Photorespiration in Air and High CO₂-Grown Chlorella pyrenoidosa

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ABSTRACT

Oxygen inhibition of photosynthesis and CO₂ evolution during photorespiration were compared in high CO₂-grown and air-grown Chlorella pyrenoidosa, using the artificial leaf technique at pH 5.0. High CO₂ cells, in contrast to air-grown cells, exhibited a marked inhibition of photosynthesis by O₂, which appeared to be competitive and similar in magnitude to that in higher C₃ plants. With increasing time after transfer to air, the photosynthetic rate in high CO₂ cells increased while the O₂ effect declined. Photorespiration, measured as the difference between ¹⁴CO₂ and ¹³CO₂ uptake, was much greater and sensitive to O₂ in high CO₂ cells. Some CO₂ evolution was also present in air-grown algae; however, it did not appear to be sensitive to O₂. True photosynthesis was not affected by O₂ in either case. The data indicate that the difference between high CO₂ and air-grown algae could be attributed to the magnitude of CO₂ evolution. This conclusion is discussed with reference to the oxygenase reaction and the control of photorespiration in algae.

Gas exchange measurements of photorespiration suggest that unicellular algae grown on high CO₂ concentrations (20–50 ml l⁻¹; high CO₂ cells) can have photorespiratory characteristics different from those of algae grown on air levels of CO₂ (330 μl l⁻¹; air-grown cells). Algae grown at high CO₂ have CO₂ compensation points similar to higher C₃ plants (9, 28), whereas those grown under air have CO₂ compensation points close to zero (3, 9, 16, 21, 25, 26). High CO₂ cells have been shown to evolve CO₂ into CO₂-free air (9) while air-grown cells have not (16). However, contradictory reports have appeared concerning the presence or absence of an O₂ effect on photosynthesis (15, 26, 29).

Air- and high CO₂-grown cells are now known to differ primarily in their affinity for inorganic carbon as a substrate for photosynthesis (2, 11–13). Since high CO₂ algae quickly become adapted when transferred to air (9, 10), it seems likely, as suggested by Turner and Brittain (29), that early studies of O₂ inhibition may have been complicated by this factor.

This study, therefore, was undertaken to compare the effect of O₂ on photosynthesis and CO₂ evolution in both high CO₂- and air-grown Chlorella pyrenoidosa.

MATERIALS AND METHODS

C. pyrenoidosa was grown and harvested as described previously (25). When high CO₂-grown cells were required, an air stream enriched with 2% CO₂ was bubbled through the culture at a rate of 34 ml min⁻¹.

Photosynthetic CO₂ fixation was determined in an open gas analysis system (20), using the artificial leaf technique as previously described (16, 25). Determination and calculation of TPS,² APS and PR using the ¹⁴CO₂/¹³CO₂ technique are well established (18).

RESULTS

In the first experiment, high CO₂-grown C. pyrenoidosa was allowed to equilibrate in the open gas analysis system for 1 h under 0.5% CO₂ (this CO₂ level was chosen to maintain high CO₂ condition but still allow rapid conversion to lower levels of CO₂), before photosynthetic CO₂ fixation was measured under air levels of CO₂ (Fig. 1). The rate of photosynthesis was initially low but increased as the cells became adapted to air levels of CO₂ (Fig. 1). After 100 min, the photosynthetic rate was similar to that found with air-grown Chlorella under comparable experimental conditions (25). This phenomenon has been noted previously (10) and is generally associated with the induction of carbonic anhydrase activity (8, 12, 22).

A marked influence of O₂ on photosynthesis was observed in the high CO₂ algae after the CO₂ level was switched to 350 μl l⁻¹ (11.7 μM) (Fig. 1). CO₂ fixation at 2 and 50% O₂ was 164 and 53%, respectively, of the rate obtained just previously in 21% O₂. The rate of photosynthesis increased with time and would probably lead to an overestimate of the stimulation by 2% O₂ and an underestimate of the inhibition by 50%. The magnitude of the effect of O₂ on photosynthesis declined with time and increasing photosynthesis, suggesting that adaptation occurred during the experiment.

