Contributions of Respiratory and Photosynthetic Pathways during Growth of a Facultative Photoautotrophic Cyanobacterium, *Aphanocapsa* 6714

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Received for publication January 27, 1981 and in revised form June 3, 1981

ABSTRACT

Comparison of the growth parameters and photosynthetic capacities of cells of *Aphanocapsa* 6714 under various growth conditions led to the following conclusions: (a), no enzymic regulation of CO₂/glucose assimilation takes place in this strain; (b), functioning of photodependent phosphorylating pathways turns off oxidative ATP synthesis; (c), no efficient regulation of pigment synthesis exists in these cells; (d), most modulations of photosynthetic activities probably occur through structural modifications of the photosynthetic membranes (a small proportion of the pigments might appear as a nonintegrated pool in the cell and be sensitive to synthesis regulation); and (e), photosystem II activity would be dependent on light intensity in a discontinuous way, the consequence of this property being the appearance of two successive exponential phases during phototrophic growth in adequate light conditions.

A number of cyanobacteria are known to photoassimilate organic substrates (6). The role of these substrates has not been well defined, since CO₂ assimilation continues simultaneously. Only when inhibition of the photosynthetic electron transfer, which results in a block of noncyclic ATP and NADPH⁺ production, does not prevent growth in the presence of an organic substrate, can the organism be considered as capable of photoheterotrophic growth (18, 25). Some strains (5, 6, 9, 24, 25) can use the organic substrate as both carbon and energy sources to promote chemoheterotrophic growth. The reasons for this restriction are still unexplained, since the existence of the respiratory chain and the presence of the corresponding enzymatic machinery seem to be constant in cyanobacteria (19).

*Aphanocapsa* 6714, a unicellular strain, grows exclusively on glucose in the dark (1, 25), the metabolic pathway being through the oxidative pentose-phosphate pathway (19). No ATP synthesis has been demonstrated through substrate phosphorylations or fermentation. The respiratory chain functions during chemoheterotrophy and PSI-dependent cyclic photophosphorylations take place during true photoheterotrophic growth (20, 22).

Very little is known about the capacities of cyanobacteria to modulate their photosynthetic activity as a result of varying external conditions (10). To some extent, they can adapt their overall pigment contents to light conditions and, sometimes, modify the ratio of the two main types of pigments, phycobilines and Chl, but no uniform response can be demonstrated (9, 18, 24, 28). Nothing was known about *Aphanocapsa* 6714 capacities for modification of its overall pigment content, except that it does not perform chromatic adaptation (28).

The purpose of the work reported here was to determine possible modulations of the phosphorylating processes and the carbon assimilation pathways in *Aphanocapsa* 6714, when the cells were submitted to growth conditions in which several modes of metabolism could take place simultaneously. A parallel study of the photosynthetic capacities of the cells maintained in these growth conditions has given information on the state of the photosynthetic apparatus. A stepwise dependence of PSII activity on light intensity has been observed. The meaning of this unusual phenomenon is discussed.

MATERIALS AND METHODS

Organism and Growth Conditions. The strain, *Aphanocapsa* 6714 (ATCC 27 178), was provided kindly by Dr. R. Y. Stanier (Institut Pasteur, Paris, France) (26). Mineral medium (13) was used with twice the amount of NO₃⁻ (1). When stated, glucose was added after sterilization, at the indicated concentrations. Growth conditions were as described by Astier (1). Temperature was 34 C, light intensity 1.5 × 10⁻³ w·cm⁻² (3.5 × 10³ lux), and CO₂ enrichment approximately 5% (v/v) in air. Photoheterotrophy was obtained either by addition of 10⁻³ M DCMU or by aeration with a N₂ + O₂ atmosphere (80 + 20%). Dark conditions were achieved by wrapping the flasks with aluminum foil.

O₂ Evolution and Uptake. O₂ concentrations were determined using a Clark polarographic electrode, the cuvette being maintained at 34 C. O₂ evolution was measured under saturating light.

Pigment Concentration Measurements. Chl concentration was determined either from the absorption at 665 nm of acetone (80%, v/v) extracts using an extinction coefficient of 85.2 ml·mg⁻¹·cm⁻¹ (11) or by Bennet and Bogorad's procedure (2). Pc was extracted from the soluble fraction of the same preparation (2) centrifuged at 144,000g for 2 h (8) to eliminate additional membranephotochlorophyll (from Ref. 28, slightly modified). Absorption spectra of these preparations are shown in Figure 1.

Determination of the absorption peaks and of the extinction coefficients for Chl and phycocyanin lead to the following formulae:

Absorption 678 nm = 67.2 [Chl mg·ml⁻¹] + 0.2 [Pc mg·ml⁻¹]

Absorption 622 nm = 22.3 [Chl mg·ml⁻¹] + 5.9 [Pc mg·ml⁻¹]

Overall pigment composition was determined from absorption spectra of whole cells on a Cary 14 spectrophotometer.

