Effects of CO₂ and O₂ on the Photosynthetic O₂ Evolution of Spirodela polyrrhiza Turions

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ABSTRACT

Net photosynthetic rates of Spirodela polyrrhiza turions, at low O₂ levels, were 6.2 and 38.8 micromoles O₂ per gram fresh weight per hour at 1 millimolar HCO₃⁻ and CO₂ saturation, respectively, and much lower in a regular low-pH growth solution. Air equilibration O₂ concentrations decreased rates considerably, except at CO₂ saturation. The surfacing rate of turions in various inorganic carbon surroundings correlated positively with their photosynthetic rates, but were the same at high and low O₂ levels. The relevance of these findings in relation to environmental conditions conducive to germination of autotrophically growing turions is discussed.

Duckweeds, like other aquatic angiosperms, are known to form over-wintering structures called turions. These are usually formed at times when environmental conditions are unfavourable for growth. Within the duckweed family, Spirodela polyrrhiza is known to form turions readily at a variety of growth conditions (Hensen, cited in [3, 12]) and as an apparent response to surrounding levels of ABA (13, 14, 16). Little is known about the physiological state of Spirodela turions. Cytologically, they differ from the mother fronds by their thick cell walls, small vacuoles, and large amount of starch grains (3, 15). The conspicuous lack of air spaces cause the turions to sink.

Although the biology of turion formation has been studied for a long time (for a review see Bhalla et al. [2]), less has been reported on their germination. The initiation of this process may depend on an array of interacting factors including temperature (11), osmotic potential (9), desiccation (8), nutrient and hormone levels (5, 9) and, in heterotrophically growing plants, the exogenous organic carbon source (7).

It was early observed that germination of turions was preceded by bubble formation in the light causing the turions to float up to the surface (Jacobs, cited in [3]). Although bubble formation may also occur in the dark (11), the relationship between light, buoyancy, and germination in autotrophically growing plants indicates the importance of photosynthetic O₂ production for surfacing as an initial step in the germination process. The photosynthetic capacity of turions has not previously been evaluated.

In this work, photosynthetic rates of Spirodela turions were measured in relation to CO₂, HCO₃⁻, and O₂ concentrations. The aim was further to investigate whether conditions conducive to high photosynthetic rates correlated with rates of surfacing of the turions.

MATERIALS AND METHODS

Spirodela polyrrhiza (L.) plants were grown on a one-tenth strength Hoagland solution (4) at 25°C, 100 μE m⁻² s⁻¹ and a 16-h photoperiod. Turions were readily obtained when growing the plants at the same conditions except for a lower temperature (20°C).

Photosynthetic O₂ evolution was measured at 25°C and 100 μE m⁻² s⁻¹ using a 2-ml water jacket equipped with a Radiometer O₂ probe and, sometimes, also with a pH electrode. Both O₂ levels and pH were recorded continuously on a dual pen recorder. Batches of 20 turions (about 0.01 g fresh weight) yielded readily measurable photosynthetic responses in this system. In some experiments, fronds of Spirodela which had been water infiltrated under vacuum were used. Measurements were started either at the air equilibrium O₂ concentration (266 μM) or at lower O₂ concentrations (about 50 μM) obtained by N₂ sparging just prior to placing the plants in the measuring system.

Two types of experimental solutions were prepared for the photosynthesis experiments: (a) a regular one-tenth Hoagland medium and (b) the same medium to which NaHCO₃ had been added to a final concentration of 1 mM. In order to equilibrate gases with the atmosphere, the solutions were then bubbled with air containing 300 to 330 μl/l CO₂ (determined by IR gas analysis) for several hours. The sparging away of excess CO₂ in solution 2 was reflected in a gradual pH increase. The equilibration process was assumed to be completed when a stable pH had been reached. The final HCO₃⁻ concentration of solutions 1 and 2, the latter with a final pH of 8.0, were 2 and 910 μM, respectively, as calculated by the computer program CARBON (cf. Beer and Wetzel [1]; the program is available upon request). The final CO₂ concentration of both solutions was 10 to 11 μM (also calculated by the program).

In order to vary CO₂ concentrations in a medium containing little HCO₃⁻, small aliquots of a solution saturated with CO₂ (35 mM at 25°C) were injected into the water jacket while measuring steady state photosynthesis in solution 1. As the pH of this solution was always below 5.5, less than 20% of the CO₂ formed HCO₃⁻. The actual CO₂ concentration in the system following CO₂ injection was calculated as the injected amount minus the fraction transformed into HCO₃⁻ at each pH reached according to program CARBON for 'closed system' calculations.

Another way to alter CO₂ concentrations in the system was to lower the pH by injecting diluted HCl into experimental solution 2, which initially held approximately 1 mM HCO₃⁻, during steady state photosynthesis. The CO₂ and HCO₃⁻ concentrations at each pH in this closed system were calculated by the computer program.

The time course of turions to float up to the water surface was followed by enclosing about 50 water-infiltrated (under vacuum) turions in 200-ml Erlenmeyer flasks containing 100 ml Hoagland solution with (a) 1 mM NaHCO₃ (solution bubbled with air to restore the air-equilibrium CO₂ concentration), (b) CO₂ added in the head space of the flask yielding an approximate concentration of 2.7 mM soluble CO₂ as estimated by the pH decrease, (c) no additions made; solutions contained 10 to 11 μM CO₂ and...
about 20 μM HCO₃⁻ (pH 5.5), and (d) the same as (a) to (c) but with O₂ lowered to about 50 μM by N₂ sparging. The experiments were performed in the same growth chamber where the plants were grown (see above). Some flasks were gently shaken by a shaker (75 5-cm strokes/min). The fraction of turions floating on the surface was recorded at time intervals.

