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# Effect of Light Intensity on Macromolecular Synthesis in Cyanobacteria

Allan Konopka and Marilyn Schnur

Department of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907

**Abstract.** The light-dependent incorporation of $NaH^{14}CO_3$ into low molecular weight compounds, polysaccharide, or protein was determined in cultures of the cyanobacterium *Merismopedia tenuissima* incubated at a series of light intensities. There was an inverse relationship between incorporation into polysaccharide and protein. At light intensities of 90 $\mu E/m^2/sec$ or above, relative incorporation of radioisotope into polysaccharide was greatest and relative incorporation into protein was lowest. Optimal relative protein accumulation occurred in samples incubated at 20 $\mu E/m^2/sec$. A broader optimum of light intensity for maximal protein accumulation was found if ammonia rather than nitrate was the nitrogen source. Physiological adaptation of cultures to growth at a particular light intensity did not alter the pattern of macromolecular incorporation when those cultures were tested over the series of light intensities. The response of cultures of *Oscillatoria rubescens* to light intensity was similar to that of *M. tenuissima*, although incorporation into low molecular weight compounds was significantly greater.

The effect of light intensity on macromolecular synthesis in a natural population of *Oscillatoria rubescens* was also determined. A pattern similar to that observed in batch cultures of *O. rubescens* was occasionally found, but in other experiments there was no increase in relative protein incorporation when light intensity was decreased.

Whereas a good deal is known about the primary carbon fixation reactions in the Calvin cycle (2) and the rate of net photosynthetic production of organic matter (22), the intermediate steps, that is, the distribution of fixed carbon to biosynthetic pathways, have not been thoroughly investigated. It is known that the flow of carbon to different biosynthetic pathways is modulated through regulation of several enzyme activities. For example, by regulating the activity of fructose bisphosphatase, the distribution of fixed carbon to carbohydrate synthesis or amino acid biosynthesis can be controlled (1).

Although the factors that influence the distribution of fixed carbon to macromolecules could be studied by examining the effectors of enzyme activities, the effect of environmental conditions on the labeling pattern of macromolecules after photosynthetic incorporation of $^{14}CO_2$ is also useful. However, few investigators have used this

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approach with photosynthetic microorganisms. Fogg (4) found that actively growing cells of *Navicula pelliculosa* synthesized primarily protein, whereas nitrogen-deficient algae synthesized fats. In *Chlorella pyrenoidosa*, the addition of ammonium stimulated the short-term incorporation of $^{14}CO_2$ into amino acids, and decreased the amount of labeled sugar phosphates (10). Morris et al. (14) investigated the effect of several environmental factors on the rate of protein synthesis by marine phytoplankton. They found that low light intensities and low temperatures increased the proportion of carbon found in the protein fraction. This work was extended to include natural algal populations and similar results were obtained (15).

We are interested in the physiological ecology of planktonic cyanobacteria in Indiana lakes. Physiological parameters (such as the pattern of $CO_2$ incorporation into macromolecules) will be used to monitor these natural populations because such measurements provide significant information about the physiological state of organisms which is not obtained by measurement of total photosynthetic carbon fixation, the technique most often used by phytoplankton ecologists. In this report, a pure culture of *Merismopedia tenuissima* was used to develop a method for separating macromolecular fractions of cyanobacteria and to determine the effect of light intensity and physiological adaption to light intensity on labeling of macromolecular fractions. In addition, the effect of light intensity on carbon fixation into macromolecules by a natural population and culture of *Oscillatoria rubescens* was determined.

## Methods

An axenic culture of *Merismopedia tenuissima* Lemmerman (strain BL4) was isolated from Beaver Dam Lake, Indiana, and a culture of *Oscillatoria rubescens* (not bacteria free) was isolated from Crooked Lake, Indiana. Isolation and subsequent cultivation were in MAME medium, which was similar to that of Gerloff and Skoog (5), but also included 1 mM $NaHCO_3$. Cultures were grown at 30°C (*M. tenuissima*) or 22°C (*O. rubescens*) and illuminated by cool-white fluorescent lamps. Batch cultures were grown in culture flasks agitated in a shaker water bath or in 1-liter bottles that were bubbled with sterile air (250 ml/min). Cell growth was monitored by the optical density of the culture at 540 nm, measured in a Turner model 350 spectrophotometer. Chlorophyll and protein determinations were performed as described previously (12). Carbohydrate was measured with the anthrone test (8).

