Photosynthetic gas exchange under emersed conditions in eulittoral and normally submersed members of the Fucales and the Laminariales: interpretation in relation to C isotope ratio and N and water use efficiency

Misni B. Surif* and John A. Raven
Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, UK

Summary. Ten species of brown macroalgae (five eulittoral and one submersed species of the Fucales; four submersed species of the Laminariales) from a rocky shore at Arbroath, Scotland, were examined for characteristics of emersed photosynthesis in relation to the partial pressure of CO2 and O2. The five eulittoral species of the Fucaleae were approaching CO2 saturation for light-saturated photosynthesis at normal air levels of CO2 (35 Pa) in 21 kPa O2. The normally submersed algae are further from CO2 saturation under these conditions, especially in the case of the four members of the Laminariales. The rate of net photosynthesis in the Fucaleae is O2-independent in the range 2-21 kPa O2 over the entire range of CO2 partial pressure tested (compensation up to 95 Pa). For the other five algae tested, net photosynthesis is slightly inhibited by O2 at 21 kPa relative to 2 kPa over the entire range of CO2 partial pressures tested (compensation up to 95 Pa). CO2 compensation partial pressures are low (< 0.5 Pa) for the Fucaleae and independent of O2 in the range 2-42 kPa. For the other five algae, the CO2 compensation partial pressure are higher, and increased with O2 partial pressure in the range 2-42 kPa. These gas exchange data show that the Fucaleae exhibit more C4-like characteristics of their photosynthetic physiology than do the other five species tested, although even the Laminariales and Halidrys siliquosa are not classic C3 plants in their photosynthetic physiology. These data suggest that, in emersed conditions as well as in the previously reported work on submersed photosynthesis, a "CO2 concentrating mechanism" is operating which, by energized transmembrane transport of inorganic C, accumulates CO2 at the site of RUBISCO and, at least in part, suppresses the oxygenase activity. Work with added extracellular carbonic anhydrase (CA), and with a relatively membrane-impermeant inhibitor of the native extracellular CA activity (acetazolamide), suggests that, in emersed conditions as well as in the previously reported work on algae submersed in seawater at pH 8, HCO3 is the major inorganic C species entering the cell. At optimal hydration, the rate of emersed photosynthesis in air is not less than the rate of photosynthesis when submersed in seawater, at least for the Fucaleae. δ13C ratios of organic C for the Fucaleae are slightly more negative than is the case for the other five algae; these data are consistent with substantial (half or more of the entering inorganic C) leakage of CO2 from the accumulated pool, and with some contribution of atmospheric CO2 to the organic C gain by the eulittoral algae. The predicted increase in N use efficiency of photosynthesis in the Fucaleae, with their more strongly developed CO2 concentrating mechanism, is consistent with data on emersed, but not submersed, photosynthesis for the algae collected from the wild and thus at a poorly defined N status. The more C4-like gas exchange characteristics of photosynthesis in the eulittoral Fucaleae may be important in increasing the water use efficiency of emersed photosynthesis from the limited capital of water available for transpiration by a haptophyte.

Key words: Fucales – Laminariales – Emersed photosynthesis – Carbon isotope ratio – Nitrogen use efficiency

The Phaeophyta are important components of the intertidal and subtidal marine flora of temperate and frigid rocky shores, and are major contributors to primary productivity in these habitats. A significant ecophysiological distinction may be made between those algae which normally live essentially completely submersed and those which normally spend a part of all (or most) tidal cycles in the emersed state.

The emersed state imposes a number of potential benefits and constraints on the intertidal algae. In terms of resource acquisition, emersed populations have a higher incident photon flux density than do the submersed, but the absence of intermittency of illumination due to wave action may reduce its effectiveness for photosynthesis. They lack nutrient supply from seawater while emersed; the only major chemical resource supplied under emersed conditions is CO2 from the air. However, the loss of water from these poikilohydric haptophytic plants while they are emersed may restrict the time available for photosynthesis: after an initial increase in the rate of photosynthesis as water is lost, the rate declines to, and then below, respiratory compensation with further desiccation. Resource loss is in-
fluenced in a number of ways by emersion; the time available for grazers which are active when submerged is, of course, decreased, although any terrestrial herbivores which fancy a snack of seaweeds have longer mealtimes in the upper intertidal. The ultimate resource loss is death of the whole plant, an end which would be facilitated by desiccation intolerance on the part of the plant, at least in the case of upper intertidal algae which may be emersed for days at a time during neap tides. The intertidal algae are desiccation-tolerators rather than desiccation-avoiders (Jones and Norton 1979; Schonbeck and Norton 1979a, b, 1980; Brown and Dring 1982) with the exception of succate waterstorers (Oates 1985, 1986).

