Control of Phycoerythrin Synthesis during Chromatic Adaptation

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ABSTRACT

Chromatic adaptation is the process by which blue-green algae alter the rates of biliprotein synthesis in response to changes in the color of available light. We have examined the control of phycoerythrin synthesis during the early stages of chromatic adaptation in *Fremyella diplosiphon* using fluorescence spectroscopy and ^35^S-labeling of polypeptides. Phycoerythrin synthesis begins within 45 to 90 minutes after transfer of cells from red to green light, but is blocked by rifamycin. Transfer of cells from green to red light stops phycoerythrin synthesis with a t1/2 = 45 minutes, as does the addition of rifamycin in green light. Transfer from green light to darkness slows but does not stop phycoerythrin synthesis. Gel electrophoresis of labeled polypeptides, both soluble and membrane-bound, shows that the synthesis of some polypeptides other than phycoerythrin are also affected by changes in light. These data suggest that chromatic adaptation involves gene regulation at the transcriptional level.

Blue-green algae (Cyanophytes) contain phycobiliprotein pigments which serve as the major accessory pigments for photosynthesis. Cyanophytes characteristically synthesize PC and APC. In addition, many species also synthesize PE. Among those species which contain PE, some synthesize different relative proportions of PE and PC depending upon the spectrum of available light. This is called complementary chromatic adaptation (2).

Although it has been shown to occur in a wide variety of algae (4, 13), recent studies of chromatic adaptation have been carried out primarily with the species *Tolypothrix tenuis* and *Fremyella diplosiphon*. In both of these organisms adaptation is achieved primarily by quantitatively or qualitatively altering the synthesis of PE, although the PC content is also affected (1, 7). Chromatic adaptation involves the synthesis of new biliproteins (1).

The time course of chromatic adaptation has been examined over periods of many hours to several days for both *Tolypothrix* (12) and *Fremyella* (1, 8). Although these studies have elucidated the over-all changes in pigment synthesis which accompany adaptation, previously employed methods have not been sufficiently sensitive to reveal the early events in this process which would be expected to give some insights to the mechanisms controlling the synthesis of the phycobiliproteins.

We have taken advantage of the sensitivity of fluorescence spectroscopy to examine early stages of PE production during chromatic adaptation by *Fremyella*. Gel electrophoresis of proteins labeled during adaptation was used to confirm the spectroscopic data, as well as to examine some changes in nonpigment polypeptides.

MATERIALS AND METHODS

Cultivation of Cells. *F. diplosiphon* cells were grown in 1-liter liquid cultures as previously described (1). Cells were kept in the logarithmic phase of growth by periodically diluting the cultures with fresh medium. Illumination conditions used were similar to those described by Haury and Bogorad (8). The wavelength of the G used was from 425 to 600 nm, while the R was from 625 nm up.

Chromatic Adaptation–Fluorescence Measurements. For the chromatic adaptation experiments, cells were grown in R to midlog phase (approximately 0.5 g wet weight of cells/liter). They were transferred to G, and 5- or 10-ml samples were taken for analysis at various intervals. In experiments measuring PE induction, sampling began at the time of transfer to green light. In experiments measuring the suppression of PE synthesis, cells were incubated in G for 5 h prior to sampling and incubation with rif or label. In either case, the cells were pelleted by centrifugation, resuspended in 5 ml water, and sonicated for three 15-s periods using the microtip of a Branson sonicator at 100 W. This broke more than 99% of the cells. The homogenate was centrifuged at 50,000 g for 15 min to remove particulate material. The supernatant was removed, and either used directly for fluorescence determinations or stored at −20 C.

The PC content of R-grown cells was measured in cell extracts by spectrophotometry as described previously (1). During the early phase of adaptation, when there were only traces of PE in the presence of large amounts of PC, PE was measured fluorometrically with a Perkin-Elmer fluorometer. Excitation was with 380 nm light and emission was recorded between 500 and 715 nm. The fluorescence emission maximum of PE was found to be at 567 nm while PC fluorescence emission was at 647 nm. At phycobiliprotein concentrations below 0.1 A unit at 620 nm (PC), the relative fluorescence intensity was proportional to the biliprotein concentration. For PE measurements the sensitivity range was S10 to S30. Both excitation and emission slits were open to position 10 and the diode current was 650 v.