Photosynthesis was measured by the incorporation of $NaH^{14}CO_3$ into particulate material. One $\mu$Ci of $NaH^{14}CO_3$ (10 $\mu$g/$\mu$Ci; New England Nuclear, Boston, Mass.) was added to 25 ml of culture in an 18 × 150 mm screw cap tube. The culture media contained 1 mM $NaHCO_3$ and had a pH of 8.4. Incubations were conducted in duplicate at appropriate light and temperature conditions for 1 h (cultures) or 2 h (natural samples). Cultures were incubated at 30°C and light intensities of 0, 2, 5, 20, 45, 90 or 180 $\mu E/m^2/sec$ (fluorescent lights). The contents were immediately filtered through a glass fiber filter (Reeve Angel 981H), rinsed three times with distilled water, and further processed to separate macromolecular fractions. The filter was placed in 3 ml of cold 5% trichloroacetic acid (TCA) and incubated for 30 min on ice. The liquid was refiltered; the filtrate represented the low molecular weight fraction. Particulate material was added to 3 ml of 10% TCA, heated at 100°C for 10 min, and again filtered. The filtrate contained polysaccharide and nucleic acids; particulate material contained protein. Incorporation into RNA could be assessed in separate samples by hydrolysis of the cold TCA particulate matter in 0.5 N NaOH at 37°C for 30 min. The NaOH filtrate was neutralized and counted. Preliminary experiments indicated that RNA incorporation represented only a small fraction of radioactivity in the hot TCA filtrate. The fractions were counted by liquid scintillation techniques in a Beckman 100S scintillation counter; liquid fractions were added to Aquasol-2 counting fluid (New England Nuclear), and the particulate fraction was counted in a toluene-based cocktail which contained 4 g of 2,5-diphenyloxazole and 0.1 g of *p*-bis[2-(4-methyl-5-phenyloxazole)] benzene per liter of toluene. Counting

efficiencies for cell fractions ranged from 76 to 82%, and were determined by the addition of an internal standard ($^{14}$C-toluene) to selected samples.

Thin-layer chromatography was used to identify the radioactive compounds in the hot TCA particulate and soluble fractions. The fractionation steps were performed on two 25 ml culture samples of *M. tenuissima*, one labeled with 2 $\mu$Ci $NaH^{14}CO_3$/25 ml for 30 min and the other unlabeled. The hot 10% TCA filter from each group was placed in 5 ml of 8 N $H_2SO_4$, and autoclaved for 3 h to hydrolyze macromolecules. The solutions were neutralized with $Ba(OH)_2$, and the precipitate removed by centrifugation. The supernatants were evaporated in an evaporating dish. The precipitates were resuspended in methanol, and spotted along with standard isoleucine and standard glucose samples onto cellulose thin-layer chromatography sheets (Eastman Kodak, Rochester, N.Y.). The sheets were developed in a phenol:$H_2O$:KCN(75 g: 25 ml: 20 mg) solvent for approximately 3 h. This solvent separated amino acids from carbohydrates. The presence of carbohydrate was detected in the unlabeled cells by the method of Trevelyan et al. (19). Amino acids were detected by spraying the chromatogram with 0.25% (wt/vol) ninhydrin in acetone (3). By comparison of $R_f$ values, the areas which contained carbohydrate and amino acids on the $NaH^{14}CO_3$-labeled chromatogram were scraped into 10 ml of toluene-based scintillation fluid and counted.

