Fructose Uptake and Influence on Growth of and Nitrogen Fixation by Anabaena variabilis

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Fructose is specifically taken up by nitrogen-fixing cultures of Anabaena variabilis in the light and lowers the doubling time from 24 to 8 h. The kinetics for both fructose-dependent growth and fructose uptake are exponential. The apparent $K_m$ for fructose uptake in N$_2$-fixing cultures is 160 $\mu$M for cells not previously exposed to fructose and 50 $\mu$M in cells adapted to fructose. Picomolar amounts of $[^{14}\text{C}]$fructose are scavenged from the medium and accumulate in filaments. Heterocysts of fructose-adapted filaments accumulate $^{14}$C from fructose within 20 min. Short-term experiments with fructose-starved cultures provide evidence that nitrogenase activity, protein, and chlorophyll content change within one generation time upon addition of fructose. In long-term experiments, the amount of fructose initially present in the medium determines heterocyst number and packed-cell volume. Photosynthetic oxygen evolution and amounts of chlorophyll decrease with exogenous fructose concentrations greater than 20 mM.

Nitrogen fixation requires a large input of reducing power and ATP for the production of NH$_3$+$. In cyanobacteria, energy to form reductant can be derived from photosynthesis, but the concomitant O$_2$ release by photosystem II can also interfere with O$_2$-sensitive nitrogenase. To overcome this problem some filamentous cyanobacteria have developed thick-walled cells, termed heterocysts, which are unable to evolve oxygen, are possibly less permeable to oxygen, and are specialized to carry out nitrogen reduction. These cyanobacteria have developed an exchange system between heterocysts and vegetative cells. Heterocysts export fixed nitrogen in the form of glutamine to vegetative cells (13), whereas CO$_2$ is fixed aerobically in vegetative cells and an unidentified compound subsequently is transferred to heterocysts (14).

If one were able to feed the carbon compound transferred or its precursor, then one might conceivably enhance the rates of N$_2$ fixation by free-living cyanobacteria. Earlier reports indicated that Anabaena variabilis, grown with NO$_3$ as the nitrogen source, can assimilate glucose or sucrose without a change in its growth rate (6, 10). The A. variabilis strain used in this investigation, however, had previously been shown to grow in the dark with fructose as a carbon source (15). Another report indicated the importance of a higher C/N ratio for heterocyst formation and subsequent nitrogenase activity in A. cylindrica (7). Since an exogenous sugar might be used to increase the C/N ratio, A. variabilis is a suitable organism to study the effects of carbon on photoheterotrophic growth and nitrogen fixation. Kinetic data and autoradiography presented here show that $^{14}$C-labeled fructose is rapidly and thoroughly removed from the medium in light, that it appears rapidly in vegetative cells of A. variabilis, and that $^{14}$C from fructose accumulates in heterocysts.

Fructose effects profound changes in short-term as well as long-term experiments on parameters such as cell yield, protein, chlorophyll formation, photosynthesis, and nitrogen fixation.

MATERIALS AND METHODS

A. variabilis Kütz was grown in 100-, 250-, or 500-ml Erlenmeyer flasks (culture volumes 50, 100, or 200 ml, respectively) or in screw-cap culture tubes (15 by 150 mm) at 24°C on a shaker. The medium of Allen and Arnon (1) was used at one-half or one-eighth strength (A ½ or A ¼, respectively) and supplemented with 6 mM phosphate (pH 7) for buffering. For CO$_2$ enrichment, the cultures were sparged with 0.5% CO$_2$ in air under low-intensity fluorescent light (3,000 lux). For measuring growth, optical density was determined at 750 nm (OD$_{750}$) after 1-ml samples of filaments were sonicated to generate three to six cell filaments, and the resultant homogeneous suspension was diluted to an OD$_{750}$ of ≤0.55 before reading. Light scattering was linearly proportional to cell density up to an OD$_{750}$ of 0.55. For determination of packed-cell volume (pcv), 2-ml graduated microcentrifuge tubes (model Assistent; Hecht, Bernhause, West Germany) of 80-ml

