Photosynthetic Utilization of Bicarbonate in Zostera marina Is Reduced by Inhibitors of Mitochondrial ATPase and Electron Transport

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When Zostera marina was irradiated after a period of darkness, initiation of photosynthetic O₂ evolution occurred in two phases. During a lag phase, lasting 4 to 5 min, photosynthesis was supported by a diffusive entry of CO₂. Photosynthesis then rapidly increased to its full rate. Tris buffer, at a concentration of 50 mM, completely inhibited this increase without affecting CO₂-supported photosynthesis during the lag phase. These results verify that the increase in photosynthesis after the lag phase depended on an activation of bicarbonate (HCO₃⁻) utilization through acid zones generated by proton pumps located to the outer cell membrane. In similar experiments, 6.25 μM of the mitochondrial ATPase blocker oligomycin inhibited photosynthetic HCO₃⁻ utilization by more than 60%. Antimycin A, a selective blocker of mitochondrial electron transport, caused a similar inhibition of HCO₃⁻ utilization. Measurements at elevated CO₂ concentrations verified that neither oligomycin nor antimycin interfered with linear photosynthetic electron transport or with CO₂ fixation. Thus, a major part of the ATP used for the generation of acid zones involved in HCO₃⁻ utilization in Z. marina was derived from mitochondrial respiration.

The prerequisites for photosynthetic carbon uptake differ considerably between marine and terrestrial habitats. Unlike the atmosphere, the oceans contain ionic carbon species in addition to CO₂. At the normal pH of seawater (8.0–8.2), the dominant inorganic carbon species is bicarbonate (HCO₃⁻), while CO₂ is present at very low concentrations. The availability of CO₂ is therefore a potential limiting factor for marine photosynthesizers (Raven et al., 1990). However, the majority of the aquatic plants can make use of HCO₃⁻ as a source of CO₂ (Raven, 1997; Axelsson and Beer, 2001). The utilization of HCO₃⁻ can occur via either nonenergy-requiring processes or a CO₂-concentrating mechanism, comprising at least one energy-consuming step (Badger et al., 1994; Giordano et al., 2005). The inorganic carbon can enter both through direct uptake of HCO₃⁻ and after a transformation of HCO₃⁻ to CO₂ outside the plasma membrane; CO₂ then diffuses or is actively transported into the cell (Sültemeyer, 1997). The HCO₃⁻ that is transported into the cell can be transformed to CO₂ in the proximity of Rubisco (Raven, 2003).

Land plants, including angiosperms, have evolved from algae. The closest living aquatic relatives to terrestrial plants are the Charophytes (Karol et al., 2001), which are assumed to have a CO₂-concentrating mechanism located to their outer cell membranes. Thus, the Charophyte Chlara corallina has pericellular acid zones where HCO₃⁻ is transformed to CO₂ for use in photosynthesis (Price and Badger, 1985). These acid zones are accompanied by alkaline zones where calcification takes place, creating protective crusts (McConnaughey, 1991). Internodal cells of C. corallina seem to be dependent on plasma membrane H⁺-ATPase both for the dehydrogenation of HCO₃⁻ to CO₂ in acid zones and for direct uptake of HCO₃⁻, especially at high pH values (Mimura et al., 1993). Chara tomentosa is likely to have ATP-driven proton pumps, which are involved in the photosynthetic utilization of HCO₃⁻ (Ray et al., 2003). Although at least some species of unicellular eustigmatophyte algae appear to need mitochondrial ATP for their HCO₃⁻ utilization (Huertas et al., 2002a, 2002b), photosynthesis is generally assumed to be the ATP source for active HCO₃⁻ utilization in algae (Kaplan and Reinhold, 1999). However, the ATP source for maintenance of the acid zones in C. corallina is not known. Although land plants have evolved in a completely different environment, they have maintained the ability to create acid zones by the release of protons, which aids in their nutrient uptake (Raven, 2000; Shen et al., 2004). As nutrient uptake occurs in nonphotosynthetic parts, these acid zones are obviously supported by mitochondrial ATP.

Seagrasses, like Zostera marina, descend from flowering land plants that have adapted to submerged conditions. These plants have maintained many characteristics of land plants, such as roots and rhizomes, and a gas phase in the lacunae (Hemminga and Duarte, 2000). Z. marina and Zostera noltii are two

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buffered with 50 mM Tris at pH 8.4 (Tris). B, In NSW adjusted to different sufficient medium (Mes; NSW buffered at pH 6.15), and in NSW buffered at pH 8.4 (Tris). B, In NSW adjusted to different pH values, as indicated. Temperature, 15°C; dissolved inorganic carbon concentration, 2.0 mM.

