Photosynthesis in Ulva fasciata

V. Evidence for an Inorganic Carbon Concentrating System, and Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase CO2 Kinetics

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ABSTRACT

Evidence of an inorganic carbon concentrating system in a marine macroalga is provided here. Based on an O2 technique, supported by determinations of inorganic carbon concentrations, of experimental media (as well as compensation points) using infrared gas analysis, it was found that Ulva fasciata maintained intracellular inorganic carbon levels of 2.3 to 6.0 millimolar at bulk medium concentrations ranging from 0.02 to 1.5 millimolar. Bicarbonate seemed to be the preferred carbon form taken up at all inorganic carbon levels. It was found that ribulose-1,5-bisphosphate carboxylase/oxygenase from Ulva had a K_m(CO2) of 70 micromolar and saturated at about 250 micromolar CO2. Assuming a cytoplasmic pH of 7.2 (as measured for another Ulva species, P. Lundberg et al. [1988] Plant Physiol 99: 1380–1387), and given the high activity of internal carbonic anhydrase (S Beer, A Israel [1990] Plant Cell Environ [in press]) and the here measured internal inorganic carbon level, it was concluded that internal CO2 in Ulva could, at ambient external inorganic carbon concentrations, be maintained at a high enough level to saturate ribulose-1,5-bisphosphate carboxylase/oxygenase carboxylation. It is suggested that this suppresses photosynthesis production and optimizes net photosynthetic production in an alga representing a large group of marine plants faced with limiting external CO2 concentrations in nature.

Seawater provides an environment of 2.7 mM Ci, only some 12 μM of which is in the form of CO2 (at 20°C). Since the K_m (CO2) of Rubisco from marine macroalgae is in the 30 to 60 μM range (6, 8), it seems likely that these plants have developed a system not only to assure high resupply rates of CO2 (12), but also to actually concentrate this carbon form to the site of fixation via Rubisco. Gas exchange features such as O2 insensitive photosynthetic rates and low CO2 compensation points have also indicated the operation of such a system, and it has been suggested that HCO3- uptake or, more rarely, C4 photosynthesis (cf. 11, for a recent review) could form its basis. Unfortunately, the only measurements of Ci in a marine macroalga (Chondrus crispus) so far (12, 13) did not support the theory of a CO2 concentrating system for this plant group.

Ulva is a ubiquitous macroalga which apparently has

1 Abbreviations: Ci, inorganic carbon; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; Ci, external Ci; Ci, internal Ci.

adapted well to the marine environment. This is evidenced by its O2 insensitive photosynthesis in spite of performing photosynthetic Ci fixation via the C3 pathway (2), and low CO2 compensation point (5). Ulva is a good HCO3- user, its photosynthesis saturating at seawater [Ci] (1), and it was proposed that this leads to the high internal [CO2] necessary to suppress photorespiration and optimize carboxylation, and thus production, rates. In this work, we determined [Ci] of Ulva and compared them with Rubisco carboxylation kinetics to further clarify the photosynthesis properties of this plant at ambient and CO2 limiting conditions.

MATERIALS AND METHODS

Intertidal plants of Ulva fasciata Delile were collected at the Mediterranean shore just north of Tel Aviv. Plants were maintained in seawater on the laboratory window sill until used for experiments (within 4 d). Circular discs of 8 mm diameter, punched with a stopper borer, were used throughout. These discs weighed 5.5 ± 0.6 mg each (n = 17); their volume was further determined by [3H]H2O incubations (0.5–30 min yielding the same results) to 5.3 ± 1.3 μL (n = 7). [14C]Sorbitol incubations (90 s followed by a 1 s rinse) yielded a periplasmic volume of 0.83 ± 0.12 μL (n = 5) which is 16% of the total disc volume. Thalli were, on the average, 76 μm thick, including two cell layers of 30 μm each. The 79% cross-section area thus occupied by cells was close to the 84% sorbitol impermeable space.

Ulva discs were stored in the light for at least 1 h in a simple synthetic seawater medium (500 mM NaCl, 10 mM KCl, 10 mM CaCl2, and 30 mM MgSO4) lacking HCO3-. Thereafter, they were transferred to equal media to which NaHCO3 had been added to various final concentrations. All solutions were presparged overnight with outside air whereby stable final pHs (6.6–8.1) were reached; for the lowest Ci level, 5 mM Mes-buffered seawater (without HCO3-) was used. Exact Ci levels of these solutions were determined by IRGA as described below. Inorganic carbon was allowed to assimilate in the light (PPFD = 300 μE m−2 s−1) for 0.5 to 1 h.