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capacity were used. Readings of pcv were taken after centrifugation for 5 min at 3,000 x g. We found a linear relationship between pcv and an OD_{750} from OD_{750} = 0 to OD_{750} = 4.5 with pcv = 2.5 ul/ml corresponding to OD_{750} = 1. Since chlorophyll was highly variable, depending on age and the amount of fructose in the culture and protein also varied with fructose present, pcv was generally used as the parameter for cell mass. The kinetics of the uptake of radioactively labeled fructose (U^{14}C, 333 mCi/mmol) were monitored in the culture tubes. The analysis of the cellular absorption of the [^{14}C]fructose was performed with 1-ml culture samples and sampling with time for direct counting and for autoradiography. For scintillation counting, samples were collected on 0.22-μm glass-fiber filters and washed with several volumes of fresh A ½ by gentle aspiration. After drying in air, the filters were immersed in scintillation cocktail (8) for counting. Samples for autoradiographic analysis were washed gently as above and transferred to glass microscope slides in contact with dry ice. After stabilizing the frozen filaments by lyophilization, the slides were dipped into molten NTB-2 autoradiographic emulsion (Kodak) and exposed for 1 to 2 weeks in dry darkness. The autoradiograms were developed in D-76 developer (Kodak) and viewed by light microscopy and photomicrography (14).

The assay of nitrogenase activity was by acetylene reduction in samples with 10% acetylene in an argon gas phase kept for 30 min in the light at 24°C. The analysis of heterocyst glycopolipids I and III by thin-layer chromatography was as previously described (5). Chlorophyll was determined in methanol extracts (9), and protein was assayed with the Folin-Ciocalteu reagent after NaN{sub}OH hydrolysis of the cells (3). For hydrogen evolution 1.5 ml of algal culture containing 5 μg of chlorophyll per ml was placed into 10-ml rubber stopper serum vials, and the H{sub}2 evolved was measured with 50-μl gas samples (12). Net oxygen evolution and respiratory O{sub}2 uptake in the dark were measured as described earlier (11). Saturating red light was provided through a 610 nm cut-off filter (Corning Glass Works, Corning, N.Y.) at 1.6 kW/m{sup 2}.

**RESULTS**

**Effect of carbon on growth.** Cultures in screw-cap tubes (15 by 150 mm) were grown under nitrogen-fixing conditions and saturating light intensity with various fixed carbon sources (C{sub}3, C{sub}6, C{sub}8, and C{sub}12 sugars). At 6 days, cell yield was monitored in terms of OD_{750}. Table 1 shows that of the C{sub}3 and C{sub}6 sugars tested, only fructose (10 mM) yielded an appreciable increase in cell yield (10-fold) over the control without addition. Other carbon sources, including α-ketoglutarate, formate, succinate, acetate, and pyruvate, showed slight inhibition of growth or no difference compared to the control. Growth rate was calculated in terms of doubling time (t2). Air-grown cells had a doubling time of about 24 h (not shown) which could be decreased to 16 h by sparging the culture with air-0.5% CO{sub}2 (Fig. 1) and further decreased to 9.6 h in the presence of fructose (10 mM) with air sparging (Fig. 1) or 8 h without sparging (not shown). The combination of 0.5% CO{sub}2-10 mM fructose in air yielded a doubling time of 16 h, indicating that the addition of CO{sub}2 to a fructose-rich medium will not further enhance, and may even inhibit, growth. Three-week-old cultures under air or in CO{sub}2-enriched air (0.5% CO{sub}2) attained an OD_{750} of 2.5 and 3, respectively, whereas in the presence of fructose (10 mM) alone, cell yield was 5 OD_{750} units. Therefore, supplementation with fructose reduced the generation time by approximately 60% and maximized final cell yield.