Natural populations of *Oscillatoria rubescens* were obtained with a Van Dorn bottle from a depth of 10 m in Crooked Lake, Noble County, Indiana. Samples were taken to a laboratory and photosynthesis measurements (described above) were initiated within 30 min of sample collection. A range of light intensities (0, 1, 5, 10, 20, 50, or 100% of incident radiation) was obtained by using neutral density filters; experiments were conducted with midday solar radiation. Light intensity was measured with a Lambda quantum sensor (LiCor Instruments, Lincoln, Nebraska). Subsequent processing of these samples differed in one aspect from the scheme described above: 80% ethanol (70°C) was used to extract low molecular weight compounds rather than cold 5% TCA. Preliminary experiments indicated that these treatments were comparable.

## Results

### *Separation of Macromolecules*

A procedure was devised, based on that of Roberts et al. (17), to separate the organic matter of cyanobacteria into low molecular weight, polysaccharide and nucleic acid, and protein fractions. The ability of the procedure to differentiate between polysaccharide and protein was determined by chemical analysis of the hot TCA particulate and dissolved fractions.

The particulate fraction contained 95% of the protein and 9% of the anthrone-reactive material (polysaccharide). The hot TCA filtrate had 5% of the protein and 91% of the polysaccharide. Contamination of the hot TCA particulate material with polysaccharide after radioactive labeling was a concern because it would cause an overestimate of the rate of protein accumulation. Therefore, *M. tenuissima* was labeled with $NaH^{14}CO_3$ and macromolecular fractions were separated. The hot TCA particulate material was hydrolyzed with 8N sulfuric acid in an autoclave for 3 h. The material was neutralized, concentrated, and thin-layer chromatographed under conditions that separate reducing sugars from ninhydrin-reactive material (amino acids). Areas of the chromatogram to which reducing sugars and amino acids migrated were scraped and the radioactivity of each was determined. Ninety-seven percent of the added radioactive material was from amino acids, and only one percent was carbohydrate.

Further analyses indicated that most of the radioactivity associated with the hot TCA filtrate can be attributed to polysaccharide. Incorporation into RNA represented only about 10% of the counts in the hot TCA filtrate. Thus changes in the labeling of this fraction can be attributed to differences in polysaccharide accumulation.

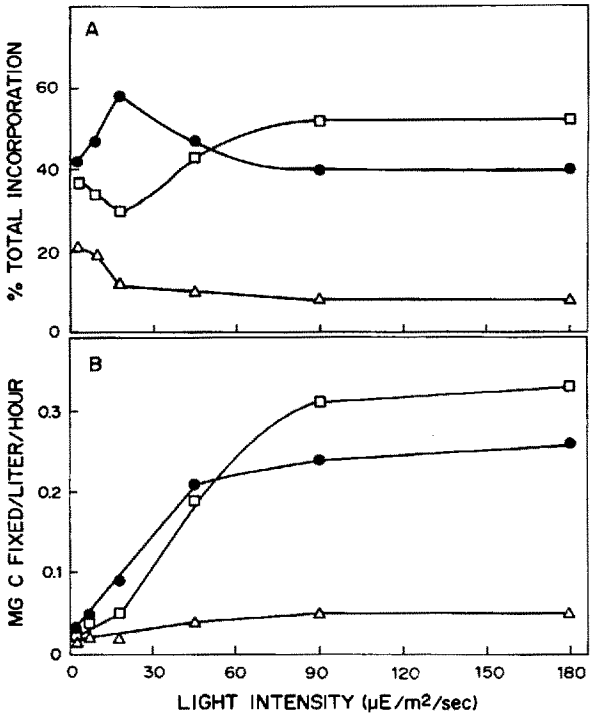


**Fig. 1.** Effect of light intensity on **(A)** relative incorporation and **(B)** absolute incorporation of $^{14}C$-bicarbonate into (●) protein, (□) polysaccharide, or (△) low molecular weight fractions in *M. tenuissima*. The culture used in the experiment was grown in MAME medium and had an $OD^{540}$ of 0.02.

