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Action Spectra for Chromatic Adaptation in *Tolypothrix tenuis*[1]

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ABSTRACT

The dark synthesis of biliproteins in the blue-green alga *Tolypothrix tenuis* is controlled by brief light treatments. Green light potentiates synthesis of phycoerythrin and red light potentiates synthesis of phycocyanin. Red reverses the effect of green and vice versa. Action spectra for the red and green effects were obtained for the wavelength region 320 nanometers to 710 nanometers, at 10-nanometer intervals. The principal action band in the red peaks at 660 nanometers, with a half-band width of 58 nanometers and an accompanying shortwave band at 360 nanometers. The green action band peaks at 550 nanometers, with a half-band width of 76 nanometers, and a shortwave band at 350 nanometers. Chromatic adaptation and another photomorphogenic response in the blue-green algae are discussed in terms of possible regulation by a photoreversible pigment recently isolated from *Tolypothrix*.

Chromatic adaptation is the name of the phenomenon in which the relative levels of different biliproteins in certain red and blue-green algae are controlled by light quality. In green-rich light, such as may be found at some depth below the surface of natural waters, the green-absorbing biliprotein PE[2] is preferentially synthesized, whereas in red-rich light, closer to the surface, the red-absorbing PC predominates. The biliproteins are important photosynthetic accessory pigments, and the ability to adapt chromatically confers an obvious ecological advantage in that the organism is able to make maximum photosynthetic use of the available light.

Chromatic adaptation, although widely recognized as a phenomenon, has been elucidated in detail by only one research group, using the blue-green alga *Tolypothrix tenuis* (5, 6, 7, 9). Dark synthesis of biliproteins occurs in this organism, provided the dark incubation is preceded by prolonged incubation at a relatively high irradiance (preillumination) in a medium devoid of combined nitrogen and at a temperature below the optimum for growth (11). During preillumination under photosynthetic conditions an alkali-soluble polysaccharide accumulates which then gradually disappears during dark incubation. The polysaccharide may be identical to the polyglucan which accumulates in *Tolypothrix* as a result of supplying exogenous glucose (4). The disappearance of the polysaccharide is accompanied, after a 5-hr lag, by biliprotein synthesis (8). The type of biliprotein synthesized can be predetermined by a brief light treatment given at the beginning of the dark incubation; red light potentiates formation of PC, and green light, PE. If brief light treatments of alternating red and green follow each other in succession, the type of biliprotein synthesized depends on the quality of the last light treatment alone (8). Thus, red light reverses the effect of green and vice versa. Action spectra for the green and red effects have been determined for the wavelength region 475 to 700 nm and show action maxima at approximately 540 and 640 nm, respectively. The action spectra did not correspond to the absorption spectra of PE and PC (7).

A model for control by light of biliprotein synthesis was formulated on the basis of these and other results. It was postulated that early precursors of PE and PC are mutually photointerconvertible, green light causing conversion of PC precursor to PE precursor, and red light causing conversion in the opposite direction. The model, in addition, invoked photodestruction of more immediate precursors of PE and PC by red and green light, respectively (9).

MATERIALS AND METHODS

Growth Conditions. *Tolypothrix* was grown on 1% agar slants in weak cool white fluorescent light at 22 C for 1 to 2 months on a medium containing the following components per liter: KNO_3, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Na_2HPO_4, 0.2 g; $CaCl_2$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 0.02 g; Arnon's A 5 micronutrient solution, 1 ml. The matted filaments were blended, and the resulting suspension was used to inoculate 950-ml prescription bottles containing 750 ml of growth medium. The bottles were placed 17 cm from a 150-w incandescent lamp and bubbled continuously with air containing approximately 1% CO_2 at 500 ml per min. Temperature of the cultures was approximately 32 C. After 7 to 26 days, the algae were harvested by centrifugation and washed three times in a nitrogen-free medium identical to the growth medium, except that 2.6 g per 1 K_2SO_4 was substituted for the KNO_3.