**Kinetics of fructose uptake.** Fructose was rapidly taken up from the medium and accumulated within the filaments (Fig. 2). A semilog plot of the time course with [U-{sup}14]C]fructose as tracer indicates that exponential growth correlated with exponential utilization of fructose. By extrapolation of the curve for fructose disappearance, it becomes evident that the initial concentration of 10 mM fructose was exhausted in less than 4 days. After 5 days, greater than 90% of the [^{14}C]fructose supplied was recovered in the filaments, which were separated from the medium by centrifugation. Thus, only a small fraction was lost as CO{sub}2 or excreted back into the medium. In this experiment, the sample was thoroughly stirred before being taken for measurements every day, i.e., aerated vigorously. Thus, the generation time was 1.5 days, much longer than that of an undisturbed culture. In another experiment, the apparent Michaelis-Menten constant (K_{m}) for fructose uptake was determined for a fructose-adapted and a nonadapted culture. In an initial experiment, it was found that fructose uptake was linear for at least 60 min. Therefore, 30-min time points were used

<table>
<thead>
<tr>
<th>Sugar</th>
<th>OD_{750} after 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.22</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.09</td>
</tr>
<tr>
<td>Gluconate</td>
<td>0.18</td>
</tr>
<tr>
<td>Fructose</td>
<td>2.1</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.22</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.26</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Growth was carried out in 15 × 150 mm screw-cap Pyrex tubes (15 by 150 mm) under 3,000-lux continuous light, and the OD_{750} was measured after 6 days. The gluconate sample at 5 mM was buffered with 10 mM potassium phosphate (pH 7); all other samples were at 10 mM sugar. For other conditions see the text.
to determine the uptake of $^{14}\text{C}$-labeled fructose in a concentration series. With 5 µg of chlorophyll per sample and 10 µM fructose, only 36% of added $[^{14}\text{C}]$fructose was taken up after 30 min, showing that fructose was not limiting. The apparent $K_m$ for fructose assimilated was 140 µM by cells grown previously in air-0.4% CO$_2$ and 50 µM by cells adapted to fructose and washed free of unlabeled fructose 10 h before the uptake experiment. Maximum rates for both adapted and nonadapted cultures were 21 µmol/h per mg of chlorophyll (equivalent to 73 µmol/h per ml of pcv).

Adaptation of cellular metabolism to fructose in short-term and long-term experiments. Since the generation time of A. variabilis can be considerably decreased by fructose, it was of interest to follow cellular changes accompanied by a shift of a fructose-adapted N$_2$-fixing culture to carbon-starved conditions or to an NH$_4^+$/NO$_3^-$ medium within one generation of 24 h (Table 2). Growth, chlorophyll, and nitrogenase were monitored in this short-term experiment. Before the transfer, the fructose-adapted, N$_2$-fixing culture was washed and starved for 9 h to deplete endogenous pools. Withdrawal of the main reductant (fructose) resulted in stagnant growth and in a loss of nitrogenase activity of >90% after 10 h. To these carbon-starved cultures, fructose, chloramphenicol, combined nitrogen, or a combination of these was added. The chloramphenicol samples were included as background controls with protein synthesis arrested. The results of these experiments are shown in Table 2. Increase in cell mass (pcv) was only slight (19%) with no addition or with NH$_4^+$/NO$_3^-$, whereas fructose alone stimulated growth by 78% in 24 h. Chlorophyll per cell increased 10% in the sample without fructose, 23% in the presence of combined nitrogen, and 47% in the sample with fixed carbon and combined nitrogen. The culture that was shifted to combined N plus fructose increased the cell mass only by 24%. It appears that part of the fructose carbon may have gone into chlorophyll formation since chlorophyll content is highest in this sample. On the other hand, adding only fructose to a nitrogen-fixing culture resulted in a 13% loss of chlorophyll. These data show that chlorophyll formation is rather sensitive to changes in both carbon and nitrogen. Increase in soluble protein compared to the chloramphenicol control was significant in all samples and highest (+88%) in the sample with fructose. Nitrogenase activity was monitored as acetylene reduction and as H$_2$ production in the presence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] to inhibit photosynthetic O$_2$ evolution since oxygen interferes with H$_2$ evolution from nitrogenase in fructose-adapted cells. It
should be noted that DCMU does not prevent further growth, provided a suitable carbon source is present. Inhibition of nitrogenase by combined nitrogen can be expected within one generation, but a threefold increase in \( \text{C}_2\text{H}_4 \) formation and a stimulation of \( \text{H}_2 \) production from 2 to 148 \( \mu \text{mol/ml of pcv} \) per h in the absence of combined nitrogen suggest an important role for nitrogenase in \( \text{N}_2 \) reduction.

