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Circular Dichroism and Polarized Fluorescence Characteristics of Blue-Green Algal Allophycocyanins†

Ora D. Canaani and Elisabeth Gantt*

ABSTRACT: Allophycocyanin (APC), the terminal pigment in the phycobiliprotein transfer sequence, isolated from dissociated phycobilisomes of *Nostoc* sp., was fractionated on calcium phosphate columns into four spectral forms: APC I, II, III, and B. These forms had distinctive isoelectric points of 5.15, 4.68, 4.82, and 4.98, respectively. The APC forms differed in their secondary structure as suggested by the varying percentages of their α helix and β-pleated sheets. APC II and III are short-emitting forms with a fluorescence maximum at 660 nm, while APC I and B are long-emitting forms with a maximum at 681 nm (at 24 °C in 0.1 M phosphate buffer). The maximum of APC I and B at −196 °C in 0.1 M phosphate shifted to 685 nm, and in 60% potassium glycerol phosphate and 20% glycerol shifted to 688 nm. Fluorescence polarization spectra suggest that there are at least two groups of chromophores responsible for the absorption of APC I and similarly of APC B. In APC II and III, the fluorescence was mostly depolarized. Circular dichroism (CD) revealed extensive positive and negative ellipticity band multiplicities in the chromophore absorption region of APC I and B, but not in APC II and III. Two main CD extrema in APC B, a negative band (668 nm) and a positive band (680 nm), are probably the result of exciton coupling of phycocyanobilin chromophores absorbing at longer wavelength. In APC I three different peaks are revealed in the absorption spectrum and four ellipticity bands in the CD spectrum at −196 °C. These can best be explained as being due to the combined interactions of the chromophore with the protein and exciton coupling between chromophores. The CD signal of APC I was shifted by disruption of strongly coupled chromophore–chromophore interaction with a chaotropic agent treatment (LiCl). It was almost abolished by disrupting both chromophore–protein and chromophore–chromophore interactions upon denaturation with sodium dodecyl sulfate ($NaDodSO_4$). Exciton interaction implies a unique structural feature where some of the chromophores in APC I and also in APC B are at a close range to each other and, therefore, most likely at the contact region between subunits. Energy transfer in some of the chromophores of the far-emitting forms is by a delocalized exciton mechanism in contrast to the short-emitting forms. Both APC I and B display an absorption peak at 680 nm (−196 °C), which is considered to be the emitting state in these long-emitting forms. APC I and B fluorescence maxima were identical with intact phycobilisomes, thus suggesting that both are final emitters of phycobilisomes. The exciton mechanism provides the bridging pigments, APC I and B, with more efficient energy transfer to chlorophyll due to increased spectral overlap between the APC fluorescence and chlorophyll absorption in vivo.

In the blue-green alga *Nostoc* sp., as in other red and blue-green algae, the light harvesting accessory pigments of photosynthesis are water-soluble phycobiliproteins contained within large aggregates called phycobilisomes that are attached to the photosynthetic membranes. In vivo, most of the excitation energy of the phycobiliproteins is transferred via allophycocyanin (APC)[1] to chlorophyll *a* with high efficiency (reviewed in Gantt, 1975). The phycocyanobilin chromophores in APC are covalently attached to the apoproteins (review by Bogorad, 1975). Allophycocyanins can be classified into two main groups according to their fluorescence wavelength emission spectra: those with a short emission at ca. 660 nm and those with a long emission at ca. 680 nm. A short-emitting form with an absorption peak at 650 nm has been isolated in many laboratories. It is a common form that is relatively easily obtainable and has been extensively studied (Bogorad, 1975; Brown et al., 1975; Brown & Troxler, 1977; Cohen-Bazire et

† From the Radiation Biology Laboratory, Smithsonian Institution, Rockville, Maryland 20852. *Received December 6, 1979.* This investigation was supported in part by a Smithsonian Postdoctoral Fellowship, awarded to O.D.C., by the Office of Academic Studies, Smithsonian Institution, and in part by Contract ASO5-76ER04310 from the Department of Energy.

[1] Abbreviations used: APC, allophycocyanin; CD, circular dichroism; $NaDodSO_4$, sodium dodecyl sulfate.

al., 1977; MacColl et al., 1978; Gysi & Zuber, 1979). The long-emitting forms generally occur in lesser amounts. One present in the smallest concentration is APC B; it has a double absorption peak at 618 and 671 nm (Glazer & Bryant, 1975; Ley et al., 1977). Another is APC I, with a single peak at 654 nm (Zilinskas et al., 1978), which is probably similar to that described by Gysi & Zuber (1976).

The primary function of the short-emitting forms is in light harvesting, whereas long-emitting forms, in addition to being involved in light harvesting, are also possible bridging pigments between the phycobilisome and the chloroplast lamellae.

