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Isolation and Function of Allophycocyanin B of *Porphyridium cruentum*[1]

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ABSTRACT

Allophycocyanin B was purified to homogeneity from the eukaryotic red alga *Porphyridium cruentum*. This biliprotein is distinct from the allophycocyanin of *P. cruentum* with respect to subunit molecular weights, and spectroscopic and immunological properties. The purified allophycocyanin B has a long wavelength absorption maximum at 669 nm at room temperature and at 675 nm at −196 C while the fluorescence emission maximum is at 673 nm at room temperature and 679 nm at −196 C. The emission spectrum of allophycocyanin shifted only 1 nm, from 659 to 660 nm, on cooling to −196 C, and was the same with allophycocyanin crystals as it was with pure solutions of the pigment. Phycobilisomes from *P. cruentum* have a major fluorescence emission band at 680 nm at −196 C which emanates from the small amount of allophycocyanin B present in the phycobilisomes. Light energy absorbed by the bulk of the biliprotein pigments is transferred to allophycocyanin B with high efficiency.

Phycobilisomes of red and blue-green algae contain several different phycobiliproteins which function together as an accessory pigment system to absorb light not otherwise absorbed by the photosynthetic apparatus, and to transfer the excitation energy to the Chl of PSII. The phycobilisomes are attached to the outer surface of the thylakoid membranes (6, 7) and energy transfer from the shorter wavelength-absorbing biliproteins (phycoerythrin, phycocyanin, and allophycocyanin) to the longest wavelength-absorbing pigment (allophycocyanin B) directs the excitation energy toward the photochemical apparatus in the membranes (5, 10).

Glazer and Bryant (15) recently isolated and purified a new long wavelength-absorbing phycobiliprotein (λ_{max} = 671 nm), denoted allophycocyanin B, from cyanobacteria. They suggested that the allophycocyanin B, which was present in the phycobilisomes in very small amounts, might serve to accept excitation energy absorbed by the other phycobiliprotein pigments and to transfer that energy to the Chl of PSII. Such an energy transfer role was also inferred from studies of fluorescence of intact cells of *Porphyridium cruentum* at low temperature (24). In fact, it was concluded, from the presence of the 680 nm emission band of allophycocyanin B in the spectrum of the fluorescence of variable yield at −196 C, that excitation energy could be transferred not only in the direction from allophycocyanin B to PSII Chl but also in the reverse direction from PSII Chl back to allophycocyanin B (especially in PSII units which had closed reaction centers) (24). Wang and Myers (27) also reported a reverse transfer of energy from Chl to allophycocyanin from measurements on *Anacystis nidulans* at room temperature.

However, the presence of allophycocyanin B in *P. cruentum* was only inferred from fluorescence measurements on intact cells. Until now, the presence of this pigment in a eukaryotic alga had not been demonstrated. The purpose of the present work was to isolate and purify allophycocyanin B from *P. cruentum*, to compare the physical and immunological properties of allophycocyanin B from *P. cruentum* with those of the pigment isolated from cyanobacteria, and to confirm the postulated role of allophycocyanin B in the phycobilisomes of *P. cruentum*.

MATERIALS AND METHODS

P. cruentum B, obtained from Scripps Institution of Oceanography, La Jolla, Calif., was grown axenically in the medium of Jones *et al.* (22) to a density of 2.5 to 3 g/l. A unialgal culture of *Porphyridium aerugineum*, originally from the collection of R. Y. Stanier, was obtained from E. Gantt. Cells were grown in the medium of Pinter and Provasoli, as described by Gantt *et al.* (8), to a density of 2.5 to 3.5 g/l. An axenic culture of *Anabaena* sp. 6411 was obtained from the Berkeley collection (25), and cultured in medium Bg11, as previously described (25).

Chemicals. Preswollen Whatman microgranular DEAE-cellulose DE52 was obtained from Reeve Angel, Clifton, N.J. Ampholytes (40%, w/v, Bio-Lyte) were obtained from Bio-Rad, Richmond, Calif. Electrophoresis grade acrylamide was obtained from Eastman Organic Chemicals, Rochester, N.Y. All other chemicals were reagent grade.

SDS-Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide gel electrophoresis was performed in gels (0.6 × 10.5 cm) prepared with 10% acrylamide and double the normal amount of cross-linker, as described by Weber and Osborn (28). Electrophoresis was performed at 4 mamp/gel for 16 hr.

Isoelectric Focusing. Isoelectric focusing was performed on photopolymerized 7.5% polyacrylamide gels (0.6 × 10.5 cm) in the pH range 4 to 6 according to the procedure of Bio-Rad Laboratories (Technical Bulletin 1030, April, 1975). Isoelectric points were determined by eluting the protein bands into 0.01 M KCl containing 5 mM NaN_3.

Purification of Proteins. The pure *P. cruentum* biliproteins R-

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phycocyanin, b-phycoerythrin, and B-phycoerythrin were the kind gift of C. S. Hixson and had been prepared as previously described (18, 19). Crystalline allophycocyanin from *Anabaena* sp. 6411 was prepared as described by Bryant *et al.* (1).