*Effect of Light Intensity on Macromolecular Incorporation*

An exponential phase culture of *M. tenuissima* grown at a light intensity of 80 $\mu E/m^2/sec$ was labeled with $NaH^{14}CO_3$ at seven light intensities from 0 to 180 $\mu E/m^2/sec$. Figure 1 illustrates that the fate of photosynthetically fixed carbon was dependent on light intensity. There was an inverse relationship between relative protein and polysaccharide incorporation. At high light intensities (90–180 $\mu E/m^2/sec$), polysaccharide accumulation was preferred, and at lower light intensities (20–50 $\mu E/m^2/sec$) relative protein incorporation increased, apparently at the expense of polysaccharide synthesis. The fraction of carbon incorporated into low molecular weight compounds was relatively constant from 20 to 180 $\mu E/m^2/sec$, but increased at the lowest light intensities. The absolute amount of incorporation into each fraction increased as light intensity increased; protein incorporation approached its maximum value at 45 $\mu E/m^2/sec$, whereas polysaccharide accumulation increased up to 90 $\mu E/m^2/sec$ (Fig. 1B).

At very low light intensities (below 20 $\mu E/m^2/sec$) the proportion of carbon incorporated into protein decreased. To determine if energy limitation at low light intensities restricted uptake and reduction of nitrate (the nitrogen source in MAME medium) and hence protein synthesis, *M. tenuissima* cells were grown in liquid MAME medium with $NH_4Cl$ substituted for $NaNO_3$. Figure 2 summarizes the effect of ammonium as a nitrogen source on the proportion of fixed carbon present in the polysaccharide and protein fractions. A broader optimum for the incorporation of carbon into protein was observed (10–50 $\mu E/m^2/sec$), but there was still a decrease in the

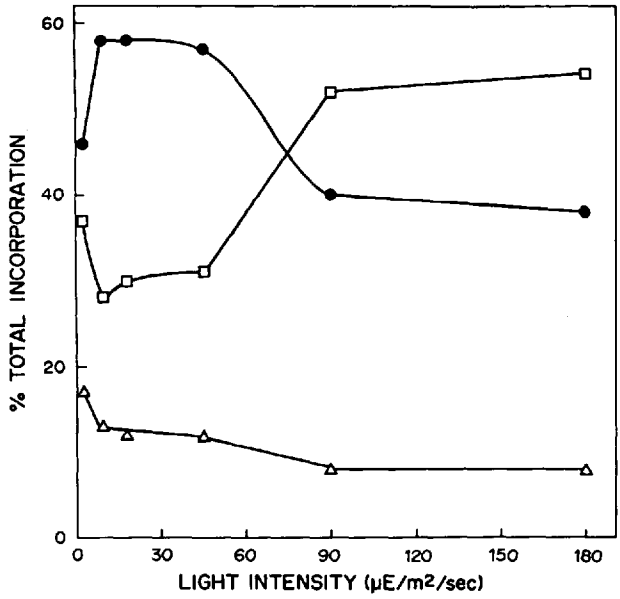


**Fig. 2.** Effect of light intensity on relative incorporation of $^{14}C$-bicarbonate into (●) protein, (□) polysaccharide and nucleic acids, or (△) low molecular weight fractions in *M. tenuissima*. The cells were grown in MAME medium with $NH_4Cl$ substituted for $NaNO_3$. The rate of carbon fixation was 2.35 mg C/liter/h at 180 $\mu E/m^2$/sec light intensity in the culture ($OD^{540} = 0.02$).

protein fraction at 2 $\mu E/m^2$/sec. The absolute amount of protein incorporation reached its maximum value at 90 $\mu E/m^2$/sec light intensity in these experiments.

