Light-induced Changes in Allophycocyanin

ITZHAK OHAD, HANS-JÖRG A. W. SCHNEIDER, STEVEN GENDEL, AND LAWRENCE BOGORAD
The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Received for publication January 25, 1979 and in revised form August 9, 1979

ABSTRACT

Several lines of evidence indicate that allophycocyanin is the previously unidentified "phyochrome" observed in extracts of blue-green algae.

Fractions containing phycoerythrin, phyocyanin, and allophycocyanin and exhibiting light-induced absorbance changes were prepared from extracts of Nostoc muscorum and Fremyella diplosiphon by isoelectric focusing. Illumination of such fractions with red light (650 nanometers) causes a reduction in absorbance at 620 nm (=1 to 2%) and an increase at 560 nm. The effect, (previously observed by Björn and Björn [1976 Physiol Plant 36: 297-304]) is reversible, upon illumination with green light (550 nm). Selective immunoprecipitation of the phycobiliproteins indicates that allophycocyanin is the photosresponsive pigment.

At pH 4.0 to 4.2, allophycocyanin purified from the same algae or from Phormidium luridum exhibits a light-induced absorbance change at 620 nm, which coincides with its absorption maximum at this pH; the fluorescence emission of allophycocyanin under these conditions is at 647 nm and its S0.05= 2.28, compatible with an a0,b1 polypeptide composition. At neutral pH (5.8 to 7.0), allophycocyanin aggregates have a sedimentation coefficient of 4.8 (=a0,b1) and an additional absorption peak at 640 nm appears while that at 620 nm remains unaffected. The fluorescence emission maximum of the larger aggregate is at 667 nm and the light-induced change in its absorption is shifted to 650 nm. The effect of pH changes in the range 4.0 to 7.0 on the spectral and aggregation properties of allophycocyanin is completely reversible. Changes in pH which affect allophycocyanin aggregation have parallel effects on absorption and fluorescence maxima as well as on the light-induced absorbance changes of the biliprotein.

No evidence is provided to resolve whether this phyochrome plays the role of an adaptochrome.

PBPs are the principal photoreceptors for photosynthesis in blue-green, red and some other groups of algae. APC and PC are usually present in blue-green algae. PE, a red PBP with a bile pigment chromophore different from the one common to PC and APC, is present in a number of blue-green algae. The polypeptides of PE, PC, and APC are all different. The pigment composition of some algae depends upon the color of light in which the organisms are growing. Adaptation to light is complementary. Algae growing in red light do not synthesize PE and tend to accumulate more PC and APC; those growing in green light accumulate PE in addition to APC (4).

Fujita and Hattori (6) found 541 nm light most effective for promoting PE production and 641 nm light most favorable for promoting PC synthesis in photobleached Tolypothrix tenus. Diakoff and Scheibe (5) found the major maxima in the action spectrum to be at 550 and 660 nm, with much smaller responses to 350 and 360 nm light, for PC and PE production, respectively. The action spectrum maxima for PC production by Fremyella diplosiphon are at 463, 641, and 575 nm, while those for PE production are at 387 and 550 nm (10).

The photoreceptor(s) for chromatic adaptation, i.e. "adaptochromes" (4), have not been identified, but Scheibe (12) observed the A of a fraction of an extract of T. tenus to increase at 650 nm and decrease at 520 nm, when irradiated with green light. Subsequent illumination with red light reversed these changes. Björn and Björn (3) extended these observations on phyochromes, i.e. the algal pigments which exhibit these light-induced A changes in vitro, with extracts of several algae fractionated by isoelectric focusing. We have reproduced the observations of Björn and Björn (3) with extracts of Nostoc muscorum and have also studied preparations from F. diplosiphon and Phormidium luridum. The responsive fractions prepared by isoelectric focusing contain mixtures of PBPs. The responses persist after removal of PC and PE by immunoprecipitation, but are abolished by removal of APC. Purified APC exhibits a red light-induced decrease in A at 620 to 650 nm which is reversed by green light but no light-induced A change at about 520 nm occurs.

