Utilization of Exogenous Inorganic Carbon Species in Photosynthesis by *Chlorella pyrenoidosa* \(^1\)

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**ABSTRACT**

The nature of the inorganic carbon utilized during photosynthesis by *Chlorella pyrenoidosa* was investigated using three experimental techniques (open gas analysis system with "artificial leaf" or "aqueous" chambers and O\(_2\) electrode system) to measure carbon assimilation. Photosynthesis was studied as a function of pH and CO\(_2\) concentration. The CO\(_2\) concentration was inadequate to meet the requirements of photosynthesis only when HCO\(_3^-\) was added at high pH. Under all other conditions, the low and constant \(K_m\) (CO\(_2\)), in contrast to the highly variable \(K_m\) (HCO\(_3^-\)), suggested that CO\(_2\) was the major species utilized.

Higher rates of photosynthesis were observed under limiting CO\(_2\) conditions above pH 7.5 but rates of hydration of CO\(_2\) were not consistent with the view that the stimulation in photosynthesis was supported by HCO\(_3^-\). In the same pH region lower rates of photosynthesis were observed under saturating CO\(_2\) conditions. These conflicting changes seemed not to be related to pH but to some as yet undetermined effect of bicarbonate. No support was obtained for the view that the quantum efficiency was different under conditions where CO\(_2\) was assimilated compared to conditions where CO\(_2\) and HCO\(_3^-\) could be assimilated, although at saturating CO\(_2\), lower maximal rates of photosynthesis were observed in the latter conditions.

As air-grown *Chlorella* do not exhibit any characteristics of photorespiration it is important to determine whether it can utilize HCO\(_3^-\) and whether this HCO\(_3^-\) uptake is sufficient to suppress photorespiration. Here, we report on the nature of the inorganic carbon sources used by *Chlorella*, using three experimental systems to measure carbon assimilation.

**MATERIALS AND METHODS**

**Plant Material.** Axenic cultures of *Chlorella pyrenoidosa* Chick (strain 252), obtained from the Culture Collection of Algae at the University of Texas (Austin) were grown in a semicontinuous system (36) in 6-liter Erlenmeyer flasks containing 3 liters Gorham's culture medium No. 11 (19). Cultures were maintained in a growth cabinet (model 16S, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) at a temperature of 25 C, illuminated continuously with a quartz flux density of 185-235 \(\mu\)E m\(^{-2}\) s\(^{-1}\). An air stream was bubbled through the continuously stirred culture at a rate of 790 ml min\(^{-1}\). Cultures were harvested during exponential growth, centrifuged at 6000g for 10 min, washed with deionized H\(_2\)O, resuspended in 25 ml of the appropriate medium (except where stated otherwise), and stored at room temperature.

**Gas Exchange Techniques.** Photosynthetic CO\(_2\) fixation was determined in the open gas analysis system described by Canvin (10) using both the artificial leaf technique of Lloyd et al. (22) and an aqueous method which allowed measurements to be obtained from algal cells suspended in liquid medium. For the artificial leaf, cells equivalent to 100 \(\mu\)g Chl were suspended on 16 cm\(^2\) of filter paper (Whatman No. 3MM) and the rate of gas flow over the leaf was 235 ml min\(^{-1}\).

The photosynthesis chamber (Fig. 1) used for the aqueous method was made of Plexiglas and consisted of: (a) the main section housing a thin inner sample chamber (6 mm thick \(\times\) 116 mm wide \(\times\) 112 mm high to give a total volume of 78 ml) with outlet and thermocouple ports (brass machined); and (b) an end section which when attached to the main section by 10 inset screws (separated by a rubber gasket prepared from an inner tube), covered the end of the sample chamber. The chamber had water jackets on both sides to assist in temperature control. The gas mixture was passed at a flow rate of 1.08 liters min\(^{-1}\) through the end into the sample chamber via a course sintered glass tubular filter (15.8 mm dia \(\times\) 102 mm length of fritted section (171 mm total length), Corning Glass Works, Corning, New York) which fitted securely into the base of the inner chamber (prepared specifically to fit the filter tightly against a piece of rubber). The filter was changed after every set of measurements on a single sample of algae. The assembled chamber was supported with retort stands and the algal suspension (360 \(\mu\)g Chl in 25 ml) was illuminated from one side.

