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PHOTOSYSTEM II REGULATION OF MACROMOLECULE SYNTHESIS
IN THE BLUE GREEN ALGAE APHANOCAPSA 6714

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ABSTRACT

Polymers synthesized by heterotrophically growing (glucose-fed) cultures of *Aphanocapsa* 6714 were compared with polymers synthesized in photosynthetically grown cultures. Loss of photosystem II by dark incubation, or inhibition of light grown cells with the PS II specific inhibitor dichlorophenymethylurea (DCMU), caused an 80% to 90% reduction in the rate of lipid and RNA synthesis, and more than a 90% reduction in the rate of protein synthesis. In contrast, glycogen synthesis was reduced only about 50% in dark cells and less than 30% in DCMU inhibited cells. After longer heterotrophic growth, glycogen became the major component, whereas in photosynthetically grown cultures protein was the major constituent. Carbon-14 assimilated into protein by heterotrophically grown cells was found in amino acids in nearly the same proportions as in photosynthetically grown cells. Thus, all of the principle pathways of intermediary metabolism were functional in heterotrophic cultures, but the supply of carbon precursors to those pathways was reduced.

The limited biosynthesis in heterotrophic cells was not due to a limitation for cellular energy. The adenylates were maintained at nearly the concentrations (and hence the energy-charge also) as in photosynthetic cells. The concentration of reduced NADPH was higher in heterotrophic (dark) cells than in photosynthetic cells.

From rates of CO₂-fixation and/or glycogen biosynthesis it was determined that stationary phase cells expended approximately 835, 165, and less than 42 nanomoles ATP/mg dry weight algae/30 minutes during photosynthetic, photoheterotrophic and chemoheterotrophic metabolism, respectively.

Analysis of the soluble metabolite pools in dark heterotrophic cultures by double labeling experiment revealed rapid equilibration of carbon-14 through the monophosphate pools, but much slower movement of label into the diphosphate pools of fructose-1,6-diphosphate and sedoheptulose-1,7-diphosphate (FDP and SDP).
Carbon did flow into PGA in the dark; however the initial rate was low and the concentration of this metabolite soon fell to an undetectable level. In photosynthetic cells, carbon-14 quickly equilibrated throughout all the intermediates of the reductive pentose cycle, in particular, into PGA.

Analysis of glucose-6-phosphate dehydrogenase in cell free extracts showed that the enzyme was very sensitive to product inhibition by NADH.

INTRODUCTION

The unicellular blue-green algae have limited heterotrophic potential. Most strains are photoautotrophs and the limited assimilation of reduced carbon is strictly coupled to photosynthetic metabolism and autotrophic CO₂-fixation (11).

However, several strains, mostly found in typological Group IIa (classification according to Stanier et al. (12)) are capable of photoheterotrophic growth, and in some cases, true chemoheterotrophic growth in the dark (10). Invariably, heterotrophic growth is slow in comparison to photosynthetic growth.

In the work to be described, we have compared photosynthetic, photoheterotrophic and chemoheterotrophic (dark) metabolism and growth in Aphanocapsa 6714, a member of typological Group IIa. This organism fixes CO₂ by the reductive pentose (Calvin) cycle during photosynthesis and utilizes glucose via the reactions of the oxidative pentose cycle during heterotrophic metabolism. That the first enzyme of the latter pathway is inhibited by ribulose-1,5-diphosphate (RuDP) probably is of physiological significance since the oxidative pathway is blocked during photosynthesis. It is noteworthy that RuDP is only detectable during photosynthesis (6).

Previous work on the assimilation of carbon-14 suggests that a block in metabolism between the sugar monophosphates and 3-phosphoglyceric acid
(PGA) was induced by placing cells in the dark, or by inhibition of light incubated cells with the herbicide DCMU (5,6). We have continued this study by measuring the distribution of assimilated carbon-14 into the polymers of the cell under both photosynthetic and heterotrophic growth conditions. Double labeling experiments with carbon-14 and phosphorous-32 were carried out in an effort to estimate the relative rates of carbon flow through the various parts of the oxidative and reductive pentose cycles. We have also estimated the relative capacity of Aphanocapsa 6714 to synthesize ATP using the three metabolic options available to the cell: photosynthetic electron transport (photosystem I and II both in operation); cyclic electron transport (photosystem I only); and respiratory oxidation of glucose. As part of the latter studies we have measured the levels of the adenylates and reduced pyridine nucleotides.

