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Action Spectra for Phycobiliprotein Synthesis in a Chromatically Adapting Cyanophyte, *Fremyella diplosiphon*[1]

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ABSTRACT

The action spectra for phycocyanin production by the cyanophyte *Fremyella diplosiphon* shows maxima at 463 and 641 nm. The action spectrum for phycoerythrin production includes maxima at 387 and 550 nm. The maxima are based on a relative response rate well within the linear ascending portion of the dose response curves; the positions of the maxima are independent of the relative response rates chosen for reference over a 3-fold range although the comparative effectiveness of light at pairs of wavelengths varies with the standard used for comparison. These action spectra differ from those reported previously for *Tolypothrix tenuis* by Fujita and Hattori (Plant Cell Physiol. 3: 209–220) and by Diakoff and Scheibe (Plant Physiol. 51: 382–385) in that blue light strongly promotes phycobiliprotein synthesis in *F. diphosiphon* but has been reported to have little or no effect on *T. tenuis*.

These data suggest that the photoreceptor or photoreceptors which regulate phycobiliprotein metabolism in *F. diplosiphon* may be either one or two metalloporphyrin complexes or double pigment systems consisting of a red or green light-absorbing photoreceptor which can be activated directly or by energy transferred from a blue light-absorbing receptor.

Blue-green algae characteristically contain two phycobiliproteins (PBPs),[3] PC and APC, but some cyanophytes also produce the red bile pigment protein complex, PE. Complementary chromatic adaptation is the term used to describe an alga's accumulation of pigments complementary in color to incident irradiation. This phenomenon was first described by Engelmann (4) and Gaidukov (6) in 1902. Photoreceptive pigments for CCA have been designated adaptochromes (2).

Fujita and Hattori (5) and Diakoff and Scheibe (3) have determined action spectra for the production of PE and PC by the cyanophyte *Tolypothrix tenuis*. *Fremyella diplosiphon*, another filamentous alga of the family Scytonemataceae, forms APC and PC but not PE when grown in light of wavelengths longer than about 600 nm. When grown under fluorescent lamps, *F. diplosiphon* produces large amounts of PE as well (1). The latter light source is relatively rich in blue and green wavelengths and relatively poor in red wavelengths. We report here action spectra in the range 365 to 730 nm for the production of PC and PE by cultures of *F. diplosiphon*.

MATERIALS AND METHODS

Experimental Organism and Culture Conditions. *F. diplosiphon* (B. and F.) Drouet obtained from the Indiana University Culture Collection (No. 481) was grown axenically in liquid media and maintained on 1% solidified agar slants (9). Medium C (7) was used as described by Bennett and Bogorad (1) without sodium citrate, with KH_2PO_4 substituted for K_2HPO_4 and KNO_3 at 1 g/l. Growth conditions were also as described by Bennett and Bogorad (1) except that cultures were maintained at 35 ± 2 C and that red illumination was provided by six 60 w red incandescent light bulbs (GE 60A21/R) without further color filtration. Two incubation chambers (model G27, New Brunswick Scientific Co., New Brunswick, N.J.), one with continuous illumination provided by fluorescent lamps as previously described (1) and one with red incandescent lamps, were used to maintain cultures of PE-rich (fluorescent light-grown) and PC-rich (red light-grown) cells. The emission spectra of these two sources are shown in Figure 1. The physiology and morphology of *F. diplosiphon* in culture have been described by Wyatt *et al.* (10) for fluorescent light-grown cells. Bennett and Bogorad (1) have described the pigmentation and CCA of this cyanophyte starting from both red and fluorescent light-grown cells as well as the morphology of cells and filaments grown under these two conditions.

Dry Weight and PBP Assay. The algal cell mass and the PBP content were measured optically, the former by turbidity and the latter by light absorption at the peak absorption wavelength for the specific accessory pigment. These techniques are described by Bennett and Bogorad (1) giving the necessary extinction coefficients and simultaneous equations for calculation of PBP content.