RESULTS

marine angiosperms that depend on active HCO₃⁻ utilization for expressing full photosynthetic rates. This utilization is assumed to occur via an excretion of protons through the outer cell membrane, thus creating acid regions, or compartments, where the conversion of HCO₃⁻ to CO₂ is favored (Hellblom et al., 2001; Mercado et al., 2003). The CO₂ formed there may then be taken up actively or, more likely, diffuse through the plasma membrane. A similar mechanism occurs in many aquatic angiosperms with leaves featuring pH polarity, for example, Potamogeton lucens (Prins et al., 1982; van Ginkel and Prins, 1997). The brackish angiosperm Ruppia cirrhosa has been reported to have substantial photosynthetic rates at pH values as high as 9.5, where almost all carbon is present as HCO₃⁻ and carbonate. Also in this plant, the mechanism for HCO₃⁻ utilization depends on acid compartments, although external carbonic anhydrase activity is involved in the HCO₃⁻ dehydration (Hellblom and Axelsson, 2003). The same mechanism has been suggested for the brown macroalga Laminaria saccharina (Axelsson et al., 2000; Mercado et al., 2006).

Z. marina has characteristics of both terrestrial plants (from which they evolved) and marine algae (with which they share the marine environment). We therefore thought it relevant to examine the role of mitochondrial activity for maintaining photosynthetic rates in Z. marina and in particular in relation to their utilization of HCO₃⁻.

Figure 1. Oxygen electrode tracings by Z. marina before and after onset of irradiation, showing the patterns for induction of O₂ evolution. A, In NSW at pH 8.4 (NSW), in NSW with oligomycin added (Oligo), in CO₂-sufficient medium (Mes; NSW buffered at pH 6.15), and in NSW buffered at pH 8.4 (Tris). B, In NSW adjusted to different pH values, as indicated. Temperature, 15°C; dissolved inorganic carbon concentration, 2.0 mM.

RESULTS

Leaves of Z. marina, dark acclimatized for more than 1 h, were irradiated in natural seawater (NSW; dissolved inorganic carbon, approximately 2.0 mM; pH, approximately 8.4). After an initial period of 4 to 5 min of constant low rate photosynthesis (lag phase), there was a rapid increase to full photosynthetic rate (Fig. 1A, NSW). The duration of this lag phase differed slightly between plants collected at different occasions. In one experiment, the lag phase lasted 4.4 ± 0.23 min, while the half-time of the increase to full photosynthesis was only 1.62 ± 0.48 min (mean ± SD, n = 6; compare Fig. 1A, NSW). When the leaves were irradiated in CO₂-sufficient medium (NSW buffered to pH 6.15), there was no lag at the onset of photosynthesis but only an induction period with a half-time of the increase to full photosynthetic rates of 2.63 ± 0.15 min (mean ± SD, n = 6; compare Fig. 1A, MES).

In NSW adjusted to different pH values, the photosynthetic rate during the lag phase was highest at the lowest pH (Fig. 1B). This rate was proportional to the calculated CO₂ partial pressure of the medium, suggesting that photosynthesis during the lag phase depended on a diffusive uptake of CO₂ (Fig. 2). The maximal rates of photosynthesis varied little with pH and were more or less proportional to the HCO₃⁻ concentration of the medium, except for a slightly higher rate at the lowest pH (6.9). These results indicate that photosynthesis at higher pH was limited by the CO₂ supply from the HCO₃⁻ utilization mechanism and that the higher CO₂ concentration at pH 6.9 was sufficient to abolish this limitation. Despite large differences in photosynthetic rates, the duration of the lag phase was almost the same (no significant differences, compare Fig. 1B). In this experiment, the lag phase lasted between 6.4 ± 0.7 min (pH 7.5) and 5.6 ± 0.4 min (pH 8.9; mean ± SD, n = 5). The presence of Tris buffer reduced photosynthesis to the same level as during the lag phase in nonbuffered seawater of the same pH (Figs. 1A and 3); thus, the difference in rates between lag phase and fully activated photosynthesis disappeared. These results strongly support the earlier