Preillumination. The washed filaments were pelleted at 12,000*g* for 10 min and resuspended in nitrogen-free medium to a final concentration of 2 to 3 g/l. Prescription bottles containing 750 ml of filament suspension were placed in a water bath at 22 C and illuminated from the side at 1800 ft-c (daylight fluorescent bank) for 22 to 25 hr with continuous aeration with 1% CO_2.

Determination of the Action Spectra. The preilluminated filaments were collected by centrifugation and resuspended to 1 g of filaments per 100 ml in the nitrogen-free medium to which casein hydrolysate was added to a final concentration of 0.15% (w/v). The suspension, in a thin layer, was irradiated for 15 min with either a broad band red or green source

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[2] Abbreviations: PE: phycoerythrin; PC: phycocyanin; APC: allophycocyanin; P_R: red-absorbing form of the photoreversible pigment; P_G: green-absorbing form of the photoreversible pigment.

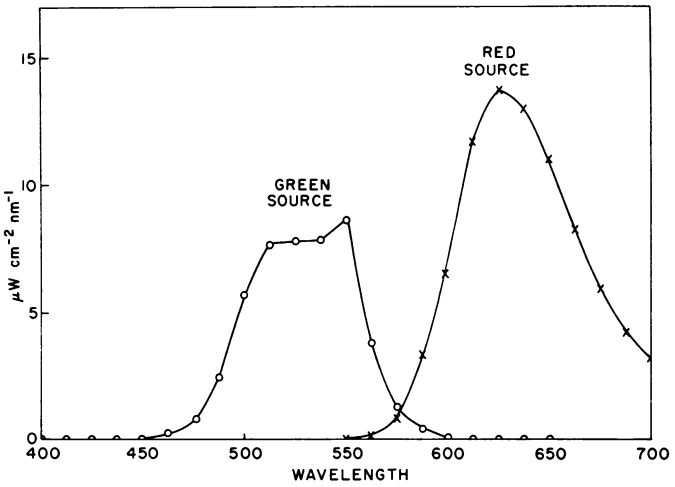

FIG. 1. Emission spectra for the broad band green and red sources. The unfiltered light source was a bank of ten 15-w "daylight" fluorescent tubes.

(Fig. 1) which light-saturated subsequent dark synthesis of PC or PE, respectively. Six-ml samples of filaments in 150-ml beakers were placed at designated wavelength stations of a diffraction grating spectrograph (1) for various exposure times, then shaken in the dark at 32 C for 22 to 24 hr, after which they were transferred to an ice bath. At least five different exposure times were used at all wavelength stations. The filaments were pelleted by centrifugation at 12,000*g* at 2 C for 10 min, then resuspended in 3 ml 0.3 M tris, pH 6.8, 0.02 M EDTA. A 2-ml aliquot was frozen at −70 C overnight, then thawed and shaken at 3 C for 6 to 7 hr to release soluble components from the broken cells. The broken cell suspensions were centrifuged, and the absorbancies of the supernatants at 565, 620, and 650 nm were taken on a Cary Model 14 spectrophotometer. By using the published extinction coefficients for each biliprotein (5), the concentration of PC and PE in each sample was determined.

The irradiance at each wavelength station of the spectrograph was determined using an Eppley thermopile (Table I). The energy dose in μjoule cm^{-2} for each treatment was computed by multiplying the irradiance in μw cm^{-2} by the time in seconds. The ratio of PE synthesized to PE plus PC synthesized [PE/(PE + PC)] was plotted against the logarithm of the dose and straight lines were drawn through the points. The reciprocal of the dose required to produce either a 25% increase (green action) or 25% decrease (red action) in PE/(PE + PC) was normalized to the two standard wavelengths, 500 and 680 nm, and plotted against wavelength.

Photoreversible Pigment. The photoreversible pigment was isolated from *Tolypothrix* and partially purified as described in reference 15.