Chl from leaves and turions was extracted in N,N-dimethylformamide for 24 h and measured spectrophotometrically according to Moran ([10], formulae 17–18). Dry weight was measured following oven-drying at 90°C for 24 h.

RESULTS

Figure 1 shows the net photosynthetic response of turions to CO₂ concentration. The HCO₃⁻ present in the experimental media was, at the most, 20% of the CO₂ level, and thus never exceeded 0.12 mM (at 0.6 mM CO₂). At lower CO₂ levels, the very low HCO₃⁻ concentrations were not likely to contribute significantly to photosynthetic rates. A saturating CO₂ concentration was reached at 0.2 to 0.3 mM. Increasing CO₂ concentrations by decreasing pH in the closed system during photosynthesis (+, Fig. 1), gave the same response as did the increase by CO₂ injection. Thus, no pH effect per se on photosynthetic rates could be observed between pH 8.0, 7.2, and 6.4. In control experiments, pH was lowered further (from 6.4 to 5.0, all in the CO₂ saturation range) without noticeable effects on photosynthetic rates. The effect of O₂ was apparent only at subsaturating CO₂ concentrations, and depressed rates by some 50% as compared to those at the higher O₂ level.

Chl contents, dry weight/fresh weight ratios, and maximal photosynthetic rates of turions and fronds of Spirodela are listed in Table 1. The Chl content was higher in the fronds and the dry weight/fresh weight ratio was 3.5 times higher in the turions. Photosynthetic rates at CO₂ saturation were considerably higher than in the normal Hoagland solution and at 1 mM HCO₃⁻.

The surface rate of turions at various conditions of CO₂, HCO₃⁻, and water movement (shaking) is presented in Figure 2. Turions experiencing CO₂ saturation floated up to the surface most quickly. Those at 1 mM HCO₃⁻ showed a slower rate of surfacing, followed by turions immersed in regular one-tenth Hoagland solution. Shaking always decreased the amount of turions surfacing: the regular Hoagland and HCO₃⁻ treated turions surfaced at a very low rate while the high CO₂ treated ones floated up following a long time period of about 3 d. The O₂ level of the media had no effect on surfacing rates.

It was observed that turions floated up to the surface following the formation of a gas bubble which remained attached. No turions floated up during the night. Once floating, no turions sank down again, even during darkness.

DISCUSSION

Turions differ from the mother fronds by their much higher dry weight content. As a result, the difference in maximal photosynthetic rates at CO₂ saturation and 1 mM HCO₃⁻, respectively, between the two morphological forms, diminishes when calculated on a dry weight basis. Thus, in spite of large morphological and cytological differences (cf. 3, 15), the physiological state of turions with respect to photosynthetic gas exchange characteristics per dry weight differs little from that of the mother fronds under the conditions investigated.

Net photosynthetic rates were markedly inhibited by air equilibration concentrations of O₂ at the normal and 1 mM HCO₃⁻.
conditions. This is probably due to the CO$_2$ concentration at the site of fixation via ribulose-1,5-bisP carboxylase not being high enough to prevent photorespiration. On the other hand, high CO$_2$ levels did prevent the negative effect of high O$_2$ levels, most likely by revoking the oxygenase activity of this enzyme. Low O$_2$ levels are thus conducive to high photosynthetic rates at 1 mM HCO$_3^-$ as well as subsaturating CO$_2$ levels. The higher photosynthetic rates in the presence of 1 mM HCO$_3^-$ than in the normal Hoagland solution could indicate HCO$_3^-$ utilization by the turions. However, solving equations for uncatalyzed CO$_2$ formation rates from the ionic carbon forms present in the medium (cf. 6, assuming a photosynthetic quotient of 1.0) revealed that maximal release rates were higher than observed photosynthetic rates within the system (2.7 10^{-3} versus 1.7 10^{-2} mol C s^{-1}). Thus, it is possible that the higher photosynthetic rates in the presence of HCO$_3^-$ are due to an enhanced supply rate of CO$_2$.

Photosynthetic rates of submerged turions at normal (low HCO$_3^-$ and CO$_2$), 1 mM HCO$_3^-$, and CO$_2$ saturation conditions correlated positively with their surfacing rate. In each case, surfacing was preceded by the formation of a gas bubble of sufficient size attached to the turion. It is apparent that those bubbles consisted of photosynthetically evolved O$_2$ in the dark no turions became buoyant. If so, the lower surfacing rates obtained under conditions of water movement around the turions (shaking) may be explained by either facilitated O$_2$ diffusion, past a declining unstirred layer, into the solution or by bubbles breaking loose from the turions before being large enough to float them to the surface. Once floating, turions of all treatments did not sink again during the time of these experiments. Although the low O$_2$ level caused markedly increased photosynthetic rates of turions at normal as well as 1 mM HCO$_3^-$ conditions, it did not increase the rate of surfacing. This is probably due to the sharp O$_2$ concentration gradient causing partial of the O$_2$ to escape from the turion surface to the surrounding water rather than being used for bubble formation.

In nature, germination of Spirodela turions mainly occurs under favorable temperature and light. In such conditions, the first phase of the process, at least in autotrophically growing turions (cf. 11), may be their light-dependent surfacing as brought about by photosynthetic O$_2$ production. As apparent from the present results, the dissolved inorganic carbon composition is of importance to the rate of surfacing. While high HCO$_3^-$ concentrations, brought about in nature by high pH, stimulate the process, the effect of CO$_2$ is stronger. In the spring, as water temperature rises, bacteria respiration may bring about enriched CO$_2$ concentrations. The concomitant decrease in O$_2$, although increasing photosynthetic rates, would not speed up surfacing rates. Similarly, water movement would hinder turions readily becoming buoyant.

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LITERATURE CITED

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