MATERIALS AND METHODS

Cultivation of Algae, Preparation and Fractionation of PBPs. F. diplosiphon was grown and harvested as described by Bennett and Bogorad (1). Cells were stored at -20 C. Frozen N. muscorum and P. luridum were both generous gifts from Dr. H. W. Siegelman of the Brookhaven National Laboratory, Upton, N.Y.

PBPs were harvested as described by Bennett and Bogorad (1) except that sonication at 0 C was accomplished by three bursts of 15 s each with intermittent cooling of the sonicator tip. The supernatant of the extract obtained after centrifugation at 8,000g for 60 min was dialyzed against 30 volumes of distilled H2O overnight at 4 C. The dialysate was brought to pH 4 by the addition of acetic acid or Ampholine (pH 4.0-6.0 to 1%). If a precipitate resulted it was removed by centrifugation at 10,000g for 15 min. The clear supernatant, which was made to 1% pH 4.0 to 6.0 Ampholine (if Ampholine had not already been added), was used for electrofocusing as described by Björn and Björn (3). Electrofocusing was at 0.5 w constant power (ISCO 493 power supply) with the anode at the top of the 440 ml LKB column. Light-induced A changes were exhibited by the lower bluish band at the pH 4 end of the column. This material was collected in the initial 10 to 14 fractions of 5 ml each. The active fractions were pooled and stored at 4 C.
Purified APC was prepared from *F. diplosiphon* cells as described by Bennett and Bogorad (1). Purified APC from *P. luridum* was kindly supplied by Dr. H. W. Siegelman.

**Analytical and Sucrose Density Gradient Centrifugation.** Sedimentation velocity determinations were made in a Beckman model E ultracentrifuge equipped with a photoelectric scanner. Centrifugation was at 21°C at 47,440 rpm for about 2.5 h and it was monitored at 280, 360, 620, and 660 nm. The PBP-containing solution was at pH 3.6; its A was about 0.7 to 0.8 at 620 nm. Sucrose density gradient centrifugation was carried out in the SW 56 rotor of the Beckman model L2-65B centrifuge at 40 to 50,000 rpm for 18 to 20 at 5°C.

Linear sucrose gradients (5–20%) were prepared in 25 mm Na-phosphate adjusted to the desired pH below 5 by addition of acetic acid. Purified *F. diplosiphon* PE, prepared according to Bennett and Bogorad (1), and Cyt c (Sigma) were used as markers.

**Spectrophotometric Measurements.** Absorption spectra were obtained with an Aminco DW-2 spectrophotometer operated in the split beam mode. Light-induced A changes were measured as absorption spectra between a reference aliquot kept continuously in darkness and a green (560 nm) or red (645 nm) light-illuminated sample. The time course of light-induced A changes of PBP fractions or purified APC was recorded at 615 or 620 nm. A 20 nm half-bandwidth Baird Atomic interference filter with transmission maximum at 615 nm was used to protect the photomultiplier. Exciting light was filtered through either a 560 or 645 nm interference filter. The sample was illuminated at 90° by a 0.5-cm diameter light pipe mounted at a port on the side of the cuvette holder delivering light from a 150-w tungsten-halogen lamp. The appropriate filter was located between the end of the light pipe and the cuvette containing the sample. The light intensities at the cuvette surface were about 50 and 150 μW/cm²-nm at the filter’s transmission peak for the green and red filters, respectively.

Action spectra for light-induced A changes were calculated from the kinetics of changes elicited at various light intensities by light passed through interference filters.

**Fluorescence Emission and Excitation Spectra.** Fluorescence spectra were recorded using a Perkin-Elmer spectrofluorometer (Norwalk, Conn.). For emission spectra the photomultiplier current was 700 to 800 volts. The spectrophotometer was operated in the “ratio-mode” with both excitation and emission slits at position 10. At the PBP concentrations used, a reasonable spectrum could be obtained in the instrument’s sensitivity range 3 to 10. Excitation was at 380 nm. Emission spectra were recorded using slits at position 4 with photomultiplier current below 1,000 volts. Emission was recorded at 680 nm.

**Gel Electrophoresis.** APC preparations from *P. luridum* and *F. diplosiphon* were tested for purity by gel electrophoresis in SDS using 12% polyacrylamide in 22-cm-1 slab gels at 25 mmamp at room temperature for 6 h (1).