Allophycocyanin and allophycocyanin B were purified from an already highly purified allophycocyanin fraction from *P. cruentum* (also a gift from C. S. Hixson). This fraction had been obtained by chromatography of the biliproteins of *P. cruentum* on DEAE-cellulose at pH 5.5, repeated chromatography on hydroxylapatite, and gel filtration on Sephadex G-200. This fraction, containing about 30 mg of protein, was dialyzed exhaustively against 0.02 M sodium acetate (pH 5.3) containing 1 mM 2-mercaptoethanol and 1 mM NaN_3, centrifuged for 10 min at 27,000*g* to remove any precipitated protein, and applied to a column (1.5 × 20 cm) of DEAE-cellulose DE52 preequilibrated with 0.02 M sodium acetate buffer (pH 5.3). The column was then developed with a linear gradient of NaCl (0–0.15 M, total volume 200 ml) in the 0.02 M Na-acetate buffer at pH 5.3. The resulting elution profile is shown in Figure 1.

The fractions enriched in a component absorbing at about 670 nm were pooled (as indicated by the bar labeled I in Fig. 1) and this material was dialyzed against 0.05 M ammonium acetate (pH 6.8) containing 1 mM 2-mercaptoethanol and 1 mM NaN_3. The protein solution was concentrated by ultrafiltration with an Amicon cell equipped with a UM-10 membrane to approximately 5.5 mg/ml. Aliquots (500 μg protein) of this solution were then applied to each of 12 isoelectric focusing gels prepared with ampholytes in the pH range 4 to 6 and were focused for about 18 hr. Two blue bands were separated upon isoelectric focusing (Fig. 2A).

These protein bands were cut from the gels, eluted with 0.01 M KCl containing 5 mM NaN_3, reconcentrated by ultrafiltration, and run again on isoelectric focusing gels (30 μg protein/gel; pH 4–6). The allophycocyanin B eluted from the lower zone (pH 5.45), was found to be homogeneous by isoelectric focusing (Fig. 2B). The eluate from the broad allophycocyanin zone, with an average pH of 4.95 was concentrated by ultrafiltration and refocused under the conditions described above to remove a trace of residual allophycocyanin B. The allophycocyanin fraction eluted from these gels was homogeneous as determined by isoelectric focusing in the pH range 4 to 6 (Fig. 2D) and by SDS-polyacrylamide gel electrophoresis (Fig. 2E).

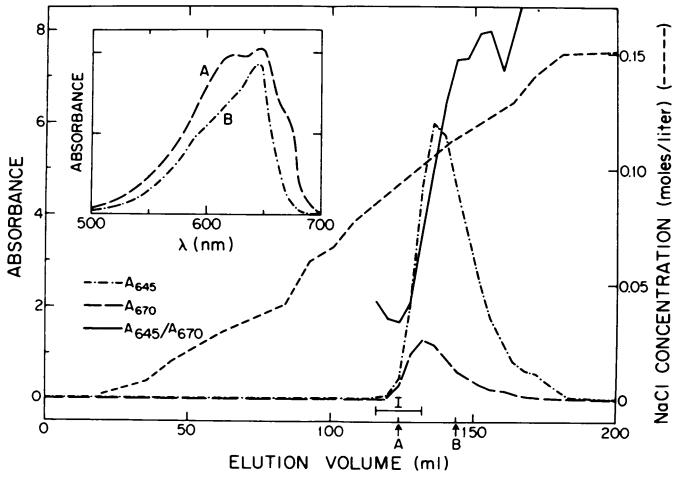

FIG. 1. Chromatographic fractionation of *P. cruentum* allophycocyanins on a column (1.5 × 20 cm) of DEAE-cellulose DE52 with a linear gradient of NaCl (0–0.15 M) in 0.05 M Na-acetate buffer at pH 5.3. The fractions indicated by the bar labeled I were pooled and concentrated for subsequent electrophoresis (Fig. 2). Spectra A and B, shown in the inset, were recorded for fractions collected from the column at the elution volumes indicated by arrows A and B, respectively. For other experimental details, see text.