Carbon fixation into macromolecules by an exponentially growing batch culture of *Oscillatoria rubescens* was also measured. Qualitatively, the response to light intensity was similar to that observed with *M. tenuissima*. Relative protein incorporation increased and relative polysaccharide incorporation decreased as light intensity decreased (Fig. 3A). Note that there was no incorporation into the polysaccharide fraction in samples incubated at 2 $\mu E/m^2$/sec. Also, a larger proportion of carbon was found in the low molecular weight fraction of *O. rubescens* cultures than was observed with *M. tenuissima*. Photoinhibition occurred in the *O. rubescens* culture—the photosynthetic maximum was found at 45 $\mu E/m^2$/sec light intensity, and photosynthesis declined at higher light intensities (Fig. 3B).

### *Effect of Light Adaptation on Macromolecular Synthesis*

The results shown in Fig. 1 indicated that light intensity did have an effect on macromolecular synthesis. Physiological adaptation to growth at a particular light intensity might have an additional effect. To test this hypothesis, *M. tenuissima* was grown in batch cultures at 180 or 10 $\mu E/m^2$/sec light intensities (generation times were 6 and 16 h, respectively), and samples were labeled with $NaH^{14}CO_3$ at light intensities from 0 to 180 $\mu E/m^2$/sec. The cultures responded similarly to one another in that polysaccharide accumulation was greatest at high light intensity and protein incorporation was optimal at 20 $\mu E/m^2$/sec (Table 1). In cultures grown at high light intensity, relative synthesis of protein was higher and of polysaccharide lower than in low-light grown cultures. The maximum photosynthetic rate in cultures grown at high

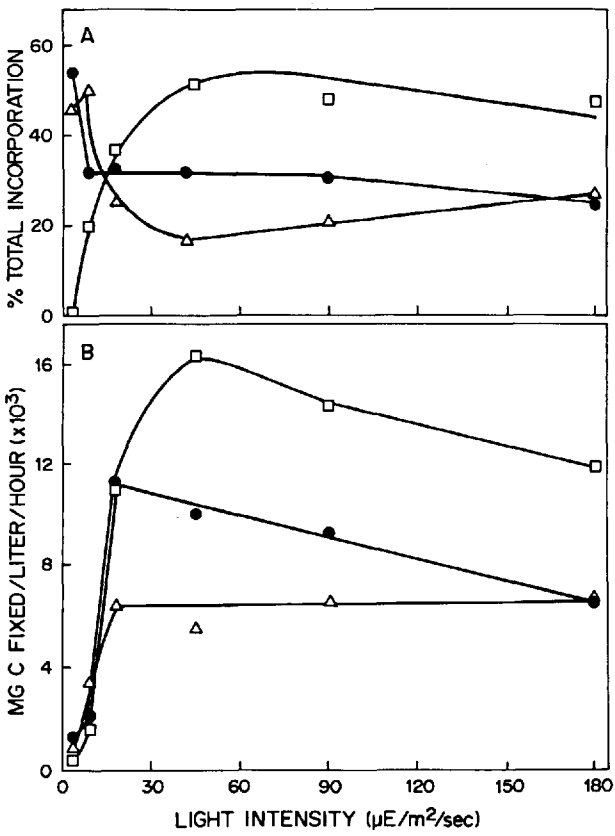


**Fig. 3.** Effect of light intensity on **(A)** relative incorporation and **(B)** absolute incorporation of $^{14}C$-bicarbonate into (●) protein, (□) polysaccharide, or (△) low molecular weight fractions in cultures of *O. rubescens*.

**Table 1.** Effect of light adaptation on macromolecular incorporation of $NaH^{14}CO_3$ at different light intensities

| Light intensity ($\mu E/m^2/sec$) | Macromolecular incorporation of $NaH^{14}CO_3$ by cells grown at: | | | |
|---|---|---|---|---|
| | 180 $\mu E/m^2/sec$ | | 20 $\mu E/m^2/sec$ | |
| | Polysaccharide | Protein | Polysaccharide | Protein |
| 180 | 49 | 44 | 52 | 40 |
| 90 | 42 | 50 | 52 | 40 |
| 45 | 27 | 64 | 43 | 47 |
| 20 | 21 | 67 | 30 | 58 |
| 10 | 28 | 56 | 34 | 47 |
| 2 | 28 | 55 | 37 | 42 |

*M. tenuissima* cultures were grown at 180 or 20 $\mu E/m^2/sec$ light intensity, and samples were incubated with $NaH^{14}CO_3$ at light intensities from 0 to 180 $\mu E/m^2/sec$ for 1 h. Results are expressed as % total incorporation.