**Immunoprecipitation of Phycobiliproteins.** Antibodies obtained from rabbits immunized with purified PE, PC, or APC from *F. diplosiphon* (1) were adsorbed on protein A-Sepharose (Pharmacia). Solutions containing mixtures of PBPs obtained by electrofocusing were passed through one or more columns of bound antibody (6 mm × 8–10 mm high).

**RESULTS**

Light-induced A Changes in PBP Fractions Obtained by Electrofocusing Algal Cell Extracts. As described by Bjorn and Bjorn (3), a large number of pigmented bands appear during electrophoresis of dialyzed extracts of blue-green algae. In all preparations from extracts of cells containing PE, PC, and APC, the blueish band in the pH range 4.0 to 4.3, i.e. in the lower part of the column, was enriched in APC but also contained large amounts of PC and variable amounts of PE. The bands in the pH 4.3 to 5.0 region contained mostly PC with varying amounts of PE. The bands in the upper, i.e. higher pH, region of the column contained mostly PE with varying smaller amounts of PC. One PC-enriched band was also usually found in the upper part of the column.

Among the fractions containing various mixtures of PBPs, light-induced A changes were detected only in the APC-containing fraction in the pH range 4.0 to 4.3. A was found to increase in the 550 to 560 nm region and to decrease at about 640 to 650 nm (Fig. 1) following illumination of the sample with 645 nm light. The light-induced A change at about 550–560 nm will be referred to as the “555 nm” change for convenience. These effects were reversed by illumination with green light (560 nm). In all preparations obtained by electrofocusing *Nostoc* or *Fremyella* extracts, the
magnitude of the photoinduced $A$ change at 620 to 650 nm was equivalent to about 0.8 to 2% of the solution's $A$ at that wavelength region. The red light-induced decrease in $A$ shifted from about 620 to 650 nm toward slightly longer wavelengths in consecutive fractions starting from pH 4 toward higher pH values.

The ratio of the response at a to b (Fig. 1) varies among fractions in the same pH zone. It is higher in the intermediate than in the earlier or later fractions (Fig. 1). This could indicate that the positive and negative $A$ changes are the responses of two different pigments which vary in relative abundance among fractions. A second possibility is that the pH of the sample, which differs slightly from fraction to fraction, influences the response. Or, both pH and composition could vary. The effects of pH on the photoinduced $A$ changes are described in a later section of this paper.

The pigment composition and $a/b$ ratio of active fractions prepared by electrofocusing extracts of the same cells differed from column to column.

To identify the photoresponsive species, photoreactive samples obtained by electrofocusing were adjusted to pH 7 with phosphate buffer and passed through a column of antibody against PC, PE, or APC bound to protein A-Sepharose. Depleted solutions were analyzed for residual photoinduced changes. Photoresponsive characteristics of the sample were unaltered after immunodepletion of PE or PC (Fig. 2). In some samples with small initial responses the anti-PE treatment appeared to reduce the 555 nm response, perhaps because of dilution during the treatment. Immunochemical removal of APC eliminates the light-induced $A$ changes at both 650 and 555 nm. These results indicate that APC, or, at the minimum, a component removed by antibody against APC, is required for photoinduced $A$ changes.

**APC Purified by Density Gradient Centrifugation or Gel Electrophoresis Shows $A$ Changes.** Fractions obtained by electrofocusing were adjusted to pH 4 by addition of glacial acetic acid and centrifuged on 5 to 20% sucrose gradients (25 mm phosphate) in a Beckman model L2-65B for 18 h at 58,000 rpm using an SW 40 rotor. The upper blue band enriched in APC exhibited the reversible decrease in $A$ at about 620 to 650 nm induced by alternate red and green illumination but the changes shown by the initial sample at 555 nm did not occur. The bands enriched in PC and PE located in about the upper one-third of the gradient did not exhibit light-induced $A$ changes.

PE and PC separated from other PBPs by electrophoresis in 1.5 to 2% polyacrylamide tube gels at pH 6.8 exhibited no response in gel slices or after elution. On the other hand, APC contained within the gel behaved as described above for the sucrose gradient purified material. These loose gels were used to permit migration of native PBPs.

