HCO$_3^-$ as an exogenous carbon source for *Ruppia cirrhosa* (PETAGNA) GRANDE

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With 3 figures in the text

Abstract

The aquatic plant *Ruppia cirrhosa* was investigated for its ability to utilize HCO$_3^-$ and CO$_2$ as exogenous carbon sources for photosynthesis. In NaHCO$_3$ solutions *R. cirrhosa* increased the pH to a maximum of 9.85 corresponding to a CO$_2$ compensation point of 0.55 mmol m$^{-3}$ CO$_2$. Measured photosynthetic rates cannot be explained only by the uptake of CO$_2$. Photosynthetic rates decreased at high pH but did not decline to zero until pH 11.5. Furthermore, photosynthesis was increased by higher HCO$_3^-$ concentrations at constant CO$_2$ concentration. It is concluded that *Ruppia cirrhosa* has the capability to utilize HCO$_3^-$ which helps to explain its wide distribution in alkaline saline waters.

Introduction

Inorganic carbon is present in water as a mixture of free CO$_2$ (with a very small amount of H$_2$CO$_3$), HCO$_3^-$ and CO$_2^2-$ in proportions that depend on the pH. In natural waters, the pH often ranges between 7.0 and 8.5 and therefore the HCO$_3^-$/CO$_2$ ratio is between 4 and 140 (REBSDORF 1972). All plants are able to use CO$_2$. Many algae and angiosperma can also use HCO$_3^-$`. The mechanism by which plants do so is still unsolved (BOSTON et al. 1989). Some algae, angiosperma and the bryophytes were thought to be incapable of using it (references in RAVEN 1970, BADGER 1987 and BOSTON et al. 1989), although recently PEÑUELAS (1985) showed that aquatic bryophytes grown in alkaline stagnant waters were capable.

We now describe tests of the ability of *Ruppia cirrhosa* to use HCO$_3^-$`. It grows in habitats with generally high levels of inorganic carbon and other nutrients, where competition among plants limits the availability of these resources. Species respond with adaptations that allow rapid growth and biomass accumulation. *Ruppia cirrhosa* is then more likely to be able to take up HCO$_3^-$ from the water efficiently.

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Material and methods

*Ruppia cirrhosa* was collected in winter 1988 and spring 1989 from the Tancada lagoon in the Ebre delta. It was colonized by very few epiphytes and it was not significantly encrusted with carbonates. Previous to the experiments it was carefully rinsed. Plants were maintained in darkness in an aquarium at 20 °C for up to 24 h until the experiments were conducted in order to get rid of as much oxygen as possible in aerial spaces. Experiments were performed in closed Winkler bottles of 250 cm³. Approximately 1.5 g of leaves were used in each Winkler bottle. Dry weight (dw) was determined after each experiment by drying to constant weight at 65 °C. Conductivity, temperature, oxygen and pH were measured before and after adding HCl or freshly prepared NaOH. Mixing was provided by stir bars. The bottles were placed at 20 ± 1 °C in a thermostatic bath.

In experiments similar to those of Ruttner (1947) and Bain & Proctor (1980), the increase in the pH at different concentrations of NaHCO₃ solutions containing *Ruppia cirrhosa* growing under a photon flux density of 700 μE m⁻² s⁻¹ was observed for various days.

In other experiments like the ones described in Peñuelas (1985) and in Prins & Elzenga (1989), photosynthetic rates were measured at different NaHCO₃ concentrations and at different pH values. The use of these NaHCO₃ solutions has advantages over the use of natural waters. Calcium is absent, so precipitation of calcium carbonate at high pH is prevented and where CO₂ is taken up by plants, the total cation concentration remains constant (Stumm & Morgan 1981). Alkalinity depends only on the carbonate species with no interference from weak acids other than carbonic acid and bicarbonate. Furthermore, changes in pH as a result of uptake of nitrogen sources as NO₃⁻ or NH₄⁺ are prevented (Brewer & Goldmann 1976).

