Effects of temperature on glycolate metabolism in Chlorella

Yasunori Nakamura¹ and Shigetoh Miyachi¹,²

¹ Radioisotope Centre and ²Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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The effects of temperature on the products of photosynthetic carbon metabolism were studied in Chlorella vulgaris 11h cells which had been grown with air enriched with 2–3% CO₂. During the test period, 300 ppm of 14CO₂ was used. On raising the temperature from 20 to 36°C during photosynthetic 14CO₂ fixation, 14CO₂-incorporation into glycolate immediately stopped whereas that into sucrose was greatly enhanced. When the temperature was lowered from 36 to 20°C, 14C-glycolate formation was greatly enhanced, but sucrose formation slowed. No significant change in the rate of total 14CO₂ fixation was induced by either temperature change. At 20°C the radioactivity incorporated into glycolate was about 20% of the total 14C fixed, but it decreased to less than 2% at 32°C. When a-hydroxy-2-pyridinemethane-sulfonate was added, the percent incorporation of 14C into glycolate was enhanced to 30% at 20°C as well as at 32°C. The increase in 14C-incorporation into glycolate was accompanied by a decrease in sucrose and glucose-polymer, although the total 14CO₂ fixation was not significantly affected by this inhibitor; most of the labelled glycolate appeared in the algal medium.

Key words: Chlorella — CO₂ fixation — Glucose-polymer — Glycolate — Sucrose — Temperature.

Glycolate is an early photosynthetic product in algae, and most algae excrete glycolate outside their cells during photosynthesis. The amount of excreted glycolate is favored by a high O₂ concentration, a low CO₂ concentration, and a high light intensity or red light as compared with blue light (4, 10, 13). However, of the environmental factors which influence the level of glycolate excretion, little has been reported about the effect of temperature (2, 6).

In a previous paper (6), we reported the dependency of photosynthesis on temperature in Chlorella vulgaris cells grown with CO₂-enriched air (high-CO₂ cells) at various 14CO₂ concentrations. At 300 ppm 14CO₂ the incorporation of 14C into glycolate decreased greatly when the temperature was raised from 20 to 37°C. At high concentrations of 14CO₂ (3,000 ppm), 14C-incorporation into glycolate was not significant at any temperature. Our preliminary experiments showed that the effects of temperature on 14C-incorporation into glycolate in C. vulgaris cells grown with ordinary air (containing 300 ppm CO₂; low-CO₂ cells) were similar to those observed in high-CO₂ cells. However, the level of 14C-glycolate in low-CO₂

Abbreviations: α-HPMS, α-hydroxy-2-pyridinemethanesulfonate; pcv, packed cell volume; P-esters, phosphate esters; ser + gly, serine + glycine.
cells was much lower than that in high-CO_2 cells. Therefore, we have studied
temperature-effects on glycolate metabolism with high-CO_2 cells and under low
CO_2 conditions.

Our present results suggest that the formation of glucose-polymer via the
glycolate pathway is enhanced greatly by a rise in the experimental temperature.
In contrast, the rate of this process under low temperatures is limited by the step of
glycolate oxidation (to glyoxylate). Since the formation of glycolate was not
affected, the excretion of glycolate was significant only at low temperatures.

Materials and methods

*Chlorella vulgaris* 11h cells were grown photoautotrophically in an inorganic
culture medium under the bubbling of air enriched with 2–3% CO_2 by volume.
Cells were harvested and used in the photosynthetic experiments with 300 ppm
^{14}CO_2 as described previously (6). The analysis of the fixation products also was
as described previously (6).

In most of the experiments shown in Fig. 1, 2 and 3 and Table 1, all the ^{14}CO_2-
fixation products were measured whether they were present inside or outside of the
cells. In the experiments shown in Fig. 4 and Table 2, which were carried out to
analyze the excreted compounds, samples of the reaction mixture were placed in
centrifuge tubes after photosynthetic ^{14}CO_2 fixation for 20 min under the bubbling
air containing 300 ppm ^{14}CO_2. The suspension was centrifuged briefly (for 1.5
min), after which 4 volumes of methanol was added to the supernatant and 80% methanol to the precipitate. The total radioactivity was determined with duplicate
portions taken from each fraction. Then both fractions were subjected to two-
dimensional paper chromatography, as described previously (6).

Results

Effects of temperature on the level of ^{14}C-glycolate

Fig. 1 shows that although the total amount of ^{14}CO_2 fixed was almost the same
in the temperature range from 20 to 36°C, the level of ^{14}C-glycolate sharply declined
with increasing temperatures within a comparatively narrow range above 20°C.