Chromatic Adaptation–Radioactive Labeling. For labeling experiments, R-grown cells were harvested by centrifugation and resuspended in fresh medium at 5 to 10 times the original density, to approximately 0.5 g wet weight to 100 ml. The cells were incubated for 1 h at this density prior to the addition of 2.5 to 3.0 mCi of ^35^Sulfate (New England Nuclear) per 100 ml of cell suspension. The cells were incubated with label for 45 min, harvested by centrifugation, washed, and pelleted again. The soluble proteins were obtained by sonication of cells and centrifugation as for the fluorescence measurements. Photosynthetic membrane fractions were isolated by layering the sonicated homogenate on 30-ml linear sucrose gradients (0–60% sucrose in 30 mM Tris [pH 7.4], 20 mM KCl, and 2 mM MgCl₂) and centrifuging at 35,000 rpm in a Beckman SW 40 rotor for 120 min. The resulting green band was collected, diluted with the same buffer without sucrose, and pelleted at 50,000 g for 30 min (9). The pellet was used for electrophoresis.
In experiments measuring suppression of PE synthesis, R-grown cells were incubated in G for 5 h prior to concentration and labeling.

**Electrophoresis.** Gel electrophoresis was by the method of Laemmli (10). The gels were 20 × 25 cm in size and contained 12% acrylamide for the soluble polypeptides and 18% for the membrane-bound polypeptides (30:0.8 acrylamide-bisacrylamide). All samples were loaded at the same total radioactivity determined by trichloroacetic-acid-precipitable counts. After electrophoresis the gels were stained with Coomassie Blue (6), photographed, and impregnated with PPO (3). Fluorographs were made on Kodak XR-1 x-ray film.

**RESULTS**

Excitation of a mixture of PE and PC at 380 nm produced a peak of PE fluorescence at 647 nm and of PC fluorescence at 647 nm. The λ maximum for PE was at 565 nm and for PC was at 620 nm. The magnitude of PE fluorescence was proportional to the concentration of PE in a dilute solution (Fig. 1) and could be used as a quantitative measure of this biliprotein. Since the cells used for fluorescence determinations contained a large amount of PC, the concentration of which did not change significantly over the time course of these experiments, the PE content was expressed as the ratio of PE to PC fluorescence and thus was normalized for variations in cell number and processing between samples.

*Fremyella* cells grown in R contained no detectable PE (1). When these cells were transferred to G, PE production was detected after 90 min in the experiment shown in Figure 2. In a series of experiments of this type PE was first detected from 45 to 90 min after transfer. PE production increased rapidly for 2 to 3 h and then became constant for up to 24 h (Fig. 2). Addition of rif at the time of transfer blocked the appearance of PE. Rif-resistant strains of *Fremyella* (J. Raiss, unpublished) underwent normal PE induction in the presence of rif at the same concentration (data not shown). In experiments similar to those shown in Figure 2, cells undergoing chromat adaptation were labeled with [35S]sulfate and the soluble proteins were analyzed by SDS-PAGE electrophoresis. Autofluorography of the gels (Fig. 3) showed labeled PE polypeptides after 90 min of induction in G, indicating *de novo* synthesis of PE. No labeled PE was seen in cells grown in R or in cells grown in G but treated with rif (data not shown). Phycobiliproteins comprise between 45 to 60% of the soluble proteins in these cells and are the major polypeptides visible after gel electrophoresis (1).

Cells transferred from G to R ceased PE synthesis, but did not actively degrade the PE which had been produced (1). The shut-off of PE synthesis was also examined by fluorescence spectroscopy. Cells which had been induced to synthesize PE at a constant rate were either kept in G or transferred to R. Figure 4 shows the decay of PE synthesis in R. This decay had a t1/2 of 40 to 50 min. The addition of rif to cells kept in G (Fig. 4) resulted in a drop in PE production which was indistinguishable from that produced by transfer to R. Addition of chloramphenicol (10 μg/ml) in G stopped PE synthesis within 5 min (data not shown).

The cessation of PE synthesis in cells transferred from G to R could reflect a response either to the presence of R or the absence of G. This was examined by transferring cells from G to darkness, and measuring PE synthesis by fluorescence spectroscopy. Following transfer to darkness, PE synthesis continued at a constant, but reduced, rate as compared to cells kept in G (Fig. 5). The lower rate of synthesis in darkness probably reflected the limitation in energy available in the absence of photosynthesis.

In the previous experiments, [35S]sulfate was added to cell suspensions 90 min after the cells had been transferred to test conditions. Spectroscopy (Fig. 4) showed that biliprotein synthetic patterns had changed completely by this time. The pattern of protein synthesis by cells during the transition was examined in the following experiment. Cells were induced to synthesize PE at a constant rate. They were then transferred to either darkness or R for 45 min. Control cells were kept in G. Rif was added to block further RNA synthesis and [35S]sulfate labeling was done in G to allow uniform photosynthesis. The pattern of polypeptide labeling in these cells reflected the condition of the cells at 45 min after

**Fig. 1.** Fluorescence emission versus absorbance for PE solutions at two sensitivities of the fluorescence spectrometer. (●): S3; (▲): S10. Fluorescence was measured at 567 nm.
DISCUSSION

Previous studies of chromatic adaptation have focused on the changes which take place in phycobiliprotein pigment metabolism over the course of several hours to several days (1, 7). We have taken advantage of the sensitivity of fluorescence spectroscopy to study changes which occur during the early phase of adaptation.