Experimental Illumination. Monochromatic light was provided by interference filters with average half-bandwidths of 10 nm (Baird Atomic, Bedford, Mass., and Jena Glaswerk, Mainz, W. Germany) in combination with Airequipt series 100-slide projectors, 500 w General Electric projection bulbs (model DAK), and voltage regulators. All interference filters were checked for light transmission characteristics across the visible spectrum and calibrated for half-bandwidth and wavelength of maximum transmission on a Cary 14 recording spectrophotometer. No side bands with greater than 0.002% transmission were detected. The intensities of monochromatic light ranged from approximately 1×10^{-4} to 10 nE per cm² per sec, or 0.5 to 30,000 erg per cm² per sec, at 550 nm. Red and green plastic transparent filters (No. 2423 and No. 2092, respectively, Rohm and Haas Co., Philadelphia, Pa.) were used for broad band colored illumination in preliminary dose response experiments.

An ISCO spectroradiometer (Instrumentation Specialties Company, Lincoln, Neb., model SR), equipped with a sliding interference wedge of 15 nm half-bandwith, was used to measure incident light intensity at each wavelength station. The spectroradiometer was calibrated using a standard light source also

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[3] Abbreviations: CCA: complementary chromatic adaptation; PBP: phycobiliprotein; PC: phycocyanin; PE: phycoerythrin; APC: allophycocyanin.

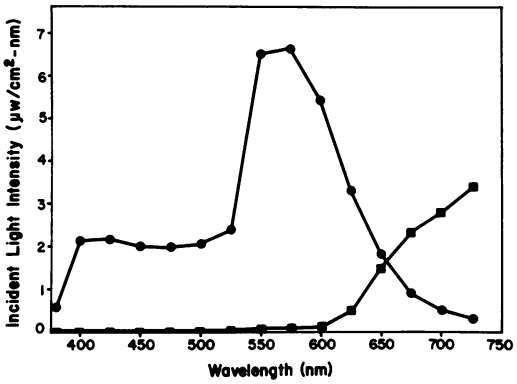

FIG. 1. Emission spectra of red (■——■) and fluorescent (●——●) lamps used for growing *F. diplosiphon*. Intensity was measured with the ISCO spectroradiometer probe positioned at the level of the cultures on the shaker platform itself. Total energy (summed for each 25 nm between 380 and 725 nm) was $\mu w/cm^2 \cdot nm$ for red light and 38 $\mu w/cm^2$ for fluorescent light. Sources of red and fluorescent illumination are described in the text.

supplied by ISCO (model SRC). For intensity measurements at 365 nm, a wavelength beyond the range of the spectroradiometer, an Epply thermopile (Newport, R.I.) was used in combination with a Keithley microvolt ammeter model 150 B (Cleveland, Ohio). This thermophile was calibrated against the spectroradiometer.

Cell Preparation and Responsivity Normalization. One-liter cultures in 2,800-ml Fernbach flasks grown in either the red or the fluorescent light chamber were transferred directly to the other light chamber for a 12- to 15-hr period prior to monochromatic illumination. From this culture duplicate 20-ml samples of cells in growth medium were placed in 2.5-cm-diameter test tubes and positioned 9 to 30 cm in front of the slide projector lens equipped with the appropriate interference filter. All experimental monochromatic illuminations were for 6 hr during which the light covered the entire 20-ml sample uniformly. A heating manifold consisting of stainless steel tubes connected in series to a constant temperature water bath and pump were immersed in each sample to maintain the temperature at 36 to 39 C. Humidified air was bubbled through each sample for aeration and stirring. Sterile conditions were maintained up to the time of aeration. All manipulations were carried out under red or fluorescent light, whichever was the last growth light for the culture. At the time of monochromatic illumination, the cells were actively growing and cultures used were between 0.05 and 0.15 mg dry wt/ml.