In this paper, we extend the characterization of APC forms from *Nostoc* sp. phycobilisomes and report on the mechanism of energy transfer in these forms. For the first time, evidence is presented for strong exciton coupling of chromophores unique to the long-emitting forms (APC I and B). A similar type of interaction has been reported in other light harvesting proteins, such as bacteriochlorophyll–protein (Philipson & Sauer, 1972; Olson et al., 1973), peridinin–chlorophyll *a*–protein (Song et al., 1976), and cryptomonad phycocyanin (Jung, 1978). We also show that the long-emitting forms emit through a single fluorescing species corresponding to a long-wavelength absorption maximum at 680 nm. Furthermore, by fluorescence polarization–excitation spectra, it is shown that at least two groups of chromophores are responsible for the absorption bands of APC I and B.

Materials and Methods

Phycobiliprotein Preparation. Phycobilisomes were isolated (Gray & Gantt, 1975; Gantt et al., 1979) from 10–14-day-old *Nostoc* sp. cells grown in white light. Phycobilisomes were dialyzed overnight against 10 mM potassium phosphate buffer, pH 7.0, at room temperature. Allophycocyanin forms were separated on calcium phosphate columns (Zilinskas et al., 1978). Recovery of APC was monitored by its 650-nm absorbance. Respective peak fractions of the various allophycocyanin forms (APC I, II, III, and B), were combined. Five-milligram protein samples of each APC form were dialyzed for 4 h with two changes of distilled water. Subsequently, the samples were subjected separately to isoelectric focusing on an LKB 8101 ampholine column (110 mL) with 1% ampholine in sucrose, in the pH range 4–6, at 10 °C for 48–60 h, at 500 V. Isoelectric points of respective bands were measured with a Radiometer pH meter at 24 °C. Only by isoelectric focusing was APC B completely separable from APC II. The APC content is expressed as milligrams per milliliter based on protein determinations according to Lowry et al. (1951) with bovine serum albumin (fraction V, Sigma) as standard.

Molecular Weight Determination. Elution on a Sephacryl S200 column was used to estimate the molecular weights. The column (0.9 × 60 cm) was run at a flow rate of 5 mL/h and was equilibrated and eluted with 0.1 M potassium phosphate buffer, pH 7.0, containing 0.1 M NaCl. The following molecular weight markers were used: myoglobin (17 000), carbonic anhydrase (30 000), bovine serum albumin (68 000), B-phycoerythrin (265 000).

$NaDodSO_4$–urea polyacrylamide electrophoresis was carried out by a procedure described in Canaani & Sauer (1977). $NaDodSO_4$ slab gels contained 5 and 10% acrylamide in the stacking and separating gels, respectively. Electrophoresis was carried out at 20 °C overnight at a current of $i = 8$ mA. Samples containing 0.05–0.1 mg of protein in 1% $NaDodSO_4$ and 1% β-mercaptoethanol were boiled for 3 min prior to electrophoresis. Subsequently, the gels were fixed in 12.5% trichloroacetic acid (30 min, 55 °C), stained in 0.1% coomassie blue in 5:1:5 methanol–acetic acid–water, and destained in the same mixture, but with omission of the dye. The following proteins were used as molecular weight calibration markers: bovine serum albumin (68 000), carbonic anhydrase (30 000), lactoglobulin (17 500), myoglobin (17 000), and lysozyme (14 300).

Spectrophotometric Measurements. Absorption spectra were measured on a Cary Model 17 spectrophotometer. For low-temperature spectra, at −196 °C, the absorbances of samples were measured in an optical Dewar using a 4-mm path length cell. The sample was suspended in a mixture consisting of 2 parts 75% potassium glycerol phosphate, 1 part sample, and 0.5 part glycerol. Under these conditions, a clear glass was formed. Fluorescence spectra and fluorescence polarization excitation measurements (24 °C) were recorded on an Aminco-Bowman spectrofluorometer equipped with an automatic corrected spectrum attachment (Grabowski & Gantt, 1978).

Degrees of the fluorescence polarization were calculated by the relation

$$p = (I_{VV} - TI_{VH})/(I_{VV} + TI_{VH})$$

where I_{VV} and I_{VH} are the fluorescence intensities with the exciting polarizer in the vertical (V) position and the emission polarizer in the vertical and horizontal (H) positions, respectively. T is the relative transmission factor of the analyzing monochromator ($T = I_{HV}/I_{HH}$; see Azumi & McGlynn, 1962). The relative transmission factor T was evaluated for a given wavelength of emission before every measurement. The value of T was constant within the accuracy of ±0.5% with respect to the wavelength of excitation.