METHODS

Growth. Aphanocapsa 6714 was grown on medium BG-11 as described previously (11) except for the following modifications: the gas phase was supplemented with 4% (v/v) CO₂; the liquid medium was supplemented with 30 ml of a 1.0 M sodium bicarbonate solution autoclaved separately before addition to BG-11.

Labeling experiments. Cells were grown to early log phase (klett reading of 50 optical density units, absorbance at 620 nm), then supplemented with radioactive tracers as follows: (1) photosynthetic cells, sodium bicarbonate-\textsuperscript{14}C, 10 mM, 0.33 uCi/umole plus 0.25% w/v glucose-\textsuperscript{14}C, 2.0 uCi/umole; (2) heterotrophic cells, 0.25% w/v glucose-\textsuperscript{14}C, 2.0 uCi/umole.

Cells were grown in 1 liter side-arm flasks, 50 ml per flask. The photosynthetic culture was capped with a sterile number 12 rubber bung. The photoheterotrophic culture was kept in the light after adding enough DCMU to bring the total concentration to \(5 \times 10^{-5}\) M, which was sufficient to completely inhibit non-cyclic electron transport and photosystem II.
The chemotrophic (dark) cultures were kept dark by carefully wrapping a side-arm flask to exclude all light. The side-arm was covered with black felt that could be removed to make optical density readings. Samples were withdrawn with sterile 10 ml pipettes and the cells washed free of tracers and degraded, as described below.

In the experiment done to estimate rates of ATP synthesis, the following specific activities were used: $^{14}\text{CO}_2$, 7.13 uCi/umole; glucose-$U-^{14}\text{C}$, 6.54 uCi/umole. Stationary phase cells were harvested and resuspended in BG-11. They were then placed in the steady-state machine, described in detail elsewhere (4,6). The tracers were added and cells withdrawn and killed in 80% methanol at specific intervals. Total incorporation of label into cell material was determined after two dimensional paper chromatography as previously described (3). The activity of labeled products is expressed as concentration/mg dry weight algae in all figures. In that part of the latter experiment which requires anaerobic conditions, an oxygen trap of Oxysorbant (Sorel Corporation) was introduced into the closed system of the steady-state machine and the concentration of oxygen was reduced to less than 5 ppm before labelled substrate was added.

Specific activities of $^{14}\text{CO}_2$ were determined by the following procedure. At the end of the experiments, cells were removed by centrifugation, the supernatant solution was made alkaline to a pH of approximately 10 and stored under $\text{CO}_2$-free $\text{N}_2$ gas in a stoppered flask. Aliquots of 1 to 6 mls of the alkaline supernatant fraction were added to enough $\text{CO}_2$-free water in the reaction vessel of the steady-state machine to make a total of 50 ml. The water in the reaction vessel was adjusted to pH 1.0 with concentrated $\text{HCl}$, thus converting most of the carbonate and bicarbonate to $\text{CO}_2$. Total $^{12}\text{CO}_2$ was determined by an infrared $\text{CO}_2$ analyzer; $^{14}\text{CO}_2$ was determined by a Cary ionization chamber. Both instruments are components of the steady-state apparatus. The specific activity of glucose-$U-^{14}\text{C}$ was determined by measuring
the total glucose concentration by the glucostat method (Worthington Biochemicals) and the carbon-14 by liquid scintillation in Aquasol.

**Stepwise degradation of cells.**

1. **Total Lipids**

   Total lipids were extracted by the method of Bligh and Dyer (1); the $^{14}$C contents of 5, 10, and 20 µl aliquot samples of the solubilized lipids in aquasol were determined by liquid scintillation counting.

2. **Protein**

   Half of the lipid-free pellet from step (1) was washed four times with distilled water and then resuspended in 2 ml of 0.05 N triethanolamine (TEA) buffer, pH 7.5 containing 50 micrograms per ml of Pronase (Sigma Biochemical Corporation). A drop of toluene was placed over each sample, the samples were tightly stoppered and placed at 37°C for 24 h to allow complete hydrolysis of cellular protein. Then 250 microliters of each hydrolysate was removed and chromatographed (see below) and the amino acids identified and counted for carbon-14 by gas flow spectrometry, as previously described (6).