The algae were permitted to equilibrate in the open systems at a temperature of 25 C, a carbon flux density of 400 \(\mu\)E m\(^{-2}\) s\(^{-1}\), a RH in excess of 90%, and an inlet CO\(_2\) concentration of 350 \(\mu\)l 1\(^{-1}\) (11.7 \(\mu\)M dissolved CO\(_2\)). Measurements were started when the

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A concentration of Mes buffer higher than 25 mM was necessary to maintain pH 5 in the aqueous system but no difference in photosynthetic rates were observed between cells suspended in 0.05 and 0.1 M Tricine at pH 8.5. Subsequently, cells were suspended in 0.1 M buffer at the appropriate pH. Of the buffers tested (Mes, citrate, Tes, Mops, Hepes, Taps, Tricine, Caps, glycine), only citrate and Caps substantially reduced the rate of CO2 fixation. The presence of growth medium with the buffer and the order of exposure to CO2 concentrations had no effect on the CO2 response curves for photosynthesis.

Since the ratio of CO2 to HCO3- present in solution is altered by changing pH, the measurement of photosynthesis as a function of inorganic carbon concentration and pH allows one to infer which form (CO2 or HCO3-) is utilized in photosynthesis (4, 30). Rates of photosynthesis of Chlorella were measured as a function of pH at different CO2 or added HCO3- (total inorganic carbon) concentrations (Figs. 2-4). In all experimental methodologies, the photosynthetic rates at saturating or near saturating CO2 concentrations were higher at pH 5.0 than at pH 8.5. At CO2 concentrations equal or below atmospheric levels (10 μM) CO2 fixation was greatest at pH 8.5.

CO2 compensation points, determined by interpolation to the point of zero net photosynthesis (22), were about 0.3 μM (Table I) regardless of the pH, except where CA was added. When CA was added the compensation point declined to about 0.11 μM CO2, though the variation was large in the case of the aqueous system (Table I). In the O2 electrode system, the photosynthetic rate extrapolated to zero CO2. CO2 evolution into CO2-free air in the light (22) was low, amounting in most cases to less than 10% of the photosynthetic rate under normal atmospheric conditions (Table I).

Assuming full equilibration of CO2 and HCO3-, the Michaelis-Menten kinetic parameters for photosynthesis (Vmax, the maximum rate of photosynthesis; Km, the concentration of CO2 at which the rate of photosynthesis attains one-half of its maximum value) were calculated using nonlinear regression analysis according to Wilkinson (39) (Table II). In the open systems, the Km (CO2) remained fairly constant over the pH range 5.0-8.5, al-

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**RESULTS**

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**FIG. 1.** Side view of chamber for determining photosynthesis of algae in liquid suspensions using the open gas analysis system. A: main section; B: end section; C: inner sample chamber (volume 6 mm × 116 mm × 112 mm = 78 cm³); D: water jacket (only front water jacket is shown); E: gas entrance; F: gas exit; G: sintered glass tubular filter; H: water inlet port; I: water outlet port; J: thermocouple port; K: sample removal port (normally clamped off); L: rubber “O” ring seals; M: rubber piece; N: rubber gasket; O: inset screws; (→) shows direction of gas flow.

CO2 depletion on the trace remained constant, which was about 1 h after the algae had been placed in the leaf chamber, and continued for a period of 2 h. All experiments were conducted at 21% O2, pH on the artificial leaf was determined with a combination pH microelectrode (Bio-Rad).

Photosynthesis was also measured as O2 evolution in an O2 electrode chamber (Yellow Springs Instruments, model 5331) using a light intensity of 600 μE m⁻² s⁻¹ provided by a projector (Hanimex 500). A constant temperature circulator bath maintained the cuvette temperature at 25 °C. Assays were performed in a total volume of 2.0 ml which included 100 μl Chlorella (20 μg Chl) in the appropriate 50 mM buffer and pH. These buffers were prepared CO2-free as described by Lorimer et al. (23). After the algae depleted the endogenous CO2 (contained in the medium or as a pool in the cells [4]) the background rate of O2 evolution, which remained constant for periods of at least 1 h, was typically 4.5 μmol O2 mg⁻¹ Chl h⁻¹. Since similar rates of HCO3-—dependent O2 evolution were obtained at any time during this period, HCO3- was routinely added 5-10 min after the CO2 was depleted.