**pH and Concentration-related Changes in Properties of Purified APC from P. luridum or F. diplospihon.** To test further whether APC is responsible for the light-induced decrease in $A$ at 650 nm, properties of purified APC were investigated in more detail.

The $A$ spectrum of purified APC in neutral or slightly acidic solution (pH 5–7) includes a maximum at 650 nm and a prominent shoulder with inflections at 618 to 620 and 595 nm. At pH 3.5 the $A$ peak at 620 nm is virtually the same but the peak at 650 nm is completely absent.

At pH 4.2 to 4.4, $A$ at 650 was about equal to that at 620 nm (Fig. 3; cf. ref. 1). Such variations in $A$ spectra were also observed with pure PC from P. luridum, F. diplospihon, T. tenus, and Calothrix membranacea.

The fluorescence emission spectrum of purified APC is drastically affected by pH. Only one fluorescence emission peak was observed at all pH values tested, but its maximum was shifted reversibly from 647 nm at pH 3.5 to 667 nm at pH values between 5.5 and 7.5. At pH values 4.2 to 4.4 and above 8.0 the fluorescence

![Figure 2](attachment://image1.png)

**Fig. 2.** Effect of antibodies against PE, PC, and APC on the light-induced response of biliproteins from Nostoc. Absorption spectrum (A) and light-induced response (A') of the effluent from an anti-PC column; absorption spectrum (B) and light-induced response (B') of the effluent from an anti-PE column; absorption spectrum (C) and light-induced response (C') of the effluent from an anti-APC column. 1: Difference spectra of sample minus reference following illumination of sample with green light (550 nm); 2: as above, after illumination with red light (665 nm).

![Figure 3](attachment://image2.png)

**Fig. 3.** Difference $A$ spectra of purified APC from P. luridum at different pH values. Reference sample was at pH 7.5 (in 25 mm phosphate). $A$ of the initial solution was 0.3 at 625 nm. pH values of the sample solutions are shown.
The maximum was at 662 nm. Fluorescence yields were drastically decreased in the more alkaline solutions (Fig. 4).

Purified APCs of *P. luridum* or *F. diplosiphon* exhibit a red (645 nm) light-induced decrease in *A* at 620 to 650 nm which is reversed by illumination with green light (550-560 nm). As shown in Figure 5, the *A* maxima and the photoinduced *A* changes coincide. Both vary with the pH of the APC solution. At pH 3.6, the greatest *A* change upon illumination with red light is at about 620 nm. At pH 4.2, the maximal change is at about 652 nm. At pH 4.4, the broad decrease in *A* has characteristics of the changes observed at both pH 3.6 and 7.2.

The reversibility of the photoinduced effects became incomplete in solutions of *P. luridum* APC at pH values above 7.5. Similar effects were observed with *F. diplosiphon* APC and photoresponsive PBP mixtures obtained by electrofocusing total cell extracts.

To determine whether the pH-dependent spectral changes are related to the association/dissociation of APC subunits or to intramolecular changes in conformation, we investigated the effect of pH on sedimentation and fluorescence excitation spectra. The *S*<sub>20w</sub> of *P. luridum* APC is 2.28 and at pH 3.5, but 4.86 at pH 6.8. (The *S*<sub>20w</sub> value at pH 4 was close to that found at pH 3.5.) The calculated *S*<sub>20w</sub> values were similar using data obtained at 620 or 650 nm.

APC in 5 to 20% (w/v) sucrose gradients at pH 3.5 sedimented faster than Cyt c and at about the same rate as *Fremyella* PE at pH 10 but more slowly than PE at pH 6.8. PE is probably in the αβ<sub>1</sub> form at pH 10. Apparently APC is more aggregated at higher than at lower pH values (Fig. 6).

Since the magnitude of the 650 nm *A* peak varies with pH (being entirely absent at lower pH values) while the *A* peak at 618 nm is essentially constant, it seemed possible that each might belong to a separate molecular entity. For example, the *A* at 618 could be by αβ<sub>1</sub> aggregates and *A* at 650 nm by more highly aggregated forms would be added to *A* at 618 nm. The fluorescence excitation spectrum of the APC solution at pH 4.2 to 4.4, a range in which the *A* peaks at 618 and 650 nm are about equal, could give information on excitation energy transfer between the two forms. Energy transfer should be affected by the concentration of the two species if the *A* peaks belong to separate molecules. The excitation maximum was at 620 nm for fluorescence emission at 680 nm (associated with the 650 nm *A* peaks) in *P. luridum* APC solutions at pH 4.4 over a wide concentration range (*A* of APC solutions at 620 nm from 2×10<sup>-1</sup> to 7.5×10<sup>-5</sup>). These data indicate that energy exciting the 618 to 620 nm absorbing species is transferred to the 650 nm absorbing form. Thus, both absorbing species are in a single aggregate.