When measurements were taken immediately after transfer of the high CO₂ cells to a range of lower CO₂ concentrations, photosynthesis was always influenced by O₂ (Fig. 2). The magnitude of the effect appeared to decrease with increasing CO₂, although, once again, the exact magnitude was difficult to determine because of the continuously increasing rate of photosynthesis. The response of CO₂ fixation to O₂ was reduced when measurements were taken 40 min after transfer of the algae to low CO₂ levels (Fig. 3).

A net evolution (8.5 μmol CO₂ mg⁻¹ Chl h⁻¹; recorder trace not shown) of CO₂ occurred at 200 μl l⁻¹ (6.7 μM) CO₂, while a net uptake of CO₂ occurred at 250 μl l⁻¹ (8.4 μM) CO₂ (Fig. 2). Thus, the CO₂ compensation point appeared to be in excess of 200 μl l⁻¹, a value higher than those previously reported for high CO₂ Chlorella (45 μl l⁻¹ at 20°C) (28) and Scenedesmus (55 μl l⁻¹ at 25°C) (9). The rapidly changing photosynthetic rates precluded any estimation of the O₂ sensitivity of the compensation point.

In air-grown Chlorella, CO₂ evolution into CO₂-free air in the

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² Abbreviations: TPS, true photosynthesis; APS, apparent photosynthesis; PR, photorespiration; RuBP, ribulose 1,5-bisphosphate; FEP, phosphoenolpyruvate.
light was less than it was in the dark, and it was insensitive to CO2 (16). At pH 5.0, where active HCO3− uptake probably does not influence the final CO2 compensation concentration (see "Discussion" below), the compensation point was low (10.7 µl l−1) (26) and, therefore, relatively unaffected by the amount of residual dark respiration which might continue in the light. Similarly, dark respiration of this magnitude in the light does not seem likely to be the entire explanation for the enhanced CO2 compensation point in high CO2-grown cells. Together, these observations suggest that photosynthetic CO2 fixation in high CO2-grown Chlorella is inhibited by O2 and that this inhibition is competitive and similar in magnitude to that in higher C3 plants.

Using the 14CO2/12CO2 technique (18), CO2 evolution during photosynthesis was not evident in several species of air-grown algae, including *C. pyrenoidosa* (16). However, in these experiments, the pH of the resuspension medium was likely in the alkaline range. Under this condition, evolved CO2 could be converted immediately to HCO3− and be reassimilated via a pump (2, 9), thus preventing the measure of CO2 evolution. Consequently, photorespiration was measured in both air and high CO2-grown algae with the 14CO2/12CO2 technique using the artificial leaf at pH 5.0. The low pH should reduce any conversion or reassimilation problem. For these experiments, it was desirable also to increase the amount of CO2 depletion and increase the amount of 14CO2 uptake. Therefore, the Chl concentration was increased from previous experiments involving the artificial leaf (Figs. 1–3) (25, 26). It has been shown that CO2 depletion is not linear with Chl concentration or cell layers (24). This result was apparently due to shading, since, in the aqueous system (25) where light limitation would not be a factor, CO2 depletion was linear up to at least 360 µg Chl (24). Thus, while the photosynthetic rates were lower than previously (Figs. 1–3) (25, 26), the magnitude of the response to O2 was unchanged (Table I). Photosynthesis by air-grown cells at 2 and 50% O2 was 109% and 94%, respectively, of that in 21% O2 (Table I), values similar to those found earlier (26). Once again, high CO2-grown cells exhibited, in contrast to air-grown algae, a larger influence of O2 on CO2 fixation (Table

**Fig. 1.** The influence of time and O2 on photosynthetic rates of high CO2-grown *C. pyrenoidosa* at air levels of CO2. Experiments were conducted using the artificial leaf technique at 25°C, quantum flux density of 400 µE m−2 s−1, an inlet CO2 concentration of 350 µl l−1 (11.7 µk), and about 100 µg Chl. Measurements began at the experimental CO2 concentration after a 1-h equilibration period at 0.5% CO2. Rates of CO2 fixation at 21% O2 (bottom) were taken before and after each change of O2 concentration. Rates at 2 or 50% O2 (top) are expressed as a percentage of the previous rate at 21% O2.