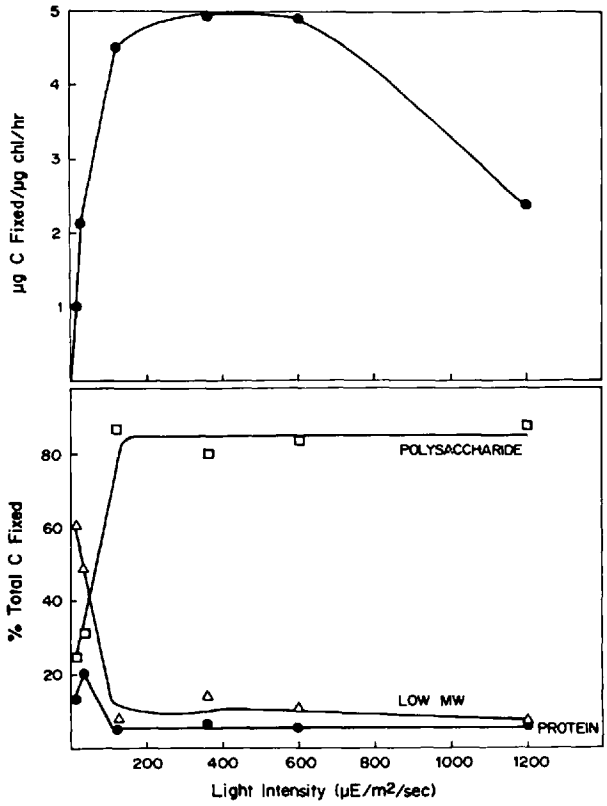


**Fig. 4.** Effect of light intensity on **(A)** photosynthetic rate and **(B)** relative incorporation into (△) low molecular weight, (□) polysaccharide, or (●) protein fractions of a natural population of *Oscillatoria rubescens* on June 11, 1979.

light was twice that of low light cultures. This increase is due in part to three-fold greater activity of ribulose bisphosphate carboxylase in cells grown at high light intensity (unpublished observations).

### *Natural Populations*

Natural samples of a metalimnetic layer of *Oscillatoria rubescens* from Crooked Lake (Noble County, Indiana, U.S.A.) were collected throughout the summer of 1979. The effect of light intensity on macromolecular synthesis was tested on five dates during 1979. Samples were exposed to a range of intensities from 1 to 100% incident solar radiation. The maximum photosynthetic rate occurred at a light intensity which was 10–20% of full sunlight, and inhibition of photosynthesis occurred in samples exposed to full sunlight (Fig. 4 and Table 2). Although the response of total photosynthetic carbon fixation to light intensity was the same on all five dates, the pattern of macromolecular incorporation varied. On two dates (June 11 and August 7) the natural populations exhibited a response similar to that of cultures; as light intensity was decreased, relative polysaccharide accumulation decreased from 83% to less than 30% (Fig. 4). Relative protein incorporation increased twofold at the lowest light intensities. However, on June

**Table 2.** Effect of light intensity on the products of photosynthesis of a natural population of *Oscillatoria rubescens* on August 23, 1979

| Light intensity ($\mu E/m^2/sec$) | Photosynthetic rate ($\mu gC/\mu g$ Chl/h) | % Carbon fixed into | | |
|---|---|---|---|---|
| | | Low MW[a] | Polysaccharide | Protein |
| 1500 | 1.2 | 6 | 79 | 15 |
| 750 | 2.4 | 5 | 79 | 16 |
| 450 | 2.9 | 4 | 84 | 12 |
| 300 | 2.6 | 5 | 81 | 14 |
| 150 | 2.3 | 4 | 79 | 17 |
| 30 | 1.0 | 3 | 81 | 16 |
| 15 | 0.6 | 1 | 81 | 18 |

[a]Low MW = low molecular weight fraction.