The concentrated algal cells (1 μg Chl/5 μl) were stored at room temperature in deionized H2O and bubbled with air. They maintained their photosynthetic rate over an 8-h period.

HCO3- and CO2 concentrations in the open and O2 electrode systems were calculated according to Buch (9). Kinetic constants were calculated using a nonlinear regression computer program following the methods of Wilkinson (39). Chl was extracted with boiling 100% methanol and MgCO3 and the concentration determined using the extinction coefficients for Chl a and b (18).

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**FIG. 2.** CO2 response curves for C. pyrenoidosa photosynthesis as a function of pH using artificial leaf technique in the open gas analysis system. Measurements were conducted at 25 °C, a quantum flux density of 400 μE m⁻² s⁻¹ and 21% O2. Mes, Hepes, and Taps buffers (0.1 M) were used at pH 5.0, 7.0, and 8.5, respectively. [CO2] represents the average dissolved CO2 concentration. Bars are ±SE of mean with the number of measurements shown beside each point. (●): measurements where sample number was less than 3. (□): fitted according to Wilkinson (39).

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2 Abbreviations: Mops: morpholinopropane sulfonic acid; Taps: tris(hydroxymethyl)methylaminopropansulfonic acid; Caps: cyclohexylaminopropansulfonic acid; CA: carbonic anhydrase.

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though when CA was added in the aqueous technique, the $K_m$ (CO$_2$) decreased to a value of 3.2 μM as compared to 11.9 μM at pH 5.0. In contrast, the $K_m$ (HCO$_3^-$) changed by several hundred-fold when the pH was changed from 5 to 8.5. When O$_2$ evolution as a function of pH was examined in the closed system, the $K_m$ (CO$_2$) decreased and the $K_m$ (HCO$_3^-$) increased with increasing pH, but the change in $K_m$ (CO$_2$) was much less than the change in $K_m$ (HCO$_3^-$). All of these results suggest that CO$_2$ was the carbon species utilized in photosynthesis (4, 30) although there may be some contribution from HCO$_3^-$ at the high pH, especially in the O$_2$ electrode system, where the inorganic carbon was added as HCO$_3^-$.

The addition of CA, which facilitates the establishment of the equilibrium between CO$_2$ and HCO$_3^-$, appeared to cause a reduction in the $K_m$ (CO$_2$) (Table II). The effect of CA addition was investigated further using the artificial leaf with the cells suspended in buffer and in fresh medium plus buffer. (Table III). Without CA, the $K_m$ (CO$_2$) in each resuspension condition was the same at pH 7 and 8.5 whereas the $K_m$ (HCO$_3^-$) changed over 20-fold. With CA addition, the change in the $K_m$ (CO$_2$) between pH 7 and 8.5 was again much less than the change in $K_m$ (HCO$_3^-$).

These results are again consistent with CO$_2$ being the carbon species utilized in photosynthesis (4). Both the $K_m$ (CO$_2$) and $K_m$ (HCO$_3^-$) were reduced by the addition of CA (Table III). The stimulation of photosynthesis at low CO$_2$ concentrations by addition of CA to the suspension medium has been noticed before (5, 25) and in one case (5) it was suggested that the cells were using HCO$_3^-$ rather than CO$_2$. With CA present, the HCO$_3^-$ concentration may be higher and the utilization of HCO$_3^-$ may be increased, thereby resulting in a decrease in the $K_m$ (CO$_2$) and $K_m$ (HCO$_3^-$). This possible explanation rests on the assumption, that without CA, the system was far out of equilibrium and the HCO$_3^-$ concentration present was not that shown (Table III) but was less than that observed when CA was present. Although the system without CA may not be in equilibrium, the large change in $K_m$ (HCO$_3^-$) between pH 7 and 8.5 suggests that any differences in HCO$_3^-$ concentration brought about by the addition of CA