Circular dichroism (CD) spectra were recorded at 24 °C on a Cary Model 61 spectrophotometer equipped with a thermostated cell holder. CD spectra were obtained for each sample between 720 and 300 nm. Appropriate blanks were subtracted from each sample and molecular ellipticities were calculated according to the equation

$$[\theta_\lambda] = \theta_{obsd}MW/10lc$$

where λ is the wavelength, θ_{obsd} is the observed ellipticity in degrees, MW is the molecular weight of each APC form, l is the path length in centimeters, and c is the protein concentration in grams per milliliter. Typically, a 1-cm path length cell and 0.8 optical density unit at the absorption maximum were used. CD spectra at −196 °C were made in a spectrophotometer built by Sutherland et al. (1974). For the determination of secondary structure, CD spectra were recorded on a Cary 60 spectrophotometer. Mean residue ellipticity, $[\theta]$, in deg cm^2 dmol^{-1} was determined using mean residue weights of 106 for all APC forms. The CD spectra of the APC forms from 207 to 243 nm were analyzed by the CYC method of Chen et al. (1972). The errors for $[\theta]_{222}$ were within ±2%.

Chaotropic Agent Treatment. Lithium chloride (10 M in water) was added to a final concentration of 1 M to each APC form (0.2 mg/mL) suspended in 10 mM potassium phosphate buffer, pH 7.0. The sample was frozen in a slurry of acetone and crushed frozen CO_2 at approximately −70 °C. Thawing at room temperature and subsequent refreezing were done on each sample.

Results

Biochemical Characteristics. A recovery of 95% of the APC layered on the brushite column was achieved. The distribution of the allophycocyanin forms obtained from the fractionation on brushite is shown in Table I, with APC II being present

Table I: Characteristics of Allophycocyanin Isolated from *Nostoc* sp. Phycobilisomes

APC type	rel %	p*I* at 22 °C[a]	est MW	est polypeptide MW	ext coeff (mL mg^{-1} cm^{-1})	% α helix	% β-pleated sheet
B	<1	4.98	98 000	α 16 500 β 20 000	3.3[b]	18.1	2.0
I	21	5.15 (5.33; 5.27 5.17; 5.04)	145 000	α 17 000 β 18 400 γ 37 000	3.9	43.0	13.0
II	47	4.68 (4.53)	105 000	α 17 000 β 18 400	5.4	33.7	9.4
III	31	4.82	105 000	α 17 000 β 18 400	5.2	37.1	8.4

[a] Minor bands are given in parentheses. [b] Determination of the extinction coefficient was performed on isoelectrofocused APC B. Under the same conditions, a 30% decrease from the original absorption occurred with APC II.

in the largest and APC B in the smallest quantity. A summary of the APC characteristics is given in Table I. It shows that their isoelectric points are distinct and range from 4.68 to 5.33. The minor bands appearing in APC I and II exhibited absorption and fluorescence emission peaks similar to the principal bands. Analysis on all APCs was carried out only on material obtained by isoelectric focusing.

APC B obtained previously from *Nostoc* sp. on the brushite column (Zilinskas et al., 1978), and further purified by gel electrophoresis, appears to be a mixture. When such an APC B enriched preparation was electrofocused, it separated into APC II (p*I* = 4.68) and APC B (p*I* = 4.98). The purified APC B was very similar in its spectral properties, molecular weight, and subunit characteristics to that from *Porphyridium cruentum* and several blue-green algae (Glazer & Bryant, 1975; Ley et al., 1977).

Sodium dodecyl sulfate gel electrophoresis of all APC forms showed no impurities. On $NaDodSO_4$–urea polyacrylamide gel electrophoresis, all forms showed α and β subunits in the molecular weight range of 16 500–20 000. The α and β polypeptides were blue in color, as was the γ polypeptide of APC I. Overloaded gels (300 μg of protein) of APC I showed two faint additional bands, but they were too scarce for further characterization. The molecular weight results and extinction coefficients for APC I, II, and III are in agreement with those obtained by Zilinskas et al. (1978). Determination of extinction coefficients is problematical because of some fading during the purification. With APC B, the time required for purification is longest; thus, the fading is perhaps more extensive and the extinction value subject to greater possible error.

The APC forms also exhibited variations in their secondary structure as suggested from CD signals at 220 and 208 nm. Computer analysis of the CD spectra (Chen et al., 1972) suggests that APC I apoprotein is comprised of approximately 43% α helix and 13% β-pleated sheet. APC II and III, respectively, had 33.7 and 37.1% α helix plus 9.4 and 8.4% β-pleated sheet, suggesting a highly similar structure. APC B had 18.1% α helix and 2% β-pleated sheet (Table I). Variation of the secondary structure may occur in different species. The only other APC studied by a similar method (Brown et al., 1975), probably APC II as judged from its spectrum, showed less random structure because it contained 60% α helix and 40% β form.

