Phycocyanin from the Red Alga *Anotrichium tenue*: Modification of Properties by a Colorless Polypeptide (M_r 30 000)†

Bruce A. Watson,*,‡ Susan D. Waaland,§ and J. Robert Waaland‡

Department of Botany, University of Washington, Seattle, Washington 98195, and Department of Biology, University of Puget Sound, Tacoma, Washington 98416

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ABSTRACT: Two species of R-phycocyanin, distinguished by chromatographic and spectroscopic qualities, were purified from isolated phycobilisomes of the red alga *Anotrichium tenue* (C. Agardh) Naegeli. Both species have the same ratio of bound chromophores. The species with an A_{max} of 617 nm is composed solely of α (M_r 18 500) and β (M_r 23 000) subunits. The other species with an A_{max} of 624–625 nm is distinguished from the first by having an additional colorless polypeptide (M_r 30 000). This polypeptide appears to be a linker protein and has an isoelectric point of 8.5–8.7. Association of this presumptive linker polypeptide with the chromophoric subunits seems to be responsible for red shifting the A_{max} by 7–8 nm and red shifting the fluorescence emission maximum by 9 nm.

Phycobilisomes are the photosynthetic light-harvesting antennae for red algae and cyanobacteria. These large, multimolecular complexes contain the chromophoric biliproteins phycoerythrin, phycocyanin, and allophycocyanin, and accessory linker proteins (Lipschultz & Gantt, 1981; Yu et al., 1981; Lundell et al., 1981; Zilinskas & Howell, 1983). These linker molecules are thought to mediate both the assembly of biliproteins into the highly ordered arrays found in phycobilisomes and the interaction between the phycobilisome and the adjacent chlorophyll-containing thylakoid membrane. The structure of phycobilisomes and of the component biliproteins has been reviewed recently (Glazer, 1982, 1983, 1984).

Characterization of the interaction between linkers and biliproteins is important for understanding the mechanism of physobilisome assembly, the pathway of energy transfer, and environmental regulation of the photosynthetic apparatus. To date, the molecular architecture of phycobilisomes has been characterized most extensively in a few cyanobacteria (Glazer, 1982). The interactions within red algal phycobilisomes are less well understood. Analysis of intact and partially dissociated phycobilisomes from red algae has revealed a variable number of associated colorless proteins. Several of these have been implicated as linkers between different chromophoric molecules, e.g., phycocyanin to phycoerythrin or allophycocyanin to reaction-center chlorophyll (Morschell, 1982; Lipschultz & Gantt, 1981; Redlinger & Gantt, 1981a,b, 1982).

Positive identification and biochemical characterization of such linkers are important not only for studies of phycobilisome architecture but also for studies of the molecular genetics of the photosynthetic apparatus. While recent studies have shown that the phycobiliproteins are chloroplast encoded (Steinüller et al., 1983; Belford et al., 1983; Egelhoff & Grossman, 1983; Lemaux & Grossman, 1984), preliminary evidence suggests that certain putative linker proteins are nuclear encoded (Egelhoff & Grossman, 1983).

Phycobilisomes of higher red algae usually contain several phycobiliproteins including r-phycoerythrin, R-phycoerythrin, R-phycocyanin, and allophycocyanin. R-Phycocyanin molecules are composed of an α and a β subunit. To the α subunit is covalently bound the bilin chromophore phycocyanobilin (PCB)[1] (1 mol of PCB/mol of protein). The β subunit binds 1 mol of PCB and 1 mol of a second bilin chromophore, phycoerythrobilin (PEB), per mole of protein (Glazer, 1982). In this paper, we report the isolation and characterization of two species of R-phycocyanin from the marine red alga *Anotrichium tenue*. A colorless, 30K linker protein copurifies with one of these R-phycocyanin species. Binding of this linker protein to R-phycocyanin changes the biochemical and spectroscopic properties of the phycobiliprotein.

Materials and Methods

Materials. DEAE-Bio-Gel A was obtained from Bio-Rad (Richmond, CA), hydroxylapatite was from Calbiochem (San Diego, CA), the Sephacryl S-200 and phenyl-Sepharose were from Pharmacia Fine Chemicals (Piscataway, NJ).