### Table I. Compensation Points and CO$_2$ Evolution Into CO$_2$-Free Air in the Light by C. pyrenoidosa

<table>
<thead>
<tr>
<th>pH</th>
<th>CO$_2$ Evolution</th>
<th>Compensation Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>6.4 ± 1.4 (7)*</td>
<td>0.36 ± 0.14 (10)</td>
</tr>
<tr>
<td>7.0</td>
<td>7.8 ± 1.7 (7)</td>
<td>0.32 ± 0.08 (9)</td>
</tr>
<tr>
<td>8.5</td>
<td>7.2 ± 1.9 (8)</td>
<td>0.29 ± 0.07 (10)</td>
</tr>
<tr>
<td>8.5 + CA*</td>
<td>5.1 ± 1.1 (4)</td>
<td>0.12 ± 0.03 (4)</td>
</tr>
<tr>
<td>Aqueous technique</td>
<td>5.0</td>
<td>7.1 (1)</td>
</tr>
<tr>
<td></td>
<td>8.5 + CA*</td>
<td>6.1 ± 1.9 (4)</td>
</tr>
</tbody>
</table>

* Wilbur-Anderson units (260) of CA from bovine erythrocytes were added to the resuspension medium.

### Table II. Influence of pH upon Kinetic Parameters of Photosynthetic Carbon Dioxide Fixation and Oxygen Evolution by C. pyrenoidosa

<table>
<thead>
<tr>
<th>pH</th>
<th>$K_m$ (Total)</th>
<th>$K_m$ (HCO$_3^-$)</th>
<th>$K_m$ (CO$_2$)</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>10.7</td>
<td>0.5</td>
<td>10.2 ± 2.9*</td>
<td>177.3 ± 3.6b</td>
</tr>
<tr>
<td>7.0</td>
<td>48.1</td>
<td>39.3</td>
<td>8.8 ± 0.9</td>
<td>181.5 ± 5.9b</td>
</tr>
<tr>
<td>8.5</td>
<td>765.3</td>
<td>743.1</td>
<td>5.4 ± 0.6</td>
<td>148.2 ± 4.9b</td>
</tr>
<tr>
<td>Aqueous technique</td>
<td>5.0</td>
<td>12.4</td>
<td>0.5</td>
<td>11.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>8.5 + CA*</td>
<td>439.3</td>
<td>3.2 ± 0.7</td>
<td>164.0 ± 9.7b</td>
</tr>
<tr>
<td>Oxygen electrode</td>
<td>5.0</td>
<td>22.5 ± 1.6</td>
<td>0.8</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>97.7 ± 0.0</td>
<td>9.49</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Kinetic constants were calculated from data in Figures 2-4, according to Wilkinson (39); compensation point was subtracted from the CO$_2$ concentration before calculation.

b Mean ± SD.

c Numbers in parentheses represent the sample number.

d Equivalent to 10.7 µl $^{-1}$ CO$_2$ in the gas phase.

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**Figure 3.** CO$_2$ response curves for *C. pyrenoidosa* photosynthesis as a function of pH using the aqueous technique in the open gas analysis system. Measuring conditions and explanations as for Figure 2. CA (260 Wilbur-Anderson units) from bovine erythrocytes (Sigma) were added to experiments at pH 8.5.

**Figure 4.** Bicarbonate-dependent O$_2$ evolution for *C. pyrenoidosa* as a function of pH. Measurements were performed at 25 C, 600 μE m$^{-2}$ s$^{-1}$ and an O$_2$ concentration of 18-21% using a sealed O$_2$ electrode system. The background rate was subtracted from the HCO$_3^-$-dependent rate of O$_2$ evolution. [Total inorganic carbon] represents the concentration of added HCO$_3^-$; the [CO$_2$] in equilibrium with the HCO$_3^-$ is shown for comparative purposes. Remainder of explanation as for Figure 2.
Table III. Influence of Addition of CA to the Resuspension Medium upon Kinetic Parameters of Subsequent Photosynthetic Rates by C. pyrenoidosa Using the Artificial Leaf

Measurements were performed at 25 C and 400 µE m⁻² s⁻¹. Experiments on ± CA conditions were performed on the same day. Remainder of explanation as for Table II.