Following the incubations, [Ci] were determined as follows: Two discs were rinsed in 5 mM pH 5.5 Mes-buffered synthetic seawater, and then closed into an illuminated O2 electrode system (comprised of a Yellow Springs O2 electrode in a 1.25 mL temperature controlled water-jacketed vial, a polarizing voltage unit and a chart recorder) containing the same Mes-
buffered solution. A stable resolution of the electrode tracing was ±2 μM O₂. By measuring the total amount of photosynthetic O₂ released, and subtracting that contributed by the Mes-buffered sodium (Ci[Mes]), the [Ci] in the cellular volume of the discs (in mM) could be calculated according to:

\[
[Ci] \text{ (mM)} = (\text{[nmol O}_2\text{ total}} - \text{[nmol O}_2\text{ Ci[Mes]})/(10.6 \times 0.84)
\]

where 1.25 is the mL system volume, 10.6 the average μL volume of two Ulva discs, and 0.84 the cellular volume fraction of the discs. For these calculations, a photosynthetic quotient of 1 was assumed.

Exact [Ci] of all media used were measured by IRGA. For the Mes-buffered solution, 0.5 mL was injected into a small (10 mL) gas stripping chamber, on line with an IRGA, containing 1 mL 2 M phosphoric acid over a glass grid. Nitrogen gas was passed through the grid at a rate of 400 mL/min, and then through the full length analytical cell of an ADC/225 MK3 IRGA. The sensitivity was set to maximum, yielding a stable resolution of ±0.5 μM Ci. The instrument was calibrated against 0.5 mL samples of a 190 ppm (7.9 μM at 20°C) CO₂ gas. It was found that peak height readings of the calibration gas, as well as of CO₂ released from injected solutions, gave as exact and replicable results as did peak areas. The Mes-buffered seawater usually contained 16 to 20 μM Ci. The [Ci] of the various incubation media were also determined by injecting 0.5 mL samples (diluted), at a lower sensitivity setting of the IRGA.

Ci compensation points of Ulva were determined by letting thalli photosynthesize in magnetically stirred synthetic seawater (prepared without HCO₃⁻), with or without the addition of 10 mM Tris (pH 8.2), in a narrow beaker. The surface was covered with a 2 mm layer of degassed paraffin oil so as to minimize gas exchange with the atmosphere. The [Ci] was determined periodically by withdrawing 0.5 mL samples of the seawater with a syringe and injecting it into the IRGA system (see above). Compensation points were reached within 3 h.

Rubisco was extracted and assayed radiometrically, at pH 8.0 and 20°C, as principally described by Vu et al. (14). Plastic vials (5.4 mL) were used for the assay, the final volume of which was 0.55 mL. After the addition of plant extract and assay buffer, the vials were closed with a serum stopper through which various amounts of [¹⁴C]NaHCO₃ was injected. Following a 10 min activation period, ribulose bisphosphate was injected to a final concentration of 2 mM. Assays were run for 0.5 min. Exact [CO₂] of the assay solutions were calculated, given the total Ci added (including that added with reactants and buffers as well as CO₂ present in the gas phase before closing the vials, altogether 0.46 μmol), as the distribution of various carbon forms in the assay mixture at the given pH (= 0.7% CO₂), temperature and ionic strength, and taking into account the distribution of CO₂ between the aqueous and gas phase of the closed vial (the molar fractionation ratio being 0.88). Preexperiments had shown that CO₂ equilibria with the gas phase were completed well within the activation period. Carbonic anhydrase additions to assays did not yield higher carboxylation rates, confirming that the Ulva extract contained enough of this enzyme for fast HCO₃⁻

![Figure 1. Oxygen tracing of two U. fasciata discs, preincubated in the light in synthetic seawater prepared to contain 0.5 mM HCO₃⁻ (and 15 μM CO₂) in Mes-buffered synthetic seawater containing 20 μM Ci (determined by IRGA). Plants were inserted and the system closed at 1. The Mes-buffered seawater was replenished and the system closed again (2), and the system was then closed again (3).](image1)

![Figure 2. Inorganic carbon release from two U. fasciata discs (full line) after rinsing (time zero) and following immersion in 2 mL Mes-buffered synthetic seawater (pH 5.5), for various lengths of time. The Ci content of the measuring medium, before inserting the discs, was 16 μM (represented by the broken zero line). Data are average of three experiments; coefficient of variation was less than 10% (n = 3).](image2)
dehydration (= CO₂ supply) rates to support enzymatic carboxylation.