Spectral Comparison. According to their absorption and fluorescence characteristics, APC I and B are considered the long-emitting forms, and APC II and III are the short-emitting forms. APC II and III were very similar in their fluorescence (660 nm); both showed an absorption maximum at 650 nm and differed only in the relative absorption at 620 nm (cf. Figure 2 in Zilinskas et al., 1978). On the contrary, APC I and B, as shown in Figure 1, displayed the same fluorescence peak (681 nm, 24 °C) but had very different absorption spectra. APC I had a single maximum at 654 nm. APC B

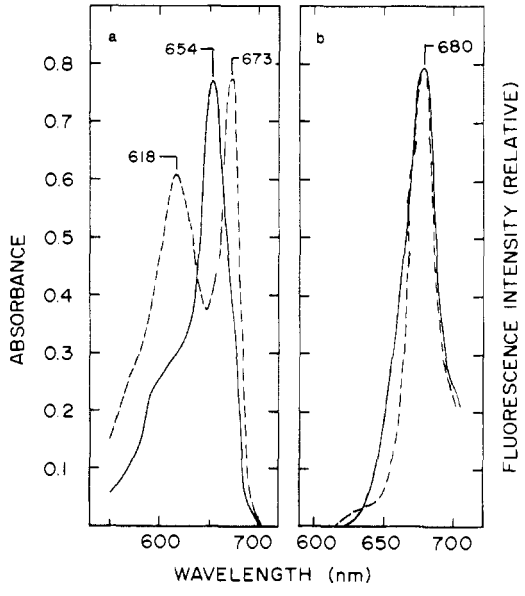

FIGURE 1: (a) Absorption and (b) fluorescence emission spectra of APC I (—) and B (---) of *Nostoc* sp. in 0.1 M phosphate buffer and 0.1 M NaCl, pH 7.0, at room temperature. Bandwidths of the excitation and emission monochromators were 11 and 2.7 nm, respectively. Samples with concentrations of approximately 25 μg of protein/mL were excited at 545 nm.

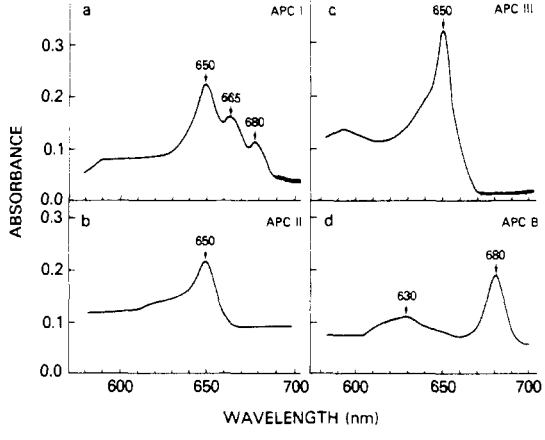

FIGURE 2: Absorption of APC forms at −196 °C in 75% potassium glycerol phosphate–sample–glycerol in the ratio of 2:1:0.5: (a) APC I; (b) APC II; (c) APC III; (d) APC B. Absorption was measured in an optical Dewar in a Cary 17 spectrophotometer.

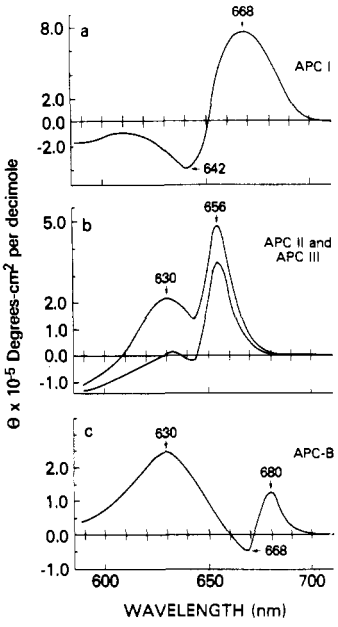

FIGURE 3: CD spectra of APC forms in 0.1 M phosphate buffer and 0.1 M NaCl, pH 7.0, at room temperature: (a) APC I; (b) APC II and III; (c) APC B. [θ], molecular ellipticity. Absorbance of the samples was 0.8 at their maximum absorption peaks.

had two major peaks at 618 and 673 nm.

Absorption spectra taken at −196 °C (Figure 2) revealed significant resolution that was not obtainable at 24 °C. The absorption band of APC I was split into several components at 680, 665, and 650 nm. APC B displayed a peak at 680 nm and another small broad peak at 630 nm. At the low temperature an absorption band at 680 nm was present in APC I and B, but was absent in the others. This long absorption band is probably responsible for the long emission of the two forms (to be discussed in more detail later).