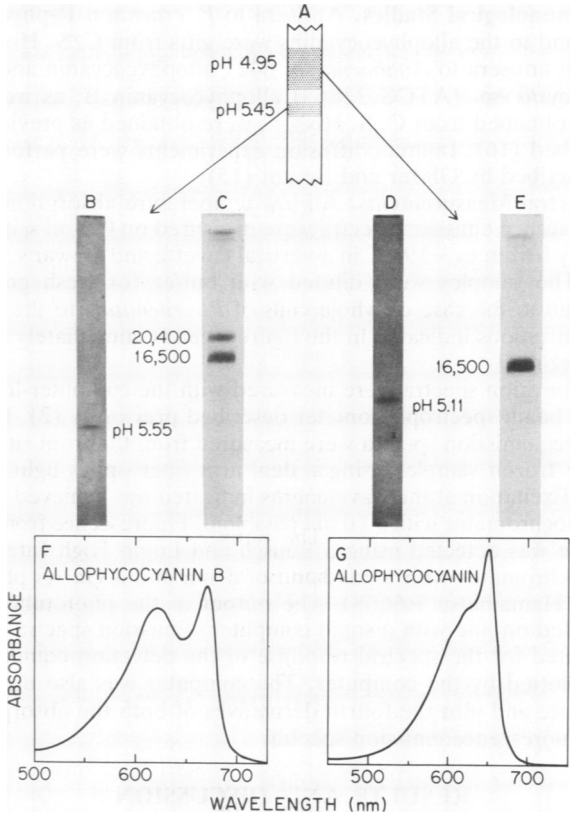

FIG. 2. A–G. purification and characterization of *P. cruentum* allophycocyanin and allophycocyanin B. A: schematic drawing showing isoelectric focusing in the range from pH 4 to 6 of the pooled fractions indicated by the bar labeled I in Figure 1. Isoelectric points are shown. For experimental details, see Figure 1 and text. B: refocusing of eluted allophycocyanin B in the range from pH 4 to 6. Protein was purified by isoelectric focusing as shown in A. C: polyacrylamide gel electrophoresis in the presence of SDS of purified allophycocyanin B. Apparent subunit mol wt are shown. D: isoelectric focusing of allophycocyanin in the range pH 4 to 6. Protein was purified by refocusing the allophycocyanin fraction obtained as shown in A and as described in text. E: polyacrylamide gel electrophoresis in the presence of SDS of purified allophycocyanin. F: visible absorption spectrum of allophycocyanin B, recorded at room temperature in 0.05 M K-phosphate (pH 7) containing 0.01 M KCl and 2 mM NaN_3. G: visible absorption spectrum of allophycocyanin, recorded at room temperature in 0.05 M K-phosphate (pH 6.85) containing 0.05 M KCl and 2 mM NaN_3.

Biliprotein fractions enriched in allophycocyanin B were also obtained with phycobilisomes as starting material. Because the content of allophycocyanin B is so low relative to that of the other biliproteins, these preparations were not carried through to homogeneity.

Preparation of Phycobilisomes. Phycobilisomes were prepared by the procedures of Gantt (9, 21) with minor modifications. All phycobilisomes were isolated in 0.75 M K-phosphate buffer (pH 7) containing 1 mM 2-mercaptoethanol and 1 mM NaN_3. Triton X-100 (1%, w/v) was used to release phycobilisomes from the thylakoid membranes. The sucrose step gradients used were prepared from the following sucrose concentrations prepared in the 0.75 M *K*-phosphate buffer: 0.25 M, 0.50 M, 0.75 M, 1 M, and 2 M. The ratio of these sucrose solutions by volume was 1:1.33:2.33:1.33:1, respectively. Sample volumes were approximately equal to the volume of 0.25 M sucrose zone. The gradients were centrifuged in the Beckman SW 41 rotor at 20 C for 5 to 6 hr at 270,000*g*. Phycobilisomes were collected from the 1 M sucrose zone of the step gradients. Contaminating membrane fragments were removed by centrifugation for 15 min at 27,000*g* after a 1:10 dilution of the phycobilisome fraction with the 0.75 M K-phosphate buffer.

Immunological Studies. Antisera to *P. cruentum* R-phycocyanin and to the allophycocyanins were gifts from C. S. Hixson. Rabbit antisera to *Anabaena* sp. 6411 allophycocyanin and *Synechocystis* sp. (ATCC 22663) allophycocyanin B, as well as those obtained from C. S. Hixson, were obtained as previously described (16). Immunodiffusion experiments were performed as described by Glazer and Bryant (15).

Spectral Measurements. All low temperature absorption and fluorescence emission spectra were measured on 0.5-ml samples rapidly frozen to −196 C in a vertical cuvette and Dewar system (2). The samples were diluted with buffer (or fresh growth medium in the case of whole cells of *P. cruentum*) to the final concentrations indicated in the figure legends immediately prior to freezing.

Absorption spectra were measured with the computer-linked single beam spectrophotometer described previously (2). Fluorescence emission spectra were measured from the front surface of the frozen samples using a dual arm fiber optics light pipe (26). Excitation at the wavelengths indicated was achieved from a monochromator with a 10 nm passband. Fluorescence from the sample was detected using a Bausch and Lomb High Intensity Monochromator with a passband of 5 nm and a Ga-As phototube (Hamamatsu R666S). The output of the phototube was recorded on line with a small computer. Emission spectra were corrected for the spectral response of the detection equipment and plotted by the computer. The computer was also used to calculate and plot the fourth derivatives of both the absorption and fluorescence emission spectra.

RESULTS AND DISCUSSION

The highly purified preparation of allophycocyanin from *P. cruentum* was found to contain both allophycocyanin and allophycocyanin B. The first protein fractions eluted upon DEAE-cellulose column chromatography of the allophycocyanin preparation contained significant amounts of a 670 nm absorbing pigment. The inset in Figure 1 shows the absorption spectra of fractions collected at the elution volumes indicated by arrows A and B. It is apparent that the earlier fraction is enriched in a pigment absorbing at 670 nm.

The fractions enriched in the 670 nm absorbing material (indicated by the bar labeled I in Fig. 1) were pooled, concentrated, and run on isoelectric focusing gels (pH range 4–6). Two blue bands were separated (Fig. 2A). The pigments eluted from these two bands were concentrated and again subjected to isoelectric focusing under the same conditions. The material from the lower band, allophycocyanin B, refocused as a single band at pH 5.55 (Fig. 2B). On SDS-polyacrylamide gel electrophoresis, this protein was shown to be composed of two subunits with apparent mol wt of 20,400 and 16,500 (Fig. 2C). A monomer structure made up of two dissimilar subunits is characteristic of biliproteins as a class (13). The aggregation state of *P. cruentum* allophycocyanin B was not examined, but the corresponding protein from *A. variabilis* was found to be a trimer, $(\alpha\beta)_3$ (1).