<table>
<thead>
<tr>
<th>Resuspension Conditions</th>
<th>( K_{0.1}^* ) (Total)</th>
<th>( K_{0.2}^* ) (HCO₃⁻)</th>
<th>( K_{0.3}^* ) (CO₂)</th>
<th>( V_{max}^* ) (umol CO₂ mg⁻¹ Chl h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>µM</td>
<td>µmol CO₂ mg⁻¹ Chl h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepe (pH 7.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CA</td>
<td>29.0</td>
<td>5.3 ± 0.6</td>
<td>105.0 ± 31</td>
<td></td>
</tr>
<tr>
<td>+ CA</td>
<td>19.7</td>
<td>3.6 ± 0.4</td>
<td>118.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Taps (pH 8.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CA</td>
<td>609.9</td>
<td>4.3 ± 0.4</td>
<td>110.7 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>+ CA</td>
<td>255.3</td>
<td>1.8 ± 0.2</td>
<td>105.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Fresh medium buffer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mops (pH 7.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CA</td>
<td>47.0</td>
<td>8.6 ± 1.5</td>
<td>162.6 ± 10.1</td>
<td></td>
</tr>
<tr>
<td>+ CA</td>
<td>33.3</td>
<td>6.1 ± 0.9</td>
<td>178.3 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>Tricine (pH 8.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CA</td>
<td>1276.5</td>
<td>9.0 ± 1.3</td>
<td>210.0 ± 10.5</td>
<td></td>
</tr>
<tr>
<td>+ CA</td>
<td>283.7</td>
<td>2.0 ± 0.1</td>
<td>178.0 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

* Eight to 11 different CO₂ concentrations with a total sample number ranging from 11 to 22, were used to calculate the kinetic constants; compensation point was subtracted from the CO₂ concentration before calculation.

(evenly at pH 8.5) are not likely to have much effect on photosynthesis. An alternative explanation for the effect of CA addition to the medium has been presented by Shiraiwa and Miyachi (34). They propose that in regions of CO₂ depletion CA can increase the CO₂ concentration by indirect supply of CO₂ from the HCO₃⁻ present. The net effect of the combination of direct and indirect CO₂ supply is that the apparent concentration of CO₂ required is decreased. This explanation would be consistent with our observations.

Another way of evaluating the relative importance of CO₂ or HCO₃⁻ to photosynthesis is to compare the rate of formation of the species not added to the rate of photosynthesis. For the data on photosynthesis at pH 8.5 in Figure 2, where CO₂ was added, the rate of formation of HCO₃⁻ can be compared to the photosynthetic rate. To calculate the production of HCO₃⁻, the rate constant of Watt and Pasche (37) was used which included both water and OH⁻ catalyzed HCO₃⁻ formation (21). For the data on photosynthesis at pH 8.5 (Fig. 4) where HCO₃⁻ was added, the rate at which CO₂ could be produced was calculated using the equations derived by Lucas (24) using constants for freshwater after Buch (9). The calculated rates would be maximum rates as the solutions involved the assumption that all CO₂ and HCO₃⁻ produced would be consumed, thus keeping their concentrations at zero.

The rates of hydration and dehydration together with the photosynthetic rates are shown in Figure 5. In the artificial leaf, HCO₃⁻ production was clearly insufficient to maintain photosynthesis. In the O₂ electrode, CO₂ production was sufficient to maintain photosynthesis above 0.5 mm total carbon. Below that concentration CO₂ production was not sufficient, suggesting that HCO₃⁻ may also be utilized.