Program CARBON (1, available from the authors) was used throughout for calculating total [Ci] of open systems as well as the distribution of various Ci forms in both open and closed experimental systems.

RESULTS

Various controls were carried out to verify the reliability of the O₂ method for Ci measurements. First, discs were depleted of their Ci by letting them photosynthesize till a zero gas exchange rate had been reached (Fig. 1). The Mes-buffered seawater was then replenished, and the discs were again allowed to photosynthesize until zero gas exchange. The molar extent of O₂ released in this second phase was always found to correspond with the Ci content of the Mes-buffered seawater as measured by the IRGA method, indicating that the O₂ release was Ci dependent and that the photosynthetic quotient was close to 1. Similarly, Ci depleted discs were allowed to reaccumulate Ci from incubation media, yielding close to original values within minutes. Additionally, it was checked whether adsorbed or carbonate-encrusted Ci could have been transferred with the plant to the Mes-buffered solution, thereby releasing Ci for photosynthesis. It was found that, following the short rinse, no Ci was released into Mes-buffered seawater for some 3 min (Fig. 2). After this time [Ci] rose, suggesting that previously accumulated Ci and/or respiratory CO₂ was released to the medium. Our Mes measurements always started within half a minute of withdrawing the discs from the incubation media, thus assuring that no Ci was released during the short rinse time and that no Ci was transferred to the measuring system, but rather that all Ci transferred had been stored in the discs.

Table I. Photosynthetic Ci Compensation Points for U. fasciata

<table>
<thead>
<tr>
<th>Assay</th>
<th>Compensation Point for</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cli µM</td>
<td>pH</td>
<td>Cli µM</td>
<td>ppm</td>
<td>Ci µM</td>
</tr>
<tr>
<td>Buffered</td>
<td>8.16</td>
<td>8.35</td>
<td>0.021</td>
<td>0.48</td>
<td>6.6</td>
</tr>
<tr>
<td>Nonbuffered</td>
<td>7.20</td>
<td>8.90</td>
<td>0.003</td>
<td>0.07</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Inorganic carbon compensation points of Ulva are shown in Table I. The [CO₃] and [HCO₃⁻] at compensation were calculated for the final pHs reached in the buffered and nonbuffered synthetic seawater solutions. As can be seen, the CO₂ concentrations varied while those of HCO₃⁻ were closer to one another at the two instances.

Internal [Ci] were found to vary considerably with the time plants were held in the laboratory, high values obtained on the day of collection and lower values during the following 3 d. Table II represents an average of 1 to 4 d old plants and shows [Ci], at three different [Clit] (the latter as measured by IRGA). Initial and final incubation pHs were recorded, and the average pH was used for calculating [CO₂] and [HCO₃⁻]. Further, internal [CO₂] were calculated assuming an average internal (excluding the vacuole) pH of 7.2 (10). Figure 3 shows internal versus external [Ci] of plants as determined on the day of collection. The lowest point on the graph was for incubations in the same Mes-medium where measurements were done, i.e. pH 5.5 containing 15 to 23 µM Ci, 73% of which was in the form of CO₂. Internal carbon accumulation leveled off at an [Clit] of about 0.6 mm.

To avoid possible adverse effects of prolonged exposure to various buffers, Ci-concentrating incubations were usually performed in synthetic seawater only (except in one case where Mes was used). By doing so, incubations at lower than natural seawater [Ci] were intrinsically paralleled by decreasing initial pH values (below 8.1, but drifting upward during incubations). However, it was observed that variations in external pH within the range used did not significantly alter photosynthetic rates. In our view, it is also unlikely that these variations in the bulk pH significantly affected Ci uptake since it has been shown that OH⁻ accumulation within the unstirred layer keeps the pH uniformly high (about 10.0) close to the plasmalemma (3). The independence of this pH from the bulk one is further indicated by recent measurements where also in 5 mm Mes buffered seawater (pH 5.5) the unstirred layer pH was close to 10 during photosynthesis (data not shown here).