Figure 2. Net photosynthesis (µmol O₂ g⁻¹ h⁻¹ fresh weight) by Z. marina in ASW adjusted to different pH values. Initial (lag) photosynthesis (measured 5 min after light on; black diamonds) and full photosynthesis (white squares). The CO₂ concentration of the medium, calculated according to Millero (1979), using the dissociation constants from Mehrbach et al. (1973), is shown by the broken line. Average of six measurements, bars indicate SD. Temperature, 15°C; dissolved inorganic carbon concentration, 2.0 mM.
DISCUSSION

Upon irradiation of *Z. marina* acclimatized to darkness, initiation of photosynthetic O$_2$ evolution occurred as two phases in our experiments. During a lag phase, there was an almost constant rate of photoproduction under the HCO$_3^-$ pool from the seawater and that both CO$_2$ fixation and photosynthetic electron transport could operate in the presence of these inhibitors. The effect of the inhibitors (obviously still present in the plant tissue) was again visible if the buffered medium was replaced by NSW (which included repeated rinsing of the electrode chamber and sample with NSW; exemplified for oligomycin in Table I).

Neither antimycin nor oligomycin caused any significant inhibition of respiratory rates in darkness (measured as oxygen consumption). To investigate if a block in the mitochondrial electron transport would cause a switch from normal to alternative respiration, the combined effect of antimycin and salicylhydroxamic acid (SHAM; an inhibitor of alternative respiration; Mikulská et al., 1998; Serikawa et al., 2000) was tested. While SHAM alone caused only a small inhibition (11% ± 9%; Table II), the presence of antimycin resulted in a pronounced increase in the inhibition (from 11% to more than 45%; Table II). The inhibition by SHAM was similar at the different concentrations of antimycin. The strong synergetic effect of SHAM and antimycin verifies that antimycin actually inhibits respiration and that the capacity for alternative respiration is substantial in *Z. marina*.

**Figure 3.** Net photosynthesis (μmol O$_2$ g$^{-1}$ h$^{-1}$ fresh weight) by *Z. marina* in NSW after inhibition of the HCO$_3^-$ utilization with 50 mM Tris buffer at two different pH values (black triangles). The value for the initial (lag) photosynthesis in nonbuffered seawater is inserted as a reference. The CO$_2$ concentration of the medium, calculated according to Millero (1979), using the dissociation constants from Mehrbach et al. (1973), is shown by the broken line. Average of six measurements, bars indicate SD, salinity, 30; temperature, 15°C; dissolved inorganic carbon concentration, 2.0 mM.

**Figure 4.** Net photosynthesis (μmol O$_2$ g$^{-1}$ h$^{-1}$ fresh weight) by *Z. marina* measured in NSW, pH 8.4 (white bars), and in NSW with inhibitors of mitochondrial ATP formation added (hatched bars) measured 5 min after light on (lag) and after full induction (full). Also shown: net photosynthesis of the control and inhibited samples after buffering at pH 6.15 with 25 mM MES buffer (Mes). A, Inhibitor oligomycin, added 4.2 h prior to irradiation, to a final concentration of 6.25 μM. Temperature, 15°C. B, Inhibitor antimycin, added more than 45 min prior to irradiation, to a final concentration of 0.01 μM (longer exposure times did not increase inhibition). Salinity, 30; temperature, 17°C. All measurements, average of three; bars indicate SD; dissolved inorganic carbon concentration, 2.0 mM.
tosynthesis, and this rate was proportional to the actual CO₂ concentration of the medium. Photosynthesis during the lag phase must consequently be assumed to depend on CO₂ uptake. The lag phase was followed by a rapid increase in the photosynthetic rate until steady state was reached. This increase was completely inhibited by Tris buffer. Further, Tris buffer did not lower the rate of photosynthesis of the plants during the lag phase, and full photosynthesis rate in the presence of Tris buffer was similar to the photosynthesis rate of the control during the lag phase. These results support the assumption that Tris buffer is an efficient inhibitor of HCO₃⁻ utilization in Z. marina (Hellblom et al., 2001) and also that the increase to full photosynthesis after the lag phase reflects the onset of HCO₃⁻ utilization. A similar situation, with an initial phase of CO₂ uptake followed by an onset of HCO₃⁻ utilization, occurs in many aquatic angiosperms featuring different pH values on the upper and lower sides of their leaves (Prins et al., 1980, 1982). The described properties of the photosynthetic induction by Z. marina made it possible to check if an inhibitor of photosynthesis acted specifically on the mechanism for utilization of HCO₃⁻. The general mechanism behind the inhibition of HCO₃⁻ utilization by buffers like Tris has been described before (Price and Badger, 1985; Hellblom et al., 2001; Mercado et al., 2006). The buffer anion competes with HCO₃⁻ for the protons excreted through the cell membrane, thereby preventing the formation of acid zones with high local concentrations of CO₂. The fast inhibition obtained by Tris addition and the fact that inhibition disappears immediately when changing to Tris-free medium suggest that the inhibition occurs extracellularly.

