polypeptide involved in anchoring the PBS to the thylakoid.

**MATERIALS AND METHODS**

Cell Culture. Cultures of *P. cruentum* were grown axenically in the medium of Jones et al. (11), using conditions already described by Gantt et al. (4, 5). Cells were harvested when the cell density was 4 g/l.

PBS Preparation. Cells were disrupted in a French pressure cell at 10,000 p.s.i. in 0.5 M phosphate buffer (pH 7.0). The homogenate was made to 1% (v/v) with Triton X-100 and stirred at 4°C for 30 min. PBS were separated from the detergent treated homogenate by sucrose gradient centrifugation after Gantt et al. (5). PBS prepared in this manner were free of any detectable Chl in an 80% acetone extract (A at λ₆80 was <0.001, and λ₅40 >2.0).

PBS Dissociation. PBS were resuspended in 33 mM Na-phosphate (pH 7.0), to a final concentration of 100 μg/ml and dialyzed at 4°C against the same buffer for 4 h. The dialysate was centrifuged at 254,000 g for 30 min. The pink supernatant (S₂) contained the initial dissociated products. The pellet (P₁) was resuspended in 10 mM Na-phosphate (pH 7.0), to a final concentration of 100 μg/ml and dialyzed at 4°C against the same buffer for 12 h. The dialysate was centrifuged as above and a bluish-pink supernatant (S₃) and a blue pellet (P₃) were obtained. Temperatures of the samples during dissociation and centrifugation procedures were maintained at 4 and 12°C, respectively.

Thylakoid Isolation. Cells were disrupted in a French pressure cell at 5,000 p.s.i. in 0.3 M sucrose, 25 mM Hepes-KOH (pH 7.5), and 1 mM MgCl₂. The homogenate was centrifuged at 10,000 g for 10 min and the green pellet was washed twice with 0.3 M sucrose, 5 mM Hepes-KOH (pH 7.5), and 10 mM EDTA. The washed membranes were purified by flotation centrifugation as described by Chua and Bennoun (2). Thylakoids were free of PBS as determined by minimal absorption at 540 to 620 nm (A at λ₆10 was about 0.1, at λ₆80 about 2.0) and by absence of visible PBS structures on vesicle preparations viewed by electron microscopy.

Native Gel Electrophoresis. Phycobiliproteins were purified from S₁ and S₂ by preparative gel electrophoresis (10) on a 5% polyacrylamide gel. The colored phycobiliprotein bands were excised and extracted from the gel as described by Redlinger and Apel (17), except that the final pigment residue was taken up in 100 mM Na-phosphate (pH 7.0).

SDS Polyacrylamide Gel Electrophoresis. Samples were analyzed on SDS (0.1%, w/v) polyacrylamide gradient gels (7.5–15%) utilizing the Laemmli buffer system (14). Samples were solubilized either at 4°C or by boiling for 2 min in 10% sucrose, 50 mM Tris-HCl (pH 6.8), 1% SDS (w/v), and 100 μM mercaptoethanol. For preparative isolations, 3-mm thick gels were used, while for analytical purposes, a 1.5-mm gel was used. Gels requiring staining were stained with Coomassie blue and destained as previously described (3). Polypeptide mol wt were calculated from protein markers: β-galactosidase 116,000; BSA 69,000; carbonic anhydrase 37,000; bovine serum albumin 67,000; and phosphorylase b 97,400.

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2 Abbreviations and definitions: PBS, phycobilisomes; PE, phycocerythrin designated as b-(small) or B-(large) native molecular weight (4); R-PC, rhodophycyan phycocyanin containing phycocyanobilin and phycocerythrin chromophores; APC, allophycocyanin.

3 Received from publication May 18, 1981 and in revised form July 20, 1981.
**RESULTS AND DISCUSSION**

**Polypeptide Composition of Phycobilisomes.** The PBS of *P. cruentum* were resolved into 18 polypeptide bands by SDS polyacrylamide gel electrophoresis (Fig. 1). Nine polypeptides were associated with phycobilin chromophores while the remaining nine were colorless and were visible only when gels were stained with Coomassie blue. Phycobiliprotein polypeptides accounted for ~84% and the colorless polypeptides for ~16% of total Coomassie stainable protein. These percentages are comparable to those in other PBS (1, 19, 20). However, most other PBS have fewer colorless polypeptides than does *P. cruentum*.

