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Role of Allophycocyanin as a Light-Harvesting Pigment in Cyanobacteria

(photosynthetic action spectra/phycobilisomes/phycobiliproteins/chlorrophyll *a*)

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ABSTRACT **Photosynthetic action spectra of several cyanobacteria show a peak at about 650 nm, the height of which is correlated with allophycocyanin content in the strains examined. Allophycocyanin harvests light more efficiently than do phycocyanin and phycoerythrin. The contribution of chlorophyll *a* absorption to photosynthetic activity is barely detectable in cells of normal pigment composition. Chlorophyll *a* becomes the major light-harvesting pigment in cells that have been physiologically depleted of phycobiliproteins.**

In cyanobacteria ("blue-green algae") and rhodophytes, water-soluble chromoproteins known as phycobiliproteins are always associated with the photosynthetic apparatus. They are localized in phycobilisomes, granules about 40 nm in diameter, attached in regular array to the outer membrane surfaces of the thylakoids (1–5). Phycobilisomes are largely (perhaps entirely) composed of phycobiliproteins (6). They function as peripheral light-harvesting organelles; that is, action spectra of photosynthesis and of the Emerson effect show that most of the light energy channelled to photosystem II in these biological groups is absorbed by phycobiliproteins (7–14). Light absorbed by the pigments in the thylakoids (carotenoids and chlorophyll *a*) makes at most a very minor contribution to photosynthetic oxygen production.

Phycobiliproteins are assignable to three special subclasses: phycoerythrins, phycocyanins, and allophycocyanins. All cyanobacteria and rhodophytes contain allophycocyanin and phycocyanin, sometimes accompanied by phycoerythrin (15). Most of the cellular phycobiliprotein absorbance is attributable to phycocyanin, phycoerythrin, or a mixture of the two pigments; and action spectra show that both are effective in harvesting light. Despite its invariable association with the photosynthetic apparatus of these two biological groups, the role played by allophycocyanin has remained unclear. This is, in part, attributable to its comparatively recent discovery (16), and, in part, to its low concentration in the cell. As a rule, allophycocyanin accounts on a weight basis for 10% or less of the total cellular phycobiliprotein. Its absorption maximum (about 650 nm) in whole cells or crude extracts is largely masked by the much greater absorbancy of phycocyanin and of chlorophyll holochromes in this region. A few published photosynthetic action spectra of cyanobacteria and rhodophytes show a slight inflection, or a very minor peak, at (or near) 650 nm, which is possibly attributable to allophycocyanin, as Halldal (13) has suggested. The best evidence for its light-harvesting role was obtained by Blinks (9) during a study of the action spectra for chromatic transient activity (one manifestation of the Emerson effect) in marine algae. He noted that the action spectrum of a rhodophyte, *Porphyra perforata*, "extends well towards 650 nm, corresponding to the high allophycocyanin content of this species."

Gantt and Lipschultz (17) have suggested that allophycocyanin plays a role in energy transfer through the phycobilisome. This proposal was based primarily on the observation that when isolated phycobilisomes of the unicellular rhodophyte *Porphyridium cruentum* are excited with light absorbed by phycoerythrin, they emit fluorescence of a much longer wavelength, attributed to allophycocyanin by these authors.

MATERIAL AND METHODS

Biological Material. The axenic cyanobacteria examined are maintained in the culture collection of the Service de Physiologie Microbienne, Institut Pasteur. The strains of *Synechococcus* 6312 and *Aphanocapsa* 6701 (described in ref. 18), were cultivated at 25° with magnetic stirring in medium BG-11 (18) and exposed to continuous fluorescent illumination at an intensity of 2000–3000 lux. *Phormidium fragile*, a marine strain originally obtained from Prof. Ralph Lewin, was cultivated in a medium of 75% (v/v) filtered seawater containing the ingredients of medium BG-11 at half strength, except for Na_2CO_3 and trace metals which were added at full strength, and further supplemented with 4 μg/liter of vitamin B_{12}. Cultures of this organism were grown without agitation at 25° and exposed to continuous fluorescent illumination at an intensity of 300–500 lux. Observations were made on cells harvested during exponential growth.

