Regulation of Photosynthetic Capacity in Chlamydomonas mundana

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Summary. A regulatory system has been described in the obligately phototrophic green alga Chlamydomonas mundana. Cells grown in acetate media are unable to fix carbon dioxide in the light but carry out a photoassimilation of acetate to carbohydrate; cells cultured with carbon dioxide as the sole source of cellular carbon carry out typical green plant photosynthesis. The control appears to take place at the level of the reductive pentose phosphate cycle. The presence of sodium acetate in the medium strongly inhibits formation of ribulose-1,5-diphosphate carboxylase, ribulose-5-phosphate kinase, and one of the 2 fructose-1,6-diphosphate aldolase activities of the cell. Ribose-5-phosphate isomerase is present in higher activity in autotrophic cells. Changes in the levels of triose phosphate dehydrogenase were also noted. The total pigment content of the cell and the photosynthetic electron transport reactions are not altered under different conditions of growth.

Little information is available in the literature concerning the presence or absence of metabolic control systems affecting photosynthetic capacity in photosynthetic green algae. A variety of sugars, organic acids, and alcohols can serve as carbon sources for growth (4), but the extent to which the organic carbon source may bring about adaptive changes in the photosynthetic capacity of the cell through inhibition and/or repression mechanisms is unknown.

In at least 2 cases it has been shown that the CO₂ fixing and O₂ evolving capacity of the cell is not altered by the nature of the growth medium and the environmental conditions under which the cells are grown. Chlorella vulgaris cells form normal amounts of chlorophyll when cultured in either the light or dark in media containing glucose, and are photosynthetically competent under all conditions of growth (16). Similar observations have been made for the wild-type strain of Chlamydomonas reinhardtii (11). The chloroplast seems to be a constitutive organelle in these 2 algal strains.

Photosynthetic O₂ evolution and CO₂ fixation by whole cells of the obligately phototrophic green alga Chlamydomonas mundana grown in a medium containing sodium acetate have been reported to be very sluggish (5, 6). However, since the organism is capable of autotrophic growth with CO₂ as the sole source of cellular carbon (with a doubling time of approximately 8 hrs.), it seemed apparent that the autotrophic cells must be able to fix CO₂ at a high rate, and that acetate was probably effecting some change in the photosynthetic capacity of the cell.

The purpose of the present communication is to demonstrate that autotrophically grown cells of C. mundana fix CO₂ and evolve O₂ in the light at high rates, and that the presence of sodium acetate in the medium appears to inhibit the synthesis of several enzymes of the reductive pentose-P cycle. Acetate apparently has no effect on the photosynthetic electron transport system.

Methods and Materials

Organism and Culture Methods. A slant of the unicellular green algal Chlamydomonas mundana Gerloff var. astigmata nov. var. (Mojave strain) was obtained from Dr. R. W. Eppley. Cells were cultured at 32° in liquid media described previously (25) to which 1.0 μg vitamin B₁₂ and 100 μg thiamine hydrochloride were added per liter. Sodium acetate (0.3%) was added where required. Cultures were grown in continuous light at 2000 ft-c. Best growth was obtained when a mixture of 5% CO₂:95% N₂ was bubbled through the medium.

Carbon Dioxide Fixation. CO₂ fixation was measured as the incorporation of NaH¹⁴CO₃. Reactions were carried out in standard 17 ml Warburg flasks in 1.0 ml reaction mixtures. After exposure to
2000 ft-c for varying periods of time, the reactions were terminated by the addition of 0.3 ml 0.5 M HCl. Aliquots were plated in duplicate at infinite thinness and counted in a Nuclear-Chicago gas flow counter.

**Photosynthetic Electron Transport Reactions.** The photoreduction of TPN and 2,6-dichlorophenol indophenol by isolated chloroplast fragments of *C. mundana* was measured in the model 14 Cary recording spectrophotometer as described by Levine and Smillie (14), using the near infrared lamp as the source of actinic and measuring light. Photosynthetic phosphorylation was measured according to the technique of Russell and Levine (19). Photosynthetic pyridine nucleotide reductase (PNR) was prepared from spinach according to the method of San Pietro (21). Chloroplast fragments were prepared by sonicication of whole cells of *C. mundana* for 10 minutes in the MSE (Measuring and Scientific Equipment Company) sonic oscillator (10 kc). Chlorophyll concentrations were determined after Arnon (1).