Circular dichroism was used in order to gain insight into the organization of the chromophores in the protein matrix. The resolving power of CD gives direct evidence for the presence of several components in the 654-nm transition of APC I and the 673-nm transition of APC B. These positive and negative CD components are interpreted as the result primarily of exciton interactions among several phycocyanobilin chromophores in APC I, as well as in APC B. The CD spectra for the four forms are shown at 24 °C (Figure 3), and for three forms at −196 °C (Figure 4). The most important feature in the CD spectrum of APC I is that the absorption band at 654 nm was split into positive and negative ellipticity bands at 668 and 642 nm, respectively (Figure 3a). Troughs also occurred at 595 and 340 nm, but are not shown. At −196 °C, in APC I the positive peak was further resolved into peaks at 655 and 669 nm and a prominent shoulder at ca. 680 nm, and the negative peak was at 645 nm. APC B showed a double CD spectrum that was positive at the long wavelength (680 nm), negative at 668 nm, and reversed the sign close to the center of the long absorption band (673 nm). In addition, there was a positive ellipticity band at 630 nm. The CD of APC B at −196 °C was similar to that at 24 °C, with an additional small positive peak at 650 nm (not shown).

Variation of the protein concentration in APC I (0.05–0.3 mg/mL) suspended in 0.1 M potassium phosphate, pH 7.0, showed no effect on the spectral properties; however, APC B in the same buffer was sensitive to protein concentration. Along with an increased absorption (over the range of 0.04–0.2 mg/mL) at 673 nm, the CD signal at 668 nm relative to 680 nm was smaller at higher protein concentration with no significant effect on the 630-nm band. We attribute these effects to a small change in protein conformation which affects the chromophore–chromophore interaction (see Discussion).

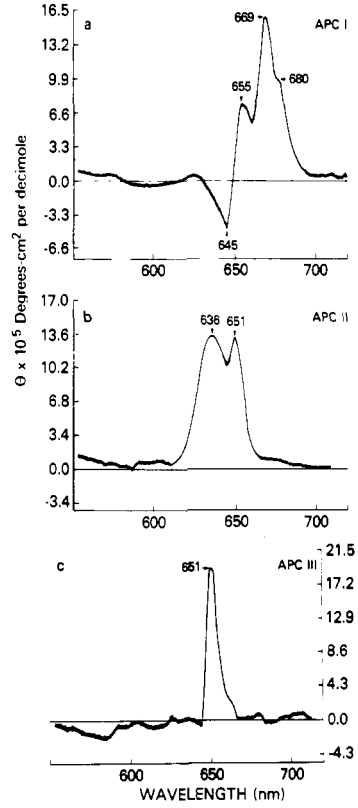

FIGURE 4: CD spectra of APC forms in a mixture of 75% potassium glycerol phosphate–sample–glycerol in the ratio of 2:1:0.5, at −196 °C. Samples were in a concentration of ~0.5 mg/mL (absorbance was ~0.45 optical density unit, path length 0.2 cm): (a) APC I; (b) APC II; (c) APC III.

The CD spectra of APC II and III at 24 °C were similar to one another, differing only in magnitude (Figure 3b) with positive peaks at 632 and 656 nm. The small troughs (not shown) were at 587 and 348 nm for APC II, and at 593 and 347 nm for APC III. At −196 °C there was a slight shift in the position and magnitude of the peaks (Figures 4b and c). In APC II, the two ellipticity bands at 638 and 651 nm correspond to the absorption bands and suggest the presence of two environmentally different chromophores. The single peak of APC III probably reflects a very similar environment for all the chromophores in this pigment. The magnitude of CD bands at −196 °C is more than double that at 24 °C in all APC forms. This is mostly due to band narrowing, which increases the amplitudes and decreases the overlap of neighboring positive and negative peaks.

Fluorescence Polarization. Fluorescence polarization–excitation spectra at 24 °C suggest that each absorption band of APC I and APC B is at least a two-component system. In APC I (Figure 5a), a comparison of the fluorescence polarization–excitation spectrum with the absorption band at 654 nm suggests that the latter is multicomponent, indicated by the different degrees of polarization of 670–675, 650–655, and 625–630 nm. The fluorescence polarization–excitation spectra of APC I were very similar at three different emission wavelengths (680, 690, and 695 nm). For APC B (Figure 5c), the fluorescence polarization–excitation spectra (3% at 620 to 10% at 675 nm) suggest that the absorption band (618 and 673 nm) is composed of at least two separate transitions. For emission at 682 and 690 nm, polarization spectra were similar in shape. The fluorescence of APC II and III was mostly

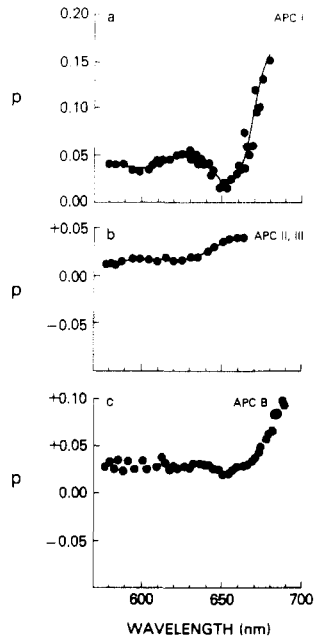

FIGURE 5: Fluorescence excitation polarization spectra of APC forms in 0.1 M potassium phosphate buffer and 0.1 M NaCl, pH 7.0, at room temperature: (a) APC I (λ_e 682 nm); (b) APC II and III (λ_e 662 nm); (c) APC B (λ_e 690 nm).