RESULTS

Dark Synthesis of Biliproteins. The cellular biliprotein levels decrease during preillumination under photosynthetic conditions. Upon subsequent dark incubation, after the addition of an appropriate nitrogen source, synthesis of biliproteins ensues following a 4- to 5-hr lag (11). Figure 2 shows the time course of dark synthesis of PE and PC at 32 C following the addition of casein hydrolysate to the nitrogen-free medium. The PE and PC curves represent synthesis following a 15-min irradiation with green or red, respectively, at the beginning of dark incubation. Pigment synthesis begins after a lag of about 4 hr and is complete on or before the 20th hr of incubation. These results are in agreement with those reported earlier (5). Under our conditions control of biliprotein synthesis by green light is incomplete. After a saturating dose in green, with either broad band (Fig. 1) or monochromatic light at 540 nm, PE subsequently synthesized constitutes only about 35% of total biliprotein synthesized, the remainder being PC. However, after a saturating dose in red, only PC is synthesized.

Since the irradiance varied among the different wavelength stations (Table I), and the dose series at each wavelength was administered by varying the duration of exposure, it was necessary to ascertain whether or not reciprocity was valid. Preilluminated cell suspensions were irradiated for 15 min with broad band red, then given a dose series at 540 nm at two different irradiances; 1, 2, and 4 min at 144 μw cm^{-2}, and 2, 4, and 8 min at 72 μw cm^{-2}. An identical time series was administered at 640 nm, at 108 and 54 μw cm^{-2}, following a 15-min initial irradiation with broad band green. The results (Fig. 3) show that the degree of potentiation (540 nm) or depotentiation (640 nm) of subsequent PE synthesis depends only on the dose of light administered and is independent of

Table I. *Irradiances at the Spectrograph Wavelength Stations*

Wavelength	Irradiance	Wavelength	Irradiance
nm	*μw cm⁻²*	*nm*	*μw cm⁻²*
710	138	510	167
700	133	500	167
690	149	490	151
680	146	480	161
670	144	470	274
660	136	460	177
650	141	450	151
640	125	440	153
630	120	430	131
620	148	420	134
610	141	410	131
600	143	400	125
590	153	390	100
580	134	380	105
570	143	370	93.5
560	149	360	95.1
550	141	350	52.5
540	161	340	24.6
530	172	330	36.1
520	174	320	14.8

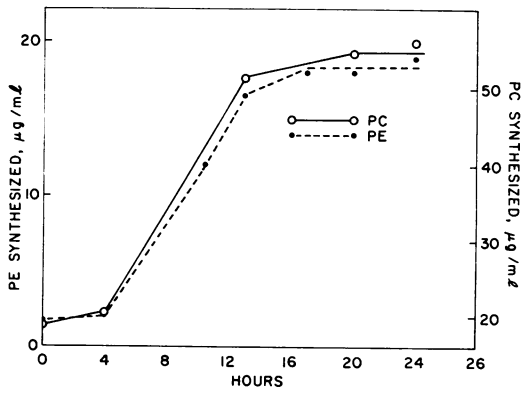

FIG. 2. Time course of dark synthesis of phycoerythrin after a 15-min green irradiation and of phycocyanin after a 15-min red irradiation. Green and red sources as in Figure 1.

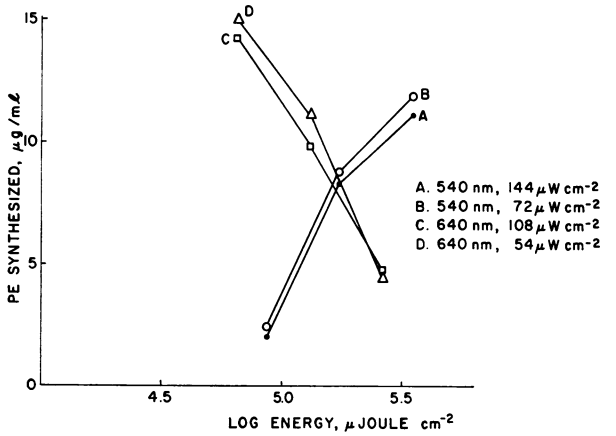

FIG. 3. Demonstration that reciprocity is valid. Amount of phycoerythrin synthesized following three different doses of green light (540 nm) replicated at two different irradiances, and following three different doses of red light (640 nm) at two different irradiances. The doses at 540 nm were immediately preceded by a saturating irradiation with broad band red, and those at 640 nm by a saturating irradiation with broad band green.