The remainder of the original 1-liter culture remained in the new light situation (red or fluorescent) during the 6-hr period that other samples were in monochromatic light. The total time in the new light was therefore 18 to 21 hr. This culture was used as a control for PBP production. The net pigment accumulation resulting from the monochromatic illumination was divided by the average net pigment increase in the control culture. The ratio of the experimental values of pigment increase to the control values was the normalized relative response which was used to form the dose response curves. If the control cells failed to show a net gain in PC under red light or in PE under fluorescent light, the experiment was discarded.

Calculations and Data for Action Spectra. A Fortran computer program was designed using simultaneous equations for calculating APC, PC, and PE contents from absorbance data and the cell dry wt relationship with optical density at 730 nm given by Bennett and Bogorad (1). The incident intensity and wavelength values were also incorporated into the computer program. After normalization to adjust for culture responsiveness, the results were stored in computer files for each wavelength. Each file was plotted as a log dose response curve. Least squares analysis of these plots yielded a straight line ascending portion for each dose response curve. A normalized relative response value of 0.555 (just over half of the response of the control and well within the linear ascending portions of each dose response curve) was chosen for comparison of all of the dose response curves. The reciprocal of the quantum flux required for this response was plotted as the ordinate in the action spectra.

The estimates of error shown in the action spectra in Figures 8 and 9 were calculated from the standard error of estimate of ordinate values on abscissa values in the log dose response curves—a measure of the scatter of data points around the regression line. To estimate the error in the action spectra, the best fit straight line was moved 1 standard error distance on the ordinate parallel to itself. The difference between the dose rate to reach the standard response on this line and on the best fit line is a measure of error. Two components are important in this measure of error: the scatter of data points around the straight line, and the slope of the straight line since steeper slopes give less variation in the x axis for the same variation on the y axis.

RESULTS

Cell Growth and Adaptation. Figure 1 shows the incident light spectra and intensities for the light sources in the two growth chambers. The light emitted by the fluorescent lamps is rich in 525 and 625 nm light; the red light source emits quanta predominantly greater than 600 nm. The growth curves in Figures 2 and 3 indicate that *F. diplosiphon* grew 1.5 to 2 times faster in fluorescent than in red light of the intensities used here. The time course of changes in PE and PC concentration by algae shifted from fluorescent to red light and from red to fluorescent light are also indicated in Figures 2 and 3, respectively. Pigment concentration changes become significant after 6 to 12 hr in the new light environment. During this period of little or no response, continuous illumination is necessary. Placing a culture in darkness at any time eliminated further significant pigment concentration increases, and intermittent light only delayed pigment accumulation by approximately the duration of the dark periods.

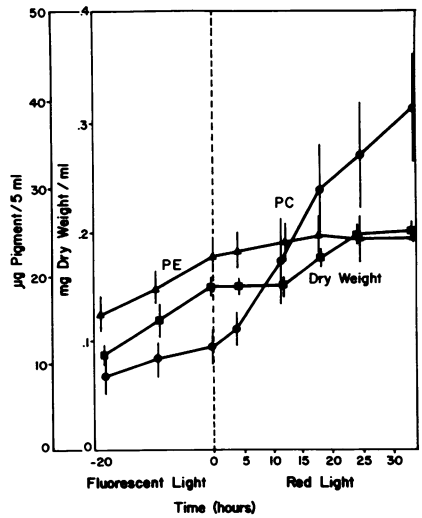

FIG. 2. Time course of complementary chromatic adaptation from fluorescent illumination to red broad band illumination. See Figure 1 for incident light spectra. One-liter cultures in 2,800-ml Fernbach flasks were grown in incubation chambers under the conditions described in the text. These curves represent the average of the results from three experiments.