Little difference between polypeptide migration during SDS electrophoresis was noted between boiled and nonboiled samples (Fig. 1). However, fading of the phycobilins was evident when samples were boiled. The similarity of results with boiled and nonboiled samples indicated that phycobiliproteins are sufficiently solubilized in 1% SDS and do not require heat for dissociation. Even so, polypeptide mol wt were determined from boiled samples and only the absorption maxima of pigmented bands were obtained from the corresponding colored band isolated from the non-boiled sample (Table I).

The phycobilisomes of *P. cruentum* contained polypeptides of molecular weights ranging from 13,300 to 95,000. Five of the nine colored bands were identified: one unresolved band with the α and β subunits of B-PE and B-PE, a pair with the α and β subunits of RPC, and another pair with the α and β subunits of APC. These identifications were made by matching the electrophoretic migration of bands of PBS polypeptides with the migration of bands from B-PE, B-PE, R-PC, and APC, which were isolated as described previously (4). The mol wt of B-PE, B-PE, and R-PC which were obtained here (Table I) agreed with previously reported values (4, 8, 12). Previously, APC of *P. cruentum* had been resolved only as a single band (4, 15), but on the gradient gel system used, it was resolved into two polypeptides of 13,300 and 95,000 daltons.

![Table I. Characteristics of *P. cruentum* Polypeptides](https://www.plantphysiol.org/)

<table>
<thead>
<tr>
<th>Band No.</th>
<th>Mol. wt. (daltons)</th>
<th>% Total PBS Protein</th>
<th>Associated Pigment Absorption (λ&lt;sub&gt;max&lt;/sub&gt;)</th>
<th>Band Identification&lt;sup&gt;a&lt;/sup&gt; or Protein Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>95,000</td>
<td>1.3</td>
<td>650</td>
<td>PBS anchor?</td>
</tr>
<tr>
<td>2</td>
<td>60,000</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>49,000</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>38,000</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>35,500</td>
<td>1.3</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>7</td>
<td>31,500</td>
<td>3.0</td>
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<td></td>
</tr>
<tr>
<td>8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>30,500</td>
<td>2.0</td>
<td>495</td>
<td></td>
</tr>
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<td>9</td>
<td>29,500</td>
<td>0.8</td>
<td>495</td>
<td>B-PE γ subunit</td>
</tr>
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<td>0.4</td>
<td>495</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>25,500</td>
<td>1.4</td>
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<td></td>
</tr>
<tr>
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</tr>
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<td>21,000</td>
<td>0.1</td>
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<tr>
<td>14&lt;sup&gt;*&lt;/sup&gt;</td>
<td>19,500</td>
<td>62.3</td>
<td>545</td>
<td>α, β subunits of B and b-PE</td>
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<tr>
<td>15&lt;sup&gt;*&lt;/sup&gt;</td>
<td>18,700</td>
<td>550</td>
<td>620</td>
<td>R-PC β subunit</td>
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<td>15,000</td>
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<tr>
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<tr>
<td>18</td>
<td>13,300</td>
<td>4.1</td>
<td>650</td>
<td>APC α subunit</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers correspond to band numbers in Figure 1. Asterisk indicates colored polypeptides.

<sup>b</sup> Protein content calculated from area under peaks of gel scans as described under "Materials and Methods."

<sup>c</sup> Pigment absorption max were measured on samples after electrophoresis on SDS-acrylamide gels and extractions.

<sup>d</sup> Band identity was based on comparison with isolated phycobiliprotein as described by Gantt and Lipschultz (4).

**Fig. 1.** Polypeptides of *P. cruentum* phycobilisomes separated by SDS polyacrylamide gel electrophoresis. PBS (100 µg protein) were solubilized in sample buffer and either boiled (100°C) for 2 min (b) or not boiled (nb) and applied to the gel slot. Bands were sequentially numbered, beginning with the high mol wt species. Bands 2 and 3 are seen as doublets when separated further. An asterisked number indicates original colored bands which had visible color before staining with Coomassie blue.

29,000; Cyt c 12,500. For estimation of relative protein content per band, stained gels were scanned at 590 nm with a Gilford spectrophotometer equipped with a gel scanner. Relative area under the peaks was integrated according to Simpson's rule. In making these estimates, it was assumed that the A at 590 nm, because of staining with Coomassie blue, was linear with protein concentration, and that the specificity of staining did not vary with the polypeptide composition.