A special procedure was used to obtain cells of *Aphanocapsa* 6701 depleted of phycobiliproteins. Exponentially grown cells were harvested by centrifugation, washed, and resuspended in medium BG-11 with the nitrogen source (KNO_3) omitted. This suspension, gassed continuously with air–1% CO_2, was exposed to continuous fluorescent illumination (3500 lux) at 25° for 72 hr.

Determinations of Phycobiliprotein Composition. Cells of *Aphanocapsa* 6701 and *Phormidium fragile* were suspended in 0.01 M phosphate buffer (pH 7.0)–0.15 M NaCl, and lysed by several cycles of freezing and thawing. The chlorophyll-bearing membranous material was removed from the lysate by centrifugation, and the absorbance of the supernatant liquid was measured at 562, 615, and 652 nm. The relative proportions by weight of phycoerythrin, phycocyanin, and allophycocyanin were calculated from the simultaneous equations of Bennett and Bogorad (19). Cells of *Synechococcus* 6312 could not be lysed by freezing and thawing, and were disrupted in a French pressure cell at 20,000 lb/inch^2. After removal by centrifugation of unbroken cells and coarse partic-

TABLE 1. *Phycobiliprotein complements of cyanobacteria examined*

Strain	Percent by weight of total phycobiliprotein		
	Phycoerythrin	Phycocyanin	Allo-phycocyanin
Synechococcus 6312	0	59	41
Aphanocapsa 6701	49	41	10
Phormidium fragile	93	6	1

ulate material, the extract was subjected to chromatography on a column of DEAE-cellulose (20), from which the phycocyanin and allophycocyanin fractions were separately eluted. Their relative proportions by weight were determined from measurements of absorbancy on the eluates at 615 and 650 nm, respectively.

Measurements of Action Spectra. The rate of oxygen evolution was measured by the modulated polarograph technique of Joliot and Joliot (21). A thin film of cells was deposited on the electrode. The circulating medium consisted of 0.05 M phosphate buffer (pH 7.5) containing 0.1 M KCl for the *Synechococcus* and *Aphanocapsa* strains, and of filtered seawater for *Phormidium fragile;* it was continuously aerated with air–1% CO_2. The modulated signal was detected by a lock-in amplifier and automatically recorded. The exciting light beam was provided by a Bausch and Lomb monochromator, with a 900-W Xenon lamp, modulated at 19 Hz. The incident light energy at each wavelength was measured with an Eppley thermopile, calibrated by the National Bureau of Standards. The band width used was selected in terms of the photosynthetic activity of the material, and is indicated in the description of each experiment.

The reference wavelength was that of maximal phycobiliprotein absorbance, except for phycobiliprotein-depleted *Aphanocapsa* 6701, for which a reference wavelength of 675 nm (maximal chlorophyll absorbance) was used. The light intensity at the reference wavelength was adjusted so that it lay within the region of linear photosynthetic response. During each experiment, the response at the reference wavelength was redetermined at regular intervals, in order to correct for any variations of activity. Data are expressed in arbitrary units, as relative rates of oxygen production per incident nanoeinstein. In the graphical presentations, action spectra have been normalized to absorption spectra at 620 nm.

Measurements of Absorption Spectra. The absorption spectra *in vivo* used for comparison with action spectra were measured at room temperature in a Cary 14 spectrophotometer, equipped with scattered transmission accessory 1462. Spectra of cells at the temperature of liquid nitrogen were measured with the device of Hoarau and Leclerc (22).

RESULTS AND DISCUSSION

The phycobiliprotein complements of the strains examined are shown in Table 1. *Synechococcus* 6312 contains only phycocyanin and allophycocyanin; the content of the latter is exceptionally high, representing 40% by weight of the total phycobiliprotein. *Aphanocapsa* 6701 contains about equal weights of phycocyanin and phycoerythrin, allophycocyanin accounting for some 10% of the total. *Phormidium fragile*