One objective of the present work is to investigate the characteristics of inorganic C acquisition during emersed photosynthesis in brown macroalgae which normally are never emersed under natural condition, and for those which are naturally emersed for varying times in the tidal cycle. As well as the rate of photosynthesis at the normal air partial pressures of CO₂ O₂, we are interested in the wider characterisation in terms of CO₂ and O₂ concentration effects to see if the characteristics of photosynthesis are such as to permit the different taxa studied to carry out varying amounts of CO₂ assimilation during desiccation while emersed (Raven et al. 1987b). This ties in with the second objective of the work, i.e. to determine the gas exchange characteristics of various brown algae during emersion to see how they fit into the continuum of photosynthetic physiologies. At one extreme is diffusive CO₂ supply to RUBISCO, resulting in a relatively low CO₂ affinity, O₂ inhibition of photosynthesis under inorganic C-limiting conditions, and a relatively high CO₂ compensation concentration (C₃ physiology and C₃ biochemistry). At the other extreme is a CO₂ concentrating mechanism which yields a higher CO₂ affinity, a negligible O₂ inhibition of even inorganic C-limited photosynthesis, and a low CO₂ compensation concentration (C₄ physiology despite C₃ biochemistry). From our earlier work on submersed photosynthesis by six members of the Fucales and four members of the Laminariales, the family Fucaceae shows characteristics closer to the “CO₂ concentrating mechanism” end of the continuum, while the Laminariales and the Fucalean genus Halidrys siliquosa (Cystoseiraceae) have characteristics which are more intermediate between those of the Fucaceae and “C₃-like physiology” (Surif and Raven 1989a, b).

Materials and methods

Plant collection

10 species of brown algae were investigated (see Table 1). The algae were collected at Arbroath, Scotland (Ordnance Survey grid reference NO 659412) at low tide. The material was maintained in a cool room (10°C) in filtered seawater in a perspex aquarium tank with fluorescent illumination (30 μmol photon m⁻² s⁻¹, 400–700 nm) supplied for 12 h in each 24 h. No reproductive material was used and all plants were free of macroscopic epiphytes and were apparently healthy. Only apical portions were used in experiments on the Fucales and basal portions of blades in those on the Laminariales, i.e. growing regions of the thalli in both orders.

Measuring of CO₂ uptake and CO₂ compensation concentration (expressed as partial pressure)

The rates of CO₂ uptake and the CO₂ compensation partial pressure in air at different O₂ tension were measured in a closed system using an IRGA (ADC Type 225 Mk 3). The IRGA was calibrated with CO₂-free air and high purity standard CO₂ (359 ppm: P.K. Morgan Ltd. Kent, England). The assimilation chamber was a 20×10⁻⁶ m³ test tube. The samples of 0.3–0.5 g fresh weight, were preilluminated by a 500 W lamp (Thorn M25 F500) giving 500 μmol photon (400–700 nm) m⁻² s⁻¹ for 30 min in filtered seawater before placed in the assimilation chamber which was covered by a black cloth to exclude light. The experimental temperature was kept constant at 10°C by circulating water from a constant temperature water bath through a jacket surrounding the assimilation chamber. The IRGA system was opened and sparged with N₂ gas until the CO₂ partial pressure approached zero Pa; it was assumed that the O₂ partial pressure was also zero, then the system was closed. Pure CO₂ and O₂ were injected through an injection port connected to the IRGA system to give the right combination of 95 Pa CO₂, 2kPa O₂; 95 Pa CO₂, 21 kPa O₂ or 95 Pa CO₂, 42 kPa O₂. The introduction of gases, including sparging with N₂, took 3–5 min after which time the black cloth was removed. The sample was illuminated with 500 μmol photon m⁻² s⁻¹ and the rates of CO₂ uptake were recorded until the CO₂ compensation partial pressure was achieved. CO₂ uptake at 42 kPa O₂ was not measured, however the CO₂ compensation partial pressure values are given for this partial pressure of O₂. CO₂ compensation partial pressure at 19°C was also measured at 21 kPa O₂.

Effect of CA and a CA inhibitor on emersed photosynthesis

The closed system IRGA was employed in this study. A sample of 0.3–0.5 g fresh weight was placed in the assimilation chamber at a constant temperature of 10°C and illuminated with 500 μmol photon m⁻² s⁻¹. The system was opened at the beginning of the experiment, and outside air was pumped into the chamber (by means of the IRGA pump) for 15 minutes before the circuit was closed. When the CO₂ compensation partial pressure was reached, the sample was taken out and put in filtered seawater for 30 min (under light saturation), blotted dry and treated with CA solution (1 kg/m² = 3,000 E.U.) until the surface of the thallus was covered by a CA solution. The thallus was then refixed in the assimilation chamber, and the rate of CO₂ uptake was once again monitored. When the CO₂ compensation partial pressure was reached, the thallus was taken out, washed 3 times with an excess of seawater and immersed in seawater for 30 min (under saturating illumination), then blotted dry and reimmersed in seawater containing 0.02 mol m⁻³ acetazolamide under illumination for another 30 min. The thallus (whose apoplast and surface film of fluid contain acetazolamide at the 0.02 mol m⁻³ supplied in the seawater) was then refixed in the assimilation chamber and the CO₂ uptake was once again monitored until the CO₂ compensation partial pressure was reached.

C and N content, and fresh weight/dry weight ratio

Samples of algae of known fresh weight were dried at 80°C in an oven and weighed after 48 h. For C and N content,