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Photosystem II Regulation of Macromolecule Synthesis in the Blue-Green Alga *Aphanocapsa* 6714

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Polymers synthesized by heterotrophically growing (glucose as carbon source) 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 photosystem II-specific inhibitor dichlorophenylmethylurea, caused an 80 to 90% reduction in the rate of lipid and total ribonucleic acid 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 dichlorophenylmethylurea-inhibited cells. After longer heterotrophic growth, glycogen became the major component, whereas in photosynthetically grown cultures protein was the major constituent. ^{14}C (from $^{14}CO_2$ and/or [^{14}C]glucose) assimilated into protein by heterotrophically grown cells was found in amino acids in nearly the same proportions as in photosynthetically grown cells. Thus, routes of biosynthesis available to autotropic cells were also available to heterotrophic cultures, but the supply of carbon precursors to those pathways was greatly reduced. The limited biosynthesis in heterotrophic cells was not due to a limitation for cellular energy. The adenylates were maintained at nearly the same concentrations (and hence the energy charge also) as in photosynthetic cells. The concentration of reduced nicotinamide adenine dinucleotide phosphate was higher in heterotrophic (dark) cells than in photosynthetic cells. From rates of CO_2 fixation and/or glycogen biosynthesis it was determined that stationary-phase cells expended approximately 835, 165, and less than 42 nmol of adenosine 5'-triphosphate per mg (dry weight) of algae per 30 min during photosynthetic, photoheterotrophic, and chemoheterotrophic metabolism, respectively. Analysis of the soluble metabolite pools in dark heterotrophic cultures by double-labeling experiments revealed rapid equilibration of ^{14}C through the monophosphate pools, but much slower movement of label into the diphosphate pools of fructose-1,6-diphosphate and sedoheptulose-1,7-diphosphate. Carbon did flow into 3-phosphoglycerate in the dark; however, the initial rate was low and the concentration of this metabolite soon fell to an undetectable level. In photosynthetic cells, ^{14}C quickly equilibrated throughout all the intermediates of the reductive pentose cycle, in particular, into 3-phosphoglycerate. Analysis of glucose-6-phosphate dehydrogenase in cell extracts showed that the enzyme was very sensitive to product inhibition by reduced nicotinamide adenine dinucleotide.

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 CO_2 fixation (12).

However, several strains, mostly found in typological group IIa (classification according to Stanier et al. [13]) are capable of photoheterotrophic growth and, in some cases, true chemoheterotrophic growth in the dark (11). 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 CO_2 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 ribu-

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lose-1,5-diphosphate (RuDP) probably is of physiological significance since the oxidative pathway is blocked during photosynthesis (6, 7, 10). It is noteworthy that RuDP is only detectable during photosynthesis (7).

Previous work on the assimilation of ^{14}C suggests that a block in metabolism between the sugar monophosphates and 3-phosphoglycerate (PGA) was induced by placing cells in the dark, or by inhibition of light-incubated cells with the herbicide dichlorophenylmethylurea (DCMU) (6, 7). We have continued this study by measuring the distribution of assimilated ^{14}C into the polymers of the cell under both photosynthetic and heterotrophic growth conditions. Double-labeling experiments with ^{14}C and ^{32}P 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 adenosine 3'-triphosphate (ATP) using the three metabolic options available to the cell: photosynthetic electron transport (photosystems 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% (vol/vol) CO_2, and 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, filter at 620 nm), and then supplemented with radioactive tracers as follows: (i) photosynthetic cells, 10 mM ^{14}C-labeled sodium bicarbonate (0.33 μCi/μmol) plus 0.25% (wt/vol) [U-^{14}C]glucose (2.0 μCi/μmol); (ii) heterotrophic cells, 0.25% (wt/vol) [U-^{14}C]glucose (2.0 μCi/μmol).