The allophycocyanin from the upper band (Fig. 2A), isolated by elution and purified further by refocusing under the same conditions, was shown to be homogeneous by isoelectric focusing (Fig. 2D), and by SDS-polyacrylamide gel electrophoresis (Fig. 2E). Allophycocyanin had an isoelectric point of 5.11, and an apparent subunit mol wt of 16,500. In contrast to the α and β subunits of allophycocyanin B (see above), the two subunits of the allophycocyanin are very similar in size, although they have been shown to be distinct in their amino-terminal sequence (14).

The absorption spectra of purified allophycocyanin B and allophycocyanin are shown in Figure 2, F and G, respectively. We estimate that the original material applied to the DEAE-cellulose column had a ratio of allophycocyanin to allophycocyanin B about 15:1.

Some of the immunological properties of allophycocyanin and allophycocyanin B are illustrated in Figure 3 (A–D). As previously reported for cyanobacterial allophycocyanins (15, 17), *P. cruentum* allophycocyanin and allophycocyanin B do not cross-react with antisera produced against R-phycocyanin (Fig. 3A) or C-phycocyanins (not shown).

P. cruentum allophycocyanin and allophycocyanin B are distinct immunologically. The crossing of the precipitin lines between wells b and c (Fig. 3B) is characteristic of a two-antigen, two-antibody system. Indeed this result is to be expected since the immunizing antigen used in obtaining the antiserum to *P. cruentum* allophycocyanin is now known to have contained allophycocyanin B.

Both allophycocyanin and allophycocyanin B cross-react with an antiserum produced against *Anabaena* sp. 6411 allophycocyanin (Fig. 3C). A spur seen behind the precipitin line formed with *P. cruentum* allophycocyanin B, but not seen in the case of

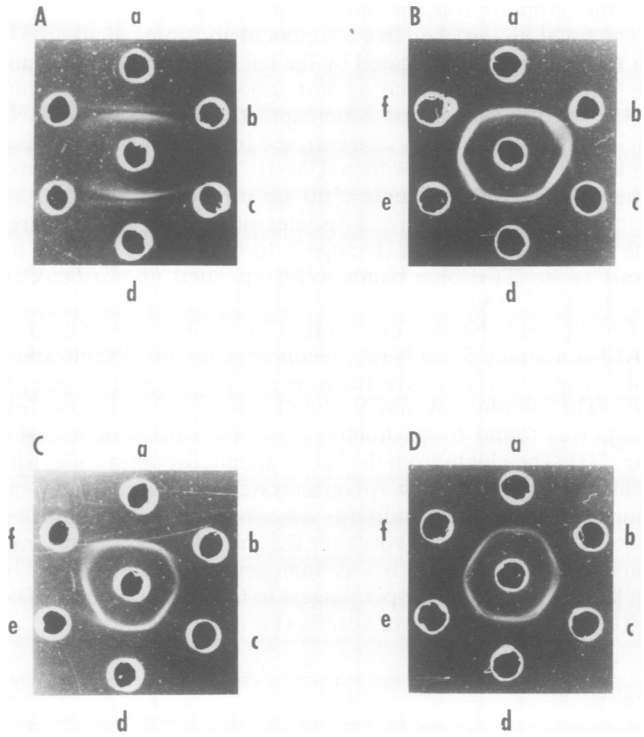

FIG. 3. Ouchterlony double diffusion experiments. In all experiments shown, antibody wells contain 20 μl of undiluted antiserum, and antigen wells contain 5 to 10 μg of protein. A: center well contains antiserum to *P. cruentum* R-phycocyanin; wells a and d contain the homologous R-phycocyanin; well b contains *P. cruentum* allophycocyanin B; well c contains *P. cruentum* allophycocyanin. B: center well contains antiserum to a biliprotein solution similar to the one chromatographed in Figure 1. (The ratio of allophycocyanin to allophycocyanin B was approximately 15:1.) Wells a and d contain the homologous antigens, allophycocyanin and allophycocyanin B (about 15:1); well b contains *P. cruentum* allophycocyanin B; well c contains *P. cruentum* allophycocyanin; well e contains *Anabaena* sp. 6411 allophycocyanin; and well f contains *P. aerugineum* allophycocyanin. C: center well contains antiserum to *Anabaena* sp. 6411 allophycocyanin; wells a and d contain the homologous allophycocyanin; well b contains *P. cruentum* allophycocyanin B; well c contains *P. cruentum* allophycocyanin; well e contains *Synechococcus* sp. 6301 allophycocyanin; and well f contains *Synechococcus* sp. 6301 allophycocyanin B. D: center well contains antiserum to *Synechocystis* sp. (ATCC 22663) allophycocyanin B; well a contains *P. cruentum* allophycocyanin B; well b contains *P. cruentum* allophycocyanin; well c contains the mixture of *P. cruentum* allophycocyanins described in B; well d contains *Synechococcus* sp. 6301 allophycocyanin; well e contains *Anabaena* sp. 6411 allophycocyanin; well f contains *P. aerugineum* allophycocyanin.