Mitochondrial ATP Supports HCO₃⁻ Utilization

At low concentrations, antimycin and oligomycin can be used to selectively inhibit mitochondrial ATP production, targeting different processes; the electron transport chain and the ATPase, respectively. These inhibitors caused a pronounced and similar inhibition of the HCO₃⁻ utilization without any effect on the CO₂-supported photosynthesis exhibited in the first phase following irradiation. Because a blockage in ATP synthesis of the chloroplast would also stop fixation of CO₂ in the Calvin cycle, the high photosynthetic rates observed at high CO₂ concentrations (NSW buffered to pH 6.16) in the presence of oligomycin and antimycin verifies that these inhibitors did not interfere with ATP synthesis in the chloroplast (with a possible exception for antimycin at the highest concentration). As the inhibition of HCO₃⁻ utilization was identical over a range of concentrations (0.1–1.0 µM for antimycin and 6–19 µM for oligomycin), maximal inhibition of the mitochondrial ATP production must have been obtained. Thus, our results show that ATP supply from mitochondria is important for the photosynthetic utilization of HCO₃⁻ in Z. marina.

The selective action of the inhibitors suggested by the above results is in line with the literature. Although there have been reports on effects of oligomycin on ATPase in, for example, isolated chloroplast envelopes from spinach (Spinacia oleracea; Wu and Berkowitz, 1992) and in pea (Pisum sativum) protoplasts at high concentrations (Romanowska et al., 2005), oligomycin at sufficiently low concentrations is a well-known selective inhibitor of mitochondrial ATPase with no effect on chloroplast ATP levels (Krömer and Heldt, 1991; Igamberdiev et al., 1998).

### Table I. HCO₃⁻ utilization, measured as the increase in net photosynthesis from lag to full rate (compare Fig. 1) by Z. marina (µmol O₂ g⁻¹ h⁻¹ fresh weight) in NSW of pH 8.4

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con 0.3 h</td>
<td>Olig 1</td>
</tr>
<tr>
<td>Mean</td>
<td>50.9</td>
</tr>
<tr>
<td>sd</td>
<td>±7.8</td>
</tr>
</tbody>
</table>

### Table II. Inhibition of the HCO₃⁻ utilization in light at different concentrations of antimycin A (remaining HCO₃⁻ utilization; percentage of control) and inhibition (percentage decrease) of respiration in the dark upon addition of SHAM to a final concentration of 15 µM

<table>
<thead>
<tr>
<th>Concentration of Antimycin</th>
<th>Remaining HCO₃⁻ Utilization</th>
<th>Decrease in Respiration by 15 µM SHAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>µM</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>70 ± 19</td>
<td>11 ± 9</td>
</tr>
<tr>
<td>0.1</td>
<td>34 ± 4</td>
<td>37 ± 7</td>
</tr>
<tr>
<td>1</td>
<td>39 ± 12</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>10</td>
<td>22 ± 3</td>
<td>42 ± 4</td>
</tr>
</tbody>
</table>
While oligomycin binds strongly to mitochondria, ATPases in chloroplasts are quite insensitive to this compound (Krömer and Heldt, 1991). Thus, oligomycin, at the concentration used on whole leaves in our experiments, should be regarded as a selective inhibitor of oxidative phosphorylation. For antimycin, there was a slightly higher inhibition of the HCO$_3^-$ utilization at 10 μM (as compared to the lower concentrations). This high concentration of antimycin probably also caused a limited inhibition of cyclic electron transport around PSI (Joet et al., 2001; Li et al., 2006). The inhibition of respiration by SHAM in the presence of antimycin was similar for all concentrations of this substance. Higher concentrations of antimycin thus did not have any further effect on respiration. Such findings are additional support for the suggestions that the higher inhibition of the HCO$_3^-$ utilization at the highest antimycin concentration was partly caused by inhibition of cyclic electron transport.