Cells were grown in 1-liter side-arm flasks, 50 ml per flask. The photosynthetic culture was capped with a sterile no. 12 rubber stopper. The photoheterotrophic culture was kept in the light after adding enough DCMU to bring the total concentration to 5×10^{-5} M, which was sufficient to completely inhibit noncyclic 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. The cells were 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: $^{14}CO_2$, 7.13 μCi/μmol; [U-^{14}C]glucose, 6.54 μCi/μmol. Stationary-phase cells were harvested and resuspended in BG-11 medium. They were then placed in the steady-state machine, described in detail elsewhere (5, 7). The tracers were added and cells were 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 (4). In all figures, the activity of labeled products is expressed as amount per milligram (dry weight) of algae. In that part of the latter experiment which requires anaerobic conditions, an oxygen trap of Oxsorbant (Sorel Corp.) was introduced into the closed system of the steady-state machine, and the concentration of oxygen was reduced to less than 5 μl/liter before labeled substrate was added.

Specific activities of $^{14}CO_2$ were determined by the following procedure. At the end of the experiments, cells were removed by centrifugation, and the supernatant solution was made alkaline to a pH of approximately 10 and stored under CO_2-free N_2 gas in a stoppered flask. Samples of 1 to 6 ml of the alkaline supernatant fraction were added to enough 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 HCl, thus converting most of the carbonate and bicarbonate to CO_2. Total $^{12}CO_2$ was determined by an infrared CO_2 analyzer; $^{14}CO_2$ was determined by a Cary ionization chamber. Both instruments are components of the steady-state apparatus. The specific activity of [U-^{14}C]glucose was determined by measuring the total glucose concentration by the Glucostat method (Worthington Biochemicals), and the ^{14}C was measured by liquid scintillation in Aquasol (New England Nuclear).

Stepwise degradation of cells. (i) Total lipids. Total lipids were extracted by the method of Bligh and Dyer (1); the ^{14}C contents of 5-, 10-, and 20-μl samples of the extracted lipids in Aquasol were determined by liquid scintillation counting.

(ii) Protein. Half of the lipid-free pellet from step (i) was washed four times with distilled water and then resuspended in 2 ml of 0.05 N triethanolamine buffer (pH 7.5) containing 50 μg of Pronase per ml (Sigma Chemical Co.). A drop of toluene was placed over each sample, and then the samples were tightly stoppered and placed at 37°C for 24 h for complete hydrolysis of cellular protein. Then 250 μl of each hydrolysate was removed and chromatographed (see below), and the amino acids were identified and counted for ^{14}C by gas flow spectrometry, as previously described (4).

(iii) Glycogen. One-fourth of the washed pellets from step (i) were resuspended in 4 ml of 1.5 N trifluoroacetic acid and placed in 10-ml "color-break" glass ampoules (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 ^{14}C was determined by radioautography.

(iv) Total deoxyribonucleic acids plus ribonucleic acids (RNAs). One-fourth of the washed pellets from

step (i) were resuspended in 2 ml of 0.05 N triethanolamine buffer (pH 7.5) plus 0.05 N $MgSO_4$ and 10 μg of deoxyribonuclease I and II per ml (Calbiochem). The suspensions were placed at 37°C for 12 h in tightly stoppered tubes. Each sample was centrifuged at 15,000 × *g* for 20 min, and the supernatant fraction was 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 × *g* for 30 min, and 5-, 10-, and 20-μl samples of the supernatant fluid containing hydrolyzed RNA were counted in Aquasol by liquid scintillation.

Labeling of cells with ^{32}P. Cells were grown from small inocula in modified BG-11 medium in the presence of 20 μCi of carrier-free [^{32}P]phosphate per ml. In the labeling experiments using ^{32}P as the only tracer, the soluble metabolites were extracted with 80% methanol, separated by paper chromatography, and then counted by radioautography (as previously described). The specific activities in the double-labeling experiments were: $^{14}CO_2$, 3.33 μCi/μmol; and [U-^{14}C]glucose, 20 μCi/μmol.

Enzyme assays. Glucose-6-phosphate (G6P) dehydrogenase was assayed by the initial rate of reduced nicotinamide adenine dinucleotide (NADPH) formation, at 340 nm, by conventional spectrophotometry. The reaction mixtures contained (in 1 ml): 55 μg of protein; 10 μmol of $MgCl_2$; 25 μmol of triethanolamine buffer (pH 7.45); 1 μmol of G6P; 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 and Firth (14).

RESULTS

Assimilation of ^{14}C: heterotrophy versus photoautotrophy. [^{14}C]glucose assimilation in photoautotrophically grown *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 $^{14}CO_2$ was almost totally inhibited. Thus, CO_2 fixation, but not glucose assimilation, was dependent on a functional photosynthetic electron transport chain. Similar results were obtained for photoheterotrophically grown (DCMU-treated) and dark-grown cells (data not shown).