**Preparation of Cell-Free Extracts.** Cell-free extracts of the organism were prepared by sonicication of whole cells for 2 minutes with the MSE sonicator. The extracts were clarified by centrifugation for 10 minutes at 10,000 × g and the clear supernatant fluids were used for the enzyme assays. Soluble protein was measured by the biuret method (10).

**Enzyme Assays.** Ribulose-1.5-P carboxylase was measured according to Fuller et al. (8). The reaction mixture contained *Chlamydomonas* extract, NaH¹⁴CO₃ (10 μmoles, 5–10 μCi), MgCl₂ (20 μmoles), EDTA (0.1 μmole), GSH (12.5 μmoles), and 0.25 μmole ribulose-1.5-P in a total volume of 2.0 ml. The reactions were run for 10 minutes, terminated with 0.5 N HCl and assayed for counts fixed as described above.

Fructose-1,6-diP aldolase was measured by coupling with triose-P isomerase and α-glycerol-P dehydrogenase (28). The colorimetric assay of Sibley and Lehninger (22) was also used for the determination of aldolase activity.

DPN and TPN dependent triose-P dehydrogenase activities were measured in the reverse direction according to the method of Wu and Racker (28).

Ribose-5-P isomerase was assayed after Axelrod (2). A standard curve was prepared using purified spinach phosphoribosylisomerase (2).

Ribulose-5-P kinase was measured according to Wu and Racker (28). Commercial pyruvate kinase and lactic dehydrogenase (Sigma) were used in the assay. Corrections were made for the endogenous reaction in the absence of substrate due to the presence of adenosine triphosphatase in the crude extracts.

**Results**

The data in table 1 clearly demonstrate that autotrophic cells of *Chlamydomonas mundana* are able to carry out green plant photosynthesis. High rates of CO₂ fixation and O₂ evolution have consistently been measured. The rate of CO₂ fixation by auto-

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Additions to reaction mixture</th>
<th>CO₂ Fixation (μmoles/hr per mg chl)</th>
<th>O₂ Evolution (μmoles/hr per mg chl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>None</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.1% Acetate</td>
<td>1.7</td>
<td>...</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.2% Acetate</td>
<td>1.3</td>
<td>...</td>
</tr>
<tr>
<td>CO₂</td>
<td>None</td>
<td>1.3</td>
<td>80</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.1% Acetate</td>
<td>101</td>
<td>...</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.2% Acetate</td>
<td>96</td>
<td>...</td>
</tr>
</tbody>
</table>

**Fig. 1.** CO₂ fixation by autotrophic cells of *C. mundana* as a function of time. Each flask contained whole cells equivalent to 87 μg chlorophyll.

**Fig. 2.** CO₂ fixation by autotrophic cells of *C. mundana* as a function of chlorophyll concentration. The reactions were run for 10 minutes.
trophic cells is linear with respect to time for at least 15 minutes (fig 1) and is linear with respect to chlorophyll concentration up to 160 μg chlorophyll per reaction mixture under the conditions of the assay (fig 2). The highest rate of CO\textsubscript{2} fixation measured with whole cells of \textit{C. mundana} is 300 μmoles per hour per mg chlorophyll (fig 2) and is more than sufficient to account for the observed growth rate in inorganic media.

The results recorded in table I also demonstrate that cells grown with acetate are essentially unable to fix CO\textsubscript{2} and evolve O\textsubscript{2} in the light, in confirmation of Epplley et al. (5, 6). The addition of sodium acetate to the reaction mixture did not restore CO\textsubscript{2} fixing capacity to cells grown on acetate, and had only a small inhibitory effect on the rate of CO\textsubscript{2} fixation by autotrophic cells.

The ability to fix CO\textsubscript{2} is rapidly restored to acetate grown cells upon transfer of the cells to inorganic media (fig 3). Within 6 hours high rates of CO\textsubscript{2} incorporation were observed. In control experiments in which acetate grown cells were resuspended in acetate media, there was no increase in CO\textsubscript{2} fixing ability. It should be noted that little if any growth (measured as increase of dry wt) took place during the first 5 hours of the adaptation from acetate to CO\textsubscript{2}. Only after 5 and one-half or 6 hours was an increase in dry weight observed.