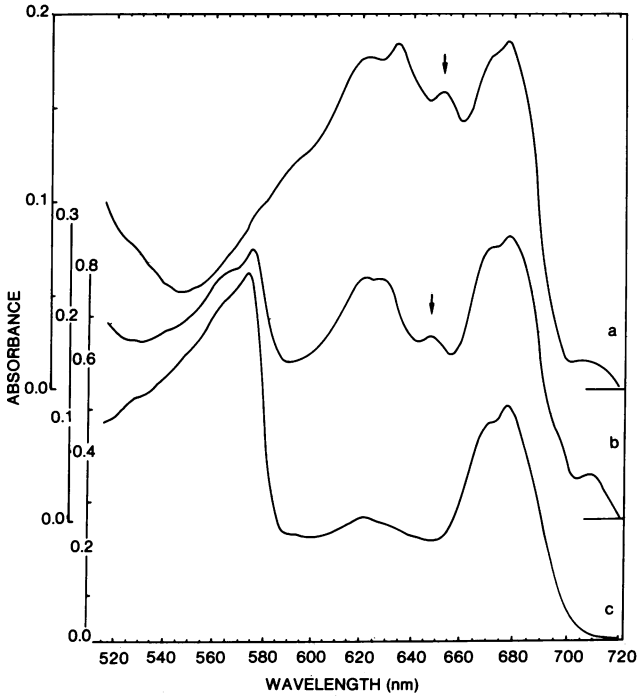

FIG. 1. *In vivo* absorption spectra at −196°. (*a*) *Synechococcus* 6312; (*b*) *Aphanocapsa* 6701; (*c*) *Phormidium fragile. Arrows,* allophycocyanin peaks at 645 nm.

contains predominantly phycoerythrin; phycocyanin accounts for 6% and allophycocyanin for 1% of the total.

Even in *Synechococcus* 6312, the allophycocyanin peak in the cellular absorption spectrum is not sharply defined at room temperature. However, an allophycocyanin peak at 645 nm is clearly resolved in this strain and in *Aphanocapsa* 6701 (though not in *Phormidium fragile*) when cellular absorption spectra are measured at the temperature of liquid nitrogen (Fig. 1).

The low-temperature spectra reveal several other features not evident in room-temperature spectra (Figs. 2–6): notably a series of peaks attributable to different chlorophyll holochromes situated between 710 and 670 nm, and a double peak (626 and 619 nm) in the region of maximal phycocyanin absorbance. Since the ratio A_{626}/A_{619} is markedly higher in

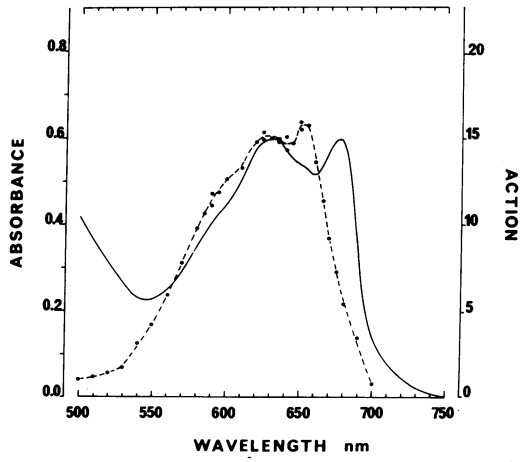

FIG. 2. Absorption spectrum (——) and action spectrum (●– – –●) of *Synechococcus* 6312. Action spectrum measured with half bandwidth of 10 nm.

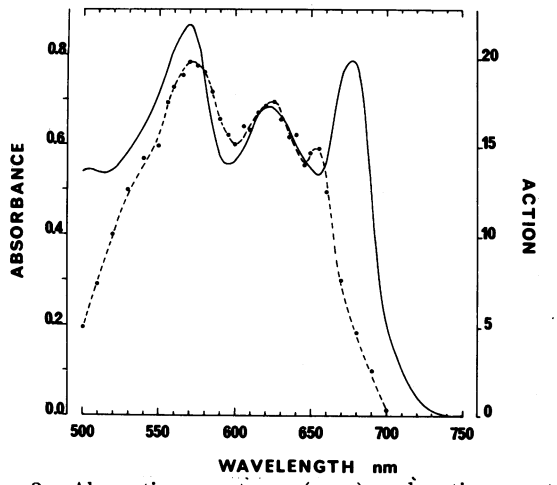

FIG. 3. Absorption spectrum (——) and action spectrum (●– – –●) of *Aphanocapsa* 6701. Action spectrum measured with half bandwidth of 10 nm.