Growth of Organism. Anotrichium tenue (C. Agardh) Naegeli (=Griffithsia *tenuis* C. Agardh) (Rhodophyta Ceramiales) was grown as previously described (Waaland & Watson, 1980), except that a photon fluence rate of 12 μM m^{-2} s^{-1} was used.

Isolation of Phycobilisomes. Phycobilisomes were isolated by a modification of the procedure of Gantt et al. (1979). Fresh samples (4–40 g) of algae (treated for 24 h prior to harvest with 40 mg/L kanamycin sulfate) were homogenized in a Waring blender with 20–100 mL of ice-cold 0.75 M NaH_2PO_4–K_2HPO_4, pH 7.0, containing 0.2 mM phenylmethanesulfonyl fluoride (PMSF) and 0.02% NaN_3. (All buffer pH values were measured at 21 °C). The homogenate was then sheared by using a French pressure cell at 1379 N/cm^2 (2000 psi). This suspension was then made 1% in Triton X-100 and stirred at room temperature fr 30 min followed by centrifugation for 5 min at 12000*g* to pellet cell

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‡ University of Washington.

§ University of Puget Sound.

[1] Abbreviations: A_{PCB}, absorption attributable to the phycocyanobilin chromophores; A_{PEB}, absorption attributable to the phycoerythrobilin chromophores; HAP, hydroxylapatite; PCB, phycocyanobilin; PEB, phycoerythrobilin; PCI, phycocyanin species with an A_{max} of 617 nm; PCII, phycocyanin species with an A_{max} of 624–625 nm; PMSF, phenylmethanesulfonyl fluoride; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; 30K peptide, peptide of M_r 30 000 which is a subunit of PCII; Tris, tris(hydroxymethyl)aminomethane; IEF, isoelectric focusing.

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wall debris. The supernate was decanted and recentrifuged for 10 min at 12000*g* to bring the membrane fraction to the surface where it was removed by suction.

The suspension of phycobilisomes was layered onto the top of sucrose step gradients [0.5 M (8 mL), 0.75 M (4 mL), 1.0 M (4 mL), and 2.0 M (6 mL), in 0.75 M phosphate buffer as above without PMSF]. Approximately 15 mL of suspension was layered on a single gradient in 2.5 × 8.2 cm tubes. The gradients were centrifuged at 20 °C for 3 h at 42 000 rpm (130000*g*) in a Beckman Ti 70 rotor. The phycobilisomes concentrated on top of the 2 M step. Phycobilisome fractions were pooled and dialyzed overnight (2 °C) against ST buffer (0.05 M NaCl, 10 mM Tris, 1 mM 2-mercaptoethanol, and 0.02% NaN_3, pH 7.5).

Separation of Biliproteins. The dialyzed and dissociated phycobilisomes were loaded onto a DEAE-Bio-Gel A column (2.6 × 40 cm) equilibrated in ST buffer. The biliproteins were retained on the first few centimeters of the column. The biliproteins were eluted with a 1.5-L gradient of 0.05–0.20 M NaCl in 10 mM Tris, 1 mM 2-mercaptoethanol, and 0.02% NaN_3, pH 7.5. The flow rate was approximately 40 mL/h, and fractions of 9.6 mL were collected. The optical density of individual fractions was read at 498, 620, and 650 nm by using a Gilford Model 2500 spectrophotometer. Salt concentration was monitored by reading the conductivity of individual fractions with a (Industrial Instruments Model RB3) conductivity meter.

Hydroxylapatite Chromatography. Pooled fractions from ion exchange were further purified by chromatography on short (3–10 cm) columns of hydroxylapatite (HAP). All biliproteins absorbed well to HAP in ST buffer and were eluted by step gradients of phosphate buffer (5–100 mM KH_2PO_4, 0.1 M NaCl, 0.02% NaN_3, and 1 mM 2-mercaptoethanol, pH adjusted to 7.0 with KOH). HAP chromatography was performed at room temperature.

Gel Filtration Chromatography. Gel filtration chromatography was performed both as a purification step and as a characterization of the molecular weight of the biliproteins. This was carried out at 2 °C in ST buffer on Sephacryl S-200 (1.5 × 90 cm). Flow rates of approximately 4 cm/h were used. The elution volume vs. molecular weight characteristics of this column was calibrated by the elution of proteins of known molecular weight: myoglobin, M_r 17 500; carbonic anhydrase, M_r 30 000; bovine serum albumin, M_r 68 000; aldolase, M_r 158 000; catalase, M_r 240 000.