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. As can be seen (Fig. 1 and 2), the fate of ^{14}C assimilated

TABLE 1. *Total incorporation of $^{14}CO_2$ and [^{14}C]glucose under three experimental conditions*

Conditions of incubation	[^{14}C]glucose[a]	$^{14}CO_2$[a]
Light	0.60[b]	1.60
Light + 5 × 10^{-5} M DCMU	0.87	0.05
Dark	0.35	0.002

[a] [^{14}C]glucose and $^{14}CO_2$ (as bicarbonate) were present at the same specific activities (2.0 μCi/μmol and 0.33 μCi/μmol) and concentrations (10 mM and 0.25% [wt/vol], respectively) as described in the Materials and Method section. The cells used were grown photoautotrophically.

[b] Units are expressed as microgram-atoms of ^{14}C per milligram of (dry) algae.

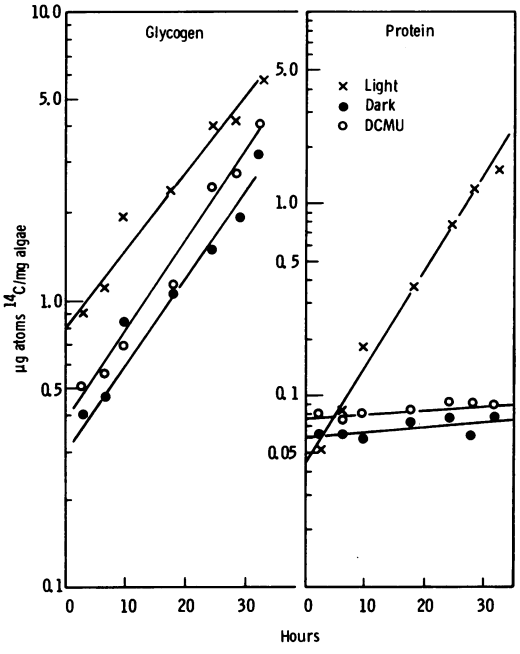

FIG. 1. *Assimilation of ^{14}C into glycogen and protein. Experimental conditions are as described in Materials and Methods. [^{14}C]glucose carbon was the source for heterotrophic (dark and DCMU-inhibited) cells; [^{14}C]glucose plus $^{14}CO_2$ were the carbon sources for photosynthetic cells.*

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 ^{14}C 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, as measured both in terms of initial velocity and 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, the pathways of intermediary metabolism and biosynthesis were still available to the tracer, but the total flow of carbon into the pathways was reduced.

Metabolite pools in photosynthetic and dark cells. In heterotrophic cells, the enhanced rates of glycogen synthesis relative to other synthetic processes suggest a restriction on the movement of carbon from the early reactions of glucose metabolism to other metabolic routes.

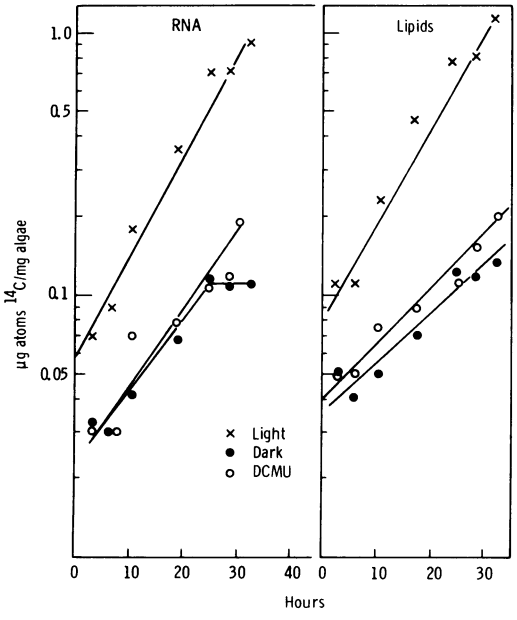

FIG. 2. *Assimilation of ^{14}C into RNA and total lipids. Same experiment as for Fig. 1.*

Previous work has shown a large accumulation of carbon in the sugar monophosphate pools of dark cells, possibly indicating a block in metabolism at the level of triosephosphates (6, 7). To study this problem further, double-labeling experiments (Fig. 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 (Materials and Methods). Then [U-^{14}C]glucose (or [U-^{14}C]glucose plus $^{14}CO_2$) was added, and the flow of ^{14}C 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. It should be noted that cells used in this experiment and in those of the remainder of the paper were light grown, harvested, and then used in the experiments described below.