The concentration of the APC solution has a remarkable effect on the *A* spectrum of the APC. At high concentrations of APC (618 nm *A* of 10<sup>-3</sup> or higher) the ratio of *A* of the 618 to the 650 nm peak was the same regardless of whether the pH of the solution was 4.4 or 7.0. At lower concentrations the height of the peak at 618 nm remained constant but that at 650 nm dropped at pH 4.4 (Fig. 7).

**Kinetics of Light-induced *A* Changes.** The kinetic data of the light-induced *A* changes were similar in solutions of APC from *P. luridum* or *F. diplosiphon* and in PBP fractions prepared by electrofocusing of *N. muscorum* or *F. diplosiphon* extracts. Maximum *A* changes were elicited by 45- to 60-s illumination at the intensity used (Fig. 8). Half-time for the rise or fall in *A* ranged between 3 and 8 s in various preparations.

The rates of changes in *A* were not significantly influenced by temperature over the range 0 to 27 C (Fig. 8). This indicates that a single molecular species is involved in the spectral changes observed.

**Action Spectra for Light-induced Changes in *A* by PBP Fractions and APC.** Action spectra for the red light-induced decrease in *A* at 620 nm have been determined for the *N. muscorum* PBP fraction obtained directly from an electrofocusing column. For equal incident energy the maximum effect of red light is at 620 to 645 nm. The effect of 600 or 656 nm light is about 50% of the maximum. The action spectrum for reversal of the red light-induced effect shows a maximum between 550 and 575 nm. Half-maximal response is at 538 and 612 nm (Fig. 9). Essentially similar effects were observed with APC from *P. luridum* or *F. diplosiphon*.

**DISCUSSION**

Earlier work by Scheibe (12), Björn and Björn (3), and Björn (2) on light-induced *A* changes in preparations from blue-green algae left unanswered the questions of: (a) the identity of photoresponsive pigment or pigments involved in the *in vitro* *A* changes; and (b) whether the photoresponsive pigment or pigments have anything to do with the photoregulation of biliprotein synthesis. We have addressed the first of these questions in the present work.

We have confirmed the observation of Björn and Björn (3) that isoelectric focusing of blue-green algal extracts yields a fraction which exhibits light-induced *A* changes. Photoresponsiveness was eliminated by immunoprecipitation of APC from active fractions of *N. muscorum* and *F. diplosiphon* extracts containing PC, PE, and APC. Removal of PC or PE by immunoprecipitation had no effect. APC separated from PC and PE in photoresponsive electrofocused fractions by gel electrophoresis or sucrose density gradient centrifugation showed light-induced *A* changes only at 620 to 650 nm. Finally, APC samples purified from three species of blue-green algae in two laboratories exhibited photoinduced *A* changes.

---

*The absorption maximum of APC in the low pH form is at about 618. In the high pH form this becomes a broad shoulder between 618 and 620. We will refer to the peak or shoulder interchangeably and as being at 618 nm.*
changes in the red region of the spectrum comparable to those displayed by electrofocused extracts of the algae after removing PE and PC. The data indicate that APC is the pigment responsible for the light-induced \( A \) changes in electrofocused fractions but the failure of purified APC to display reversible bleaching in the green region of the spectrum is puzzling.