**Fig. 2.** Recorder tracings of the influence of O2 on photosynthetic rates by high CO2-grown *C. pyrenoidosa* at different CO2 concentrations. Measurements began immediately after transfer to CO2 levels shown above each trace. Times of changing O2 concentrations are shown; the initial rate of photosynthesis was always taken in 21% O2. Control rates (21% O2) changed with time and are shown in brackets; rates at each O2 are expressed as a percentage of the previous control rate.

**Fig. 3.** Recorder tracings of the influence of O2 on photosynthetic rates by high CO2-grown *C. pyrenoidosa* at different CO2 levels. Measurements began 40 min after transfer to the CO2 levels shown. Remainder of explanation is same as in Figure 2 legend.
DISCUSSION

Gas exchange measurements of high CO₂-grown Chlorella were conducted with the same open-gas analysis system used previously for air-grown cells (25, 26). The algal cells were suspended on the artificial leaf and equilibrated at 0.5% CO₂ to minimize adaptation to low levels of CO₂. In addition, all of the experiments reported here, including those with air-grown algae, were performed at pH 5.0 to reduce the influence of active HCO₃⁻ uptake and the possibility that evolved CO₂ is immediately converted to HCO₃⁻ and reassimilated.

High CO₂-grown Chlorella exhibited a marked effect of O₂ on net photosynthesis, which appeared to be competitive with respect to CO₂ (Figs. 1 and 2) and similar in magnitude to that in higher C₃ plants (6). The response to O₂ decreased as cells became air-adapted. The magnitude and nature of this effect (Table I) are in contrast to those observed with air-grown cells, which are small and not affected by CO₂ (Table I) (26). Bowes and Berry (5) have also reported a competitive inhibition of CO₂ fixation by O₂ in Chlamydomonas.

Measurements of photorespiration using the ¹⁴CO₂/¹²CO₂ technique indicated that high CO₂-grown cells evolved a much greater amount of CO₂ (47% TPS at 21% O₂) than did air-grown cells (14% TPS) (Table II) (16). Photorespiration in air-grown cells was comparable to the 16% TPS value determined for higher C₃ plants (14, 19). In high CO₂ algae, the CO₂ evolution was O₂-sensitive (Table II), whereas, in air-grown algae, it was O₂-insensitive (Table II) (16), indicating that CO₂ evolution in air-grown algae is probably different from that in high CO₂ algae and higher C₃ plants.

Since O₂ did not affect TPS (Table II), photorespiration accounted for 100% of the O₂ inhibition of photosynthesis. Thus, in contrast to higher C₃ plants (6, 7, 14, 19), the effect of O₂ on high CO₂-grown algae appeared to be competitive due to a stimulation of CO₂ evolution without the accompanying inhibition of true photosynthesis.

The difference in photorespiration between high CO₂ and air-grown algae may be due to differences in the refixation of evolved CO₂, but the ratio of RuBP carboxylase to PEP carboxylase (21, 22) and the analysis of early photosynthetic products (11, 13) suggested a lack of involvement of PEP carboxylase and C₄-type photosynthesis in recycling CO₂ in Chlorella.