Within 6 min after the addition of the tracer to the system, ratios of $^{14}C/^{32}P$ became approximately constant for the monophosphates, G6P and sedohepulose-7-phosphate (S7P), showing the rapid movement of carbon through these pools in both photosynthetic and dark heterotrophic cells (Fig. 3). Incorporation of ^{14}C 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

TABLE 3. *Incorporation of $^{14}CO_2$ and [^{14}C]glucose into amino acids*

Amino acid	Condition		
	Light	Light + DCMU	Dark
Alanine	4.98[a]	0.41	0.71
Asparagine	1.97	0.41	0.33
Aspartic acid	1.58	0.18	0.27
Glutamate	2.94	0.30	0.46
Glutamine	3.48	0.25	0.56
Proline	1.42	0.37	0.25
Serine-glycine	0.81	0.20	0.18
Threonine	0.70	Nil	Nil
Tryptophan-valine	7.09	0.79	1.23

[a] Units are microgram-atoms of ^{14}C per milligram of algae.

TABLE 2. *Long-term incorporation of $^{14}CO_2$ and [^{14}C]glucose into polymers of Aphanocapsa 6714*

Condition	Glycogen	Protein	Lipid	RNA	DNA[a]
Light	11.3[b]	34.0	6.3	2.8	0.9
Light + 5×10^{-5} M DCMU	65.0	5.4	7.3	2.8	1.17
Dark	45.0	7.5	6.1	1.6	1.0

[a] DNA, Deoxyribonucleic acid.

[b] The specific activities and concentrations of $^{14}CO_2$ and [^{14}C]glucose were the same as in Table 1. The cells used for each of the experimental conditions were grown under that condition, as described in the text.

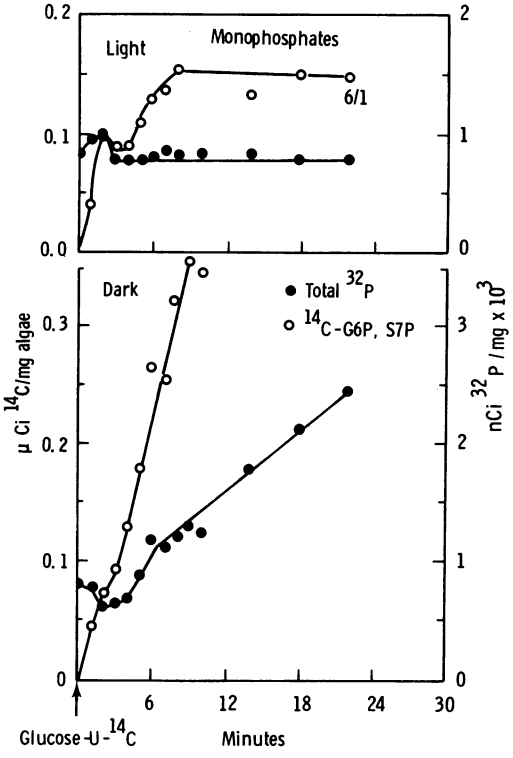

FIG. 3. *Incorporation of ^{14}C into monophosphate sugars by photosynthetic and dark heterotrophic cells. Double-labeling experimental conditions were as described in Materials and Methods. [^{14}C]glucose plus $^{14}CO_2$ were the carbon sources for photosynthetic cells; [^{14}C]glucose was the carbon source for heterotrophic cells.*

dark cells. For cells in the light, the ratio of ^{14}C/^{32}P 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 ^{14}C in the monophosphate sugars increased at an approximately 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 fructose-1,6-diphosphate (FDP) and sedoheptose-1,7-diphosphate (SDP) was noted in the dark cells (Fig. 4), whereas the corresponding concentrations in the light were very low. As expected, RuDP was the principal diphosphate sugar in photosynthetic cells, since this metabolite is the CO_2 acceptor for autotrophic CO_2 fixation by *Aphanocapsa* 6714. In contrast, no ^{14}C was found in RuDP in dark cells. It follows that flow from glucose into RuDP was completely blocked in the dark. These data are in agreement with the low rate of CO_2 fixation observed in the dark-incubated and DCMU-inhibited cells, and confirm previous results from single-labeling experiments (6, 7). It should be noted that the rate of ^{14}C accumulation in FDP + SDP in the dark was much slower than the rate of accumulation in G6P + S7P.