To examine further whether APC is the pigment responsible for photoinduced changes at 620 to 650 nm in algal extracts, we studied the effects of various conditions and agents on properties of APC and on photoinduced \( A \) changes. As summarized in Table I, APC from \( N. \) muscorum or \( F. \) diplosiphon in solution at pH 3.4 is small in size, possibly being aggregated in the \( \alpha_1\beta_1 \) form, displays an \( A \) maximum at 618 to 620 nm, and a light-induced reversible \( A \) change at about 618 to 620 nm. Its fluorescence maximum is at 647 nm. At pH 6.8 to 7.2, the \( S_{20w} \) is 4.86, the \( A \) peak at 650 nm is greater than that at 618 to 620 nm and the red light-induced \( A \) drop maximum is at 650 to 660 nm. The fluorescence emission maximum of this species is at 667 nm. In the pH range 7.0 to 7.5 the magnitude of the red light-induced \( A \) drop increases but reversibility is lost. The coincidence of alterations in light-induced

**FIG. 5.** Effect of pH on wavelength of \( A \) maxima and light-induced change in \( A \) in APC from \( F. \) diplosiphon. \( A \) and difference spectra at pH 3.6 (\( A \) and \( A' \)), pH 4.4 (\( B \) and \( B' \)), and pH 7.2 (\( C \) and \( C' \)). Curve sets 1 and 2 as in Figure 1.

**FIG. 6.** Effect of pH on sedimentation in sucrose gradients of \( P. \) luridum APC and on \( A \) spectra of APC isolated from gradients. 1: \( A \) spectra of APC from sucrose gradients after centrifugation; 2: sedimentation pattern after 9 h at 246,000g followed by 9 h at 157,000g; 3: sedimentation pattern after 17 h at 157,000g. A: APC, pH 3.5; B: APC, pH 4.0; C: APC, pH 4.2; D: APC, pH 4.6; E: APC, pH 6.8; F: Cyt c (upper band) and Fremyella PE (lower band); pH 6.8; G: Fremyella PE, pH 10.
A changes with shifts in $A$ spectra provides additional evidence that the photoresponsive species is APC. This view is further strengthened by our finding the same coincidence in APCs purified from several blue-green algal species in two laboratories.

The state of aggregation of PBP subunits has long been known to be pH-dependent (4). Our sedimentation results indicate that, opposite to the situation for PE, low pH favors disaggregation and high pH favors aggregation of APC. Figure 6 shows that the $A$ spectrum varies with degree of association or dissociation of the APC. Chemical agents which might affect aggregation influence both the $A$ and the light-induced spectral changes in the same manner as variations in pH. Results of preliminary experiments show that at neutral pH (6.8–7.0) $\beta$-mercaptoethanol at 25 to 250 nm reduces the $A$ of APC at 652 nm until this peak disappears completely. However, $\beta$-mercaptoethanol had no effect on the $A$ at 618 nm nor on the light-induced decrease in $A$, which was maximal at 618 nm, as in a solution of pH 3.5 to 4.0 without $\beta$-mercaptoethanol. In this concentration range, $\beta$-mercaptoethanol had little or no effect on the $A$ spectra of PE or PC in neutral solutions.

![Figure 7](image1)

**Fig. 7.** Changes in $A$ spectrum of *P. luridum* APC as a function of concentration at two pH values. An APC solution in 25 mm phosphate buffer was adjusted to pH 4.4 (●) or pH 7.0 (○○○○○) and $A$ at 650 and 618 nm was measured at several concentrations. Data are given as the ratio of $A$ 618/650 nm.

![Figure 8](image2)

**Fig. 8.** Kinetics of changes in $A$ at 620 nm following illumination with 645 nm light (●) or 560 light (○). A: biliprotein fraction in 10% sucrose prepared by electrofocusing *N. muscorum* extracts, 27 C; B: same as in A, 12 C; C: same as in A, $-0.7$ C; D: purified *F. diplosiphon* APC, 1 C; E: purified *P. luridum* APC, 1 C. pH of the above solutions was 6.8 for samples D and E, and 4.0 to 4.6 for samples A, B and C; vertical bar represents 0.002 $A$ units for samples A through D and 0.0005 for sample E; horizontal bar represents 20 s for samples A, D, and E and 10 s for samples B and C. Similar kinetic data were obtained when $A$ changes were measured at 550 nm in samples prepared by electrofocusing.