The short-term effects noted above were contrasted with the longer-term effects of fructose. For this purpose, 8-ml samples were grown in screw-cap tubes (15 by 150 mm) in the light with various initial amounts of fructose. Cell growth, chlorophyll, nitrogenase, oxygen evolution, and dark respiration were measured after 6 days (Fig. 3 and Table 3). At low initial concentrations of fructose (0 to 5 mM), there was an almost exponential increase of pcv, chlorophyll, and nitrogenase (Fig. 3). There was no further increase of pcv and nitrogenase above 20 mM fructose. Chlorophyll content was maximal at 10 mM fructose and then declined from 10 to 50 mM. This change in the composition of photo-

![FIG. 2. Fructose uptake and growth of A. variabilis. Algae were cultivated in 6 ml of medium A \( \frac{1}{2} \) in screw-cap tubes (15 by 100 mm) in the presence of 10 mM fructose labeled with \( ^{14}\text{C} \)fructose (20.10) cpm/ml]. Light was 3,000 lux; the temperature was 24°C. At the time indicated, after thorough mixing, 0.5 ml was withdrawn for the determination of the OD\(_{750} \), the total counts, and the counts in the supernatant and in the washed filaments. Starting cell density, OD\(_{750} \) = 0.4; Symbols: O, counts remaining in the medium; \( \triangle \), \( ^{14}\text{C} \)fructose (mM) recovered in filaments; ●, growth as OD\(_{750} \).

**TABLE 2. Changes in growth, chlorophyll, and nitrogenase activity of an air-grown culture of Anabaena 24 h after transfer to fructose or \( \text{NH}_4^+ / \text{NO}_3^- \) in air**

<table>
<thead>
<tr>
<th>Addition</th>
<th>pcv (ml)</th>
<th>Chlorophyll (mg/ml of pcv)</th>
<th>Protein (mg/ml)</th>
<th>( \text{C}_2\text{H}_4 ) reduction (( \mu \text{mol/ml of pcv per h} ))</th>
<th>( \text{H}_2 ) production (( \mu \text{mol/ml of pcv per h} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.5</td>
<td>3.4</td>
<td>0.25</td>
<td>77</td>
<td>2</td>
</tr>
<tr>
<td>Chloramphenicol, 10 ( \mu \text{g/ml} )</td>
<td>2.1</td>
<td>2.6</td>
<td>0.16</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Fructose, 5 mM</td>
<td>3.5</td>
<td>2.7</td>
<td>0.32</td>
<td>230</td>
<td>148</td>
</tr>
<tr>
<td>( \text{NO}_3^- ), 5 mM; ( \text{NH}_4^+ ), 2 mM</td>
<td>2.5</td>
<td>3.8</td>
<td>0.28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( \text{NO}_3^- ), 5 mM; ( \text{NH}_4^+ ), 2 mM; fructose, 5 mM</td>
<td>2.6</td>
<td>4.5</td>
<td>0.3</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