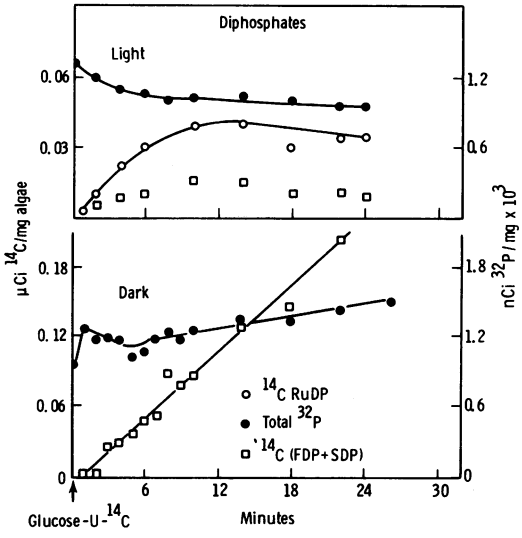

FIG. 4. *Incorporation of ^{14}C into diphosphate sugars by photosynthetic and dark heterotrophic cells. Same experiment as for Fig. 3.*

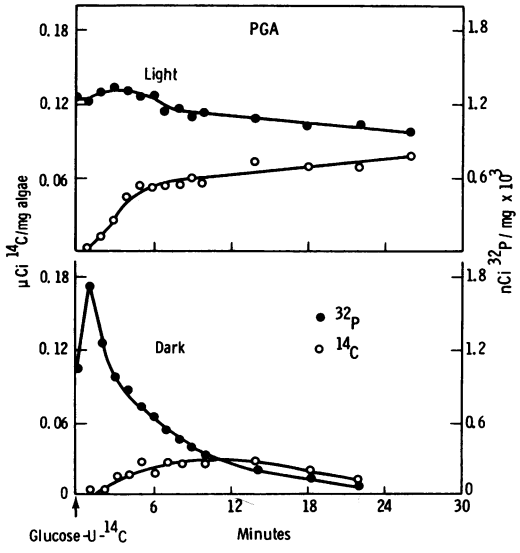

FIG. 5. *Incorporation of ^{14}C into PGA by photosynthetic and dark heterotrophic cells. Same experiment as for Fig. 3.*

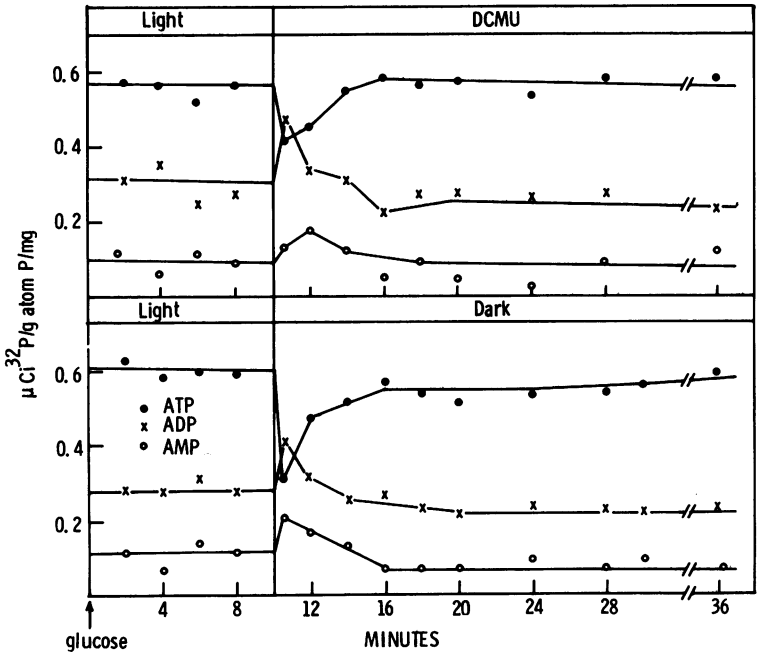

FIG. 6. *Concentrations of adenylates under three experimental conditions.*

In previous work (6, 7) we found almost no label in PGA during dark incubation of *Aphanocapsa* 6714, suggesting a block between triose phosphates and PGA. In the present experiments, some ^{14}C did flow into PGA in the dark (Fig. 5). However, relative to that in 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 amount as soon as the lights were turned off, and then rapidly decreased, becoming almost undetectable by the end of the experiment. The rapid increase in PGA concentration when the light was turned off (Fig. 5) 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 (9).