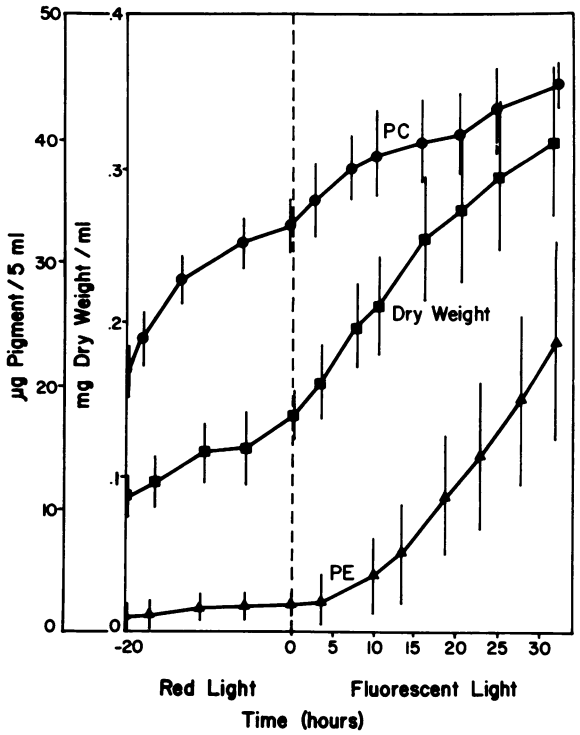

FIG. 3. Time course of pigment changes during CCA from red to fluorescent broad band illumination. Light conditions are shown in Figure 1 and the other conditions of growth are described in the text. The results presented here are the average of three experiments.

Preliminary Spectral Response Experiments. Preliminary experiments using a series of intensities of broad band red and green light from the slide projectors equipped with transparent colored plastic filters indicated that the maximum net PC and PE increase occurred with light of intermediate intensities (approximately 0.1 nE/cm^2). At low and at high intensities, the net PC or PE change/cell dry wt was low or negative. Negative values result if the dry wt increases faster than the pigment accumulation. Active cultures usually increased in dry wt by 10 to 20% in the 6-hr experiments.

Monochromatic Light Experiments and Action Spectra. A series of monochromatic illumination experiments were performed. Approximately one-third of these experiments had to be disregarded because the balance of the original 1-liter control culture maintained under fluorescent or broad band red illumination failed to show a net gain in PC or PE during the 6-hr experimental period. Pigment production in monochromatically illuminated cultures was calculated on the normalized basis described above. Figures 4 and 5 show examples of the fact that an optimum dose rate exists. Above the optimum intensity the normalized relative pigment increase/unit cell mass declines, finally reaching zero. Negative values are not plotted here, nor have they been used in slope determinations.

The data in Table I define the ascending part of the log dose response curve for each wavelength used. All of these data were used in ascertaining the two action spectra. Typical dose response plots of normalized relative PBP concentration change *versus* dose rate (which is proportional to total dose, since all experiments were for 6 hr) are shown in Figures 6 and 7. The dashed horizontal line refers to the normalized relative pigment production rate of 0.555, *i.e.* just over one-half of the response of the controls.

Table I as well as Figures 6 and 7 show that the slopes of dose response curves vary with wavelength without an apparent pattern. It seems possible that the slopes, even in their early linear rise, reflect integrated promotive and destructive effects

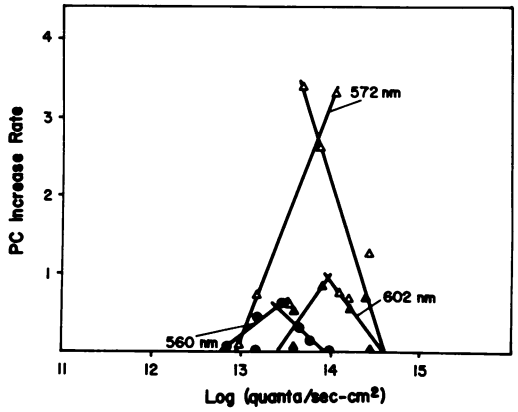

FIG. 4. Typical dose response curves for PC accumulation showing an ascending portion and a descending portion. Least squares analysis was applied to the data points on either side of the maximum response to yield one ascending and one descending straight line. PC and PE response data, Table I and Figures 4–7 are taken from both fluorescent and red light-grown cells. PC increase rate is relative to that of control under broad band illumination.