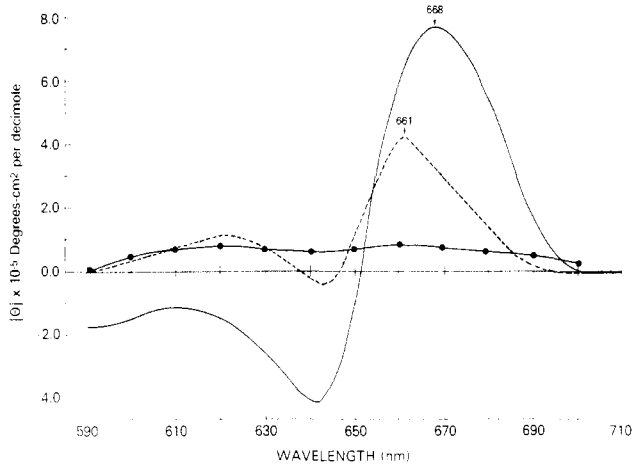

FIGURE 6: Effects of treatment with LiCl and $NaDodSO_4$ on the CD spectrum of APC I at 24 °C: APC I (absorbance 1.0 at 654 nm) (—); APC I treated with 1 M LiCl and twice frozen and thawed at −70 °C (- - -); APC I treated with 1% $NaDodSO_4$ at 24 °C (●—●).

depolarized (Figure 5b) throughout the absorption band as also reported for APC II in other algae (Grabowski & Gantt, 1978; MacColl et al., 1978). Furthermore, the polarization degree of APC II and III remained unchanged in a highly viscous solvent (90% glycerol).

Disruption of Protein Structure. Treatment of APC I with the chaotropic agent LiCl (1 M), which causes disruption of subunit–subunit interactions in proteins (Vogel & Steinhart, 1976), resulted in substantial changes in the optical properties of APC I. The absorption was reduced by about 40% with a shift from 654 to 649 nm, and an increase in the ratio of 620 to 650 nm absorption from 0.39 to 0.63. This absorption spectrum then resembled that of APC II. In the CD spectrum, a peak shift from 668 to 661 nm was observed. The intensity of CD signals at 680, 665, and 643 nm subsequently decreased by a similar extent, the 655-nm band did not change, and a new positive CD band appeared at 620 nm (Figure 6). Concomitantly, its fluorescence emission peak at 681 nm was

Table II: Variation of Fluorescence Maxima of Allophycocyanins with Temperature and Media

APC	abs at λ_{max}	fluorescence, λ			
		phosphate buffer[a]		glycerol[b]	
		24 °C	−196 °C	24 °C	−196 °C
B	0.01	681	685	685	688
I	0.01	681	685	685	688
II	0.01	660	662	662	662
III	0.01	660 (4)[c]	664	664	664

[a] In 0.1 M potassium phosphate plus 0.1 M NaCl, pH 7. [b] In 60% potassium glycerol phosphate, pH 7, and 20% glycerol. [c] At higher concentrations the fluorescence peak shifted to 664 nm.

almost abolished, and instead a fluorescence maximum appeared at 658 nm with a shoulder at 642 nm. The treatment with LiCl substantially changed the multicomponent CD spectrum, probably by disrupting exciton interaction.

The treatment with LiCl had almost no effect on the spectral properties of APC II, III, and B, which do not possess a γ subunit. It appears that the γ subunit is important in stabilizing APC I, because, when LiCl caused a partial removal of the γ subunit, the exciton interaction almost disappeared. The removal of the γ subunit was ascertained by recovering LiCl-treated APC I from the isoelectrofocusing column and analyzing it on $NaDodSO_4$–urea polyacrylamide gel electrophoresis. In this case, only the α and β polypeptides were present.

In all APC forms, treatment with 8 M urea, 6 M guanidine·HCl, or 1% $NaDodSO_4$, respectively, caused a five-, seven-, or nine-fold reduction in the absorption at 650 nm. Denaturation with $NaDodSO_4$ disrupts not only the chromophore–chromophore interaction, but also the chromophore–protein environment. This is manifested by the virtual disappearance of the CD signals of all APC forms as exemplified by $NaDodSO_4$-treated APC I in Figure 6.