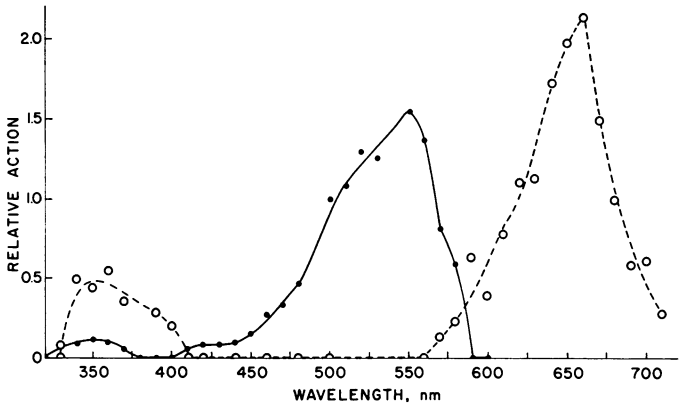

FIG. 4. Action spectra for potentiation (solid line) and depotentiation (dashed line) of phycoerythrin synthesis in the dark. See "Materials and Methods" for method of determination.

the irradiance or duration of irradiation, at least over the 2-fold range of irradiances tested.

Action Spectra. Action spectra for the potentiation (green action) or depotentiation (red action) of PE synthesis, for the wavelength region 320 to 710 nm, at 10-nm intervals, are presented in Figure 4. The two principal action maxima are at 660 nm (red) and 550 nm (green). The half-band width for the red action band is 58 nm, whereas that for the green is somewhat broader, 76 nm. This is probably due to a shoulder in the neighborhood of 510 nm. The action in the green drops more sharply with increasing wavelength above the action maximum than does that in the red, but this is probably a result of overlapping green and red action in the region 560 to 590 nm. A most interesting feature of both spectra, hitherto unreported, is the action in the near ultraviolet. The shortwave band associated with red action is broad and extends from 330 nm to 410 nm, with a maximum at about 360 nm, where the activity is approximately 25% of that at the principal action maximum at 660 nm. The action region in the near ultraviolet associated with the principal green action band extends from 320 nm to 380 nm, peaking at approximately 350 nm, where the action is 8% of that at 550 nm.

DISCUSSION

Identity of the Photoreceptors. The availability of action spectra for chromatic adaptation should aid in determining what pigments serve as photoreceptors for the red and green light effects. The principal action bands resemble the *in vitro* absorption spectra of the biliproteins PE and APC, which have absorption maxima at 565 nm and 650 nm, respectively (2, 10). Both of these biliproteins occur in *Tolypothrix*. Fujita and Hattori (7) have, however, adduced evidence showing that chlorophyll, carotenoids, PE, and PC do not serve as photoreceptors. Instead, they have proposed a model (9) which is based on the results of light and chemical perturbations to the alga administered immediately before and also during dark biliprotein synthesis. According to this model, green light converts a distant precursor of PC into a distant precursor of PE. Red light causes photoconversion in the opposite direction; that is, conversion of the PE precursor into the PC precursor. A second effect of light on biliprotein synthesis, distinct from photointerconversion of distant precursors, was photodestruction of a more immediate precursor of PC by green light, and of a more immediate precursor of PE by red light. A curiosity of the model is that both precursors in the biosynthetic sequence leading to PC absorb maximally in the green, and both precursors of PE absorb maximally in the red, whereas these spectral characteristics are switched in the final products. The model has been criticized because none of the postulated intermediates have been detected whereas the amount of appropriate biliprotein finally synthesized presupposes the existence of substantial intermediate pool sizes (3).

Photomorphogenesis in *Nostoc*. A phenomenon that may possibly be related to chromatic adaptation is the control by red and green light of morphogenesis in dark-grown cultures of the blue-green alga *Nostoc*. Lazaroff and Schiff (13) and Lazaroff (12) have shown that dark-grown cultures of *Nostoc muscorum*, which exhibit an aseriate (nonfilamentous) growth habit, can be induced to develop into filaments, characteristic of the light-grown habit, by a brief irradiation with red light. The effect of red light was completely reversed by a subsequent brief irradiation with green light. Action spectra for induction (red effect) and reversal (green effect) of filament development showed a narrow action band in the red peaking at 650 nm and a broad action band in the green peaking at