Hydrophobic Interaction Chromatography. PCI fractions were purified by chromatography on phenyl-Sepharose. The protein was loaded either in ST buffer (following gel filtration) or in phosphate buffer (following HAP chromatography). In either case, the phycocyanin was adsorbed to the top centimeter of a 1.0 × 3.0 cm column. PCI was eluted at room temperature with 0.02 M Tris-HCl, pH 8.2. Contaminating biliproteins were retained on the column under these elution conditions. The salt concentration of the eluate was brought immediately to 0.1 M by the addition of solid NaCl to stabilize the phycocyanin against dissociation.

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (1970) on 12.5% gels, 0.75 mm thick. All samples were prepared by heating at 95–100 °C for 5 min in sample buffer containing 50 mM dithiothreitol. These gels were silver stained either by the method of Morrisey (1981) or with Coomassie Blue R.

Isoelectric focusing was performed following a modification of the O'Farrell (1975) procedure for the first dimension of a two-dimensional electrophoresis. The important modification was the inclusion of 0.4% SDS in the sample buffer (Bio-Rad Bulletin 1144). Triton X-100 was present to a final concentration of 8%. This procedure resulted in tight bands in the IEF gel and prevented first-dimensional streaking when the IEF gel was employed as the first dimension of a two-dimensional gel (O'Farrell, 1975). IEF gels were Coomassie stained according to Spencer and King (1971). Two-dimensional gels were silver stained according to Morrissey (1981).

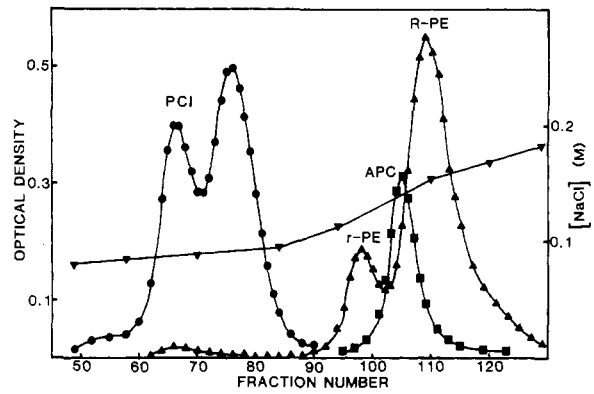

FIGURE 1: DEAE elution profile of biliproteins from *A. tenue*. Optical densities are A_{650} (■), A_{620} (●), and A_{498} × 0.1 (▲). NaCl molarity (▼).

Sedimentation Analysis. Purified phycocyanin fractions were analyzed by centrifugation on linear sucrose gradients, 5–15% in 0.05 M potassium phosphate, pH 7.0, according to the method of Yu et al. (1981). Centrifugation was carried out for 27 h (4 °C) in an SW-40 rotor. Gradients were fractionated by pumping from the bottoms of the tubes.

Spectroscopy. Absorbance spectra were recorded with an Aminco Model DW-2 recording spectrophotometer. Fluorescence spectra were recorded with a Perkin-Elmer scanning fluorometer (Model MPF-44A) using the ratio mode and an emission slit width of 1.7 nm.

RESULTS

Phycobilisomes from *A. tenue* were isolated as described under Materials and Methods. The isolated phycobilisomes were dissociated by overnight dialysis against phosphate-free buffer; the resultant free biliproteins were resolved by chromatography on DEAE-agarose. Figure 1 shows the DEAE elution profile of biliproteins obtained. The biliproteins included r-phycoerythrin, R-phycoerythrin, allophycocyanin, and R-phycocyanin. These assignments are made on the basis of the visible absorption spectra of fractions. Note, however, the presence of two distinct R-phycocyanin peaks. The first of these peaks contained an R-phycocyanin species, with an absorption maximum of 617 nm (PCI), and also a small amount of phycoerythrin. The second R-phycocyanin peak contained primarily R-phycocyanin species with absorption maxima at 624–625 nm (PCII).