P. cruentum allophycocyanin, suggests that certain of the determinants common to *Anabaena* sp. and *P. cruentum* allophycocyanins are absent from *P. cruentum* allophycocyanin B. Both allophycocyanin and allophycocyanin B show a reaction of identity when challenged with an antiserum to *Synechocystis* sp. allophycocyanin B (Fig. 3D). Collectively, the immunological data suggest that allophycocyanin and allophycocyanin B are very closely related molecules. In this context, it is worth noting that *Synechococcus* sp. 6301 allophycocyanin and allophycocyanin B have very similar amino-terminal sequences (Bryant and Glazer, unpublished observations).

The low temperature absorption spectra of the main phycobiliproteins from *P. cruentum, i.e.* b-phycoerythrin, B-phycoerythrin, and R-phycocyanin, are shown in Figure 4, A, B, and C for comparison with the low temperature absorption spectrum of the phycobilisomes from *P. cruentum* in Figure 4D. It is apparent that the absorption maxima of these phycobiliprotein pigments change very little during their isolation and purification from the phycobilisomes. Approximately 82% of the absorbance of the phycobilisomes is due to the two forms of phycoerythrin, approximately 8% is due to R-phycocyanin, and the remaining 10%, which accounts for the absorbance at wavelengths longer than 635 nm, is due to allophycocyanin. This estimate of the pigment composition of the phycobilisomes is in reasonable agreement with that determined by Gantt and Lipschultz (11). However, we see from the purification studies indicated in Figures 1 and 2 that a small amount of allophycocyanin B is also present. We estimate that there is one allophycocyanin B trimer per phycobilisome.

Absorption and fluorescence emission spectra of purified solutions of allophycocyanin and allophycocyanin B from *P. cruentum*, both at room temperature and at liquid N_2 temperature, are presented in Figure 5. In addition, the same spectral measurements were made on crystals of allophycocyanin from *Anabaena* sp. 6411 to determine whether the close packed stacking of the allophycocyanin trimers forming the crystal lattice (1) would cause the absorption or fluorescence bands of allophycocyanin to shift to longer wavelengths.

On cooling to low temperature, the 650 nm absorption maximum of allophycocyanin in solution splits to give two bands at 641 and 654 nm. The fluorescence emission band becomes considerably sharper on cooling but shifts very little. The absorption and fluorescence bands of allophycocyanin B both sharpen and shift appreciably to longer wavelengths. Thus, allophycocyanin B, which absorbs at 669 nm and fluoresces at 673 nm at room temperature, absorbs at 675 nm and fluoresces at 679 nm at −196 C. These changes are reversed upon thawing.

The absorption and fluorescence maxima of allophycocyanin B are close to those of Chl *a* which probably accounts for the efficient energy coupling of the phycobilisomes to the Chl of PSII. However, in an effort to determine if any Chl might be associated with the phycobilisomes, a preparation of phycobilisomes from *P. cruentum* was lyophilized and extracted by sonication in acetone. No Chl *a* was detected in the acetone extract by absorption or by fluorescence. We estimate, as an upper limit, that less than one molecule of extractable Chl *a* was present/100 phycobilisomes.

The fluorescence emission spectrum of *P. cruentum* cells at −196 C is shown in Figure 6A along with the fourth derivative of that spectrum. The resolution of the spectral components is greatly enhanced in the fourth derivative spectrum (3, 4) so that the emission of allophycocyanin B, which appears as only a slight shoulder at about 680 nm on the emission spectrum, is resolved as a well defined maximum at 681 nm in the fourth derivative spectrum. This fluorescence emission spectrum in *P. cruentum* cells was measured at the maximal F_M level (PSII reaction centers fully closed) so that much of the fluorescence at 680 nm is due to the reverse transfer of excitation energy from PSII Chl

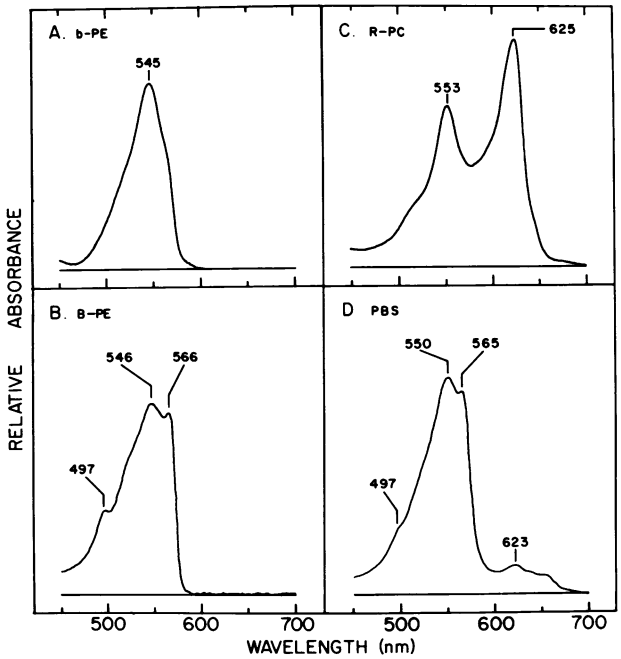

FIG. 4. Low temperature absorption spectra of phycobiliproteins and phycobilisomes isolated from *P. cruentum*. A: b-phycoerythrin (b-PE) 68 nM in 0.10 M K-phosphate buffer (pH 7). B: B-Phycoerythrin (B-PE) 0.48 μM in 0.10 M K-phosphate buffer (pH 7). C: R-phycocyanin (R-PC) 0.64 μM in 0.01 M ammonium acetate buffer (pH 6.8). D: phycobilisomes (PBS) in 0.75 M K-phosphate buffer (pH 7), 0.05 M sucrose.