Adenylate and pyridine nucleotide pools. The concentrations of the adenylates and the NADPH and NADP pools are shown in Fig. 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).

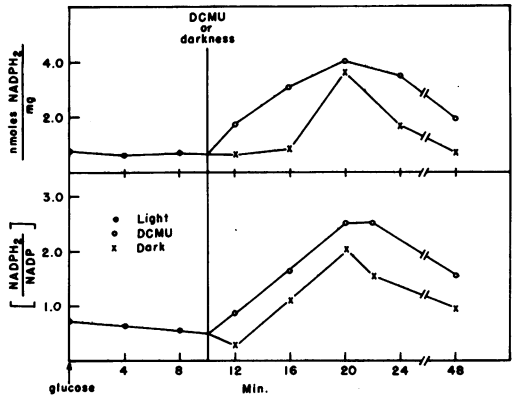

FIG. 7. *Concentrations of NADPH and NADP and ratios of reduced to oxidized pyridine nucleotides under three experimental conditions.*

This suggested a reduction in the utilization of NADPH for biosynthetic purposes in cells lacking photosystem II activity. Ratios of reduced to oxidized NADs 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 (10).

Rates of ATP synthesis: estimates under four metabolic conditions. Figures 8 and 9

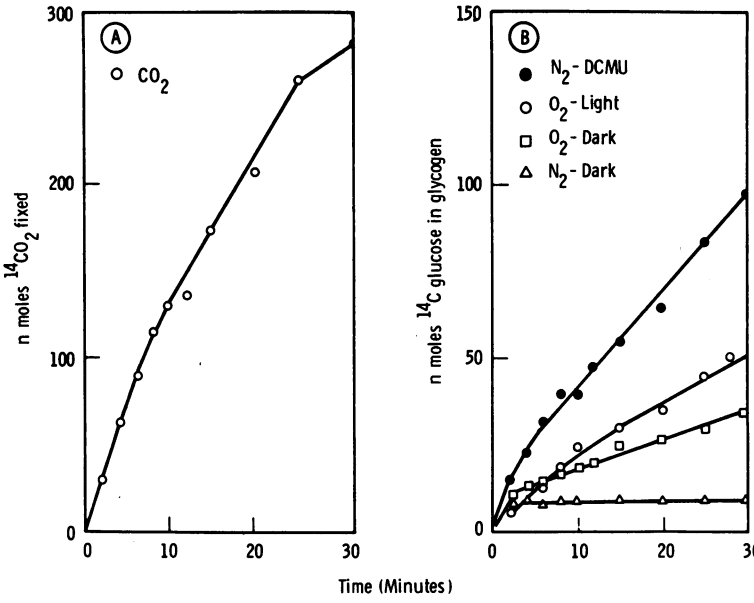

FIG. 8. *CO_2 fixation (A) and glycogen synthesis (B) under three experimental conditions. Photosynthetic cells were labeled with $^{14}CO_2$; photoheterotrophic (DCMU) cells, dark heterotrophic (aerobic) cells, and dark anaerobic cells were labeled with [^{14}C]glucose.*