3. **Glycogen**

   One-fourth of the washed pellets from step (1) were resuspended in 4 ml of 1.5 N trifloroacetic acid (TFA) and placed in 10 ml "color-break" glass ampules (Kimble Products), sealed by flame and autoclaved at 240°C for 30 min to hydrolyze glycogen. Solubilized glucose was chromatographed (see below) and the incorporation of carbon-14 determined by radioautography.

4. **DNA + RNA**

   One fourth of the washed pellets from step (1) were resuspended in 2 ml of 0.05 N TEA buffer, pH 7.5, plus 0.05 N MgSO$_4$ and 10 micrograms per ml of DNAase I and II (Calbiochem). The suspensions were placed at 37°C for 12 h in tightly stoppered tubes. Each sample was centrifuged at 15,000 x g for 20 min and the supernatant fraction counted in Aquasol by liquid scintillation.
The pellets were washed four times with distilled water and resuspended in 0.5 N sodium hydroxide and then placed in tightly stoppered tubes at 37°C, for 24 h. The remaining suspension was centrifuged at 15,000 x g for 30 min, and 5, 10, and 20 ul aliquot samples of the supernatant containing solubilized RNA were counted in Aquasol by liquid scintillation.

Labeling of cells with Phosphorous-32. Cells were grown from small inocula in modified BG-11 medium in the presence of 20 uCi/ml of carrier free 32P phosphate. In the labeling experiments using 32P as the only tracer, the soluble metabolites were extracted with 80% methanol, separated by paper chromatography, then counted by radioautography (as previously described). The specific activity of 14CO2 in the double labeling experiments was 3.33 uCi/umole; glucose-U-14C, 20 uCi/umole.

Enzyme assays. Glucose-6-phosphate dehydrogenase was assayed by the initial rate of NADPH formation, at 340 nm, by conventional spectrophotometry. The reaction mixtures contained (in one ml): 55 ug of protein; 10 umoles of MgCl2; 25 umoles TEA, pH 7.45; 1 umole of glucose-6-phosphate; NADP, as indicated; NADPH, as indicated; and other metabolites, as indicated in the text. The concentrations of the pyridine metabolites were estimated fluorometrically by the methods described by Wimpenney & Firth (13).

RESULTS

Assimilation of Carbon-14: Heterotrophy versus photoautotrophy. 14C-glucose assimilation in Aphanocapsa 6714 was roughly the same under all three experimental conditions (Table 1). However, in the absence of photosystem II activity (i.e., in DCMU-inhibited and dark-incubated cells) the fixation of 14CO2 was almost totally inhibited. Thus CO2-fixation, but not glucose assimilation, was dependent on a functional photosynthetic electron transport chain.
In this same experiment, photosynthetic growth was approximately exponential from an optical density of 50 optical density units to a reading of about 150 optical density units. Photosynthetic cells then entered a slower, approximately linear growth phase. In the two cultures lacking photosystem II activity (heterotrophic cells) growth was very slow and never approached an exponential rate. As can be seen (Figs. 1 and 2), the fate of carbon-14 assimilated by the three cultures was different. Cells incubated in the dark, or in the light with DCMU, incorporated much less tracer into synthetic polymers—i.e., lipids, protein, and RNA. In particular, protein synthesis nearly ceased in this 30 h experiment.

The composition of cells at the end of a longer term labeling experiment is shown in the data of Tables 2 and 3. The specific activities of the various polymers show that glycogen or protein were the principle products of carbon-14 assimilation under all of the experimental conditions. In photosynthetic cells the ratio of glycogen to protein was about 1/3. In heterotrophic cells, this ratio was much higher, with values of 11/1 and 8/1 for DCMU-inhibited and dark incubated cells, respectively. Thus, glycogen synthesis was the main biosynthetic process in heterotrophically metabolizing cells; both in initial velocity and also as the longer term product.

When hydrolyzed protein from the long-term labeling experiment was chromatographed (Table 3), the distribution of tracer was approximately the same for both photosynthetic and heterotrophic cells. It follows that (in heterotrophic cells) all the major pathways of intermediary metabolism were still available to the tracer, but the total flow of carbon into these pathways was reduced.