11, protein accounted for only a small proportion of total carbon fixation, so that the bulk of the $^{14}C$ incorporation at the lowest light intensities was into the low molecular weight fraction. In the other three experiments, neither polysaccharide nor protein incorporation changed significantly at different light intensities (Table 2).

## Discussion

The results presented herein indicate that separation of the products of photosynthesis into physiologically meaningful fractions is feasible and that the procedure provides useful information on the physiological state of the organism. Separation of protein and polysaccharide was considered to be the most important step, and treatment with hot 10% TCA was very effective in this regard. The fractionation procedure used in this work is similar to that of Morris et al. (14). In our procedure, cold 5% TCA (instead of hot ethanol) was used to extract low molecular weight compounds from *M. tenuissima*. Incorporation into lipid (measured by chloroform-soluble radioactivity) was very low (less than 5% of total incorporation) in *M. tenuissima*, so that the results should be comparable to those of Morris and his colleagues. The low molecular weight compounds were extracted from natural populations of *O. rubescens* with hot ethanol because it was more convenient in the field studies. The other difference in methodology was an increase in the concentration of hot TCA from 5 to 10% and a decrease in incubation time in the procedure; this method yielded more consistent results with cyanobacteria.

Experimental samples were labeled for 1 to 2 h with $^{14}C$-bicarbonate. This period is not sufficient for the organisms to attain isotopic equilibrium with the external medium. The results should not be construed as indicating net rates of macromolecular synthesis, because the turnover rates of these fractions are unknown. However, the technique does approximate gross rates of synthesis and indicates physiological changes within the cell.

The rationale for examining the products of photosynthesis rather than total photosynthetic carbon fixation is that the former provides some information on the nutritional status of the organism. Measurements of total carbon fixation do not necessarily reflect nutritional status in short-term experiments (6, 7); incident light intensity and the previous history of the cells (as it affects the amount of photosynthetic

pigments, electron transport carriers, and carbon fixation enzymes) will determine the photosynthetic rate of a sample. Nutritional status will affect the concentrations of the photosynthetic components in the cell, but these changes occur over a longer time span than is measured in a 1–2 h incubation with $NaH^{14}CO_3$.

From previous studies on photosynthetic carbon metabolism (1, 4, 10), we infer that labeled $CO_2$ is first incorporated into the low molecular weight fraction. If other required nutrients are available (e.g., N and P), these low molecular weight compounds can be converted into catalytically active biomass (protein and nucleic acid). If nutrients are not available, low molecular weight compounds may accumulate or be converted into polysaccharide. Thus the proportion of total $^{14}CO_2$ incorporation found in each macromolecular fraction reflects the relationship between the rates of carbon fixation and nutrient assimilation. If the two processes occur at comparable rates, relative protein incorporation should be high, and polysaccharide incorporation low. In this presentation, the proportion of carbon fixed into each fraction rather than absolute incorporation is emphasized because relative incorporation reflects changes in the metabolism of the immediate products of photosynthesis.

The inverse relationship between relative polysaccharide and protein incorporation found in batch cultures of *M. tenuissima*, in which polysaccharide accumulation was greatest at high light intensity, can be explained by the pathways of photosynthetic carbon metabolism described above. At high light intensities, the rate of carbon fixation exceeded the velocity of nitrogen assimilation; excess carbon and energy derived from photosynthesis was stored as polysaccharide. Note that the absolute amount of protein incorporation was saturated at a lower light intensity and had a lower maximum value than polysaccharide accumulation. Lower light intensities limited the rate of $CO_2$ fixation and ATP production, so that the amount of organic carbon and energy produced did not greatly exceed what could be assimilated into protein. When *M. tenuissima* was grown with $NH_4Cl$ instead of $NaNO_3$ as nitrogen source, the absolute amounts of protein and polysaccharide incorporation were saturated at the same intensity (90 $\mu E/m^2/sec$), but polysaccharide accumulation attained a greater maximum rate. This resulted in the same overall response to light intensity as in nitrate-grown cells, but a broader optimum for relative protein incorporation. Preferential incorporation of $CO_2$ into protein at low light intensities and into polysaccharide at higher light intensities has previously been found with cultures (4, 14, 20) and natural populations (15, 16) of photosynthetic microorganisms.