When cells which were grown in R were transferred to G, PE synthesis was detected within 45 to 90 min after the transfer (Fig. 2). The apparent lag could reflect either the inability of the fluorescence spectroscopy to detect small amounts of PE made earlier, the time necessary for the cells to actually begin PE production, or some of each.

The biological processes leading to PE formation probably comprised a significant component of the lag since we were unable...
to detect earlier synthesis of PE when using the fluorometer at three times greater sensitivity.

When cells which had been induced to synthesize PE were transferred from G to R PE synthesis stopped with a $t_{1/2}$ of about 45 min. It was completely halted by about 90 min after transfer (Fig. 4). No earlier changes were seen using the fluorometer at higher sensitivity. The fact that the cells required about equal times to turn PE synthesis on suggests that both processes were controlled at about the same step in PE synthesis.

Gel electrophoresis of the soluble proteins from cells labeled shortly after the induction of PE synthesis (Fig. 3) showed that new PE polypeptides were being made. This implies that the regulation of PE synthesis was either at the level of transcription or translation. This correlates with previous studies which have shown that no free precursor PE polypeptides or chromophore could be detected immunochemically in R-grown Fremyella (A. Bennett and L. Bogorad, unpublished).

The control of PE synthesis was studied by the use of the inhibitor, rif. At the concentrations used in these experiments rif was a specific inhibitor of RNA polymerase in these cells (11). Rif-resistant strains of Fremyella were able to undergo normal chromatic adaptation in the presence of concentrations of rif greater than used in these experiments. It has been shown (S. Miller, M. Neuberger, and L. Bogorad, unpublished) that the rif resistance in these strains derived from rif-insensitive RNA polymerase. Since the resistant strains were unaffected by rif, it is unlikely that the rif (at these concentrations) had any nonspecific effect on processes other than RNA synthesis in the rif-sensitive cells. Therefore, it appears that rif affected chromatic adaptation by blocking RNA synthesis in the wild type Fremyella.

The addition of rif to R-grown cells at the same time as transfer to G blocked the appearance of PE (Fig. 2). This suggests that RNA synthesis was necessary for chromatic adaptation. When rif was added to cells which had been induced to synthesize PE (Fig. 4), the shut-off of PE synthesis had the same kinetics as transfer to R. This suggests that rif and R acted to block PE synthesis at the same level, transcription. Since the addition of chloramphenicol, an inhibitor of protein synthesis, blocked PE synthesis much more rapidly than did either rif or R, it is unlikely that translational controls were acting during adaptation.

The fact that R, rather than just the lack of G, blocked PE synthesis was shown in Figure 5. When cells which had been induced to synthesize PE in G were put into darkness PE synthesis continued, whereas R blocked it. This indicates that R and G acted antagonistically on a regulatory system which, in turn, controlled PE synthesis. A similar pattern of R and G control of PE formation has been seen in dark-grown T. tenuis (7).

The possibility that PE synthesis is controlled at the level of RNA synthesis was further examined by labeling cells early in chromatic adaptation after blocking further RNA synthesis. Cells
kept in G or darkness for 45 min had the capacity to synthesize large amounts of PE, whereas cells kept in R could synthesize considerably less PE (Fig. 6). This strongly suggests that PE mRNA was synthesized in G and darkness, but not in R. The pattern of labeling is consistent with the $t_{1/2}$ of 45 to 50 min for PE mRNA which was suggested by the fluorescence data. One other possibility which can not be distinguished by these experiments is that PE mRNA may have continued to be synthesized but was destroyed before translation in cells growing in R.

In vivo PE and PC are located in phycobilisomes in the cell matrix. It has been reported that in addition to the biliprotein pigments these phycobilisomes contain a number of other polypeptides, some of which appear to be altered during chromatic adaptation (5, 14). Figure 3 shows that there were differences in polypeptide-labeling patterns in R and G other than those attributable to the pigments. Figure 7 shows that labeling differences were also found in the membrane-associated polypeptides. Bennett and Bogorad (1) observed differences in filament length and cell morphology between F. diplosiphon cultured in R and G. This suggests that the activity of a number of genes in addition to those for PE were regulated during chromatic adaptation. At this time our knowledge of gene regulation in cyanophytes is too limited to speculate on the possibility that operon-type control systems exist in these organisms, but clearly some system of coordinate control of gene expression must exist.

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LITERATURE CITED