Metabolite pools in photosynthetic and dark cells. In heterotrophic cells, the enhanced rates of glycogen synthesis relative to other synthetic processes suggests a restriction on the movement of carbon from the early reactions of glucose metabolism to the rest of the metabolism. Previous work
has shown a large build-up of carbon in the sugar monophosphate pools or dark cells possibly indicating a block in metabolism at the level of triose phosphates (5,6). To study further this problem, double labeling experiments (Figs. 3 through 5) were done. The metabolite pools of the cells were first prelabeled with $^{32}$P-phosphate until steady-state levels of $^{32}$P-labeled metabolites were obtained (Methods). Then glucose-$^{14}$C (or glucose-$^{14}$C plus $^{14}$CO$_2$) was added and the flow of carbon-14 through the system was measured by the ratios of $^{14}$C/$^{32}$P. In time, steady-state levels of $^{14}$C labeling of metabolite pools occurred and thus a constant ratio of $^{14}$C/$^{32}$P in each metabolite was obtained.

Within six min after the addition of the tracer to the system, ratios of $^{14}$C/$^{32}$P became approximately constant for the monophosphates, glucose-6-phosphate (G6P) and sedoheptulose-7-phosphate (S7P) showing the rapid movement of carbon through these pools in both photosynthetic and dark heterotrophic cells (Fig. 3). Incorporation of carbon-14 from glucose into G6P and S7P requires the enzyme sequence: hexo(gluco)kinase, phosphohexose isomerase and transaldolase. Accordingly, these enzymes are very active in both light and dark cells. Moreover, in the light cells, the $^{14}$C and $^{32}$P labels each became constant after 6 min, indicating a steady-state in which further conversion of these sugar monophosphates just equals their rate of formation. However, in the dark the total concentration of carbon-14 in the monophosphate sugars increased at approximately a linear rate during this experiment. Thus flow of carbon into these pools was not balanced by the exit of carbon metabolites into other pathways.

A slow increase in the concentration of FDP and SDP was noted in the dark cells (Fig. 4), while the corresponding concentrations in the light were very low. As expected, RuDP was the principle diphosphate sugar in photosynthetic cells, since this metabolite is the O$_2$ acceptor for autotrophic
CO₂-fixation by Aphanocapsa 6714. In contrast, no carbon-14 was found in RuDP in dark cells. It follows that flow from glucose into RuDP was completely blocked in the dark. This data is in agreement with the low rate of CO₂-fixation observed in the dark-incubated and DCMU inhibited cells, and confirms previous results from single labeling experiments (5,6). It should be noted that the rate of carbon-14 accumulation in FDP+SDP in the dark was much slower than the rate of accumulation in G6P+S7P.

In previous work (5,6) we found almost no label in PGA during dark incubation of Aphanocapsa 6714, suggesting a block between triose-phosphates and PGA. In these present experiments some carbon-14 did flow into PGA in the dark (Fig. 5). However, compared with photosynthetic cells, the rate of incorporation was sharply reduced. PGA was maintained at nearly a constant level in photosynthetic cells. In dark cells PGA increased in total concentration as soon as the lights were turned off, then rapidly decreased becoming almost undetectable by the end of the experiment. The rapid increase in PGA concentration when the light is turned off probably represents the continued carboxylation of RuDP at a time when reduction of PGA has stopped. The kinetics of this process have been analyzed in detail elsewhere (8).

Adenylate and Pyridine Nucleotide Pools. The concentrations of the adenylates and the NADPH and NADP pools are shown in Figs. 6 and 7. As can be seen, neither the concentration of ATP nor ratios of adenylates (e.g., the energy-charge) had appreciably lower steady-state values in dark or DCMU treated cells, although transient changes were seen when the light was turned off. The concentration of NADPH and the ratios of reduced to oxidized pyridine nucleotides actually increased in dark and DCMU treated cells, relative to comparable values in the photosynthetic cells (Fig. 7). This suggested a reduction in the utilization of NADPH for biosynthetic purposes in cells lacking photosystem II activity. Ratios of NADH/NAD⁺ remained essentially unchanged in this experiment (data not shown). It should be
noted that NADP is the pyridine nucleotide of major importance in the metabolism of *Aphanocapsa* 6714; both as a biosynthetic electron donor and also as the first electron carrier in respiration (9).