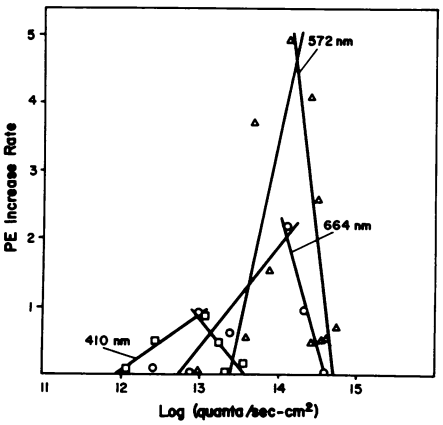

FIG. 5. Typical dose response curves for PE accumulation showing an ascending portion and a descending portion.

Table I. Slopes and Y Values at X=10 for Log Dose Response Curves.

The slope is equal to the normalized relative rate of pigment increase per quantum per sec per cm^2. The Y value is the normalized relative rate of pigment increase. NR means no response at this wavelength.

Wavelength (nm)	Phycocyanin		Phycoerythrin	
	Slope	Y value	Slope	Y value
730	NR	NR	0.68	-1.75
700	NR	NR	54.39	-174.15
680	4.98	-16.11	1.03	-3.33
664	2.66	-8.05	1.42	-3.89
641	0.88	-1.94	1.62	-6.55
621	2.20	-7.78	NR	NR
602	1.62	-5.55	1.60	-5.28
572	3.12	-9.16	5.97	-20.55
560	0.93	-2.58	2.88	-7.78
550	NR	NR	9.14	-21.94
500	1.00	-3.25	4.28	-14.89
482	NR	NR	1.83	-5.14
463	3.24	-8.05	1.17	-3.08
441	0.95	-2.50	1.15	-2.50
430	1.49	-3.69	0.69	-1.25
410	12.04	-38.88	0.81	-1.56
387	NR	NR	1.67	-3.58
365	NR	NR	2.62	-7.89

of light. Over a great range these variations introduce no ambiguity regarding wavelengths of maximum effectiveness for PC and PE production. PC production is promoted most by 463 and 641 nm light while PE production is most rapid in 387 and 550 nm light regardless of whether the comparisons are made at normalized relative response values of 0.278, 0.555,

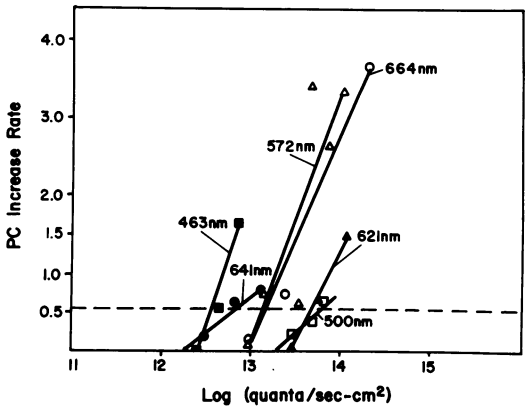

FIG. 6. Typical ascending dose response lines for PC increase. The response, PC increase rate, is PC increase of sample/PC increase of control under broad band illumination. As in Figures 4 and 5, least squares analysis yields the straight lines. (- - -): normalized relative response of 0.555. This response was used as a standard for comparing all wavelengths to draw the action spectrum for PC increase shown in Figure 8. See Table I for parameters of ascending portions of all dose response lines obtained and used in the preparation of Figure 8.

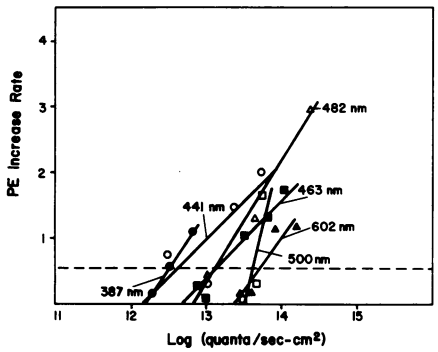

FIG. 7. Typical ascending dose response lines for PE increase. See Figure 6 legend for details.

or 0.833. (The dashed horizontal lines in Figures 6 and 7 are at the 0.555 level.)