When photosynthetic rate and the hydration rate of CO₂ are determined over the pH range 5–10.5, it is clear that the rate of production of HCO₃⁻ is not sufficient to maintain photosynthesis (Fig. 6). In contrast the rate of production of CO₂ from HCO₃⁻ was sufficient to maintain photosynthesis at all pH values below pH 8.5 (Fig. 7). Above this pH photosynthesis would appear to depend on HCO₃⁻ utilization (Fig. 7). At saturating inorganic carbon concentrations, photosynthesis was depressed at the higher pH values (Figs. 6A and 7). It seems unlikely that this is a direct effect of pH on photosynthesis because at limiting CO₂ concentrations, a stimulation of photosynthesis at higher pH values was observed (Fig. 6B). It is tempting to attribute the stimulation in the rate of photosynthesis observed above pH 7.5 (Fig. 6B) to the increased utilization of HCO₃⁻ in addition to CO₂, but HCO₃⁻ production was not sufficient to accomodate the increase in pho-
electrode system these drying, HCO$_3^-$.

FIG. 7. Rates of dehydration (----) and photosynthesis of *C. pyrenoidosa* (- - -) as a function of pH. Measurements were performed at 25 C, 600 $\mu$E m$^{-2}$ s$^{-1}$, and 21% $O_2$ by addition of 0.5 mM HCO$_3^-$ to the $O_2$ electrode. The background rate of $O_2$ evolution was subtracted from the HCO$_3^-$-dependent rate. (O): Mes; (>): Hepes; (A): Taps. Errors quoted are the se of the mean. Sample number was 6-10 for each pH.

tosynthetic rate.

At saturating CO$_2$ concentrations, photosynthesis was higher under conditions where only CO$_2$ was available than under conditions where both CO$_2$ and HCO$_3^-$ was available (Figs. 6A and 7). Other authors, who have also observed a similar phenomenon, suggested from light intensity experiments, that the lower rate of photosynthesis in the presence of HCO$_3^-$ indicates that more light was required to fix a mole of carbon supplied as HCO$_3^-$ than was needed for a mole supplied as CO$_2$ (24, 29). For the conditions used in this study, it was found that especially at high light intensities, photosynthesis was greater when CO$_2$ was supplied (Fig. 8). However, photosynthesis was saturated at a light intensity of 400 $\mu$E m$^{-2}$ s$^{-1}$ at both pH values and in the region of limiting light similar quantum efficiencies were observed. The quantum efficiencies calculated on the basis of incident light (0.02 mol CO$_2$ F$^{-1}$) were low, probably because all of the light was not absorbed by the thin layer of algae.

DISCUSSION

Investigation of photosynthetic rates as a function of pH and several CO$_2$ concentrations in the open gas analysis system (Figs. 2 and 3 and Table II) show that comparable results were obtained using the artificial leaf and aqueous techniques. There is no evidence that results with the artificial leaf are unreliable due to drying, pH changes, or limitation in CO$_2$ diffusion during the course of the experiments. Measurements on photosynthesis in these two systems were comparable to those obtained with an $O_2$ electrode system where the inorganic carbon was supplied as HCO$_3^-$.

With the three systems, the response of photosynthesis to inorganic carbon concentration as a function of pH (Figs. 2-4) indicated that in most circumstances CO$_2$ and not HCO$_3^-$ was the species taken up by the cells to be used in photosynthesis. The $K_m$ (CO$_2$) usually ranged from about 2 to 12 $\mu$M regardless of the pH, and this $K_m$ was similar to that found for ribulose-1,5-bisP carboxylase activity in isolated chloroplasts (20) and purified enzyme preparations (23). Only in the $O_2$ electrode measurements where the inorganic carbon was added as HCO$_3^-$ did the $K_m$ (CO$_2$) fall outside those values. In contrast to the observed stability of the $K_m$ (CO$_2$), the $K_m$ (HCO$_3^-$) changed many hundred fold from pH 5 to 8.5 (Table II). Such changes in the observed affinity of the cells for the two inorganic carbon species are more consistent with CO$_2$ being the species utilized (4, 30). Earlier work (8, 12, 25-27, 38) also indicated a low $K_m$ (CO$_2$) for *Chlorella* photosynthesis and a lack of dependence of photosynthesis on HCO$_3^-$ concentration. These authors had also concluded that CO$_2$ was the carbon species utilized by *Chlorella*. This conclusion is further supported in the present work, because when CO$_2$ was supplied to the cells, the rate of formation of HCO$_3^-$ in all conditions was not adequate to supply the carbon fixed in photosynthesis (Figs 5 and 6).