![Graph](medium, rate in cells per hour, CO\textsubscript{2})

**Fig. 3.** CO\textsubscript{2} fixation by whole cells of \textit{C. mundana} after transfer from acetate medium to inorganic medium. Acetate cells were harvested, washed once with inorganic medium, and resuspended in inorganic medium under the usual growth conditions. At various times samples were removed and assayed for their ability to fix CO\textsubscript{2}. Each reaction mixture contained whole cells equivalent to 50 to 100 μg chlorophyll. The reactions were carried out for 10 minutes. The values given in the figure are corrected for dark fixation.

It seemed that acetate might exert its effect either at the level of the photosynthetic electron transport system or at the level of the reductive pentose-P cycle, the pathway of CO\textsubscript{2} fixation in photosynthesis. Chloroplast fragments of both acetate grown cells and autotrophic cells were found to carry out photosynthetic electron transport reactions at roughly similar rates (table II). The Hill reaction (measured as the photoreduction of 2,6-dichlorophenol indophenol), the photoreduction of TPX in the presence of spinach PPNR, and cyclic photosynthetic phos-

| Table II. Photosynthetic Reactions by Chloroplast Fragments of Autotrophic and Photoheterotrophic Cells |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Reaction                                                      | Carbon source for growth                                      |
|                                                               | Acetate CO\textsubscript{2} (μmoles/hr per mg chl)            |
| Photoreduction of TPN                                          | 20                                                            |
| Hill reaction                                                 | 46                                                            |
| Photosynthetic phosphorylation                                 | 100                                                           |

Table II. Photosynthetic Reactions by Chloroplast Fragments of Autotrophic and Photoheterotrophic Cells

To assay for TPN production, the reaction mixture (0.8 ml) contained chloroplast fragments (10-15 μg chlorophyll), an excess of purified spinach PPNR, and the following in μmoles: TPN, 0.2; MgCl\textsubscript{2}, 0.015; and Tris buffer (pH 7.5), 30. The reaction was measured as the increase of OD at 340 μm in the Cary recording spectrophotometer.

The Hill reaction was assayed as follows: the reaction mixture (0.8 ml) contained chloroplast fragments (3-4 μg chlorophyll) and the following in μmoles: phosphate buffer (pH 6.8), 10; KCl, 5; and 2,6-dichlorophenol indophenol, 0.05. The reaction was measured as the decrease of absorption at 600 μm in the Cary recording spectrophotometer.

Photophosphorylation was assayed as follows: the reaction mixture (3.0 ml) contained chloroplast fragments (100 μg chlorophyll) and the following in μmoles: Tris buffer (pH 7.5), 100; MgCl\textsubscript{2}, 10; K\textsubscript{2}HPO\textsubscript{4}, 10; ADP, 10; and phenazine methosulfate, 0.15. The reaction was measured as the disappearance of Pi (19).

These data represent the average of 2 assays.

Phosphorylation with phenazine methosulfate took place at approximately the same rates with preparations of both kinds of cells. The presence of acetate in the growth medium does not appear to exert a major effect on the photosynthetic electron transport system of the cell. In addition, there is no major difference in the chlorophyll content of cells grown under both sets of conditions; acetate cells and autotrophic cells both contain about 2.5 to 3.0% of the dry weight of the cell as chlorophyll.

A survey of some of the enzymes of the reductive pentose-P cycle revealed major differences between acetate grown and autotrophic cells. In contrast to autotrophically grown cells, only low levels of ribulose-5-P kinase and ribulose-1,5-diP carboxylase were detected in extracts of cells grown with acetate (table III). Ribulose-5-P kinase was difficult to measure because of the presence of adenosine triphos-
phosphatase activity in the crude extracts; the data for this activity are less reliable than the data for ribulose-1,5-diP carboxylase. Mixing experiments give no evidence for an inhibitor of ribulose-1,5-diP carboxylase in extracts of acetate cells, or for an activator of the enzyme in autotrophic cells (table IV). Ribulose-1,5-diP carboxylase is either not present in acetate cells or is present in a totally inactive form.