The percentage of carbon fixed into protein decreased at the two lowest light intensities used in the experiments. Assimilation of nitrate, the nitrogen source in MAME medium, requires energy, and perhaps the extent of energy limitation imposed on the cells at these intensities drastically reduced nitrate uptake. Experiments in which ammonia was the nitrogen source are consistent with this explanation, because under these conditions maximum relative rates of protein synthesis were observed at a lower intensity (10 $\mu E/m^2/sec$). Protein incorporation still declined, however, at an intensity of 5 $\mu E/m^2/sec$ (Fig. 2).

Gas vacuolate cyanobacteria, which exist in lakes that are not subject to turbulence, can stratify in the water column; this might result in physiological adaptation to the incident light intensity (9, 18). It was necessary to determine if physiological adaptation affects the response of macromolecular incorporation to light intensity. The results in Table 1 indicate that there was no qualitative difference in the response of organisms grown at low or high light intensities. The greater capacity for protein incorporation

observed in cultures grown at high light is probably a consequence of the faster growth rate of these cultures, because the cellular concentration of ribosomes has been found to increase as growth rate increases (13). In terms of studies on natural populations, light adaptation would not be expected to significantly change the effect of light intensity on relative macromolecular synthesis.

The experiments with *M. tenuissima* indicated that light intensity did affect the metabolism of photosynthetically fixed carbon. To determine if natural populations responded to light intensity in the same way, natural samples of *Oscillatoria rubescens* were tested. As a control, the products of photosynthesis of a culture of *O. rubescens* (isolated from the habitat) incubated under illumination conditions identical to those used in the experiment with *M. tenuissima* were determined; the response to light intensity was similar, although relative incorporation into low molecular weight compounds was twofold higher.

Of five experiments with natural samples, in only two was the change in macromolecular products at different light intensities similar to what was observed in cultures (Fig. 4). In the other experiments there was little change in the incorporation pattern as a function of light intensity (Table 2). Note that in experiments with cultures the highest light intensity used was 180 $\mu E/m^2/sec$, whereas solar radiation was employed for the natural samples, so much higher light intensities were tested. The response of *Oscillatoria rubescens* to changes in light intensity was interesting in relation to its ecology. *O. rubescens* stratified at a depth of 10 m in Crooked Lake. The light intensity at this depth was 15–30 $\mu E/m^2/sec$ on a sunny summer day. Light intensity limited the rate of photosynthesis at this depth (Fig. 4), and it was expected that relative protein incorporation would be greatest at low light intensities, as was observed with cultures (Fig. 3). Stratification of *O. rubescens* deep in the water column was due to regulation of buoyancy conferred by gas vesicles. The regulatory mechanism has been hypothesized to respond to light and nutrient concentrations such that the rate of photosynthesis does not exceed the rate of growth imposed by the limiting nutrient (11, 21). Thus from this consideration one might also expect that a greater proportion of carbon would be fixed into protein at the preferred low light intensity and polysaccharide synthesis would predominate at higher intensities. However, this response was not consistently observed. One difference between the cultures and natural populations was that the cultures were not nutrient limited, whereas the natural populations probably were. When *M. tenuissima* was grown in a nutrient-limited chemostat, relative protein incorporation was not enhanced at low light intensity (manuscript in preparation). Morris and Skea (15) found that the effect of reduced light on relative protein incorporation by marine phytoplankton varied seasonally. Thus the results of experiments on natural populations were probably influenced by the nutritional status of the population, and this type of experiment may be a good technique to determine nutrient limitation of natural algal populations.

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