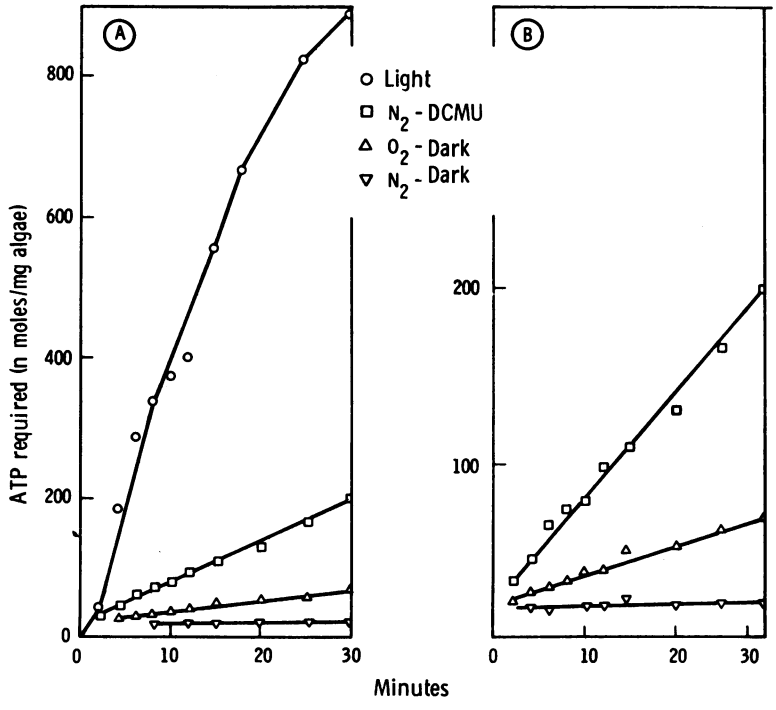

FIG. 9. *ATP synthesis estimated from data of Fig. 8.*

show assimilation of $^{14}CO_2$ and [U-^{14}C]glucose into glycogen. Previous work with *Aphanocapsa* 6714 has shown that the potential for glycogen synthesis in stationary-phase cells is always high (8). 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-in-

hibited cells. It should be noted that DCMU-inhibited cells were kept under anaerobic conditions to block any respiratory contribution to the synthesis of ATP (see below). Thus, ATP required for glycogen synthesis probably came from photophosphorylation via photosystem I (i.e., cyclic phosphorylation). In both the aerobic-dark cells and photosynthetic cells, glycogen synthesis was somewhat inhibited (relative to the value in DCMU-treated cells), except for a short burst at the beginning of the experiment. In anaerobic-dark cells, glycogen synthesis was almost completely inhibited. It should also be noted that $^{14}CO_2$ fixation occurred only in O_2-light cells.

Since the energy requirements for glycogen synthesis and CO_2 fixation are known—i.e., 2 ATPs per glycosyl unit derived from hexose monophosphates, and 3 ATPs 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 ^{14}C recovered in glycogen (Fig. 8) it can be calculated that approximately 840, 165, and about 40 nmol of ATP per mg of algae per 30 min were expended for CO_2 fixation and/or glycogen synthesis (Fig. 9). This was done as follows. First, Fig. 8A shows total $^{14}CO_2$ incorporation(O_2-light), the bulk of which accumulated in glycogen after the first 2 min of the experiment (Fig. 8B). Figure 8B also shows glycogen synthesis in the heterotrophic cultures that are unable to fix $^{14}CO_2$ via the Calvin cycle. In the photoautrotropic (O_2-light) culture, from the amount of $^{14}CO_2$ fixed (3 ATPs per CO_2 fixed) and the amount of glycogen synthesized (1 additional ATP for G6P coming from the Calvin cycle), we were able to estimate total ATP expended on CO_2 fixation plus glycogen synthesis. This figure is 840 μmol/mg of algae after 30 min (Fig. 9A). Using a similar procedure, glycogen synthesized in heterotrophic cells consumed 2 ATPs per glycosyl units added to glycogen. Thus, at 30 min the values of 165 and 40 μmol were obtained for DCMU (N_2-DCMU) and dark (O_2-dark) cells (Fig. 9A and B). The values of Fig. 9A are replotted on an expanded scale in Fig. 9B to show the degree of linearity obtained in the experiment. This suggests a uniform rate of glycogen synthesis and ATP utilization. Essentially no ATP was consumed by fermentative metabolism of glucose in anaerobic-dark cells. If we assume that the relative capacities for ATP synthesis are not greatly altered in the stationary phase, then potentials for ATP synthesis by cyclic, noncyclic, and respiratory electron transport are related by the ratio 840:165:40. These data suggest the capacity for energy synthesis in aerobic-dark cells was 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 G6P dehydrogenase. Table 4 shows the effect of various metabolites on the activity of G6P dehydrogenase in cell extracts of *Aphanocapsa* 6714. As can be seen, only NADPH and RuDP inhibited enzyme activity. On a molar basis, as an inhibitor, NADPH was much the stronger of the two metabolites. This regulation of G6P dehydrogenase by NADPH, amplified by RuDP, may be similar to the regulation of this enzyme in spinach chloroplasts (3). NADPH also inhibits the enzyme of *Anacystis*, but no RuDP inhibition was demonstrated (2).