Fructose-1,6-diP aldolase activity was detected in extracts of both types of cells, but it appears that the cell contains 2 distinct enzymes, one of which is formed under each set of growth conditions. Autotrophic cells contain an aldolase which has a high affinity for fructose-1,6-diP, is not inhibited by chelating agents, and is not stimulated by Fe²⁺ (table V). Acetate cells contain an enzyme which is inhibited by EDTA and o-phenanthroline, is markedly stimulated by Fe²⁺, and has a much lower affinity for fructose-1,6-diP in crude extracts (table V).

**Discussion**

The results reported here demonstrate very clearly that the inclusion of an organic carbon source in the medium of C. mundana brings about adaptive changes in the photosynthetic capacity of the cell. In the presence of sodium acetate, several of the enzymes involved in the reduction of CO₂ to carbohydrate are formed in greatly reduced amounts. Under these conditions, the cells carry out a light dependent assimilation of acetate (6). similar to that reported for Rhodospirillum rubrum (23), other photosynthetic bacteria (9), and the acetate requiring obligately phototrophic green alga Chlamydomonas.

### Table IV. Comparison of Ribulose-1,5-diP Carboxylase Activities

<table>
<thead>
<tr>
<th>Source of extract</th>
<th>Mg protein in extract</th>
<th>Enzyme activity (µmole) fixed/10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate cells</td>
<td>0.57</td>
<td>0.5</td>
</tr>
<tr>
<td>Acetate cells</td>
<td>1.15</td>
<td>1.3</td>
</tr>
<tr>
<td>CO₂ cells</td>
<td>0.82</td>
<td>58</td>
</tr>
<tr>
<td>CO₂ cells</td>
<td>1.64</td>
<td>125</td>
</tr>
<tr>
<td>Acetate cells</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ cells</td>
<td>0.82</td>
<td>62</td>
</tr>
</tbody>
</table>

### Table V. Comparison of 2 Fructose-1,6-diP Aldolases from Chlamydomonas

The effects of EDTA, o-phenanthroline, p-CMB and Fe²⁺ were measured using the colorimetric assay of Sibley and Lehninger (22). Each reaction mixture contained Chlamydomonas extract, Tris buffer (pH 8.0), 20 µmole MgCl₂, 0.1 µmole EDTA, 12.5 µmole GSH, 0.25 µmole ribulose-1,5-diP, and 10 µmole NaHCO₃ in a total volume of 2 ml. The values given below are corrected for fixation in the absence of added substrate.

<table>
<thead>
<tr>
<th>Carbon source for growth</th>
<th>Acetate</th>
<th>CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kₘ (Fructose-1,6-diP)</td>
<td>2 × 10⁻³ M</td>
<td>5 × 10⁻⁵ M</td>
</tr>
<tr>
<td>Effect of 10⁻³ M EDTA</td>
<td>95–100% inhibition</td>
<td>None</td>
</tr>
<tr>
<td>Effect of 10⁻³ M o-phenanthroline</td>
<td>95–100% inhibition</td>
<td>None</td>
</tr>
<tr>
<td>Effect of 10⁻³ M Fe²⁺</td>
<td>3–5-fold stimulation</td>
<td>None</td>
</tr>
<tr>
<td>Effect of 10⁻⁵ M p-CMB</td>
<td>15% inhibition</td>
<td>97% inhibition</td>
</tr>
</tbody>
</table>
(17). Eppley et al. (6) have reported the adaptive formation of isocitrate lyase, an enzyme of the glyoxylate cycle, in cells of C. mundana cultured with acetate. Extracts of autotrophic cells show very little isocitrate lyase activity. Upon transfer to inorganic media, the cells quickly adapt to typical green plant photosynthesis and are capable of growth with CO\textsubscript{2} as the sole source of carbon. The evidence presented here suggests that acetate or some metabolite derived from acetate inhibits enzyme formation, but definitive evidence for de novo synthesis during adaptation has not been given. Acetate or an acetate derivative may inhibit enzyme action.