**Fluorescence and Absorption Measurements.** Fluorescence emission spectra were measured at −196°C in an Aminco-Bowman fluorometer with corrected emission (5). Absorption spectra were measured at room temperature in a Cary 17 spectrophotometer.
Fig. 2. Absorption (---) and corrected fluorescence (----) emission spectra for the electrophoretically purified 95,000-dalton polypeptide. Samples were measured in 100 mM Na phosphate buffer (pH 7.0), containing 30% glycerol. For fluorescence measurements, the solution was adjusted to an $A$ of 0.05, frozen to $-196^\circ$C, and excited at 640 nm. Absorption measurements were at $23^\circ$C.

Fig. 3. Absorption spectra of electrophoretically purified polypeptides: bands 8, 9, and 10. Samples were measured in 100 mM Na-phosphate buffer (pH 7.0), containing 30% glycerol. Solutions were adjusted to an $A$ of 0.3 to 0.5.

Fig. 4. SDS-polyacrylamide gel electrophoresis of PBS fractions, $S_1$, $S_2$, and $P_2$. The two soluble fractions ($S_1$ and $S_2$) and an insoluble fraction ($P_2$) were obtained by the fractionation procedure as described under "Materials and Methods." One hundred $\mu$g protein was applied to each slot. The numbers on the left side refer to the numbered polypeptides in Figure 1.

15,000. These latter polypeptides were present in approximately a 1:1 ratio as estimated by absorbance of stained gels.

Polypeptide 1 was blue, and had a mol wt of 95,000. The room temperature absorbance spectrum has $\lambda_{max}$ at 610 and 650 nm with a shoulder at 667 nm (Fig. 2). The low temperature fluorescence spectrum has a single emission peak at about 680 nm (Fig. 2) which is characteristic of long wavelength-emitting APC (15), and appears to be the final emitter of intact PBS (7). The mol wt of this polypeptide remained the same when it was extracted from the gel, boiled in 2% SDS and 1% $\beta$-mercaptoethanol. The possible significance of this polypeptide will be discussed below.

Polypeptides 8, 9, and 10 were orange, and had mol wt of 30,000, 29,500, and 29,000, respectively. Each had an absorption maximum at 495 nm (Fig. 3), which suggests that the chromophore is phycourorubin (9, 16). Absorption ascribable to phycoerythrobilin was not evident in the spectra. Phycoerythrobilin, as well as phycourorubin, have been reported to occur in the $\gamma$-polypeptide of B-PE (9). Isomerization of phycoerythrobilin to phycourorubin might account for the observed difference (9); however, no indication of phycoerythrobilin isomerization was present in the spectrum of band 14. Although it is to be expected that at least one, or
Dissociation of composition. The gel was PBS and as nm) all ionic strengths were judged by uv absorption of uncolored discrepancies apparent peptide (Fig. 4-6). Dissociation. To determine the possible association of uncolored polypeptides with specific phycobiliproteins, the PBS were subfractionated by controlled partial dissociation in low ionic strength buffer. This procedure, based on earlier studies (6) in P. cruentum PBS, had shown an ordered phycobiliprotein release (PE → R-PC → APC) under lowered ionic conditions with time (Figs. 4-6).

Two highly colored soluble fractions were obtained, S1 by dissociation of PBS in 33 mM phosphate buffer, and S2 by further dissociation of (P)ellet, in 10 mM phosphate buffer (see "Materials and Methods"). The insoluble material remaining after the second dissociation was designated P2. The S1 and S2 fractions were enriched in PE (λmax 545 nm) relative to APC (λmax 620 and 650 nm) as judged by absorbance spectra (not shown) and polypeptide composition. The P2 was enriched in APC. The P2 assumed to represent the PBS core (6), was enriched for all of the colorless polypeptides, except for polypeptide 6 when compared to PBS, S1, or S2 (Fig. 4). However, S1 and S2 contained detectable quantities of colorless polypeptides 3–9. This indicates that colorless polypeptides 2, 7, and 11 to 13 are associated with P2, the PBS core, and directly or indirectly with APC. In addition, colored polypeptide 1 (95,000) is specifically associated with P2 and thus, seems to

be part of the PBS core. Enrichment of the S1 fraction by the colorless polypeptide 6, relative to S2, P2 or PBS is of interest because it strongly suggests that it is near the periphery of the PBS with PE.

In a previous investigation on the pigment analysis of P. cruentum, colorless polypeptides were not detected in phycobiliproteins isolated by using non-denaturing gel electrophoresis and SDS electrophoresis (4). Therefore, the phycobiliproteins were reexamined here using a higher resolution SDS-gel system.