**Rates of ATP synthesis: Estimates under four metabolic conditions.**

Figures 8 and 9 show assimilation of $^{14}$CO$_2$ and glucose-U$^{14}$C into glycogen. Previous work with *Aphanocapsa* 6714 has shown the potential for glycogen synthesis in stationary phase cells is always high (7). On the other hand, stationary cells do not synthesize significant quantities of other polymers. As can be seen (Fig. 8b), glycogen synthesis was most rapid in DCMU-inhibited cells. It should be noted the DCMU-inhibited cells were kept under anaerobic conditions in order to block any respiratory contribution to the synthesis of ATP (see below). Thus, ATP required for glycogen synthesis came totally from photophosphorylation via photosystem I (i.e., cyclic phosphorylation). In both the aerobic-dark cells and photosynthetic cells, glycogen synthesis was somewhat inhibited (compared with DCMU-treated cells), except for a short burst at the beginning of the experiment. In anaerobic dark cells, glycogen synthesis was almost completely inhibited.

Since the energy requirements for glycogen synthesis and CO$_2$-fixation are known—i.e., 2 ATP per glycosyl unit derived from hexose monophosphates, and 3 ATP required for each CO$_2$ fixed via the reductive pentose cycle—it is possible to compare the capacity of these stationary phase cells to synthesize ATP under each of the four physiological conditions.

From carbon-14 recovered in glycogen it can be calculated approximately 840, 165, and about 40 nanomoles ATP per mg algae per 30 min were expanded for CO$_2$-fixation and/or glycogen synthesis (Fig. 9). Essentially no ATP was consumed by fermentative metabolism of glucose in anaerobic-dark cells. If we assume the relative capacities for ATP synthesis are not greatly altered in the stationary phase, then potentials for ATP synthesis by cyclic, non-cyclic and respiratory electron transport are related by ratios of 840:165:40. These
data suggest the capacity for energy synthesis in aerobic dark cells is drastically reduced, although as shown by the data of Fig. 6, the cellular concentrations of ATP (also the other adenylates) were not greatly altered. Thus the overall rate of energy consumption was probably reduced to approximately the same extent as the decrease in ATP synthesis.

**Inhibition of Glucose-6-Phosphate Dehydrogenase.** Table 4 shows the effect of various metabolites on the activity of glucose-6-phosphate dehydrogenase in cell free extracts of *Aphanocapsa* 6714. As can be seen, only NADPH and RuDP inhibited enzyme activity. On a molar basis, inhibition of NADPH was much the stronger of the two metabolites. This regulation of glucose-6-phosphate dehydrogenase by NADPH, amplified by RuDP, may be similar to the regulation of this enzyme in spinach chloroplasts (2).

Figure 10 shows the inhibition of glucose-6-phosphate dehydrogenase at fixed concentrations of NADPH as the substrate of NADP was increased in concentration. At the highest concentration of NADP (4.4 x 10^{-4} M) 50\% inhibition of the enzyme was observed at about 10^{-4} M NADPH. Thus glucose-6-phosphate dehydrogenase, the first enzyme of the oxidative pentose cycle is strongly inhibited by NADPH. In view of the relatively high intracellular concentrations of NADPH found in heterotrophic cells (Fig. 7), the level of this metabolite may be an important factor regulating the oxidation of glucose-6-phosphate and hence the flow of carbon from the monophosphates during heterotrophic metabolism.

**DISCUSSION**

The slow heterotrophic growth of *Aphanocapsa* 6714 is marked by a disproportionate increase in the amount of glycogen synthesized as compared with the biosynthesis of growth polymers such as protein, lipids, and nucleic acids. Photosynthetically competent cells—i.e., cells having
both PS I and PS II activity—in addition to a much faster growth rate, show a much more balanced distribution of carbon into growth polymers, especially protein. Evidently, carbon from glucose has freer access to the major biosynthetic pathways in photosynthesizing cells than in heterotrophically metabolizing cells. The simplest explanation for these observations is that the reduced rate of biosynthesis under heterotrophic conditions is mainly a consequence of a reduced supply of precursor molecules resulting from a block early in the intermediary metabolism of glucose. Moreover, this block would occur early in the intermediary metabolism of glucose so as not to limit the synthesis of glycogen. The block cannot be absolute since carbon-14 derived from glucose during heterotrophic metabolism finds its way into all of the amino acids of protein in approximately the same proportions as in the more rapidly growing photosynthetic cells. However, as indicated above, total uptake into protein is much reduced during heterotrophic metabolism.