On the other hand, the relative effectiveness of light at the pairs of action maxima varies considerably depending upon the rate of PBP synthesis taken for comparison. Thus, for normalized relative PE production at a standard of 0.833, the ratio of effectiveness of 387 nm/550 nm light is 0.7; at a standard of 0.555, it is about 0.9; and at 0.278, it is about 1.3. For PC at a reference level of normalized relative production of 0.833, the ratio of effectiveness of 463 nm/641 nm is 2.6; at 0.555, it is 1.8; and at 0.278, it is 0.9. The inverse order of these ratios for PE and PC production is noted, but we offer no explanation for this relationship here.

The action spectrum in Figure 8 is a plot of wavelength *versus* reciprocal of the quantum flux required to attain a normalized relative response value of 0.555. Figure 9 is the corresponding action spectrum for PE production. The estimates of error at certain wavelengths suggest that some smaller peaks with large error estimates may be insignificant, *e.g.* PC induction at 572 nm and between 430 and 441 nm.

DISCUSSION

F. diplosiphon cells adapted to growth under red light (wavelengths longer than 600 nm) are free of PE (1). In the present experiments, *e.g.* Figure 3, the red incandescent lamps used emitted some light below 600 nm; consequently, PE was detectable in cells grown in "broad band red light." For the first 6 to

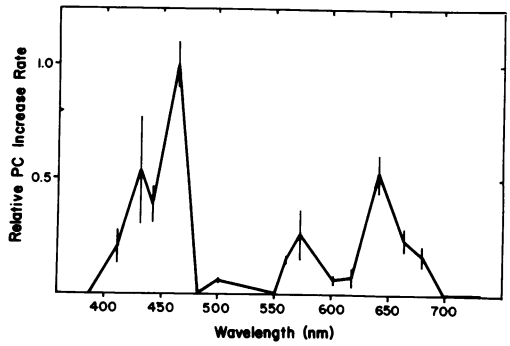

FIG. 8. Action spectrum for PC increase during CCA. Ordinate values were determined as the inverse of the quantum flux (quanta per sec per $cm^2)^{-1}$, at which the dose response line for PC (see Fig. 6) reached a normalized relative standard response of 0.555. Values were adjusted after equating the highest values on the ordinate to 1.0; in this case, the response at 463 nm was assigned a value of 1.0.

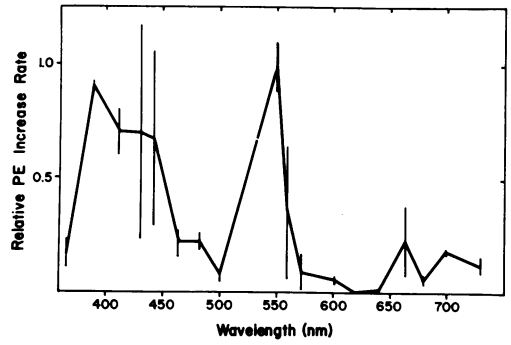

FIG. 9. Action spectrum for PE increase during CCA. Ordinate values were defined as for Figure 8, using the dose response lines of Figure 7. Adjustment on the ordinate was carried out with the response at 550 nm being set equal to 1.0.

12 hr after transfer to light which stimulates PE production, no pigmentation changes are significant. At the end of this lag period, PE accumulation becomes significant, and PC continues to increase at a lower rate. The kinetic data of pigment production resulting from shifting PE-rich cells to red light are comparable. Continuous illumination is required throughout the lag phase regardless of the direction of CCA, *i.e.* from either the PC-rich or PE-rich condition to the opposite state. We found it convenient for technical reasons to use broad band illumination during the lag phase and early into the pigment change period before transfer of adapting cells to monochromatic light.

To minimize the effects of photosynthesis (and growth) on the action spectra for phycobiliprotein synthesis, all pigment concentrations were divided by the simultaneously determined cell mass. A positive response value resulted only if the rate of biliprotein synthesis outpaced that of increase in fresh wt. This does not eliminate the possibility that photosynthesis could influence our data, but the action spectra maxima found here do not correspond to that for photosynthesis.