Alkalinity was measured by potentiometric titration and [DIC], [CO₂], [HCO₃⁻] and [CO₃²⁻] were computed according to Mackenth et al. (1978) by a computer program (WATEQ).

In all experiments, bottles without plants were used as controls and dark ones to measure respiration. Each experiment was repeated at least twice. The same values and patterns were obtained in the different replicates, except in the absolute values of replicates carried out at different dates (Fig. 1 A), likely because of seasonal plant development.

Results

*Ruppia cirrhosa* caused the pH of 2.5 mol m⁻³ NaHCO₃ solutions to rise to a maximum of 9.85 during the afternoon and to decrease afterwards. Oxygen followed the same pattern as pH. If the plant uses only CO₂ and light is saturating, then the maximum pH reached corresponds to the CO₂ compensation point. For *Ruppia* the CO₂ compensation point was calculated as 0.55 mmol m⁻³. The ratio [DIC]/alkalinity when the pH reached the maximum was 0.69—0.76.

The photosynthetic rate decreased at pH values from 7 to 9, but it did not reach zero as it would be expected for “strict CO₂ users”. A total photosynthetic rate of zero was not reached until pH values around 11.5 (Fig. 1). These results suggest utilization of HCO₃⁻, although with less affinity than CO₂ uti-
Fig. 1. Rates of photosynthesis as a function of pH for *Ruppia cirrhosa* in different initial concentrations of NaHCO₃. A, replicates carried out in different dates; B, in the same date.

Utilization (photosynthetic rate was 2—10 times higher at lower pH values). The curves of photosynthetic rate as a function of [CO₂] support this conclusion: at minimal and insufficient CO₂ concentrations, the photosynthetic rate was positive. Furthermore, at the same [CO₂], plants in solutions with higher [DIC] showed a higher photosynthetic rate because of higher [HCO₃⁻] (Figs. 1 and 2). Saturation occurred at about 150—250 mmol m⁻³ CO₂ corresponding to total photosynthetic rates of 14 to 4 mg O₂ g⁻¹ dw h⁻¹ depending on the HCO₃⁻ concentration and the seasonal state of the studied plant specimens.

The total photosynthetic rates at pH 8—10 increased in parallel to the [HCO₃⁻] increases (Figs. 1 and 3). Under such conditions, total photosynthet-
Fig. 2. Rates of photosynthesis as a function of different [CO$_2$] induced by changing pH of the same following initial solutions of Fig. 1.

ic rates were 2 to 10 times lower than when the CO$_2$ was supplied at saturating concentrations under lower pH values. The representation of total photosynthetic rate versus [HCO$_3^-$] pointed to an approximate HCO$_3^-$ compensation point of about 0.10 mol m$^{-3}$ (Fig. 3). Those concentrations were not in equilibrium with any photosynthetically substantial amount of CO$_2$ in the used solutions because of their high pH values.
Fig. 3. Rates of photosynthesis as a function of different [HCO$_3^-$] induced by changing pH of the same following initial solutions of Fig. 1.

**Discussion**

The results show that *Ruppia cirrhosa* is capable of utilizing bicarbonate as a carbon source for photosynthesis. The following observations support this conclusion.
The observation that *R. cirrhosa* had higher photosynthetic rates at subsaturating and saturating free CO₂ concentrations when [HCO₃⁻] were higher is indicative of its ability to use HCO₃⁻ in addition to free CO₂.

Water in equilibrium with air contains approximately 10 µM free CO₂. The low apparent Km(CO₂) found for *R. cirrhosa*, approximately the free CO₂ concentration of water, suggests that it has a high affinity for this form of DIC. Consequently, CO₂ could comprise an important proportion of the DIC used by this macrophyte. However, HCO₃⁻ is probably still the major source of DIC because lagoon water concentrations are 5–6 fold greater than the apparent Km(HCO₃⁻) value.