Two lines of evidence suggest inhibition of glucose-6-phosphate dehydrogenase is the site of this block. First, in dark incubated cells, carbon derived from glucose accumulates rapidly in the monophosphate sugars (mainly in G6P and S7P). On the other hand, carbon flow into the diphosphates (FDP and SDP) and into PGA is strongly reduced (along with reduced biosynthesis of the polymers which ultimately depend on PGA). Since oxidation of glucose-6-phosphate is a necessary step joining metabolism of the monophosphates to PGA, triose and diphosphate sugars, a necessary condition for regulation at the glucose-6-phosphate reaction is established: restricted carbon flow on one side of the putative barrier and accumulation of carbon on the other.

These data based on measurements of pool sizes and uptake rates in vivo are in agreement with in vitro inhibition of glucose-6-phosphate dehydrogenase by reduced NADP. The pool size of this metabolite increases significantly in cells which are placed in the dark or left in the light, but inhibited with DCMU. It is thus reasonable to assume the intracellular concentration
of NADPH could serve as a "breaking" mechanism limiting oxidation of glucose-6-phosphate with the consequences to overall metabolism outlined above. Previous evidence has shown that glucose-6-phosphate dehydrogenase is inhibited by ribulose-1,5-diphosphate, which we interpreted as one means for preventing the wasteful oxidation of glucose-6-phosphate during photosynthesis (9). We interpret the evidence presented in this paper as indicating this enzyme is also inhibited to a large extent during heterotrophic growth by NADPH.

Inhibitory regulation at the second enzyme of the oxidative pathway, 6-phosphogluconate dehydrogenase, can be ruled out. Such inhibition would have resulted in accumulation of carbon in 6-phosphogluconate which was not seen.

Regulation of heterotrophic metabolism by the adenylates appears to be minimal in Aphanocapsa 6714. Both ratios and total concentrations (in particular concentrations of ATP) remained essentially unchanged in heterotrophic cells. This is clearly not consistent with either energy-charge or stoichiometric control of metabolism by adenylates.

ACKNOWLEDGEMENTS

This work was supported, in part, by the U. S. Energy Research and Development Administration.
Table 1. Total incorporation of $^{14}\text{CO}_2$ and $^{14}\text{C}$-glucose under three experimental conditions

<table>
<thead>
<tr>
<th>Conditions of incubation</th>
<th>$^{14}\text{C}$-glucose</th>
<th>$^{14}\text{CO}_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>0.60$^a$</td>
<td>1.60</td>
</tr>
<tr>
<td>Light + $10^{-5}$ DCMU</td>
<td>0.87</td>
<td>0.05</td>
</tr>
<tr>
<td>Dark</td>
<td>0.35</td>
<td>0.002</td>
</tr>
</tbody>
</table>

$^a$ Units are $\mu$g atom $^{14}\text{C}$/mg algae.
Table 2. Longterm incorporation of $^{14}$CO$_2$ and $^{14}$C-glucose into polymers of Aphanocapsa 6714

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glycogen</th>
<th>Protein</th>
<th>Lipid</th>
<th>RNA</th>
<th>DNA</th>
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<tbody>
<tr>
<td>Light</td>
<td>11.3$^a$</td>
<td>34.0</td>
<td>6.3</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Light plus 10$^{-5}$ M DCMU</td>
<td>65.0</td>
<td>5.4</td>
<td>7.3</td>
<td>2.8</td>
<td>1.17</td>
</tr>
<tr>
<td>Dark</td>
<td>45.0</td>
<td>7.5</td>
<td>6.1</td>
<td>1.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$ Units are µg atoms $^{14}$C/mg algae.
Table 3. Incorporation of $^{14}$CO$_2$ and $^{14}$C-glucose into amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Condition</th>
<th>Light</th>
<th>Light + DCMU</th>
<th>Dark</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>4.98$^a$</td>
<td>0.41</td>
<td>0.71</td>
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<tr>
<td>Asparagine</td>
<td>1.97</td>
<td>0.41</td>
<td>0.33</td>
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<td>Aspartic Acid</td>
<td>1.58</td>
<td>0.18</td>
<td>0.27</td>
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<tr>
<td>Glutamate</td>
<td>2.94</td>
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<td>Glutamine</td>
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<tr>
<td>Proline</td>
<td>1.42</td>
<td>0.37</td>
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<tr>
<td>Serine-Glycine</td>
<td>0.81</td>
<td>0.20</td>
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<td>Threonine</td>
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<tr>
<td>Tryptophan-Valine</td>
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<td>0.79</td>
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<tr>
<td>Totals</td>
<td>24.10</td>
<td>3.06</td>
<td>4.00</td>
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$^a$ Units are $\mu$g atoms $^{14}$C/mg algae.
Table 4. Inhibition of glucose-6-phosphate dehydrogenase by intermediary metabolites