back to allophycocyanin B. The major emission bands at 693 and 713 nm are due to the antenna chlorophyll in PSII and PSI, respectively, while the smaller bands at 662, 643, and 573 nm can be assigned to allophycocyanin, R-phycocyanin, and phycoerythrin (see Fig. 6C).

The low temperature emission spectrum of the phycobilisomes isolated from *P. cruentum* cells is shown in Figure 6B and the emission spectra of the purified phycobiliprotein pigments at −196 C are presented in Figure 6C. It is apparent that the long wavelength emission band of the phycobilisomes at 680 nm emanates from the very small amount of allophycocyanin B present in these structures. Allophycocyanin B serves as the sink for most of the energy absorbed by the phycobilisomes. If the phycobilisomes are connected to the photosynthetic apparatus in the thylakoid membranes, most of the energy absorbed by the phycobilisomes is transferred to the Chl *a* in PSII. If the phycobilisomes are separated from the photosynthetic apparatus, then that energy is emitted largely as fluorescence from the allophycocyanin B traps.

Gantt *et al.* (6, 12) suggested that the 675 nm emission band from phycobilisomes at room temperature was due to an aggregated form of allophycocyanin which had a longer wavelength fluorescence band because of the aggregation. However, we could not observe such effects of aggregation in crystals of allophycocyanin (see Fig. 5) where the interaction between chromophores should be maximal. On the other hand, we can identify the long wavelength emission of phycobilisomes with a specific pigment, allophycocyanin B.

Fluorescence from any of the antenna phycobilin pigments indicates that the efficiency of energy transfer from these pigments to allophycocyanin B is less than 100%. The emission spectrum of the phycobilisomes in Figure 6B shows fluorescence from phycoerythrin and from R-phycocyanin but none from allophycocyanin. Gantt *et al.* (10, 12) have shown that phycobilisomes, on standing in a low ionic strength medium, dissociate and that on dissociation the long wavelength emission band

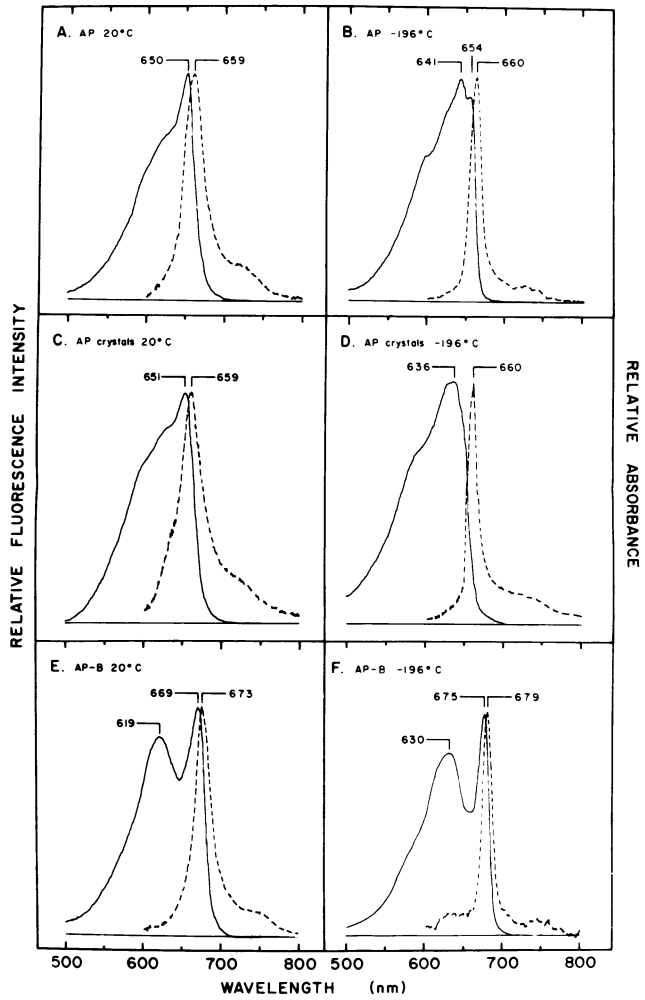

FIG. 5. Room and low temperature absorption and fluorescence emission spectra of *P. cruentum* allophycocyanin (AP) and allophycocyanin B (AP-B) and dilute suspensions of crystals of *Anabaena* sp. 6411 allophycocyanin. Absorption spectra: ——; fluorescence emission spectra; ---. The excitation wavelength for fluorescence emission measurements was 560 nm. The biliproteins, buffers, and protein concentrations used for absorption and fluorescence emission measurements respectively were: A: allophycocyanin at 20 C; 0.05 M K-phosphate buffer (pH 6.85), 0.02 M KCl; 3.1 μM; 0.31 μM. B: allophycocyanin at −196 C; 0.05 M K-phosphate buffer (pH 6.8), 0.02 M KCl; 0.15μM; 31 nM. C: allophycocyanin crystals at 20 C; 0.2 M sodium acetate buffer (pH 5.5); 0.27 μM 67 nM. D: allophycocyanin crystals at −196 C; 0.2 M sodium acetate buffer, (pH 5.5), 0.27 μM; 5.4 nM. E: allophycocyanin B at 20 C; 0.04 M K-phosphate buffer (pH 7), 0.01 M KCl; 2.3 μM; 0.23 μM. F: allophycocyanin B at −196 C; 0.04 M K-phosphate buffer (pH 7), 0.01 M KCl; 1.6 μM; 28 nM.