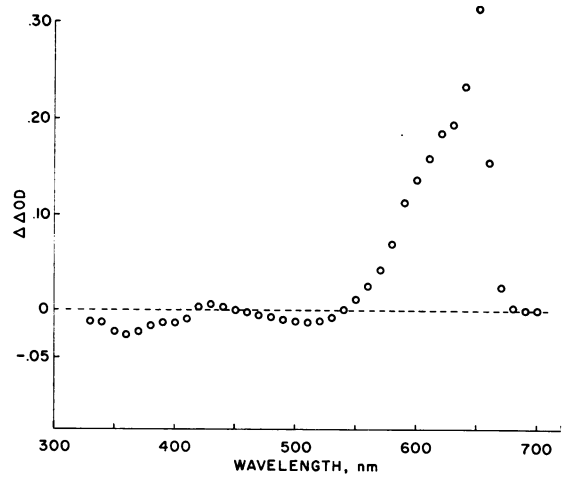

FIG. 5. Difference-difference spectrum showing spectra of the two forms of the photoreversible pigment. Positive △ △ OD values represent the red-absorbing form, negative values, the green-absorbing form.

550 nm and possessing a prominent shoulder at 570 nm. It was suggested that APC and one or more forms of PE served as photoreceptors for the red and green effects, respectively. A more recent study of a similar photomorphogenic response in dark-grown cultures of another species, *Nostoc commune* (14), showed that red light (action maximum 640 nm) caused filament differentiation and that green light (action maximum 520 nm) reversed the effect of red. Further, and most interestingly, multiple photoreversibility by green of red and vice versa was demonstrated. In a series of alternating exposures to red and green, the photomorphogenic effect depended only on the quality of the last irradiation. The strong possibility of the existence of a phytochrome-like photoreversible pigment, with the two principal absorption maxima in the green and the red, instead of the red and far-red, which had already been alluded to in reference 12, was discussed.

A Master Photomorphogenic Pigment in Blue-Green Algae? The photoreversible control of development by green and red light in *Nostoc* suggests the presence of a phytochrome-like pigment which can exist in either a red-absorbing form or a green-absorbing form. The results of an intensive series of investigations on chromatic adaptation in *Tolypothrix* (9, and earlier) are also completely compatible with the notion that such a pigment controls this phenomenon, which is distinct from photomorphogenesis in *Nostoc*. Such a green-red photoreversible pigment, having the properties of a chromoprotein, has recently been isolated from *Tolypothrix* (15). A spectrum showing the existence of the two forms appears in Figure 5. Negative values represent the spectrum of P_G and positive values that of P_R. Evidence showing that P_G is quantitatively converted to P_R by green light and vice versa by red light, with first order kinetics, is presented elsewhere (15). The spectrum of P_R shows a peak at 650 nm and a shoulder at approximately 620 nm, and thus resembles the absorption spectrum of APC. P_G has an ill defined peak at about 520 nm, with no discernible fine structure. The extinction coefficient at the absorption maximum of P_G is only about 4% of that of P_R. A small shortwave band at 430 nm accompanies the major absorption band of P_R, while P_G has a much larger shortwave band peaking at 360 nm. A comparison of Figure 5 and Figure 4 shows that the absorption spectra of the two forms bear some resemblance to the action spectra for chromatic adaptation. Points of resemblance are the presence of: (*a*) two principal long wave bands, one in the green and one in the red; (*b*) a shortwave band associated with each of the two long wave bands. Points of dissimilarity in the absorption spectra, with reference to the action spectra, are (*a*) relative extinction coefficients of the two principal long wave bands, (*b*) peak wavelength of the shortwave band associated with P_R, and (*c*) relative extinction coefficients of the shortwave bands of P_R and P_G. These dissimilarities are not minor, but may at least in part be explained as artifacts of extraction.

The question yet to be answered is whether or not the photoreversible pigment is the photoreceptor for the light control of biliprotein synthesis in *Tolypothrix* and morphogenesis in *Nostoc*, as well as perhaps other as yet unreported photoreversible red-green effects, and thus represents the blue-green algal equivalent of phytochrome.

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