1 Abbreviations: Pc, phycocyanin; DNP, dinitrophenol.
RESULTS

Four types of growth conditions were imposed upon Aphanocapsa 6714: chemoheterotrophy (dark, glucose); photoautotrophy (light, CO₂); photoheterotrophy (light, glucose); and mixotrophy (light, CO₂, glucose). The following parameters and characteristics were measured in each condition after the cultures had preadapted to the condition considered; growth rate (number of generations/h); maximal growth; growth yield (increase in CO₂ fixation/CO₂ consumption) as nmol Pₚ consumed or evolved/cell).

CHEMOTHERAPY (Fig. 2A). A classical exponential growth curve was obtained. Maximal growth increased linearly with glucose concentration up to 4.6 × 10⁻² M, from which level saturation was attained. Growth yield was constant throughout exponential growth.

PHOTOHETEROTROPHY. Utilization of glucose as the exclusive carbon source was obtained by either of two methods which appeared essentially equivalent: addition of 10⁻¹ M DCMU, known to inhibit completely photosynthetic electron transfer from PSII to PSI (3) and, thus, to prevent formation of NADPH⁺, necessary for CO₂ reduction; or deprivation of CO₂ in the gas phase and of Na₂CO₃ and NaHCO₃ in the growth medium. Here, again, growth was uniformly exponential (Fig. 2B), and the growth yield was constant up to a concentration of glucose of 3.5 × 10⁻² M.

PHOTOAUTOTROPHY. The appearance of two successive exponential phases (Fig. 2C), already described by Astier (1), has been confirmed in nine replications of the experiment. The first rapid phase, showing a generation time of 6 h, stopped reproducibly at a cell concentration of 4 to 5 × 10⁻³ M. The second phase, with a generation time of 31 h, was always followed by a very slow nonexponential phase, when a cell density of approximately 2 × 10⁻³ M was reached. This succession of exponential phases cannot be explained either by deprivation of one of the nutrients from the medium or by a phenomenon analogous to diauxy (17), since only one carbon source, CO₂, is available. The only hypothesis that can be proposed implies a threshold of accessibility of an external factor dependent on the increase in cell concentration. Only two such factors involved in photosynthesis can be considered: light intensity and/or CO₂ concentration.

Growth occurring under various incident light intensities from suspensions of identical, low initial concentrations [5 × 10⁶ cells/ml] showed that the sharp decrease in growth rate observed was not a consequence of partial CO₂ deprivation, since the first exponential growth was maintained up to higher cell densities when light intensity was increased while CO₂ concentration remained constant. On the contrary, the growth rate did depend on light intensity, according to two successive linear laws; while the increase was very weak above 4.7 × 10⁴ W/m² (1,100 lux), the variation was more important for lower intensities (manuscript in preparation). This is in accord with the fact that the second phase never ended in true stationary phase but in a slow decelerating one. Contrarily, no distortion from exponential could be observed in our experimental procedures during the first phase.

MIXOTROPHY. Growth in this condition also showed two exponential phases (Fig. 2D), with generation times of 5.5 and 17 h, respectively, followed by a true stationary phase. The change in exponential rate appeared at 6 to 8 · 10⁷ cells/ml. Maximal growth increased linearly with glucose concentrations (i.e., constant growth yield) only between 0.6 and 1.7 × 10⁻² M (Fig. 2D, inset). Quantities of glucose higher than 1.7 × 10⁻² M saturated the system; maximal growth for concentrations lower than 0.6 × 10⁻² M was identical to that obtained in photoautotrophic conditions.

PHOTOSYNTHETIC CAPACITIES OF THE CELLS IN THE VARIOUS GROWTH CONDITIONS. These capacities have been measured in cultures preadapted for at least 20 generations to the conditions considered. Both Chl and Pₚ contents remained constant (Tables I and II), whether glucose was present or not, when both photosystems were allowed to function. No difference appeared between cells taken from first or second exponential phases. Inhibition of electron transfer from PSII to PSI resulted in a decrease of both pigment concentrations, slightly greater for Pₚ. However, this did not disturb their capacity for photoautotrophy, since photoautotrophic growth characteristics appeared with no lag after transfer of the cells from DCMU + glucose to CO₂ medium. Whether this reflects the presence of an excess of pigments (possibly not included in efficient structures) in photoautotrophically growing cells, is not clear. Growth in nonphotosynthetic conditions only resulted in a slight (25%) simultaneous decrease of both pigment contents. Here, again, no adaptation was necessary to resume complete autotrophic capacities.