\( ^{14}\text{C} \)fructose-air-grown culture (pcv = 2.1; chlorophyll = 3.1 mg/ml of pcv) was washed twice and carbon-depleted for 9 h in air under light (3,000 lux) before 10-ml samples were transferred to screw-cap tubes (15 by 150 mm) with the above additions. Samples were run in duplicate. After 24 h of growth in air under light (3,000 lux), samples were taken for various measurements. Acetylene reduction in Ar-10% \( \text{C}_2\text{H}_4 \) was determined after 2 h; \( \text{H}_2 \) production in the presence of DCMU (5 \( \mu \text{M} \)) was calculated as the rate after an initial lag period of 8 h and was followed for 2 days.
synthetic pigments was also visible as a change in color from blue-green to yellow-green with high fructose content. The increase in nitrogenase activity with high fructose concentration was paralleled by a distinct rise in protein per cell and an increase in heterocyst number (Table 3). The sample with 0.5 mM fructose showed rather low activity in nitrogenase (Fig. 3) and oxygen evolution similar to the sample without fructose. Both were carbon starved. On the other hand, a culture that was sparged with air-0.5% CO₂ evolved 1,030 μmol of O₂ per ml of pcv per h, significantly more than any fructose-enriched culture. Samples grown in 50 mM fructose exhibited a more than 3-fold increase in protein and a 14-fold increase in nitrogenase over the 0.5 mM sample. In the fructose concentration series, oxygen evolution was highest at 10 mM fructose and reached a minimum at 50 mM fructose with 330 μmol of O₂ per ml of pcv per h, only 1/3 of what A. variabilis can produce under photoautotrophic conditions. Respiration in the dark under these conditions ranged from 90 to 130 μmol of O₂ taken up per ml of pcv per h, depending on how long the culture had been carbon starved before measurements. Adaptation to fructose can almost triple this value from 90 to 250 μmol of O₂ consumed per ml of pcv per h and may reach over 70% of the photosynthetic oxygen evolution rate. It appears from the divergence of the curves for pcv and chlorophyll (Fig. 3) that pcv, but not chlorophyll, can serve as a reliable parameter for growth. The 50% rise in heterocyst frequency from 8 to 12.3% of the total cells in 50 mM fructose indicates an effect of fructose even at the level of cellular differentiation. Microscopic observations show that low-fructose cultures contain heterocysts with normal polar bodies and thick envelopes. High-fructose cultures contain rather long filaments, and the heterocysts appear undeveloped, lacking strong polar bodies with thin outer cell walls. Such visual observations are not an indication of altered glycolipid content specific for the heterocyst envelope since thin-layer chromatography showed a normal complement of glycolipids I and III (for details see reference 5).

**Cellular accumulation of ^14C from fructose in adapted and nonadapted cells.** If fructose has a dual function of serving as carbon skeleton for cell growth and providing an organic reductant to nitrogenase through vegetative cells, uptake and transport of this compound ought to be rapid, and carbon derived from fructose might accumulate in heterocysts. To ascertain specifically when and where within a filament the ^14C from fructose accumulates in vegetative cells and heterocysts, we applied 1.5 nmol of carrier-free [^14C]fructose to a 1-ml culture containing 5 μg of chlorophyll and sampled filaments with increasing time of contact (20 min to 6 h) with [^14C]fructose for autoradiography (Fig. 4 and 5). The rate of [^14C]fructose uptake