Fluorescence Emission Variation. Each APC form had a single emission peak whether taken at room temperature or at liquid nitrogen temperature. The fluorescence excitation spectrum (not shown) of APC I at −196 °C displayed peaks at 650 and 665 nm and a shoulder at 680 nm, greatly resembling its absorption at −196 °C. Similarly, the fluorescence excitation spectra of APC B, II, and III at −196 °C were very similar to their corresponding absorption spectra at this temperature. These results suggest that the spectroscopically different phycocyanobilin chromophores in APC I and similarly in APC B emit through a single fluorescing species corresponding to the longest wavelength absorption maximum at 680 nm, at −196 °C. As shown in Table II, the emission peaks were affected by temperature and the suspending medium. However, the long-emitting APC I and B remained as one group and the short-emitting APC II and III as another. At lower temperature (−196 °C), only APC I and B showed a shift to longer wavelengths in potassium glycerol phosphate plus glycerol. This mixture is a cryoprotectant frequently used because it forms a "glass" on freezing and thus reduces scatter and possibly quenching. In potassium glycerol phosphate plus glycerol, emission maxima of all APCs were shifted toward longer wavelengths. The most plausible explanation is that glycerol, by reducing the activity of water, leads to a change in the conformation of the protein moiety, which in turn affects the chromophore–protein environment (Clement-Metral & Yon, 1968; Mathis & Sauer, 1972).

In a mixture prepared from purified APC II and I, the separate fluorescence peaks at 662 and 685 nm were clearly distinguishable at −196 °C, as they were also in the mixture

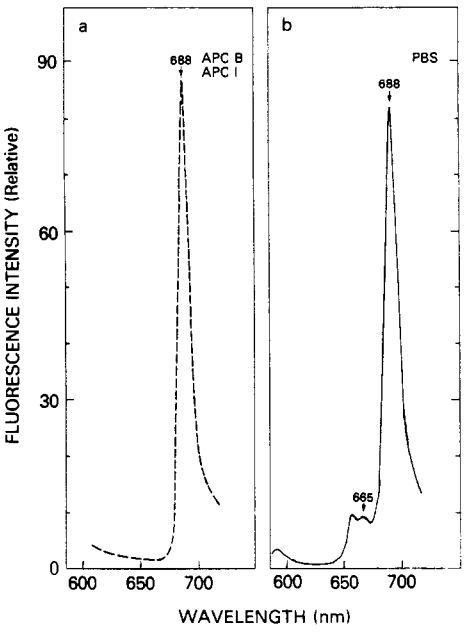

FIGURE 7: Fluorescence emission spectra at −196 °C: (a) APC I and B and (b) phycobilisomes (PBS). Samples of 25 μg of protein/mL were frozen at −196 °C in a glycerol mixture as in Figure 2. (The small emission peaks at ca. 578 and 650 nm, respectively, arise from phycoerythrin and phycocyanin.)

of APC II and B. Since the pigments had distinct isoelectric points, individual polypeptide bands on $NaDodSO_4$–urea gels, and separate absorption peaks prior to mixing, neither APC I nor APC B can be seriously considered as being contaminated. There is little doubt that the long-emitting forms are responsible for the fluorescence of the phycobilisomes (compare Figure 7a with 7b). Furthermore, both long- and short-emitting forms exist in phycobilisomes. In slightly uncoupled phycobilisome preparations, accomplished by storage or lowering the ionic strength (see Gantt et al., 1979), the short-emitting peak at ca. 665 nm and a long-emitting peak at ca. 688 nm are seen.

Discussion

The absorption and CD spectra of APC II and III are characterized by a single peak at ~650 nm with a shoulder at ~625 nm. These spectral features were previously attributed to chromophore–protein interactions (Brown et al., 1975; Brown & Troxler, 1977). The optical properties of APC I and B are more complex than those of APC II and III. Our data are best explained by a dipole–dipole exciton coupling between phycocyanobilin chromophores in APC I and in APC B. Exciton state is defined as the collective excitation of an assembly of molecules, by contrast with the localized excitation of each individual member of the assembly (Kasha et al., 1965). The interactions between transition charge distributions of like molecules will result in the splitting of energy levels. The number of the component spectral peaks observed will be equal to or less than the number of molecules interacting. The CD is then a function of the asymmetrical arrangement of the interacting molecules and is characterized by having multiple components of both negative and positive sign.