Measurement of O₂ evolution capacities (Table I) revealed a number of interesting features. Only about 20% activity was observed during the second exponential phases of both photoautotrophic and mixotrophic conditions, as compared to first phases. Besides, about 70% activity remained in mixotropic cells and 50% in chemoheterotrophic ones, as compared to photoautotrophic cultures. None of these modifications reflected the variation in pigment contents described above.

DISCUSSION

Interpretations in Terms of Carbon and Energy Sources (Tables I and II). It is clear that CO₂ and light are the only carbon and energy sources during photoautotrophy and that glucose plays both roles in chemoheterotrophy. Phototrophic cells, however, have a choice of energy sources: either glucose through the oxidative phosphorylating electron transfer chain and/or light through cyclic photophosphorylations using PSI. When photosynthetic and chemoheterotrophic were compared, the former showed higher growth rate and yield, identical maximal growth, and lower glu-
cose-limiting concentration. The data suggest a more efficient utilization of glucose in the former conditions. Since rates of ATP synthesis reflect the differences in growth rates (22), even though internal ATP concentrations are identical in this strain (20, 21), the most probable hypothesis is that light alone would serve as energy source during phototrophotrophy. The insensitivity of this means of growth (very low decrease of growth rate but no change in yield [Fig. 2B and Table I]) to DNP 10^{-3} M, which inhibits chemoheterotrophy to 90% (Fig. 2A), confirmed this hypothesis. This capacity for the cells to control their means of ATP production suggests the existence of distinct ATP synthesizing sites.

Since 3.5 \times 10^{-3} M glucose, used only as a carbon source, is necessary for maximal phototrophotrophic growth, the excess of this sugar (Fig. 2, A and B, insets) (4.6-3.5 \times 10^{-2} M, that is 25%) necessary to promote the same maximal growth in chemotrophotrophy represents the proportion that is degraded for respiratory energy production.

Comparisons of the parameters for mixo- and chemoheterotrophy are in favor of an exclusive utilization of light as energy source during mixotrophy, with both CO_2 and glucose feeding the carbon metabolism. The first part of this hypothesis is confirmed by the absence of inhibitory effect of DNP 10^{-3} M (Fig. 2D). We may then speculate as to what part photosynthetic and photomixotrophy (the two possible contributing ways of growth) play during
Table I. Growth Characteristics of Aphanocapsa 6714

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Chemoheterotrophy</th>
<th>Photoheterotrophy</th>
<th>Photoautotrophy</th>
<th>Mixotrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generations-10^3·h^{-1}</td>
<td>-DNP 10^{-3} M</td>
<td>+DNP 10^{-3} M</td>
<td>Exponential phase 1</td>
<td>Exponential phase 2</td>
</tr>
<tr>
<td>Maximum growth, 10^8 cell·ml^{-1}</td>
<td>6.0</td>
<td>0.3</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Growth yield, 10^8 cell·g^{-1} glucose</td>
<td>74 ± 2.0</td>
<td>108 ± 3.0</td>
<td>105</td>
<td>140</td>
</tr>
<tr>
<td>Chl μg·10^7 cell*</td>
<td>0.78</td>
<td>0.65</td>
<td>1.08</td>
<td>1.06</td>
</tr>
<tr>
<td>Pc μg·10^7 cell</td>
<td>10.14</td>
<td>6.25</td>
<td>13.10</td>
<td>13.10</td>
</tr>
<tr>
<td>Pc:Chl ratio</td>
<td>13.0</td>
<td>9.6</td>
<td>12.1</td>
<td>12.4</td>
</tr>
<tr>
<td>O₂ consumed or evolved, nmol·h^{-1}10^7 cells</td>
<td>290</td>
<td>510</td>
<td>90</td>
<td>380</td>
</tr>
</tbody>
</table>

*These values are determined at ±0.05 μg·10^7 cell.

Table II. Carbon and Energy Sources of Aphanocapsa 6714 in Various Growth Conditions

<table>
<thead>
<tr>
<th>Carbon</th>
<th>Glucose oxidative phosphorylations</th>
<th>Light cyclic/noncyclic phosphorylations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemoheterotrophy</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Photoheterotrophy</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Photoautotrophy</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Exponential phase 1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Exponential phase 2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mixotrophy</td>
<td>+</td>
<td>(+)*</td>
</tr>
<tr>
<td>Exponential phase 1</td>
<td>+</td>
<td>(+)*</td>
</tr>
<tr>
<td>Exponential phase 2</td>
<td>(+)</td>
<td>+</td>
</tr>
</tbody>
</table>

* (+) indicates minor utilization.

Each of the two exponential phases of mixotrophic growth.