*Ruppia cirrhosa* caused the pH of 2.5 mol m⁻³ NaHCO₃ solutions to rise to 9.85, and the CO₂ concentration to drop to 0.55 mmol m⁻³. As Prins et al. (1980) state, C₃ plants with such CO₂ compensation points never cause the pH to rise to much more than 9.0 by simple CO₂ fixation without an associated uptake of HCO₃⁻.

The [DIC]/alkalinity ratio at equilibrium is considerably less than 0.95. Values greater than this suggest that a species is unable to use bicarbonate, so, by this criterion (Maberly & Spence 1983), *R. cirrhosa* appears to be an efficient bicarbonate user. A high ratio shows that most of the [DIC] in solution is photosynthetically unavailable to the plant (Maberly & Spence 1983).

At high pH values, the [CO₂] is much lower than the minimum Km(CO₂) found in vitro for the ribulose-1,5-biphosphate carboxylase (about 10 mmol m⁻³; Jensen & Bahr, 1977). Thus, at pH 8.7–9.2 in 1–6 mol m⁻³ NaHCO₃ solutions, total photosynthetic rate should decrease dramatically. Although total photosynthetic rates decreased, positive values were maintained until much higher pH values were reached with practically no CO₂ in solution (Figs. 1 and 2). The fact that Total Photosynthetic Rates at these high pH values remained positive is not explained by inorganic carbon reserves present in the cells, since these would be exhausted within short time (less than 1 h; Steemann Nielsen 1952). Furthermore, calculations show that the measured total photosynthetic rates must have involved HCO₃⁻ uptake since the [CO₂] in solution was insufficient at these high pH values. In fact, positive total photosynthetic rates were observed at practically zero [CO₂], and total photosynthetic rates were higher at higher [HCO₃⁻] under the same [CO₂] in different [DIC] solutions.

Bicarbonate utilization is likely in this species that is widely distributed in alkaline saline waters (Verhoeven 1979). However the problem, as discussed in Peñuelas (1985), is very complex because there is always equilibrium with CO₂ and the difference between CO₂ and HCO₃⁻ utilization is not well known yet (Badger 1987, Prins & Elzenga 1989, Boston et al. 1989). We again agree with Allen & Spence (1981), Peñuelas (1985) and Sand-Jensen (1987) who stated that rather than users and non-users of HCO₃⁻, a gradation
exists, with use depending on the relative availability of the different forms of DIC. The next still unsolved questions are what controls the ability to use bicarbonate, and how it works at the physiological and biochemical level.

The higher efficiency in the utilization of CO$_2$ compared to HCO$_3^-$ is a well known phenomenon (SAND-JENSEN 1983). *Ruppia*’s relative efficiency was, however, higher than that of other HCO$_3^-$ users like the bryophyte *Fontinalis antipyretica* (PEÑUELAS 1985) as it was expected from a saline water macrophyte.

The potential rate of photosynthesis exceeds the rate of CO$_2$ supply from the surrounding medium (MABERLY & SPENCE 1985). This is particularly important at alkaline pH where a decreased proportion of the external DIC is present as CO$_2$. An organism with a relatively low affinity for CO$_2$ and an inability to directly access the HCO$_3^-$ pool would be severely limited. A second benefit of direct access to HCO$_3^-$, and therefore to an additional carbon source, comes from the possibility of utilizing limiting sources of nitrogen and light more efficiently (BADGER 1987). HCO$_3^-$ utilization compensates for slow diffusion supply of CO$_2$ and decreases photorespiration by creating high intracellular DIC concentrations. All factors together allow this species to be widely distributed in alkaline saline environments as La Tancada lagoon in the Ebre delta where it was collected. The HCO$_3^-$ utilization is very important as an adaptive mechanism for this species that usually grows in productive waters, commonly CO$_2$ depleted and with high pH. In these waters, the ability to access the HCO$_3^-$ pool is of vital significance for positive growth.

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