Ribulose-1, 5-diP carboxylase has been implicated as the enzyme directly responsible for the photosynthetic fixation of CO\textsubscript{2} both through kinetic analysis of the soluble products of CO\textsubscript{2} fixation (3) and, more recently, through investigation of mutant strain of Chlamydomonas reinhardtii devoid of carboxylase activity (15). The results reported in this paper indicate that a regulatory system exists in Chlamydomonas mundana controlling the formation of ribulose-1,5-diP carboxylase and probably ribulose-5-P kinase as well as one of the 2 fructose-1,6-diP aldolase activities of the cell. The absence of these activities during growth on acetate probably spares the cell metabolic energy needed for the synthesis of these enzymes (if the control is at the level of enzyme synthesis), or permits more efficient utilization of energy for carbon assimilation (if the control is at the level of enzyme activity).

Changes in the levels of ribulose-1,5-diP carboxylase brought about by organic carbon sources have been reported in several photosynthetic bacteria (8, 9, 13) and the chemosynthetic bacterium, Micrococcus denitrificans (12). Fuller and Gibbs (7) reported that acetate grown cells of Chlorella variegata contain very low levels of the enzyme. In this latter case, however, acetate brings about repression of the entire chloroplast, since acetate grown cells of Chlorella variegata are essentially devoid of chlorophyll. Dark grown Euglena gracilis cells contain low levels of the enzyme and are pigmentless (7). The unique feature of the C. mundana system reported in this paper, at least with respect to photosynthetic algae, is that acetate specifically affects the formation of several of the enzymes of CO\textsubscript{2} fixation without affecting the total chlorophyll content of the cell or the ability of the cell to carry out photosynthetic electron transport reactions. Since C. mundana has an absolute requirement for light for growth, the photosynthetic electron transport system may be the major source of ATP and/or TPNH for the cell.

Ribose-5-P isomerase was detected in extracts of cells cultured under both growth conditions, but was present in 5-fold higher activity in autotrophic cells. The enzyme may function in both the oxidative and reductive pentose-P cycle in cells cultured with CO\textsubscript{2}.

Both DPX and TPX dependent triose-P dehydrogenase activities are present in C. mundana under both conditions of growth and may be essential for photosynthetic as well as phototrophic growth, although TPX-dependent triose-P dehydrogenase is usually considered to be a component of the photosynthetic CO\textsubscript{2} fixation cycle (3). The activity of triose-P dehydrogenase is reduced in acetate grown cells, but not to as great an extent as ribulose-1,5-diP carboxylase and ribulose-5-P kinase. Hudock (private communication) has recently demonstrated that TPX-linked triose-P dehydrogenase is formed in the y-2 mutant of Chlamydomonas reinhardtii (yellow in the dark; green in the light) under conditions where ribulose-diP carboxylase and PPXR are greatly reduced in activity (11). The enzyme may not function in photosynthesis or may be involved in other metabolic reactions as well as reactions of photosynthesis.

The demonstration of 2 aldolase activities in Chlamydomonas mundana extends the findings of Rutter (20), who has found 2 aldolases in Euglena gracilis and Chlamydomonas reinhardtii. In C. mundana the Fe\textsuperscript{2+} activated enzyme appears to be involved in the conversion of acetate to carbohydrate through the glyoxylate cycle (6) and reversal of glycolysis. The enzyme is very similar to the aldolase of the blue-green alga Anacystis nidulans and the photosynthetic bacterium Rhodopseudomonas spheroides (26, 27). Under conditions of autotrophic growth, only small amounts of this enzyme are formed as judged by elution of aldolase activity from ion exchange columns (Russell and Gibbs, unpublished observations). Cells capable of carrying out green plant photosynthesis contain an enzyme not dependent on a metal for activity with a relatively high affinity for the substrate. Only trace amounts of the enzyme are found in acetate grown cells (table III). Stumpf (24) has described a nonmetal aldolase in pea seeds. This enzyme is not sensitive to p-CMB in contrast to the enzyme from autotrophic Chlamydomonas (table V).

Further investigation is needed to establish both the nature and specificity of the control system reported here. A study of the adaptation process in the presence of inhibitors of protein synthesis should indicate the nature of control; a comprehensive survey of the effect of a wide variety of organic carbon sources should provide information for an understanding of the specificity of control.

**Literature Cited**