A photobiological response is expected to saturate at some light intensity and then to continue maximally at higher intensities. In the present case, PBP production increased to a maximum in response to increasing intensity of illumination at each wavelength. This was generally followed by a decrease in rate of accumulation with increasing intensities. The falling part of the curve (*e.g.* Figs. 4 and 5) could result from bleaching (photodestruction) of the photoreceptor, of the free PBP chromophore, of the PBP itself, or of a PBP precursor other than its free chromophore. We have used the ascending linear portions of the response curves in drawing action spectra for PE and PC production; however, as noted, the rising linear portions of the

dose response curves differ from one another in slope. The variations may result from differences in magnitude of photodestruction at the wavelengths studied. Regardless of their cause, the variations in slope complicate attempts to interpret the action spectra and thus to use them to identify the chemical species which serve as photoreceptors. If the variations in slope indeed reflect stimulation of synthesis as well as increased bleaching at higher intensities, then the ratios of effectiveness and action spectra calculated at the lowest light doses would be expected to show the absorption properties of the photoreceptors most accurately. However, the ratio of effectiveness of blue/red light for PC production is higher at more rapid rates of synthesis whereas the ratio of effectiveness of blue/green light is lower at higher rates of PE accumulation.

Fujita and Hattori (5) and Diakoff and Scheibe (3) studied the action spectra for PE and PC formation in the cyanophyte *T. tenuis*. These algae were suspended in nitrate-free medium and bleached with high intensity light before illumination with various wavelengths of monochromatic light. The amounts of PBPs produced during a subsequent period of incubation with nitrate in darkness were measured. Fujita and Hattori (5) found 641 nm and Diakoff and Scheibe (3) found 660 nm light to favor PC production. On the other hand, 541 nm (5) and 550 nm (3) were found to most favor PE production. Diakoff and Scheibe (3) found a slight stimulation of PE production by 350 nm light, but it was only about one-eighth as effective as light of 550 nm. For induction of PC production, 360 nm light was about one-fourth as effective as 660 nm light. The major difference between the action spectra for CCA in *T. tenuis* and *F. diplosiphon* lies in the relative effectiveness of blue *versus* longer wavelengths. PE formation by *F. diplosiphon* is strongly promoted by 387 as well as by 550 nm light. PC production is promoted by 463 as well as by 641 nm light.

The action spectra for PE and PC production by *F. diplosiphon* may each reflect two interacting photoreceptive systems, one with an absorption maximum in the blue region of the spectrum and the other with an absorption peak in the green or red. For example, the blue absorbing pigment could transfer energy to a photoreceptor having a bile pigment chromophore, *e.g.* a PBP different from either PE or PC although, based on the response at 641 nm, allophycocyanin cannot be excluded as one photoreceptive partner regulating PC production. On the other hand, the blue and red action maxima may be the two absorption peaks of a single absorber, for example, a hemoprotein, a non-iron metalloporphyrin or a protein complex with the latter.

The *F. diplosiphon* and *T. tenuis* adaptochromes may be entirely different from one another. However, if an interacting two-pigment system regulates PE production and another double pigment system moderates PC production by *F. diplosiphon*, then *T. tenuis* may lack a supplementary blue absorber or this part of the system may be bleached during preparation of the latter alga for the experiments as carried out by Fujita and Hattori (5) and Diakoff and Scheibe (3). In addition, two other features noted in *F. diplosiphon* may be pertinent: (*a*) descending accumulation rates at some higher light intensities; and (*b*) changing ratios of effectiveness of blue *versus* red or green light depending upon the rate of PBP production used for comparison. Analysis of spectral changes *in vivo* or in extracts of these algae should help reveal the nature of the photoreceptors for CCA. It should be pointed out that the action spectrum for PC production by a Chl-less mutant of *Cyanidium caldarium* includes a strong maximum at about 420 nm and a weaker response in the 550 to 600 nm region (8); it has been suggested that the photoreceptor is a hemoprotein.

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