decreases while the emission bands from the individual phycobiliprotein pigments increase. The phycobilisomes used to measure the spectrum shown in Figure 6B had been stored for 24 days, and even though the conditions of storage, *i.e.* 3 C in a high ionic strength buffer, had been chosen to maintain the structural integrity of the phycobilisomes, we cannot preclude that some pigment dissociation or structural deterioration had occurred during the long period of storage. Therefore, a comparison was made between the low temperature emission spectrum of a sample of phycobilisomes stored only 4 days (the minimum storage time was appreciable since the samples were prepared in Los Angeles and measured in La Jolla) with that of a sample stored 18 days. The spectrum of the 4-day sample (Fig. 7A) showed emission from the allophycocyanin B but none from allophycocyanin or from R-phycocyanin while the spectrum from the 18-day sample (Fig. 7B) showed emission from R-phycocyanin in addition to that from allophycocyanin B. We conclude that the sample of phycobilisomes represented by the spectrum in Figure 6B had undergone some deterioration prior to the measurement. The very high degree of energy transfer in the fresh phycobilisomes was not anticipated since the emission spectrum of the whole cells (Fig. 6A) shows some emission from both R-phycocyanin and allophycocyanin. We suggest that these emission bands in the whole cells represent newly synthesized R-phycocyanin and allophycocyanin which have not yet been fully

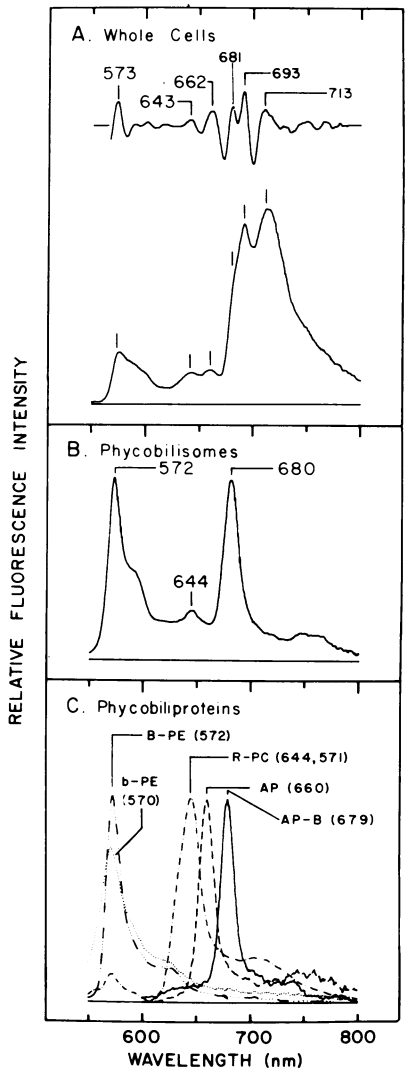

FIG. 6. Low temperature fluorescence emission spectra of isolated phycobiliproteins, phycobilisomes (24 days old), and whole cells of *P. cruentum*. Fluorescence from whole cells, phycobilisomes, and the phycobiliprotein B-phycoerythrin, b-phycoerythrin, and B-phycocyanin was excited using 500 nm light. Fluorescence from allophycocyanin and allophycocyanin B was excited using 560 nm light. A: whole cells suspended in fresh growth medium. The absorbance of the frozen suspension at 440 nm was 0.06. Also shown is the fourth derivative of the fluorescence emission spectrum. B: phycobilisomes in 0.75 M K-phosphate buffer, (pH 7). The phycoerythrin concentration was estimated to be 12 nM. C: isolated phycobiliproteins. The fluorescence emission spectrum and the location of the emission maxima for each phycobiliprotein are labeled in the figure. Protein concentrations and buffers were: B-phycoerythrin, .68 nM in 0.1 M K-phosphate buffer (pH 7); b-phycoerythrin, 48 nM in 0.1 M K-phosphate buffer (pH 7); R-phycocyanin, 0.1 μM in 0.01 M ammonium acetate buffer (pH 6.8); allophycocyanin, 31 nM in 0.05 M K-phosphate buffer (pH 6.85), 0.02 M NaCl; allophycocyanin B, 23 nM in 0.04 M K-phosphate buffer, (pH 7), 0.01 M KCl.