ATP produced in the mitochondria is likely to play a major role in providing energy for active utilization of HCO$_3^-$ by driving proton pumps at the plasma membrane. Although NADPH is produced in the chloroplast, chloroplasts have been reported to communicate with, and transport energy to, mitochondria in the light. Malate has, for example, been suggested as an energy carrier between chloroplasts and mitochondria in terrestrial angiosperms (Raghavendra et al., 1994). This would be in agreement with results on leaves of the freshwater angiosperm Elodea, where hyperpolarization decreased at high inorganic carbon concentrations and at a lowered NADPH to NADP$^+$ ratio (Elzenga et al., 1989). Inorganic carbon uptake appears to also depend on respiration for some species of eustigmatophyte microalgae (Huertas et al., 2002a). This dependency occurs regardless of the inorganic carbon species utilized, which is an obvious difference from the situation for Z. marina, in which only HCO$_3^-$ utilization depends on mitochondrial respiration.

Studies on pea protoplasts and leaves show that mitochondria are involved both in the activation of the Calvin cycle (Padmasree and Raghavendra, 1999a, 2001a, 2001b) and the activation of key chloroplast enzymes (Padmasree and Raghavendra, 2001a). There is, thus, a clear difference between terrestrial and aquatic plants regarding the relation between photosynthesis and mitochondrial ATP production. This is well illustrated by the effect of CO$_2$ concentration on the inhibition of photosynthesis by oligomycin. In terrestrial angiosperms, the inhibition of photosynthetic rates was maximal when there was abundant CO$_2$ (Padmasree and Raghavendra, 1999b), while in Z. marina the degree of inhibition instead decreased with increasing CO$_2$ concentrations. The inhibition was also much more pronounced in Z. marina. The supply of ATP for HCO$_3^-$ utilization is thus an additional function of mitochondria in angiosperms that is exclusive for aquatic plants and differs from some of the mitochondrial functions reported in terrestrial angiosperms.

### Importance of Respiration

The strong synergistic effect between SHAM and antimycin shows that alternative respiration becomes highly active upon antimycin addition. The alternative respiratory pathway bypasses energy-conserving sites, resulting in very low ATP production (Siedow and Umbach, 1995), which explains the inhibition of HCO$_3^-$ utilization with only antimycin present. Inhibition of respiratory ATP production by oligomycin is less likely to result in any decrease in the respiratory oxygen consumption. Mitochondria in both plants and animals can excrete protons through proton gates instead of passing them through ATPase (Ricquier and Bouillaud, 2000), including in the monocotyledon maize (Zea mays; Faavaro et al., 2006). Such bypasses by uncoupling proteins is believed to be important for the regulation of mitochondrial redox status and energy level (Ricquier and Bouillaud, 2000; Brandalise et al., 2003). Unfortunately, it is not possible to use SHAM to study the importance of alternative respiration for photosynthetic HCO$_3^-$ utilization. As SHAM has a pKa close to 8 and because it is applied at comparatively high concentrations, this buffer interferes strongly with the acid zones involved in the HCO$_3^-$ utilization mechanism of buffer-sensitive plants like Z. marina, resulting in a severe inhibition of photosynthesis (compare Price and Badger, 1985; Mercado et al., 2006).

#### The Lag Phase Depends on a Lag in HCO$_3^-$ Utilization

In many terrestrial plants, a lag phase in the onset of photosynthesis is known to occur when dark-acclimatized samples are exposed to light. Such lag phases have been ascribed to, for example, stomatal opening and, thus, the availability of CO$_2$ (Sacher and Burian, 1994). The lag phase in some aquatic angiosperms has been related to a lag in the onset of HCO$_3^-$ utilization (Prins et al., 1980, 1982). A coincidence between the onset of HCO$_3^-$ utilization and the increase to full photosynthesis does not necessarily imply that the lag phase is a result of a lag in the HCO$_3^-$ utilization. An alternative explanation could be that the lag depends on a shortage of CO$_2$ that is needed for the activation of Rubisco in the Calvin cycle (Kursar and Coley, 1993; Jensen, 2004) or for light activation of enzymes required for restoration of photosynthetic intermediates after a dark period (Kirschbaum et al., 2005). If so, one should expect a shorter lag phase at higher CO$_2$ concentrations as well as photosynthetic rates during the lag phase that did not depend on the CO$_2$ concentration. However, the photosynthetic rates during the lag phase varied considerably as a function of the actual CO$_2$ concentrations, while the length of the lag phase remained constant (compare Fig. 1B). Thus, our data suggest that the lag in the onset of photosynthesis in NSW by Z. marina depends on a lag before the onset of the activation of HCO$_3^-$ utilization, and there could only be a minor contribution, if any at all, from a lag in the activation of enzymes or in the formation of

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intermediates in the Calvin cycle. At high pH values and, consequently, low CO₂ concentrations in the medium, the lag phase was slightly S-shaped (Fig. 1B), implying that the Calvin cycle actually operates immediately, relying on CO₂ from the respiration accumulated within the plant tissue. The CO₂ fixation (and the O₂ production) decrease as this internal pool of respiratory released CO₂ is used up and the CO₂ fixation thus becomes dependent on diffusive entry of CO₂.