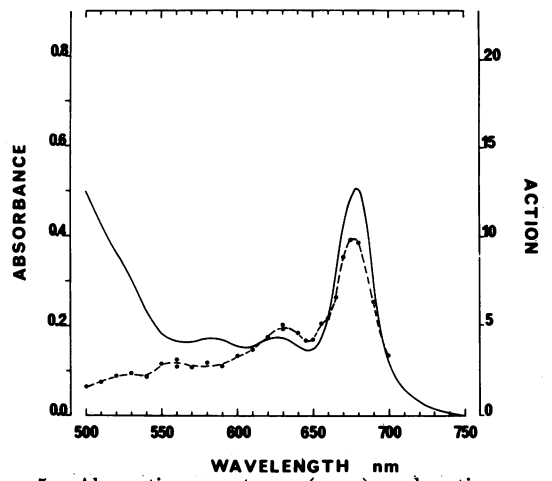

FIG. 5. Absorption spectrum (——) and action spectrum (●– – –●) of *Aphanocapsa* 6701 depleted of phycobiliproteins. Action spectrum measured with half bandwidth of 10 nm.

Synechococcus 6312 than in *Aphanocapsa* 6701, the peak at 626 nm may be largely (if not entirely) attributable to allophycocyanin; the spectrum of pure allophycocyanin shows two shoulders at wavelengths that are situated close to the absorption maximum of pure phycocyanin (23). The importance of the peak at 645 nm (Fig. 1) is correlated with the determined allophycocyanin contents of the three strains (Table 1).

Peaks at 630 and 655 nm are evident in the action spectrum of *Synechococcus* 6312 (Fig. 2). The lower action peak at 630 nm corresponds in position to the phycocyanin absorption maximum; the higher action peak at 655 nm has no counterpart in the room-temperature absorption spectrum, but corresponds in relative position to the allophycocyanin peak in the spectrum measured in liquid nitrogen (Fig. 1). At longer wavelengths, photosynthetic activity declines continuously; not even an inflection can be detected at 678 nm, where the red absorption peak of chlorophyll *a* is located. From the structure of the action spectrum, the relative efficiencies with which the three pigments contribute to photosynthetic oxygen production are clearly in the order: allophycocyanin > phycocyanin >>> chlorophyll *a*.

Peaks at 570, 625, and 652 nm occur in the action spectrum of *Aphanocapsa* 6701 (Figs. 3 and 4). The two former correspond, respectively, to the positions of the phycoerythrin and phycocyanin peaks in the absorption spectrum. The action peak at 652 nm, attributable to allophycocyanin, has no correspondence in the room-temperature absorption spectrum, and provides a further demonstration of the high efficiency with which this pigment harvests light. When the action spectrum of this strain was measured with a half bandwidth of 10 nm, the decline in photosynthetic activity at wavelengths longer than 652 nm was continuous (Fig. 3). However, a repetition of the experiment with a half bandwidth of 3 nm revealed a slight shoulder at about 675 nm (Fig. 4). Action spectra very similar in structure to those shown in Figs. 3 and 4 have been obtained with two other *Aphanocapsa* strains, 6605 and 6711, both of which resemble *Aphanocapsa* 6701 in phycobiliprotein composition.

When cyanobacteria are illuminated without a nitrogen source in the presence of CO_2, the phycobiliproteins of the cells are destroyed, although both viability and chlorophyll