The R-phycocyanin peaks overlap considerably in their elution from DEAE-agarose. The component peaks are, however, better resolved by hydroxylapatite (HAP) chromatography. Thus, all R-phycocyanin fractions were combined and fractionated on HAP as described under Materials and Methods. PCII eluted in 8–15 mM phosphate and PCI in 20–30 mM phosphate. In some preparations, PCII was composed of several very similar R-phycocyanin species marginally separable by HAP chromatography between 8 and 15 mM phosphate. The absorption maximum varied slightly for these species, from 624 to 625 nm. However, as all other properties (subunit structure and stoichiometry, molecular weight,

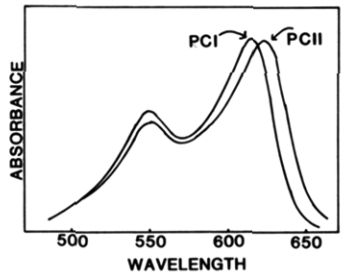

FIGURE 2: Absorption spectra of *A. tenue* R-phycocyanins in 0.1 M NaCl, 1 mM 2-mercaptoethanol, 10–50 mM phosphate (pH 7.0), and 0.02% NaN_3.

Table I: Comparative Properties of *A. tenue* R-Phycocyanins

property	PCI	PCII
wavelength of A_{max}[a] (nm)	617	624–625
wavelength of fluorescence max[b] (nm)	634	643
A_{PCB}/A_{PEB} ratio, native[a]	1.59	1.75–1.77
A_{PCB}/A_{PEB} ratio, denatured[c]	0.975	0.977–0.982

[a] Absorbance spectra were recorded in 50 mM NaCl, 10 mM Tris, 1 mM 2-mercaptoethanol, and 0.02% NaN_3, pH 7.5. [b] The fluorescence emission maximum was determined for excitation at 550 nm. [c] Denatured spectra were run in 6 M guanidine hydrochloride and 0.1 M HCl. Native spectra were run in 0.1 M NaCl, 1 mM 2-mercaptoethanol, 10–50 mM phosphate (pH 7.0), and 0.02% sodium azide.

chromophore ratio, and fluorescence emission spectra) were essentially identical, we have treated them as a single R-phycocyanin species. Small variations in properties are indicated by showing the range of measured values. PCI and PCII fractions were rechromatographed on HAP at least once. The PCI fractions from HAP were freed from contaminating phycoerythrin by hydrophobic interaction chromatography on phenyl-Sepharose as described under Materials and Methods. Final purification of these proteins was accomplished by gel filtration chromatography on Sephacryl S-200.

The (visible light) absorption spectra of the purified R-phycocyanins are displayed in Figure 2. As is typical for R-phycocyanin spectra, these show a major absorption maximum in the red, attributed to absorption by the PCB chromophore (A_{PCB}), and a minor absorption maximum in the green, attributed to absorption by the PEB chromophore (A_{PEB}) (Glazer, 1982). The wavelength of the A_{PCB} is different for the two R-phycocyanins from *A. tenue*; in addition, the wavelength of maximum fluorescence emission (excitation at 550 nm) for PCII (643 nm) is red shifted 9 nm compared to that for PCI (Table I). In contrast, the wavelengths of the A_{PEB} for both R-phycocyanins are identical (Figure 2). The ratio of A_{PCB} to A_{PEB} is greater in PCII than in PCI (Figure 2 and Table I). The A_{PCB}/A_{PEB} ratio is stable during storage at 4 °C for PCI but slowly decays to values of 1.65–1.73 for PCII. The wavelength of A_{max} is, however, stable over a period of months for both species. Apparently, the slow decay of A_{PCB}/A_{PEB} reflects a slow denaturation or conformational change in PCII. The differences in the absorption ratios between PCI and PCII could be due to differences in the ratio of PEB to PCB chromophores or to differences in the chromophore absorption spectra due to differences in the binding site environment in the different R-phycocyanins.

To determine the relative amounts of PCB and PEB bound by the two R-phycocyanins, spectra of each were measured under denaturing conditions. Under these conditions, the A_{max} for PCB shifts to 665 nm and that for PEB to 570 nm. As protein–chromophore interactions will be limited to the covalent attachment through the thioether linkage, the ratio of A_{665} to A_{570} should reflect the true PCB/PEB ratio (Glazer & Fang, 1973). The A_{665}/A_{570} ratio was essentially the same for PCI and PCII (Table I). Therefore, each has the same ratio of bound PCB to PEB. Thus, the observed differences in A_{PCB}/A_{PEB} must be due to differences between the chromophore–protein interactions.