If the constant growth yield observed in mixotrophy for glucose concentrations between 0.6 and 1.7·10^{-2} M (Fig. 2D, inset) is assumed also to be constant below 0.6·10^{-2} M, then the extrapolation of the curve shows glucose to be metabolized at least as soon as a density of 4·10^9 cells·ml^{-1} is reached. The slightly higher value of the growth rate for mixotrophic, compared to autotrophic, first exponential phases suggests that glucose is, in fact, used from the start of the growth. The similar cell concentrations for which this growth phase stops indicate that the change of growth rate is independent of glucose metabolism. Unfortunately, antimycin A, described as specific inhibitor for cyclic phosphorylations (12), was without effect on all photodependent growths and could not permit unambiguous assessment of the previous conclusions.

Almost similar maximal growths are reached in mixo- and photoheterotrophic, although limiting glucose amounts are higher in the latter (3.5·10^{-2} M against 1.7·10^{-2} M). This apparent discrepancy reflects the contribution of CO₂ (about 50%). However, the ratios of glucose to CO₂ utilization vary with the phases of growth. The slight increase in first phase compared to autotrophy suggests a low rate of glucose uptake. On the contrary, the difference in yields of the second exponential phases of mixo- and heterotrophic growths shows that 25% \( \frac{(140 - 108)}{140} \) of the carbon comes from CO₂ during that phase. Mixotrophy can, thus, be considered as the sum of photoheterotrophic and photoautotrophic growths, the former being predominant during the second exponential phase. The contribution of photoautotrophy would reflect the difference between the actual growth rate (5·10^{-3} generations·h^{-1}) and that due to photoheterotrophy (46 ± 4·10^{-3} generations·h^{-1}), that is approximately 13·10^{-3} generations·h^{-1}. The activity of the photoautotrophic pathway is then reduced to 30% of its maximal capacity (13/32; Table I).

These results tend to confirm the absence of efficient control of carbon assimilatory pathways through the nature of the carbon substrate provided, as was suggested by previous results obtained in this strain (14) and in most cyanobacteria (6). Plectonema boryanum would be an exception (24). The variations in rates of CO₂ to glucose assimilations (not higher than 30%) seem to be the consequence of regulations of the energetic pathways. Again, however, a complete shutoff was observed only for oxidative phosphorylations. Several works relate this regulation to a photosynthetically controlled production of glucose-6-phosphate and a light inhibition of glucose-6-phosphate dehydrogenase activity (4, 9, 19, 27). Both cyclic and noncyclic phosphorylations...
function throughout mixotrophic growth, though the photoheterotrophic pathway is less energy-consuming and allows faster growth rates.

**Modifications of the Photosynthetic Activities and Structure.** The inability of *Aphanocapsa* 6714 to display chromatic adaptation, not only when grown in the dark but also depending on the type of photosynthetic activity taking place, does not differentiate it from most cyanobacteria (6, 15, 18, 24). However, this clearly shows its very important phylogenetic difference with the other group of facultative prokaryotic phototrophs, the photosynthetic bacteria, which can regulate their pigment content and the development of the photosynthetic membranes as a function of external conditions (7, 16, 23).

Although parallel variations of contents of the two main types of pigments have been observed, no simple correlation can be drawn from those variations in relation to either photosynthetic activity measured as capacity of O₂ evolution, when existing, or to growth rates. In contrast, good agreement is found between those last two parameters and with the rates of CO₂ versus glucose assimilation.

These results, and the general absence of measurable adaptation period after any change of growth conditions, suggest that most of the possible modulations of photosynthetic activity take place through structural modifications of the photosynthetic membranes, independently of any de novo synthesis.

The last interesting phenomenon observed was the existence of two successive exponential phases whenever both photosynthetic could function. The cell density threshold separating these two phases is clearly defined. One hypothesis is that the growth rate is dependent upon light intensity and this, in a discontinuous manner. Light intensity received per cell in these experiments changes as a consequence of the increase of population density. While both PSII and PSI would function at their maximal rates during the first phase, PSII electron transfer would become limiting during the second phase. Parallel variations of rates of growth, O₂ evolution, and CO₂ assimilation are in agreement with this hypothesis. The sequence of O₂ evolution from cells taken in both exponential phases, measured in flash illumination experiments (J. Lavorel, personal communication), followed identical normal kinetics; however, the mean emission was lower for the more slowly growing cells. This is evidence for a lower number of normally efficient PSII centers in these cells.

We cannot assess whether the decrease of PSIII activity would be the initial cause of the later-reduced metabolism or whether a light-dependent enzymic reaction (see Ref. 19) would result in modulation of PSI activity. The kinetic characteristics of the change, and of its reversion, are also best explained by structural modifications of the photosynthetic membranes.

Acknowledgments—The authors wish to thank Professor N. G. Carr for helpful discussions and Professor Carr and Dr. M. Herdman for critical reading of the manuscript.

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