Conclusions that HCO$_3^-$ is utilized in addition to CO$_2$ seem to be based either on measuring systems where the inorganic carbon was added as HCO$_3^-$ (4, 14) or on the stimulation of photosynthesis at low CO$_2$ concentrations that is observed upon the addition of CA to the suspending medium (5). Findenegg (14) observed that the photosynthetic rate of *Chlorella fusca* as a function of pH did not follow the calculated rate of dehydration of carbonic acid. At total carbon concentrations below 0.5 mm, we confirm that CO$_2$ production from HCO$_3^-$ was not adequate to sustain the rate of photosynthesis (Fig. 5) and under these conditions HCO$_3^-$ absorption by the cells may be a source of CO$_2$ for photosynthesis. If HCO$_3^-$ is utilized by the algae it cannot be used very effectively as the apparent affinity for HCO$_3^-$ above pH 6.5 is low as the $K_m$ (HCO$_3^-$) exceeds the $K_m$ (CO$_2$) by severalfold (4; Tables II and III). If the inorganic carbon had been added as CO$_2$ to these systems an opposite conclusion may have been reached, as in that case, the hydration rate of CO$_2$ is much too slow to supply the HCO$_3^-$ that would be required for photosynthesis (Figs. 5 and 6). We also confirm that photosynthesis of *Chlorella* at low concentrations of CO$_2$ and high pH is stimulated by the addition of CA to the suspension medium (Table III). The decrease in $K_m$ values (CO$_2$ or HCO$_3^-$) suggests that, in these circumstances, HCO$_3^-$ utilization supplements CO$_2$ utilization and the resultant higher CO$_2$ concentration in the cell gives rise to higher photosynthetic
rates. At limiting CO₂ concentrations photosynthesis increases above pH 7.5 in the absence of CA (Fig. 6B) and the rate of hydration of CO₂ was not adequate to supply enough HCO₃⁻ to sustain the increase in the photosynthetic rate. Although the role of CA in the external medium can not be explained with certainty, it appears likely that it could function to indirectly supply CO₂ to the cells (24).

The stimulation of photosynthesis at pH values above 7.5 and low CO₂ concentrations does not appear to be due to a direct effect of pH on photosynthesis because at saturating CO₂ concentrations and inhibition of photosynthesis was observed (Figs. 6 and 7). It seems that low HCO₃⁻ concentrations stimulate photosynthesis and high HCO₃⁻ concentrations inhibit photosynthesis but a logical explanation of the observations is not apparent.

This inhibition of photosynthesis at saturating CO₂ and high pH has been observed by Lucas (24) and Raven (30) who suggested that HCO₃⁻ uptake required more light energy than did CO₂ uptake. Raven (30) has suggested from inhibitor studies that a metabolic HCO₃⁻ pump related to a light dependent reaction may be involved; the reaction is dependent on PSI, but independent of ATP. However, these studies show that in the light limiting region there is no difference in the quantum efficiency of CO₂ fixation at pH 5 and 8.5 (Fig. 8). This result is again consistent with the conclusion that CO₂ is the major species of inorganic carbon being utilized at each pH and that HCO₃⁻ if used at all, plays only a minor role.

Chlorella is a C₃ plant based on the ratio of ribulose-1,5-bisP carboxylase to phosphoenolpyruvate carboxylase (11, 31, 32) or the analysis of products after short periods of ¹⁴CO₂ fixation (3, 17). Yet the low CO₂ compensation points, the apparent scarcity of CO₂ evolution into CO₂-free air (Table II and ref. 22) and the apparent lack of sensitivity of photosynthesis to O₂ discussed in an accompanying paper (33) indicate that photorepiration is low or absent. An elegant case, based on studies of low CO₂- and high CO₂-grown algae, has been presented that photorepiration in low CO₂-grown algae is suppressed as a result of HCO₃⁻ accumulation and a subsequent high internal CO₂ concentration (1, 2). If, as this work shows, Chlorella uses CO₂ and has only a low affinity for HCO₃⁻, it will be necessary to examine closely this proposal as an explanation for the apparent lack of photorepiration in air-grown algae.

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