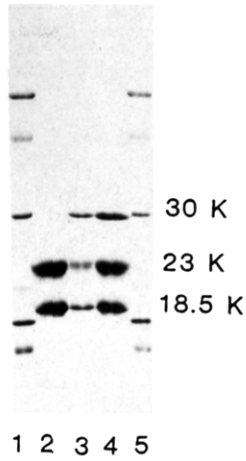

FIGURE 3: SDS–PAGE of R-phycocyanins from *A. tenue*. Lane 2 contains PCI, lanes 3 and 4 contain PCII, and lanes 1 and 5 contain the molecular weight standards cytochrome *c* (12 400), myoglobin (17 500), soybean trypsin inhibitor (21 500), carbonic anhydrase (30 000), ovalbumin (43 500), and bovine serum albumin (68 000). The gel was stained with Coomassie Blue R.

Table II: Relative Areas under the Densitometer Tracing Curves from a SDS–PAGE Gel of *A. tenue* R-Phycocyanins

protein	α subunit[a]	β subunit	30K peptide
PCI	1.00	1.01	
PCII	1.00	0.96–1.05	0.46–0.50

[a] The areas are normalized to the area under the α-subunit peak.

To determine whether the subunit compositions of these R-phycocyanins differed, we subjected samples of each to SDS–PAGE (Figure 3). The PCI species is composed of only the α(M_r 18 500) and β (M_r 23 000) subunits of R-phycocyanin. Both of these peptides were clearly visible in the unstained gel as colored bands, the α subunit being blue and the β subunit being purple-red. In addition to the α and β subunits of R-phycocyanin, PCII has a peptide, M_r 30 000 (30K peptide), which is not present in the PCI species. This band is not colored and is visible only following staining. Densitometer scans of these stained gels revealed that there were no significant differences in the ratios of α to β subunits between the two R-phycocyanins (Table II). Note also that there is no detectable contamination of these preparations with phycoerythrin subunits. The α and β subunits of R-phycoerythrin and r-phycoerythrin from *A. tenue* travel with R_f values intermediate between those for the α and β subunits of R-phycocyanin and are easily distinguished by SDS–PAGE (data not shown for phycoerythrins).

The isoelectric points of the α and β subunits of PCI and PCII and of the 30K peptide were determined. The α subunit focused as a blue band with a p*I* of 6.0, while the β subunit focused as a reddish band with a p*I* of 5.2 (these p*I*'s were determined directly by excision of the colored bands). The 30K peptide focused in a tight (Coomassie staining) band at pH 8.5–8.7 (determined by the position of the band). The molecular weight of this focused, colorless, Coomassie staining band was confirmed by two-dimensional electrophoresis by the method of O'Farrell (1975) (data not shown). The α and β subunits of PCI had the same isoelectric points as those from PCII.

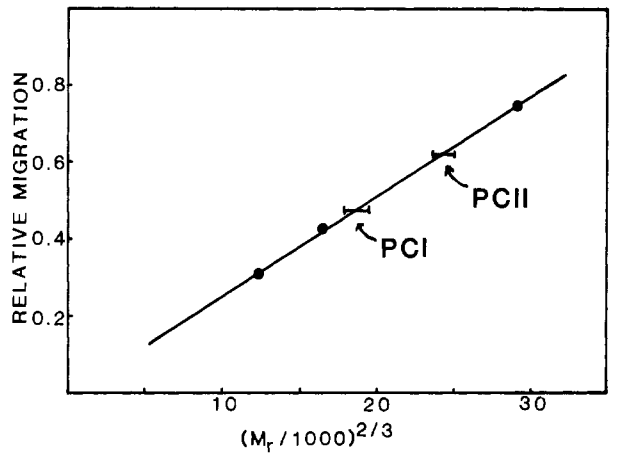

FIGURE 4: Sucrose density gradient centrifugation of PCI and PCII. Purified PCI and PCII were sedimented through 5–15% sucrose together and separately. The error bars show the range of measurements from two independent preparations of PCI and PCII. The procedure was calibrated by the sedimentation of standard proteins: ovalbumin (M_r 43 500), bovine serum albumin (M_r 68 000), and aldolase (M_r 158 000).