![Graph](image_url)
in this experiment (Fig. 4) shows a steep rise in \(^{14}\text{C}\) in the filaments after 3 h, and nearly all of the fructose, 390 \(\mu\text{mol}\) per mg of chlorophyll, was assimilated after 4.5 h. The drop in total \(^{14}\text{C}\) absorbed by filaments after 6 h of incubation can be explained by a breakdown of fructose to soluble compounds which are washed out and lost in the process of rinsing the filters. The fraction of carbon lost through respiration cannot be large, however, since in the fructose uptake experiment detailed earlier (Fig. 2), \(<10\%\) of the total \(^{14}\text{C}\) was lost by either respiration or by release of unspecified carbon into the growth medium. The kinetics of fructose uptake in the unadapted sample appeared to be similar but exhibited lower rates per microgram of chlorophyll (Fig. 4). Samples from fructose adapted and unadapted cells were treated for autoradiography. As seen in Fig. 5A to C, with increasing contact times of 20 min, 75 min, and 6 h, silver grains appeared first over and near the vegetative cells before being concentrated within heterocysts (Fig. 5C). In a fructose-adapted culture which was fructose starved 18 h before adding labeled fructose, the sequential steps of uptake by vegetative cells and subsequent accumulation of \(^{14}\text{C}\) in the heterocysts took place within only 20 min (Fig. 5D). It is notable on a qualitative basis that the concentration of \(^{14}\text{C}\) from fructose was ultimately greater in the heterocyst than in the vegetative cell, regardless of whether or not the cultures were fructose adapted.

**DISCUSSION**

We have shown that exogenous fructose stimulates light-dependent growth and nitrogenase activity in the cyanobacterium *A. variabilis*. This effect in *A. variabilis* differs from that in *Nostoc* sp. strain Mac in which neither fructose nor glucose stimulates light-dependent growth, although slow heterotrophic growth occurs (2). The 8-h generation time of *A. variabilis* in the presence of light, fructose, and \(\text{N}_2\) as the sole nitrogen source represents a threefold increase in growth rate over air-grown cells and is the most rapid rate ever reported for this strain (for example, an approximate 11-h generation time in a batch culture at 30\(^\circ\)C [15]). Thus, its growth rate is comparable to those of *Nostoc* sp. strain Mac, using \(\text{CO}_2\) and \(\text{KNO}_3\) with a generation time of 5 to 6 h (2), and to fast-growing marine \(\text{N}_2\)-fixing cyanobacteria (4). In contrast to the above *Nostoc* strain with the fastest growth under \(\text{CO}_2\)-fixing conditions, \(\text{CO}_2\) addition to a fructose-grown culture of *A. variabilis* slows down growth. Since \(\text{CO}_2\) fixation also generates \(\text{O}_2\) saturation of a culture with \(\text{O}_2\) may have two effects: (i) interference with nitrogenase expression, and (ii) inhibition of heterocyst formation, the level of which is lower in air-\(\text{CO}_2\) cultures. In fact, high \(\text{CO}_2\) (2\%) and \(\text{N}_2\) inhibit growth (H. Spiller, J. F. Haury, and K. T. Shanmugam, Genetic Engineering of Symbiotic \(\text{N}_2\)-Fixation and Conservation of Fixed Nitrogen, in press).

**Fig. 4.** Kinetics of \(^{14}\text{C}\)-fructose absorption by *A. variabilis* filaments. The algal culture containing 5 \(\mu\text{g}\) of chlorophyll (Chl) per ml was devoid of combined nitrogen. Its previous growth history was: \(\Delta\), not adapted to fructose; \(\bullet\), adapted to fructose (10 mM). At 18 h before addition of 1.5 nmol of fructose per ml, with no carrier added, filaments were washed with medium A \(\frac{3}{4}\). Samples from this experiment were used for autoradiographic localization of \(^{14}\text{C}\) within the filament.
FRUCTOSE UPTAKE IN A. VARIABILIS

The growth response of the A. variabilis strain used in this investigation is also different from the A. variabilis strain reported by Kratz and Myers (6) and Pearce and Carr (10), who state that the growth rate of their strain was not affected by either glucose or sucrose. Moreover, our strain exhibits a high endogenous respiration per pcv, which is increased when cells are grown on fructose. No other carbohydrate tested (e.g., glucose, acetate, sucrose, etc.) gave a comparable rate of O₂ uptake or growth response in adapted or unadapted cells. Thus, fructose stimulation seems to be specific for this strain. This is reflected in the comparably low $K_m$ of 140 μM for
fructose assimilation. Fructose-adapted cells had an even lower \( K_m \) with 50 \( \mu M \), which indicates a greater affinity for fructose.