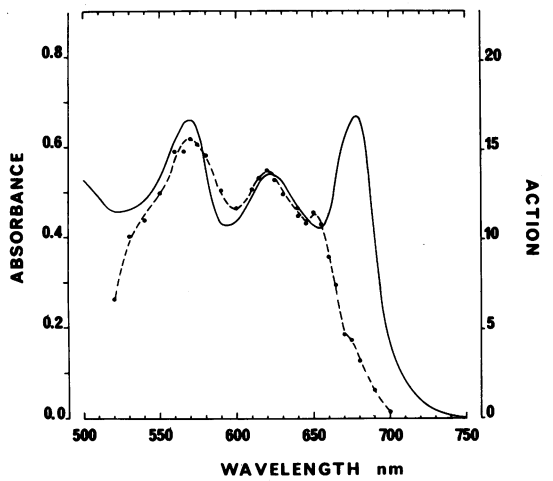

FIG. 4. Absorption spectrum (——) and action spectrum (●– – –●) of *Aphanocapsa* 6701. Action spectrum measured with half bandwidth of 3 nm.

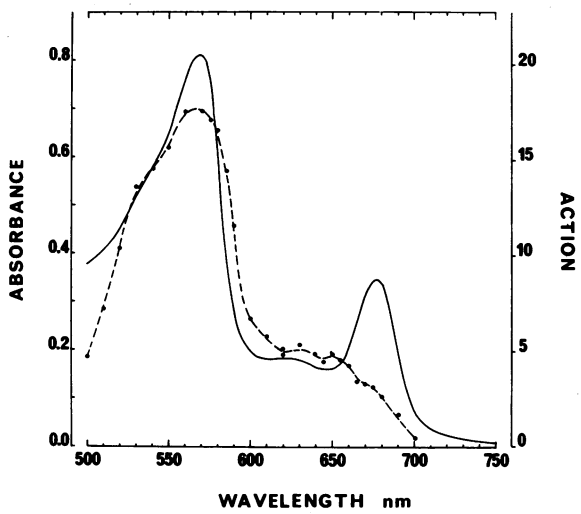

FIG. 6. Absorption spectrum (——) and action spectrum (●– – –●) of *Phormidium fragile.* Action spectrum measured with half bandwidth of 3 nm.

content remain high (24). An action spectrum was determined on cells of *Aphanocapsa* 6701 that had been depleted of phycobiliproteins by this treatment (Fig. 5). The cells still retained a substantial capacity to produce oxygen in the light; but the structure of the action spectrum was completely different from that of cells having a normal phycobiliprotein content. Chlorophyll *a* is evidently the principal light-harvesting pigment in the depleted cells, although the slight shoulder at 655 nm might be attributable to residual allophycocyanin. The results of this experiment suggest that when the cellular phycobiliprotein level becomes too low to maintain photosynthetic activity, the light-harvesting role of these pigments is assumed by chlorophyll *a*. The path of energy transfer to photosystem II in cyanobacteria can therefore undergo major physiologically induced changes. A similar change of light-harvesting properties was demonstrated by Volk and Bishop (25) in a phycocyaninless mutant of the unicellular rhodophyte *Cyanidium caldarium*.

The action spectrum of *Phormidium fragile* (Fig. 6) shows a major peak at 565 nm, which corresponds in position to the phycoerythrin peak in the absorption spectrum. Despite the very low levels of both phycocyanin and allophycocyanin characteristic of this species, minor action peaks at 630 and 655 nm, attributable to these pigments, are also evident. A small participation of chlorophyll *a* is indicated by a shoulder at 675 nm.

To summarize, allophycocyanin appears to be the most efficient light-harvesting pigment in all the cyanobacteria that we have examined. In the special case of *Synechococcus* 6312, a large fraction of the energy transferred to photosystem II is absorbed by this phycobiliprotein. However, the concentration of allophycocyanin relative to that of other phycobiliproteins in most cyanobacteria and rhodophytes is so low that this pigment cannot play a quantitatively significant role in energy capture, despite its high efficiency of energy transfer. The universal occurrence of allophycocyanin in the photosynthetic apparatus of both biological groups probably reflects a different primary function: that of an intermediate in the chain of energy transfer from the major light-harvesting pigments of the phycobilisome, phycoerythrin and phycocyanin, to chlorophyll molecules in the thylakoid. Needless to say, a high efficiency of energy transfer is essential for performance of this role. The observations of Gantt and Lipschultz (17) on the fluorescence emission spectrum of isolated phycobilisomes also suggest that allophycocyanin is an intermediate in energy transfer.

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