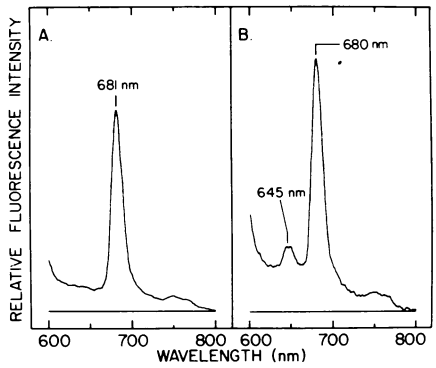

FIG. 7. Low temperature fluorescence emission spectra of *P. cruentum* phycobilisomes 4 and 18 days after isolation. Phycobilisomes were stored at 3 C suspended in 0.75 M K-phosphate buffer, (pH 7), 0.10 M sucrose at an estimated phycoerythrin concentration of 0.3 μM. Fluorescence emission was excited with 560 nm light from samples of phycobilisomes suspended in 0.75 M K-phosphate buffer (pH 7) at an estimated phycoerythrin concentration of 6 nM. A: phycobilisome fluorescence emission spectrum 4 days after isolation. B: phycobilisome fluorescence emission spectrum 18 days after isolation.

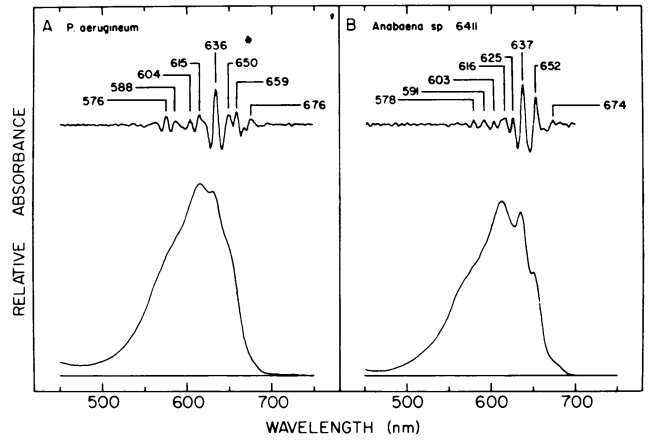

FIG. 8. Low temperature absorption spectra and their fourth derivatives of phycobilisomes isolated from *P. aerugineum* and *Anabaena* sp. 6411. Phycobilisomes are in 0.75 M K-phosphate buffer (pH 7). A: *P. aerugineum* phycobilisomes; B: *Anabaena* sp. phycobilisomes.

incorporated into mature phycobilisomes. (Unfortunately, the 4-day sample of phycobilisomes was not measured at wavelengths shorter than 600 nm so we cannot conclude much about energy transfer from the phycoerythrin from the data in Figure 7. The emission at 600 nm rises much less rapidly in the 4-day sample than in the 18-day sample so that it is likely that the emission from the phycoerythrin is appreciably less in the fresher sample.)

Phycobilisomes were also isolated from *Porphiridium aerugineum* and from *Anabaena* sp. 6411. Low temperature absorption spectra of those phycobilisomes and the fourth derivative curves of the spectra are shown in Figure 8. These organisms do not contain phycoerythrin but they do appear to contain the two forms of phycocyanin noted by Gray *et al.* (20) and *Anabaena* sp. 6411 contains some phycoerythrocyanin (1). A small amount of 675 nm absorbing form, presumably allophycocyanin B, is indicated in the long wavelength tail of the absorption spectra and by a small peak near 675 nm in the fourth derivative spectra. The fluorescence emission spectra of these samples at −196 C (data not presented) also show a long wavelength band at 681 nm.

Allophycocyanin B has been found in several cyanobacteria (15), *e.g. Synechococcus* sp., *Anabaena variabilis, Aphanocapsa* sp., *Synechocystis* sp. (ATCC 22663), *Synechococcus lividus,* and *Aphanizomenon flos-aquae,* and in the eukaryotic red algae, *Porphyridium aerugineum* (Glazer and Bryant, unpublished observations) and *P. cruentum*. We conclude that allophycocyanin B is a generally occurring constituent of the phycobilisomes of cyanobacteria and red algae.

Recent studies on the wavelength dependence of the energy distribution between PSI and PSII (24) and on the efficiency of energy transfer between the two photosystems (23) in *P. cruentum* gave specific insights into the structural organization of the photosynthesis apparatus of this red alga. It was found that almost all of the energy absorbed by Chl was distributed to PSI, indicating that at least 95% of the Chl was associated with PSI units. This led to the conclusion that the photochemical apparatus in the thylakoid membranes consisted of relatively large PSI units and rather small PSII units. In addition, the efficiency of energy transfer from PSII to PSI was found to be quite high (approximately half of the energy directed initially to PSII units was transferred subsequently to PSI even when all of the PSII reaction centers were in the open state [23]) indicating a close association between the small PSII units and the large PSI units. However, almost all of the energy absorbed by the phycobilisomes was distributed initially to PSII, indicating that the phycobilisomes are connected directly to the small PSII units in the membranes. The pathway of energy transfer from the extralamellar phycobilisomes to the intralamellar Chl *a* of PSII proceeds via allophycocyanin B. The high yield of energy transfer from the phycobilisomes to the small amount of Chl in PSII requires that allophycocyanin B be precisely located on the photosynthetic lamallae with respect to this Chl. It appears that allophycocyanin B is involved in the structural as well as energetic coupling of the phycobilisomes to the photochemical apparatus of photosynthesis.

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