Effects of a temperature change between 20 and 36°C on photosynthetic ^{14}CO_2 fixation

The rate of total ^{14}CO_2 fixation did not change significantly when the reaction
temperature was raised from 20 to 36°C (Fig. 2) or lowered from 36 to 20°C (Fig. 3)
during the experiment. When the temperature was raised, the rate of ^{14}C-incor-
poration into the soluble fraction decreased and that into the insoluble fraction
increased, whereas the rate of ^{14}C-incorporation into the insoluble fraction was
essentially unchanged when the temperature was lowered.

When the temperature was raised from 20 to 36°C, the increase in radioactivity
in glycolate stopped, then it started to decrease (Fig. 2). This decrease ceased after
about 17% of the ^{14}C-glycolate had disappeared. In contrast, the ^{14}C-incorporation
into glycolate was enhanced greatly when the temperature was lowered from 36 to
20°C (Fig. 3). The enhanced rate of glycolate formation was nearly equal to that
Effects of temperature on glycolate metabolism

Fig. 1. Effect of temperature on the level of $^{14}$C-glycolate during photosynthesis by Chlorella vulgaris. $^{14}$CO$_2$ fixation was run for 20 min. Cell density was 2 ml pcv/liter.

observed when the temperature was 20°C from the beginning of the experiment (Compare Fig. 2 and 3). $^{14}$C-Incorporation into ser+gly was similar, but somewhat higher at 20°C than at 36°C.

$^{14}$C-Incorporation into sucrose was enhanced greatly when the temperature was raised from 20 to 36°C (Fig. 2), but sucrose formation ceased when the temperature was lowered from 36 to 20°C (Fig. 3). The rate of $^{14}$C-incorporation into sucrose induced by raising the temperature from 20 to 36°C, however, was much higher than that observed when the temperature was kept at 36°C from the beginning.

Effect of $\alpha$-HPMS on photosynthetic $^{14}$CO$_2$ fixation products at high and low temperatures

When $\alpha$-HPMS, an inhibitor of glycolate oxidase (13) and glycolate dehydrogenase (7), was added, $^{14}$C-incorporation into glycolate was enhanced at 20°C (Table 1). The effect of $\alpha$-HPMS was more pronounced at 32°C. The radioactivity in glycolate at 32°C was much less than at 20°C in the absence of the inhibitor, but with $\alpha$-HPMS at 32°C $^{14}$C-glycolate increased to almost the same level as at 20°C. At both temperatures, the amount of $^{14}$C-glycine was decreased by $\alpha$-HPMS, whereas that of incorporated serine was increased (Table 1). $^{14}$C-
Incorporation into P-esters and the insoluble fraction also was lower in the presence of α-HPMS than in its absence, and the effect of this inhibitor on both fractions was more pronounced at 32°C than at 20°C.

*Excretion of $^{14}$C-glycolate into the suspending medium at various temperatures*

Most unicellular algae excrete glycolate (4, 10, 11, 13). To see whether temperature affects the excretion of glycolate, Chlorella cell suspensions, which had fixed $^{14}$CO₂ for 20 min at various temperatures, were centrifuged briefly to separate the cells from the extracellular products. At all temperatures, most of the $^{14}$C-
Effects of temperature on glycolate metabolism

Fig. 3. Effects of lowering the temperature from 36 to 20°C on the products of photosynthetic $^{14}$CO$_2$ fixation.

glycolate had been excreted from the cells (Fig. 4). At temperatures below 28°C, most of the excreted radioactivity was glycolate. In addition, Table 2 shows that irrespective of the experimental temperatures in the presence or absence of $\alpha$-HPMS, most of the $^{14}$C-glycolate was excreted from the algal cells.

During photosynthesis by Chlorella the pH value of the suspending medium changed (Fig. 5). At 20°C, which favored glycolate excretion, the pH of the medium decreased after an initial rise (A). At 32°C, when $^{14}$C-glycolate excretion was insignificant, the pH continued to increase (C). These pH changes may reflect the excretion of glycolate by Chlorella cells since the experiments shown in Fig. 4 and 5 were run at the same time with the same material. When $\alpha$-HPMS was added, the pH in the suspending medium decreased irrespective of the temperature (B and
Table 1  Effect of α-HPMS on photosynthetic $^{14}$CO$_2$ fixation by Chlorella cells at low and high temperatures