In APC B, the two absorption peaks at 618 and 673 nm suggest the presence of two groups of phycocyanobilin chromophores in the native protein. The absorption band at 618 nm probably represents noninteracting phycocyanobilin chromophores, since it is similar to the 620-nm absorption of APC II monomers (MacColl et al., 1980). The 673-nm absorption band is red shifted (~1320 cm^{-1}) with respect to the band at 618 nm. CD band splitting occurs only in the long-wavelength absorption band in APC B. This conclusion is based on the following observations: (1) the 630- and 680-nm absorption bands at −196 °C were well separated from each other; (2) the isoelliptic point (sign reversal) of the 668- and 680-nm CD bands was located at 673 nm, coinciding with the absorption maximum of APC B at 24 °C; (3) the double CD may be conservative if one takes into account a 650-nm positive CD peak (resolved only at −196 °C, Figure 2), which may partially cancel the negative peak at 668 nm; (4) with increasing protein concentration, only the 668- and 680-nm ellipticity bands were affected. The effect of protein concentration may be explained by a small shift in protein conformation which slightly alters transition energies and would result in relatively large changes in the region of the CD spectrum due to exciton interactions.

The exciton split can be roughly calculated from the maximum and minimum CD bands (680 and 668 nm) and is approximately 260 cm^{-1}. Using a simple model of a dimer for the interacting chromophores of APC B, we found that this exciton split corresponds to a distance of 11 Å between two phycocyanobilin chromophores (Kasha et al., 1965). The dimer model is consistent with only two allowed transitions in absorption and in CD and is conceivable because: only two CD bands were observed for the long-wavelength absorption band, and no splitting was observed in the 680-nm absorption band at −196 °C. It is generally accepted that in APC one chromophore is bound per polypeptide (Glazer & Fang, 1973) and this was also reported for APC B from several blue-green algae (Bryant et al., 1976). Exciton interaction then implies that some of the chromophores in APC B would be at the contact region between subunits within a range of ~11 Å.

The visible absorption spectrum of APC I is analyzed in terms of four electronic transitions represented by the peaks at 645, 655, 665 (9), and 680 nm. We suggest that the 645-, 665(9)-, and 680-nm bands are the result of strong exciton coupling among at least three phycocyanobilin chromophores, and that the 655-nm band is not part of the strong exciton coupling system. It probably is due to chromophores in a different type of interaction. These conclusions are based on the following observations. (1) The 655-nm band in APC I corresponds to the same band observed in the CD spectra of APC II and III. (2) Treatment with a chaotropic agent, LiCl, which disrupts subunit–subunit interactions, had a large effect on the 645-, 665-, and 680-nm bands and almost no effect on the 655-nm band. (3) Denaturation with $NaDodSO_4$ almost abolished all the CD signals in APC I, as well as in the other APC forms. (4) With $NaDodSO_4$, the absorption spectra of separated α, β, and γ subunits of APC I were very similar at 585–590 nm (not shown), indicating that all the chromophores are chemically identical with phycocyanobilin. (5) Phycocyanobilin shows a single allowed transition in the visible region. (6) The fifth band located at the 620- nm region is probably due to noninteracting phycocyanobilin chromophores occurring in APC I because it can be increased by chaotropic agents or by aging. From all the considerations above, we conclude that exciton interaction occurs among at least three phycocyanobilin chromophores probably in the contact region of APC I subunits.

Fluorescence polarization–excitation spectra of APC I and B were similar in appearance to those obtained for several phycobiliproteins (Dale & Teale, 1970; Teale & Dale, 1970; Grabowski & Gantt, 1978) and indicate that their absorption bands are composed of at least two groups of chromophores. The latter authors suggested that energy transfer occurs only

by the inductive resonance (Förster) mechanism from sensitizing (s) to fluorescing (f) chromophores on the same protein molecule. On the other hand, MacColl et al. (1980) suggested that in APC II and III, excitation transfer occurs by intermediate coupling rather than the very weak (Förster) type. The intermediate coupling may be a probable mechanism in APC II and III in *Nostoc* as well.

We consider the chromophore–chromophore interaction and energy transfer in the long-emitting allophycocyanin forms to occur at several levels. Energy migration in one group of chromophores in APC I and B is due to a delocalized (strong) exciton mechanism as shown above. In addition, the inductive resonance mechanism (Förster) may be involved in energy transfer among noninteracting chromophores (618-nm absorption band in APC B), as well as between the latter group and the strongly (exciton) coupled chromophores. Similarly, in APC I the Förster (1960, 1965) mechanism may operate between chromophores absorbing at 655 nm and the strongly coupled (exciton) chromophores. The lowest energy level (680 nm) resulting from the exciton splitting is the fluorescent state in both APC I and B. The principal fluorescence emission of intact phycobilisomes at −196 °C was identical with those of APC I and B, suggesting that both are the final emitters in phycobilisomes. The exciton mechanism provides the long-emitting forms with more efficient energy transfer to chlorophyll due to increased spectral overlap between the APC fluorescence emission and chlorophyll absorption in vivo.

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