The Rubisco carboxylase activity of Ulva saturated at about 250 µM CO₂ and the in vitro was 70 µM (Fig. 4). A Lineweaver-Burk plot (excluding the lowest [CO₂] point) showed a Km similar to the in vitro (68 µM), confirming that close to Michalis-Menten kinetics were obtained in these experiments.

Table II. External and Internal Ci Concentrations, and Their Ratios (Ci/Cit), for U. fasciata Incubated in the Light at Various Ci Levels

<table>
<thead>
<tr>
<th>Internal Cl</th>
<th>Cl</th>
<th>CO₂ at pH 7.2</th>
<th>Cl/Cit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td></td>
<td>µM</td>
</tr>
<tr>
<td>0 (Mes)</td>
<td>18</td>
<td>5.5</td>
<td>4</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>7.1</td>
<td>83</td>
</tr>
<tr>
<td>1,000</td>
<td>750</td>
<td>8.2</td>
<td>651</td>
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</table>

<table>
<thead>
<tr>
<th>External Cl</th>
<th>HCO₃⁻ added</th>
<th>Cl measured using IRGA</th>
<th>pH</th>
<th>HCO₃⁻</th>
<th>CO₂</th>
<th>Cl</th>
<th>CO₂ at pH 7.2</th>
<th>Cl/Cit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td></td>
<td></td>
<td>µM</td>
<td>ppm</td>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (Mes)</td>
<td>18</td>
<td>5.5</td>
<td>4</td>
<td>14</td>
<td>1,390 ± 391</td>
<td>68</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>90</td>
<td>7.1</td>
<td>83</td>
<td>7</td>
<td>3,040 ± 974</td>
<td>145</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>1,000</td>
<td>750</td>
<td>8.2</td>
<td>651</td>
<td>3</td>
<td>4,600 ± 1,740</td>
<td>222</td>
<td>6</td>
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</tr>
</tbody>
</table>

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thus these comparisons:

**DISCUSSION**

*Uln* was here found to possess a system which concentrates Ci intracellularly. The established way of concentrating CO₂ to the site of carboxylation via Rubisco in terrestrial plants is C₄ photosynthesis. However, although a biochemical system similar to the C₄ one has been described also for submerged plants (4), most macroalgae (cf. 11), including *Uln* (2), photosynthesize via the C₃ pathway. Here, like in many other submerged plants (cf. 9), it seems that active Ci transport at the plasmalemma level accounts for elevated [Ci]. The Ci concentrating capacity was highest at low [Ci], and much lower at close to ambient seawater [HCO₃⁻]. In nature, low [Ci] occur especially when *Uln* is exposed to the air during low tide. Such fluctuations in Ci availability may thus be the rationale for *Uln* to maintain its Ci concentrating system over a range of [Ci].

The C₃-like gas exchange features of *Uln*, including O₂-insensitive photosynthesis (2) and the both here and previously (5) found low compensation points, indicate that this alga can efficiently suppress photorespiration. This, in turn, indicates that intracellular [CO₂] are high enough at the site of Rubisco so as to saturate carboxylation and repress the oxygenation reaction. To estimate the effect of internal [CO₂] on Rubisco’s performance, we have assumed a cytoplasmatic pH of 7.2 for *Uln* (10). At this pH, and given a high resupply rate catalyzed by internal carbonic anhydrase (3), 4.8% of the Ci would be in the form of CO₂, i.e. internal [CO₂] would on the average be some 200 μM at 1 mm external Ci. This value is very close to saturation for the enzyme, and it is likely that internal [CO₂] therefore suppresses oxygenation in situ and thus photorespiration. Some care should however be taken in these comparisons: It is likely that internal pH, and thus [CO₂], is further compartmented within the cells, possibly in favor of even higher [CO₂] within the chloroplasts. Similarly, it is possible that the Ci concentrating transport system is partly located at the chloroplast, which would also underestimate our reported [CO₂] in the vicinity of Rubisco. Further, Rubisco may in vivo show different kinetics than in our assays.

**LITERATURE CITED**

transport of CO₂ and HCO₃⁻ by the cyanobacterium Synechococcus UTEX 625. Plant Physiol 87: 551–554