Figure 10 shows the inhibition of G6P dehydrogenase at fixed concentrations of NADPH as the substrate of NADP was increased in concentration. At the highest concentration of NADP (4.4×10^{-4} M) 50% inhibition of the enzyme was observed at about 10^{-4} M NADPH. The initial rates of enzyme activity were measured by appearance of NADPH. Thus, G6P 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 G6P and hence the flow of carbon from the monophosphates during heterotrophic metabolism.

TABLE 4. *Inhibition of G6P dehydrogenase by intermediary metabolites*[a]

Compound	Concn	% Inhibition[b]
NADPH	1.0×10^{-4} M	80
NADH	1.0×10^{-4} M	0
NADPH + RuDP	1.0×10^{-4} M + 1.25×10^{-4} M	90
RuDP	1.25×10^{-4} M	56
FDP	1.5×10^{-3} M	12
Ru5P	3×10^{-3} M	0
PGA	3×10^{-3} M	0
PEPA	3×10^{-3} M	0
ATP	4×10^{-3} M	5
ADP[c]	4×10^{-3} M	0
AMP[c]	4×10^{-3} M	+10

[a] Substrate NADP concentration was 1.0×10^{-4} M. The specific activity of the control (no inhibition) was 1.27 μmol of $NADPH_2$ formed per min per mg of protein.

[b] Inhibition was based on initial velocity measurements of absorbance at 340 nm.

[c] ADP, Adenosine diphosphate; AMP, adenosine monophosphate.

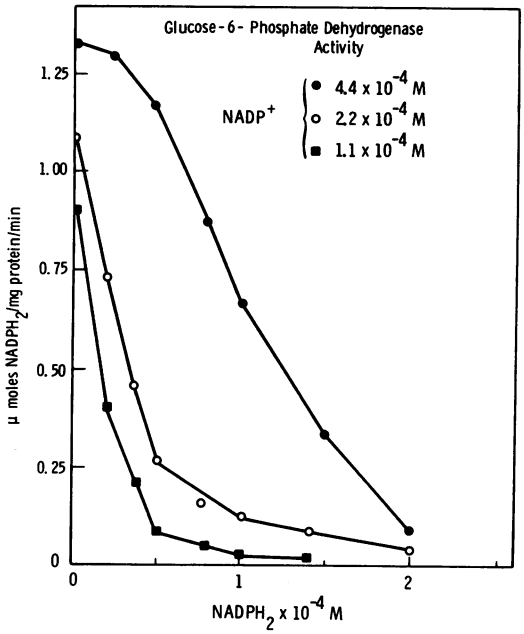

FIG. 10. *Inhibition of G6P dehydrogenase by NADPH in cell-free extracts of Aphanocapsa 6714.*

DISCUSSION

The slow heterotrophic growth of *Aphanocapsa* 6714 is marked by a disproportionate increase in the amount of glycogen synthesized relative to the biosynthesis of growth polymers such as protein, lipids, and nucleic acids. Photosynthetically competent cells—i.e., cells with both photosystem I and photosystem 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 ^{14}C 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 G6P 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 that ultimately depend on PGA). Since oxidation of G6P is a necessary step joining metabolism of the monophosphates to PGA triose and diphosphate sugars, a necessary condition for regulation at the G6P 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 G6P dehydrogenase by NADPH. The pool size of this metabolite increases significantly in cells that are placed in the dark or left in the light, but inhibited with DCMU. It is thus reasonable to assume that the intracellular concentration of NADPH could serve as a "braking" mechanism limiting oxidation of G6P, with the consequences to overall metabolism outlined above. Previous evidence has shown that G6P dehydrogenase is inhibited by RuDP, which we interpreted as one means for preventing the wasteful oxidation of G6P during photosynthesis (9). We interpret the evidence presented in this paper as indicating that 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.

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