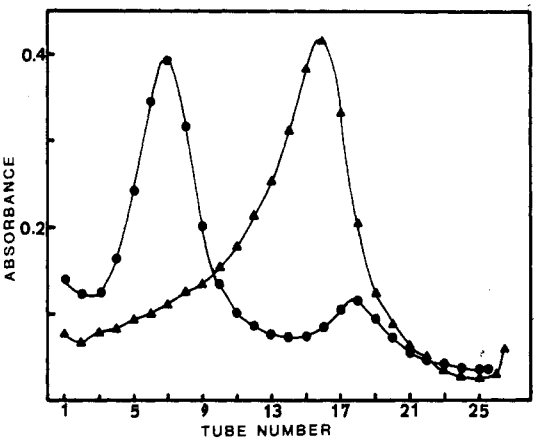

FIGURE 5: Sucrose density gradient centrifugation of standard proteins for calibration. Bovine serum albumin (M_r 68 000) was sedimented as in Figure 4, 26.5 fractions of 0.47 mL were collected, and A_{280} × 0.5 was plotted (▲). A mixture of ovalbumin (M_r 43 500) and aldolase (M_r 158 000) was sedimented, 25.5 fractions of 0.49 mL were collected, and A_{280} was plotted (●).

Both PCI and PCII had apparent molecular weights from gel filtration of approximately 130 000. They were indistinguishable by this method. However, the data from SDS–PAGE suggest a difference of molecular weight between these species. Thus, we sought to characterize the molecular weights of these species by another more sensitive method: sedimentation through linear sucrose gradients. This method has been successfully used (Yu et al., 1981) to characterize phycocyanin–linker complexes from cyanobacteria. Two independently prepared samples of PCI and two of PCII were analyzed by sucrose gradient centrifugation. Figure 4 shows these results with the range of data for the two independent R-phycocyanin preparations. PCI sedimented as a blue band 37–40 mm from the bottom of the 88-mm-high gradient and PCII as a blue band at 49–53 mm from the bottom. The two R-phycocyanins were sedimented both separately and in a mixture with identical results. A_{620} was recorded for the fractionated gradients containing the R-phycocyanins. PCI sedimented with an apparent molecular weight of 82 000, while PCII was estimated to have a molecular weight of 121 000. Figure 5 shows representative data from which the gradients were calibrated by using proteins of known molecular weight.

Discussion

Two spectroscopically and biochemically different R-phycocyanin species were isolated from phycobilisomes of *A. tenue*. PCII (A_{max} = 624–625 nm) contains an M_r 30 000 peptide not present in PCI (A_{max} = 617 nm). This peptide is apparently unpigmented; it is not visible in unstained SDS–PAGE gels, as are the α and β subunits, nor does it presence in PCII change the PCB/PEB ratio. The densitometer tracings of stained SDS–PAGE gels indicate that the ratio of α to β subunits is the same in PCI and PCII, as is suggested by the fact that both show the same PCB/PEB ratio. Furthermore, the ratio of α to β subunits appears to be 1. Our data do not allow an unambiguous determination of the molecular weights and subunit compositions of the two species. The two R-phycocyanin species were not distinguishable by their elution characteristics from gel filtration, both exhibiting apparent molecular weights of 130 000. The molecular weights determined by sucrose gradient sedimentation are substantially lower, PCI being 82 000 and PCII being 121 000. Given the apparent molecular weights of the α (18 500) and β (23 000) subunits determined by SDS–PAGE, we calculate molecular weights of 83 000 for $(\alpha\beta)_2$, 124 500 for $(\alpha\beta)_3$, 112 000 for $(\alpha\beta)_2$–30K peptide, and 154 500 for $(\alpha\beta)_3$–30K peptide. Thus the sedimentation data are in good agreement with the molecular weights of the dimeric forms, while the gel filtration data are more consistent with the trimeric forms. The densitometry data from SDS–PAGE also bear on this issue. The normalized area under the 30K peptide peak (relative to the α subunit) was found to be 0.46–0.50. The calculated value for a dimeric form $(\alpha\beta)_2$–30K peptide is 0.83; for the trimeric form, $(\alpha\beta)_3$–30K peptide is 0.55. Thus, the densitometry data support the subunit composition $(\alpha\beta)_3$–30K peptide.