<table>
<thead>
<tr>
<th>Products</th>
<th>20°C</th>
<th>20°C + α-HPMS</th>
<th>32°C</th>
<th>32°C + α-HPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg atom $^{14}$C/ml pcv (percent total)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.25 (100.0)</td>
<td>14.47 (100.0)</td>
<td>15.48 (100.0)</td>
<td>13.89 (100.0)</td>
</tr>
<tr>
<td>Insoluble</td>
<td>5.04 (38.3)</td>
<td>4.57 (31.6)</td>
<td>7.22 (46.6)</td>
<td>3.80 (27.4)</td>
</tr>
<tr>
<td>Souble</td>
<td>9.41 (61.7)</td>
<td>9.90 (68.4)</td>
<td>8.26 (53.4)</td>
<td>10.09 (72.6)</td>
</tr>
<tr>
<td>P-esters</td>
<td>0.24 (1.4)</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.04 (0.3)</td>
<td>0.01 (0.1)</td>
<td>0.04 (0.3)</td>
<td>0.02 (0.1)</td>
</tr>
<tr>
<td>Malate</td>
<td>0.20 (1.3)</td>
<td>0.12 (0.8)</td>
<td>0.23 (1.5)</td>
<td>0.07 (0.5)</td>
</tr>
<tr>
<td>Glycolate</td>
<td>2.91 (19.1)</td>
<td>4.43 (30.6)</td>
<td>0.23 (1.5)</td>
<td>4.42 (31.8)</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.45 (3.0)</td>
<td>0.25 (1.7)</td>
<td>0.29 (1.9)</td>
<td>0.18 (1.3)</td>
</tr>
<tr>
<td>Serine</td>
<td>0.58 (3.8)</td>
<td>0.96 (6.6)</td>
<td>0.70 (4.5)</td>
<td>1.43 (10.3)</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.05 (0.3)</td>
<td>0.08 (0.6)</td>
<td>0.08 (0.5)</td>
<td>0.03 (0.2)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.48 (3.1)</td>
<td>0.32 (2.2)</td>
<td>0.45 (2.9)</td>
<td>0.20 (1.4)</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.20 (1.3)</td>
<td>0.27 (1.9)</td>
<td>0.24 (1.6)</td>
<td>0.21 (1.5)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.25 (1.6)</td>
<td>0.17 (1.2)</td>
<td>0.48 (3.1)</td>
<td>0.25 (1.8)</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.87 (5.7)</td>
<td>0.82 (5.7)</td>
<td>1.85 (12.0)</td>
<td>1.31 (9.4)</td>
</tr>
<tr>
<td>Others</td>
<td>0.59 (3.9)</td>
<td>0.40 (2.8)</td>
<td>0.55 (3.6)</td>
<td>0.07 (0.5)</td>
</tr>
</tbody>
</table>

Experiments were run for 20 min with 300 ppm $^{14}$CO$_2$ in the presence or absence of α-HPMS (1 mM). For experimental conditions see Fig. 4.

D). This is evidence that when the glycolate metabolism was inhibited, glycolate was excreted into the suspending medium even at high temperatures.

Discussion

$^{14}$C-Accumulation in glycolate during photosynthesis by Chlorella vulgaris 11h is a reversible phenomenon that ceases when the temperature was raised from 20 to 36°C (Fig. 2), or that occurs when the temperature was dropped from 36 to 20°C.

Table 2  Effect of α-HPMS on glycolate excretion at 20 and 32°C

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Total fixed</th>
<th>Excreted</th>
<th>Extracellular glycolate</th>
<th>Intracellular glycolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg atom $^{14}$C/ml pcv (percent total)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20°C control</td>
<td>15.67 (100.0)</td>
<td>2.79 (17.8)</td>
<td>2.69 (17.2)</td>
<td>0.21 (1.3)</td>
</tr>
<tr>
<td>20°C + α-HPMS (1 mM)</td>
<td>14.57 (100.0)</td>
<td>3.96 (27.2)</td>
<td>3.85 (26.4)</td>
<td>0.21 (1.4)</td>
</tr>
<tr>
<td>32°C control</td>
<td>16.17 (100.0)</td>
<td>0.45 (2.8)</td>
<td>0.24 (1.5)</td>
<td>trace</td>
</tr>
<tr>
<td>32°C + α-HPMS (1 mM)</td>
<td>14.12 (100.0)</td>
<td>3.85 (27.3)</td>
<td>3.71 (26.3)</td>
<td>0.20 (1.4)</td>
</tr>
<tr>
<td></td>
<td>14.53 (100.0)</td>
<td>3.92 (27.0)</td>
<td>3.76 (25.7)</td>
<td>0.24 (1.7)</td>
</tr>
</tbody>
</table>

For experimental conditions see Fig. 4.
Effects of temperature on glycolate metabolism

Fig. 4. Amount of $^{14}$C in the medium and in the algal cells after photosynthetic $^{14}$CO$_2$ fixation at various temperatures. The duration of $^{14}$CO$_2$ fixation was 20 min. For other experimental conditions see Methods. Experiments in Fig. 4 and 5 and Tables 1 and 2 were run with the same Chlorella culture of 4.6 ml pcv/liter.