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<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% Inhibition</th>
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<tbody>
<tr>
<td>NADPH</td>
<td>$1.0 \times 10^{-4}$ M</td>
<td>80</td>
</tr>
<tr>
<td>NADH</td>
<td>$1.0 \times 10^{-4}$ M</td>
<td>0</td>
</tr>
<tr>
<td>NADPH + RuDP</td>
<td>$1.0 \times 10^{-4}$ M + $1.25 \times 10^{-4}$ M</td>
<td>90</td>
</tr>
<tr>
<td>RuDP</td>
<td>$1.25 \times 10^{-4}$ M</td>
<td>56</td>
</tr>
<tr>
<td>FDP</td>
<td>$1.5 \times 10^{-3}$ M</td>
<td>12</td>
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<tr>
<td>Ru5P</td>
<td>$3 \times 10^{-3}$ M</td>
<td>0</td>
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<tr>
<td>PGA</td>
<td>$3 \times 10^{-3}$ M</td>
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</tr>
<tr>
<td>PEPA</td>
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</tbody>
</table>
Figure 1. Assimilation of $^{14}$C into glycogen and protein. Experimental conditions described in methods section. $^{14}$C-glucose carbon source for heterotrophic (dark and DCMU inhibited) cells; $^{14}$C-glucose plus $^{14}$CO$_2$ carbon sources for photosynthetic cells.

Figure 2. Assimilation of $^{14}$C into RNA and total lipids. Same experiment as for Figure 1.

Figure 3. Incorporation of $^{14}$C into monophosphate sugars by photosynthetic and dark heterotrophic cells. Double labeling experimental conditions described in methods section. $^{14}$C-glucose plus $^{14}$CO$_2$ carbon sources for photosynthetic cells; $^{14}$C-glucose carbon source for heterotrophic cells.

Figure 4. Incorporation of $^{14}$C into diphosphate sugars by photosynthetic and dark heterotrophic cells. Same experiment as for Figure 3.

Figure 5. Incorporation of $^{14}$C into 3-phosphoglycerate by photosynthetic and dark heterotrophic cells. Same experiment as for Figure 3.

Figure 6. Concentrations of adenylates under three experimental conditions.

Figure 7. Concentrations of NADPH and NADP and ratios of reduced to oxidized pyridine nucleotides under three experimental conditions.

Figure 8. CO$_2$ fixation (A) and glycogen synthesis (B) under three experimental conditions. Photosynthetic cells were labeled with $^{14}$CO$_2$.
photoheterotrophic (DOMU) cells, dark heterotrophic (aerobic) cells and dark anaerobic cells were labeled with $^{14}$C-glucose.

Figure 9. ATP synthesis estimated from data of Figure 8.

Figure 10. Inhibition of glucose-6-phosphate dehydrogenase by NADPH in cell-free extracts of *Aphanocapsa* 6714.
Fig. 1

GLYCOGEN

μg ATOMS $^{14}_C$

HOURS

PROTEIN

μg ATOMS $^{14}_C$

HOURS

- LIGHT
- DARK
- DCMU
Fig. 2

μg ATOMS $^{14}C$

HOURS

RNA

LIPIDS

- LIGHT
- DARK
- DCMU
Fig. 3
Fig. 4

DIPHOSPHATES

LIGHT

DARK

nCi $^{14}$C / mg x $10^2$

nCi $^{32}$P / mg x $10^3$

GLUCOSE-U-$^{14}$C

MINUTES

$^{14}$C RuDP

TOTAL $^{32}$P

$^{14}$C FDP/SDP $\approx$ 2/1

$^{14}$
**Fig. 5**

Graph showing the effects of light and dark conditions on PGA production over time. The graph includes two sets of data points, one for light conditions and one for dark conditions, with measurements of \( ^{14}C \) and \( ^{32}P \) over minutes. The graph is labeled with "PGA," "LIGHT," and "DARK."
Fig. 6
Fig. 7
Fig. 9
GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY

- 4.4 x 10^{-4} M
- 2.2 x 10^{-4} M
- 1.1 x 10^{-4} M

Fig. 10
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