![Figure 9](image3)

**Fig. 9.** Action spectrum for light-induced changes in $A$ of biliprotein solution. Curves show relative effectiveness of equal energies of light-inducing (●●●) and reversing (▲▲▲) the $A$ changes at 620 nm.
Table 1. pH-dependent Properties of Allophycocyanins

<table>
<thead>
<tr>
<th>pH of Solution</th>
<th>Absorption Peak</th>
<th>Photoinduced Absorption Change</th>
<th>Fluorescence Emission</th>
<th>Probable Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ Max.</td>
<td>λ Max.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5-3.6</td>
<td>2.28</td>
<td>+</td>
<td>618</td>
<td>Yes</td>
</tr>
<tr>
<td>4.2-4.4</td>
<td>+</td>
<td>+</td>
<td>618-660</td>
<td>Yes</td>
</tr>
<tr>
<td>6.8-7.2</td>
<td>4.86</td>
<td>&lt; +</td>
<td>650</td>
<td>Reduced</td>
</tr>
<tr>
<td>7.5</td>
<td>&lt; +</td>
<td>650</td>
<td>No</td>
<td>667</td>
</tr>
</tbody>
</table>

The photoinduced spectral changes correspond in magnitude to about 2% of the A at the 620 or 650 nm maximum. It is not known whether this is a small A change exhibited by all of the APC molecules present or a response exhibited by a unique small population. Under some conditions, e.g., high pH, APC is permanently converted to an inactive form. Also, some of our preparations of APC have been inactive. We cannot exclude the possibility that only a small fraction of the APC molecules in our active preparations are responsive.

Several varieties of APC have been described in recent years. Glazer and Bryant (7) reported the presence in *Synechococcus sp.* and *Anabena variabilis* of a new APC which they designated B. Its major A maxima are at 671 nm (major) and 618 nm and its fluorescence maximum is at 689 nm. *Porphyridium cruentum* also contains APC B but with an A maximum at 669 and a fluorescence maximum at 673 (11). The A and fluorescence changes we have observed would not correspond to those of either type of APC B thus far reported. On the other hand, Gys and Zuber (8, 9) and Zilinskas et al. (13) have described APcs I, II, and III. The first two were from *Mastigocladus laminosus* (8, 9) and the latter two from *Nostoc sp.* (13). Evidence has been presented for the interconversion of APC I to APC II (8) and APC II to APC III (13). Zilinskas et al. (13) reported that APC I comprised 15 to 25% of the total from *Nostoc*. We have not resolved which if any of the three interconvertible species of APC is responsible for the light-induced A and fluorescence changes we have observed.

Scheibe (12) made the important suggestion that the reversible red and green photoinduced A changes he observed in algal cell extracts revealed two different absorbing forms of the same molecule, in analogy to the behavior of phytochrome. APC behaves in this way. Even though the purified APC retained reversible bleaching in the 620 nm region, it had lost the reversible bleaching at 550 to 560 nm seen in the crude preparations. Possible reasons for this loss include partial denaturation of the APC itself or loss of a minor pigment responsible for this reaction. In the latter case the unidentified pigment would have been trapped with the anti-body to APC, as this removed the 550 to 560 nm change in crude preparations.

Finally, is there any relationship between light-induced A changes in APC and complementary chromatic adaptation? Is the phytochrome, identified here as APC, an adaptionchrome? The light-induced A change at 620 to 650 nm exhibited by APC may be entirely gratuitous, i.e., a property unrelated to any function. In its more aggregated state APC retains an A peak at 618 but absorbs principally at 650 nm; it fluoresces at 667 nm, a wavelength closer to the A maximum of CHL a. APC in phycobilisomes transfers energy absorbed by PE or PC to CHL a. This APC would be expected to occur in very large aggregates which might not display the light-induced changes we have described. Some smaller APC aggregates which might occur either in a part of the cell whose APC concentration is lower or in combination with other substances could have a regulatory function in chromatic adaptation and photomorphogenesis. Whether APC is an adaptionchrome and how shifts in APC A may affect blue-green algal metabolism is questions which remain to be addressed. It is interesting that the photomorphogenetic pigment phytochrome, which also exhibits light-induced A changes, appears to have a chromophore quite similar to that of APC, although phytochrome is a very large single polypeptide and APC is comprised of aggregates of small polypeptides.

**Acknowledgment**—We are indebted to Ms. Suzanne Groet without whose skilled technical assistance this work would not have been possible.

**LITERATURE CITED**