Work by Tolbert and coworkers (27) has indicated that glycerate metabolism, rather than glycolate metabolism, contributes relatively more serine in high CO₂-grown Chlorella and Chlamydomonas than it does in higher C₃ plants. Low levels of serine hydroxymethyl transferase supported this interpretation and further suggested that the glycine-to-serine conversion was limiting in these algae (27). Whether the glycine-to-serine conversion is different in high CO₂- and air-grown algae remains unanswered.

According to the current scheme of photorespiration (6, 23, 27), CO₂ evolution is a consequence of glycolate synthesis. Purified RuBP carboxylase isolated from Chlorella (17) and Anabaena (1) have been shown to possess oxygenase activity, but active HCO₃⁻ uptake has been postulated to suppress oxygenase activity in air-grown algae (2, 5, 9, 21). However, with the external pH used in this study, HCO₃⁻ uptake at the plasma membrane was unlikely to occur (25). If active transport of HCO₃⁻ occurred at the chloroplast membrane (21), the CO₂ concentration at the RuBP carboxylase site and, thus, true photosynthesis would be expected to be higher in air-grown than in high CO₂-grown algae. True photosynthesis was, however, similar in both cell types (Table II), indicating that HCO₃⁻ uptake did not appear to play a role in supporting photosynthesis or in controlling photorespiration. Since O₂ did not influence true photosynthesis, it would seem that glycolate synthesis and the effect of O₂ on CO₂ evolution may not be mediated by the oxygenase reaction.

Whatever the mechanism for the reduced response of true

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### Table I. Rates of Net Photosynthesis of Air- and High CO₂-grown C. pyrenoidosa under Three O₂ Concentrations

<table>
<thead>
<tr>
<th>Growth Condition</th>
<th>Net Photosynthesis</th>
<th>2% Oxygen</th>
<th>21% Oxygen</th>
<th>50% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol CO₂ mg⁻¹ Chl h⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td></td>
<td>30.1 ± 0.4 (109)</td>
<td>27.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>27.9 ± 0.6</td>
<td>26.2 ± 0.7 (94)</td>
<td></td>
</tr>
<tr>
<td>High CO₂</td>
<td>1</td>
<td>15.3 ± 0.4 (14)</td>
<td>10.3 ± 0.4</td>
<td>7.2 ± 0.4 (70)</td>
</tr>
</tbody>
</table>

* Mean ± se (n = 10).

**Table II. Influence of O₂ Upon TPS, APS and PR in Air and High CO₂-grown C. pyrenoidosa**

<table>
<thead>
<tr>
<th>Growth Condition</th>
<th>O₂</th>
<th>TPS</th>
<th>APS</th>
<th>PR</th>
<th>PR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>µmol CO₂ mg⁻¹ Chl h⁻¹</td>
<td>%</td>
<td>TPS</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>21</td>
<td>27.4 ± 1.4*</td>
<td>23.6 ± 1.2</td>
<td>3.8 ± 1.0</td>
<td>14</td>
</tr>
<tr>
<td>50</td>
<td>32.8 ± 1.4</td>
<td>26.3 ± 0.2</td>
<td>6.5 ± 1.3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>High CO₂</td>
<td>2</td>
<td>31.2 ± 1.9</td>
<td>30.0 ± 1.8</td>
<td>1.2 ± 2.0</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>27.8 ± 1.0</td>
<td>14.6 ± 0.3</td>
<td>13.1 ± 1.0</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>29.0 ± 2.2</td>
<td>11.1 ± 0.3</td>
<td>17.9 ± 2.2</td>
<td>62</td>
<td></td>
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</tbody>
</table>

* Mean ± se (n = 10).
photosynthesis to O2 in algal cells, the differences in net photosynthesis between high CO2- and air-grown cells appears to be due to CO2 evolution. Whether the reduction in CO2 evolution in air-grown algae is due to a reduction in glycolate synthesis, a limitation in the glycine to serine conversion or a refixation of the evolved CO2 remains to be clarified. Carbon flux experiments (23) should provide important information about these options.

LITERATURE CITED