MATERIALS AND METHODS

Plant Material

Zostera marina, a marine monocotyledon, was collected at 1.0 to 2.0 m depth outside Kristineberg Marine Research Station on the Swedish west coast during May and September. The plants were allowed to acclimatize in aquaria with a flow of temperature-controlled NSW (16°C; saltwater from 35 m depth, salinity approximately 30) and at an irradiance of approximately 150 µmol m⁻² s⁻¹ (16-h photoperiod). Disregarding the youngest and oldest leaves, 20- or 25-mm-long, epiphyte-free sections were cut from the middle of leaves of similar width (5 mm) for the experiments.

Oxygen Electrode Setup

Photosynthetic O₂ evolution was measured in six 3.0-mL temperature-controlled (15°C–17°C) O₂ electrode chambers (Larsson et al., 1997). The leaf segments were inserted in a semicircle in the O₂ electrode chambers and directed like a parabola facing the light source supplied through optical fibers. The approximately 300 photons µmol m⁻² s⁻¹ irradiated on all samples were light saturating. Oxygen electrode tracings were recorded on a strip chart recorder (Seconic flat bed recorder).

Medium and Inhibitors

All measurements were carried out in NSW or in artificial seawater (ASW; salinity 30; Hellblom and Axelsson, 2003) with inorganic carbon added. The NSW, with a salinity of 30 to 32, was taken from 35 m depth by means of the deep saltwater system of the Research Station. A stock solution (1 g L⁻¹) of a mixture of oligomycin A, B, and C (Sigma chemicals), an inhibitor of mitochondrial ATPase, was prepared in 60% ethanol and was supplied to the O₂ chambers at a final concentration of 5 mg L⁻¹ (approximately 6.25 µM). Antimycin A (Sigma Chemicals) was dissolved in 96% ethanol to give three stock solutions at the concentrations of 3.3, 0.33, and 0.033 mg mL⁻¹, which were injected into the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively. SHAM (Sigma Chemicals) was dissolved in 1.0 M NaOH to give a stock solution of 1.0 M and was added to the stock solutions at concentrations of 3.3, 0.33, and 0.033 mg mL⁻¹, which were injected into the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively. SHAM (Sigma Chemicals) was dissolved in 1.0 M NaOH to give a stock solution of 1.0 M and was added to the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively. SHAM (Sigma Chemicals) was dissolved in 1.0 M NaOH to give a stock solution of 1.0 M and was added to the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively. SHAM (Sigma Chemicals) was dissolved in 1.0 M NaOH to give a stock solution of 1.0 M and was added to the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively. SHAM (Sigma Chemicals) was dissolved in 1.0 M NaOH to give a stock solution of 1.0 M and was added to the O₂ chambers to generate final concentrations of approximately 1, 0.1, and 0.01 µM, respectively.

Experimental Procedure

Measurements of the inhibitory effects on HCO₃⁻ utilization were carried out in the O₂ electrode chambers using the desired medium as follows. The Z. marina samples were exposed to irradiation until steady-state O₂ evolution was obtained. The light was then turned off, and the samples were kept in darkness until the oxygen had decreased to the same level as before irradiation (usually 2 h). The plants were then exposed to a new light/dark cycle. During the dark period of this cycle, three of the six samples were supplied with an inhibitor of mitochondrial ATP formation (oligomycin or antimycin). During the next light/dark cycle, the three remaining samples were supplied with the inhibitors. This approach made it possible to keep track of (and even correct for) any changes in the O₂ traces not caused by the inhibitors. Net photosynthetic rates were calculated directly from the O₂ tracings. HCO₃⁻ utilization was calculated by subtracting the photosynthetic rate during the lag phase from the full photosynthetic rate at saturating irradiance. SHAM is a buffer and inhibits HCO₃⁻ utilization in light and, consequently, affects photosynthesis in a manner similar to Tris buffer. Therefore, SHAM was added in darkness after a light period to avoid lower respiratory rates as a consequence of low photosynthetic rates. Respiration was calculated directly from the O₂ tracings.

LITERATURE CITED

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Bicarbonate Utilization in Zostera marina Depends on Respiration