An increased ability to incorporate fructose is also reflected in the autoradiographs of fructose-grown material which show \( ^{14}C \) accumulation in the heterocysts after a pulse of \( ^{14}C \)fructose as short as 20 min. The distinctly higher concentration of silver grains in heterocysts as compared to vegetative cells and the impermeability of heterocysts to large molecules suggest that mass transfer of carbohydrate, in whichever form, has occurred from vegetative cells to heterocysts after fructose uptake. In another experimental series it was found that within one generation (~24 h) nitrogenase activity was increased over threefold upon supplementation with fructose. This, disproportionately high accumulation of \( ^{14}C \) from fructose in the heterocysts and maximal stimulation of nitrogenase in the short-term time range seem to indicate an involvement of fructose-derived reductant in nitrogen fixation.

In the long-term time range with >20 mM fructose, pcv and nitrogenase are maximal, whereas chlorophyll decreases and oxygen evolution capacity attains a minimum. Nitrogen fixation and oxygen evolution thus exhibit an inverse correlation to one another under photoheterotrophic conditions. A similar phenomenon of a decrease in oxygen production concomitant with chlorophyll loss per cell and maximum nitrogenase activity has earlier been observed by us with young cultures (3 to 4 days old) of *Nostoc muscorum*. The rate of oxygen evolution under photoautotrophic conditions is over 1,000 \( \mu mol \) of \( O_2 \) evolved per ml of pcv per h, equivalent to 290 \( \mu mol \) of \( O_2 \) evolved per mg of chlorophyll per h. This rate is decreased to \( \frac{1}{2} \) in the presence of 50 mM fructose. Since respiration in the latter sample increased to over 70% of the measured \( O_2 \)-evolving photosynthetic rate, it can be concluded that photoheterotrophic cultures create a microaerobic environment, with low oxygen pressure establishing favorable conditions for \( N_2 \) fixation. This is in contrast to cultures supplemented with 0.4%-CO\(_2\)-enriched air. As reported elsewhere (Spiller et al., in press), nitrogenase in this culture reaches less than 78% of a culture grown with 10 mM fructose. A maximum in nitrogen fixation appears to require a higher level of preformed reductant. This, in turn, affects both photosynthesis and respiration of our *A. variabilis* strain, which makes it distinct from others (6, 10).

According to our kinetic data, 10 mM fructose is totally assimilated after 4 days by *A. variabilis*. The fructose concentration series used in our study may also possibly reflect an increasing C/N ratio. Indeed, this series reveals a similar phenomenon to the one reported by Kulasooriya et al. (7): depending on the initial amount of fructose carbon available, protein content and nitrogenase activity rise by a factor of 3, and the number of heterocysts increase by \( \frac{1}{2} \) to about 12%.

In summary, fructose seems to be substantially more important in the metabolism of *A. variabilis* and possibly in other cyanobacteria than would be indicated by its limited ability to support heterotrophic growth in the dark. The amount of fructose available influences (i) the growth rate, which can be increased threefold; (ii) nitrogenase activity, which is stimulated severalfold; (iii) respiration, which is increased from 10 to over 50% of the photosynthetic oxygen evolution rate, thereby lowering oxygen tension in the medium; and (iv) the differentiation of vegetative cells into heterocysts, which is promoted.

Although we do not know the nature of the compound derived from fructose which is transferred into heterocysts, the changes effected by fructose are striking. Thus, the important role of fructose in dark and light reactions in heterocysts and vegetative cells and its impact on the oxygen balance and on chlorophyll demand further analysis of its specific metabolism.

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LITERATURE CITED