Unfortunately, all of these techniques present uncertainties in the interpretation of the results. Values of molecular weight determined by density gradient sedimentation are influenced by molecular shape and degree of hydration. Densitometry data on SDS–PAGE gels are influenced by many factors: amino acid composition, amount of SDS remaining bound to the peptides after fixation, and efficiency of dye binding to bands of variable width (Wilson, 1979; Fishbein, 1972). In addition, Beer's law is followed only at certain ranges of protein concentration for stained bands (Fishbein, 1972).

Trimeric and hexameric disk forms of phycocyanin–linker complexes have been previously isolated from phycobilisomes and are known to have functional significance in intact phycobilisomes (Lundell et al., 1981; Yu et al., 1981; Yu & Glazer, 1982). Our data do not allow clear assignation of a dimeric or a trimeric form to PCI or PCII.

The 30K peptide is almost certainly a colorless linker protein of the type studied in a red alga by Lipschultz and Gantt (1981) and in cyanobacteria by Lundell et al. (1981), Yu et al. (1981), and *Zilinskas* and Howell (1983). It is significant that the linker peptide from *Porphyridium* studied by Lipschultz and Gantt (1981) is also of M_r 30 000 and is associated with phycocyanin and phycoerythrin.

It seems clear that the association of the 30K peptide with the chromophoric subunits is responsible for the red shift in the PCB absorption peak, the red-shifted fluorescence emission peak, and the modification of the ratio of the PCB and PEB absorption peaks which are observed in PCII. This last feature probably results from an enhancement of the PCB absorption peak. Glazer and his co-workers have found an analogous situation in phycocyanins isolated from the cyanobacterium *Anabaena variabilis*. They have shown that the association of 27K and 32.5K colorless linker polypeptides with C-

phycocyanin is responsible for the red shift in the spectral absorption maximum and a modification of the chromatographic properties of phycocyanin (Lundell et al., 1981). Gray et al. (1976) isolated two phycocyanins from the cyanobacterium *Agmanellum quadruplicatum* with absorption maxima at 637 and 623 nm. Although they did not characterize the subunit structure of these phycocyanins, our results strongly suggest that their form with a 637-nm absorption maximum was a phycocyanin–linker complex.

Association of the linker with R-phycocyanin in *A. tenue* results in a change in the chromatographic behavior of R-phycocyanin; the R-phycocyanins with attached linker elute from DEAE ion-exchange chromatography at a higher salt concentration than those without linker (Figure 1); thus, they are more tightly bound to the anionic exchange site on DEAE. This observation suggests that the association of the linker with the chromophoric subunits renders the protein complex more negative, i.e., more acidic. However, the 30K linker is, in fact, more basic than the biliprotein subunits. Lundell et al. (1981) have shown that the linker proteins of *Synechococcus* 6301 phycobilisomes are more basic than the biliprotein subunits. Glazer (1982) has suggested that this may have importance in linker–biliprotein interactions. The mechanism by which binding of a basic linker protein results in tighter binding of the R-phycocyanin subunits to DEAE remains unknown.

The observed heterogeneity of the PCII species (proteins with A_{max} of 624 or 625 nm separable from each other in some phycocyanin preparations) is probably an artifact of partial proteolysis during isolation. In early preparations from whole cell extracts, rather than from isolated phycobilisomes, there was considerable proteolysis of the 30K peptide, and A_{max} was reduced to 623–624 nm with no separation of individual species possible. This and the observed slow decay of the A_{max}/A_{551} ratio during storage of PCII suggest that the spectroscopic properties of PCII are very sensitive to small changes in the conformation of the 30K peptide. PCI exhibits spectroscopic properties which are completely stable for several months of storage at 4 °C.

This phycocyanin–presumptive linker complex is of interest because of its simplicity. The isolation of an R-phycocyanin with and without associated linker protein provides a good system in which to study the role of linker peptides in modifying the energy transfer properties of R-phycocyanin complexes. Fluorescence lifetime studies on these two R-phycocyanin species have revealed significant differences (S. C. Switalski et al., unpublished results). The isolation and characterization of specific nonpigmented phycobilisome components from red algae should also aid in studies concerned with the localization of the coding sites for different phycobilisome components and the regulation of their synthesis.

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