Fig. 5. Changes in pH-values of a Chlorella suspension during photosynthesis at different temperatures and in the presence or absence of α-HPMS (1 mM).
Most of this glycolate was excreted from the Chlorella cells (Fig. 3 and 4). When the reaction temperature was raised from 20 to 36°C, and $^{14}$C-glycolate accumulation stopped, there was, in fact, a decrease of about 17% in $^{14}$C-glycolate. Perhaps this small decrease was due to the metabolism of the glycolate that had not been excreted because the excreted $^{14}$C-glycolate was not reabsorbed by the algal cells, consistent with the report of Bruin and Tolbert (1) that Chlorella does not take up and metabolize glycolate.

There are several factors which might affect the excretion of glycolate by Chlorella during photosynthesis, such as the rate of glycolate biosynthesis, the rate of glycolate oxidation, and the transport mechanism for glycolate. Our data indicate that the rate of glycolate metabolism in the cell certainly is influenced by the temperature change. In the presence of $\alpha$-HPMS the amount of $^{14}$C-glycolate produced by the algae at 32°C increased to about the same level observed at 20°C (Table 1). Most of the $^{14}$C-glycolate produced in the presence of $\alpha$-HPMS at 32°C was excreted from the algal cells (Table 2 and Fig. 5D). These data are evidence that the rate of glycolate biosynthesis and transport were not limiting factors or were not affected by the temperature change. Thus the metabolism of glycolate seems to be enhanced greatly by a temperature rise. Assuming that the metabolism of glycolate was inhibited completely by $\alpha$-HPMS, we estimated that the amount of glycolate transformed at 32°C was about 2.6 times as large as that at 20°C during 20 min of $^{14}$CO$_2$ fixation [amount at 32°C/$\alpha$-HPMS/amount at 20°C/$\alpha$-HPMS = 
\[\frac{31.8 - 1.5}{30.6 - 19.1} = 2.6\].

$\alpha$-HPMS blocked $^{14}$C-incorporation into glycine irrespective of the temperature (Table 1). However, $^{14}$C-serine formation was enhanced by the addition of $\alpha$-HPMS (Table 1) as if the alternative pathway via glycerate to serine was used when the glycolate to glycine pathway was inhibited (9).

The addition of $\alpha$-HPMS also decreased $^{14}$C-incorporation into the P-esters and the insoluble fraction, and this effect at 32°C was greater than at 20°C (Table 1). The insoluble fraction is made up mostly of a glucose-polymer (5). The ratio of the percent of $^{14}$C in the glucose-polymer at 32 and 20°C with and without $\alpha$-HPMS was 
\[\frac{46.6 - 27.4}{38.3 - 31.6} = 2.9\] (Table 1). This 2.9-fold increase in the glucose-polymer caused by $\alpha$-HPMS at 32°C over the increase at 20°C is in close agreement with the 2.6-fold enhanced glycolate transformation at 32°C. Thus, during photosynthesis by Chlorella cells at 300 ppm $^{14}$CO$_2$, the accumulation and excretion of glycolate at 20°C may be changed mainly to the production of a glucose-polymer at 32°C via the glycolate pathway. In this connection Tolbert et al. (3, 8) reported that $^{14}$C-glycolate was converted to sucrose in the light via the glycolate and Embden-Meyerhof pathways in the leaves of higher plants. We assume that the formation of the glucose-polymer via the glycolate pathway in Chlorella vulgaris is enhanced greatly at high temperatures. The excretion of glycolate becomes significant at low temperatures when glycolate metabolism seems to be limiting. Generally, photorespiration as equated biochemically by the glycolate pathway and CO$_2$-compensation point has been used as an index of photorespiration. Tsuzuki and Miyachi (12) have reported that the CO$_2$-compensation point in high-CO$_2$ cells of Chlorella vulgaris doubled when the temperature was raised from 20 to 32°C, which would be consistent with the increased metabolism of glycolate at high temperatures.
Effects of temperature on glycolate metabolism

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References