When S1 and S2 were electrophoresed on non-denaturing gels (Fig. 5), colored bands were obtained and identified as R-PC (band a), APC (band b), B-PE (bands c, d), and b-PE (bands e–g) by measuring absorption spectra of proteins isolated from the bands and comparing absorbance spectra of previously published data (4). The multiple bands for B-PE and b-PE are probably charge isomers (12). On staining the non-denaturing gels with Coomassie blue, only those proteins stained which had been

![Fig. 5](image-url)

Separation of phycobiliproteins of phycobilisome subfractions S1 and S2 on nondenaturing gels. Separation was on a 5% polyacrylamide gel without SDS at pH 7.9. Seven colored bands were observed: a, R-phycocyanin; b, allophycocyanin; c–g, phycocerythrin.

![Fig. 6](image-url)

SDS polyacrylamide gel electrophoresis of phycobiliproteins isolated from phycobilisome subfractions S1 and S2. Phycobiliprotein samples were prepared by separating them on non-denaturing polyacrylamide gels (bands a, b, c, g, in Fig. 4), extracting them from the gel, and denaturing in the presence of sodium dodecyl sulfate. Lanes B, C, D, E are allophycocyanin, R-phycocyanin, b-, and B-phycocerythrin, respectively (each 40 μg protein). Lanes A, F are PBS at 100 and 75 μg of protein, respectively.
identified as phycobiliprotein. No additional protein bands were detected. This led to the consideration of whether or not the colorless polypeptides detected in S1 and S2 remained associated with the various phycobiliproteins displayed on nondenaturing gels of S1 and S2. To test this, bands a, b, c, and g were extracted from the nondenaturing gels and were examined by SDS gel electrophoresis (Fig. 6). The B-PE (band c, Fig. 5) was found to have a colorless band associated with it (Fig. 6), corresponding to polypeptide 6. Except for this polypeptide, the colorless polypeptides 3, 4, and 5 appear to have been lost, possibly during electrophoresis by not migrating into the nondenaturing gel.

**A 95,000 Polypeptide in Thylakoids and Phycobilisomes.** Of special interest in the energy transfer from PBS to the photosynthetic membrane is the pigmented 95,000 mol wt polypeptide because it has an emission at F680 nm (−196°C), which is characteristic of long wavelength-emitting APC (15). This long wavelength APC most probably is positioned close to PSI and PSII to enable efficient energy transfer. Also, since this polypeptide was found mainly in the core fraction (P3), its proximity to the thylakoid membrane is probable. A large polypeptide of 95,000 mol wt was also observed in thylakoids washed free of PBS (gel not shown). Highly similar trypptic digest patterns were obtained from the 95,000 mol wt polypeptide separately isolated from PBS and from washed thylakoids (18). These results strongly suggest that this polypeptide is a common component, and may serve as a link between the PBS core and membranes. In other red algae, a blue colored polypeptide with a similar mol wt has also been found in _P. sordidum_ (observations, our laboratory), and two high mol wt polypeptides (95,000 and 86,000) reported as colorless, were also suggested to be associated with the core fraction in _Rhodella_ (13).

**CONCLUSION**

Previously it had been found (4) that the entire protein content of PBS could be accounted for by phycobiliproteins. This conclusion was based on applying extinction coefficients for four phycobiliproteins to determine PBS protein content. Although a reasonable approximation at that time, it now needs to be modified in light of the present SDS gel findings. Phycobilisomes of the red alga, _P. cruentum_, like PBS of cyanobacteria also contain ~15% colorless polypeptides as determined from Coomassie stained gels. The exact role of the colorless polypeptides in the PBS is at present not understood; however, they may be involved in stabilizing phycobiliprotein associations for enhanced energy transfer. We show here that the colorless polypeptides are not randomly distributed within the PBS. Most of them are in the core fraction, while one colorless polypeptide (32,500 mol wt) was associated with B-PE, presumably at the PBS periphery. This polypeptide may function in linking PE to PE. The presence of other colorless polypeptides in the core fraction suggests that they may function in attaching R-PC to APC, and APC to APC. There may be a positive correlation between PBS complexity and the number of colorless polypeptides. _P. cruentum_ PBS with nine colorless polypeptides has the most complex composition reported to date (B-PE, b-PE, R-PC, APC, and APC-B) (4, 15). On the other hand, _Synechococcus_ 6301 PBS has a simpler composition (C-PC, APC, APC-B) and only five colorless polypeptides (20).

**LITERATURE CITED**

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