Photosynthetic Pathways in Freshwater Aquatic Plants

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Recent studies show that generalizations about photosynthetic pathways, derived from terrestrial plant studies, do not apply to aquatic plants. Crassulacean acid metabolism (CAM) photosynthesis is of selective value only in arid environments, where it enhances water-use efficiency, but also in aquatic plants of oligotrophic waters, where it enhances competitive ability in carbon acquisition. CAM photosynthesis is present in many aquatic species, but in these species it is not coupled with the specialized anatomy of terrestrial C4 plants. The ratio of the stable carbon isotopes, 13C/12C, in the biomass of terrestrial plants is a marker of their photosynthetic pathway. In aquatic environments, additional resistance to carbon-isotope fractionation make this technique of limited use in detecting photosynthetic pathways.

Much of our current understanding of photosynthetic pathways has been derived from studies of terrestrial plants, where we can distinguish three modes: C3, C4, and CAM (see Box 1 and Fig. 1). Recent studies on aquatic plant photosynthesis have revealed that many generalizations about photosynthetic pathways need to be reconsidered.

The aquatic milieu presents several factors not normally encountered by terrestrial species. Due to the viscosity of water, gases diffuse 10 times slower than in air. Consequently, in the boundary layer around leaves, gases may undergo markedly greater diurnal changes than are observed in the atmosphere. During the day, photosynthetic consumption may deplete the boundary layer of carbon dioxide (CO2) and generate elevated oxygen (O2) concentrations. Under such conditions, some aquatic species are capable of utilizing other forms of inorganic carbon such as bicarbonate (HCO3-) (Ref. 2). This, however, requires expenditure of energy, and in some environments it seems that the benefits do not balance the costs; consequently, many species have not evolved the capacity for active uptake of HCO3-.

Historically it was thought that because the total pool of inorganic carbon (CO2, HCO3-, CO32-) was orders of magnitude higher than in air, aquatic plants were seldom carbon-limited. Today, carbon limitation is considered to be a major driving force in the evolution of photosynthetic characteristics of aquatic plants.

Aquatic CAM plants

Crassulacean acid metabolism (CAM) is present in most terrestrial succulent plants of arid environments. Due to the reverse stomatal behavior (in which stomatal pores open at night and close during the day), CAM photosynthesis plays an important role in conserving water. In light of the adaptive significance of CAM in terrestrial species, the last place one might expect to find a CAM plant would be in water. However, we now know that a large number of aquatic plants have the CAM pathway. Included in this group is the rather large genus of primitive spore-bearing plants Isoetes (Isoetaceae) (Fig. 2). Other aquatic CAM species include higher plants, both monocots (Poaceae) and dicots (Crassulaceae and Plantaginaceae). It appears that ambient carbon limitation has been the driving force selecting for this pathway. Aquatic CAM plants are found worldwide, but only in certain aquatic habitats. Many are distributed in small temporary pools, which experience very marked diurnal fluctuations in CO2 concentration (Fig. 3a). Due to the fact that such environments have high irradiances and are densely vegetated, the CO2 is depleted very rapidly in the morning; at a rate in excess of the diffusion of CO2 from the atmosphere into the water. Since aquatic CAM plants are unable to use HCO3- (Refs 10–12), they are carbon-limited throughout much of the day, and carbon uptake is greatly curtailed (Fig. 3b). However, nighttime respiration by the flora and invertebrate fauna replenishes the CO2. Aquatic CAM plants take advantage of this plentiful supply of CO2, available at night by fixing it and storing it overnight as malic acid (Fig. 3c). During the day, when the ambient CO2 concentration is near zero, these plants break down the malic acid (as do terrestrial CAM plants) and thus generate their own intracellular supply of CO2.

In all aquatic plants, stomata are lacking or non-functional; therefore, in aquatic CAM plants, the balance between nighttime and daytime carbon uptake is controlled by CO2 availability rather than stomatal behavior, as in terrestrial CAM plants. Thus, the CAM pathway stands as an example of a biochemical pathway that has been selected in response to two very different sets of ecological conditions.

Temporary pools eventually dry up. When Isoetes and other aquatic CAM plants are exposed to the atmosphere they lose the CAM pathway and rely strictly on C3 photosynthesis. This loss of CAM activity occurs on a cell-by-cell basis; submerged leaf bases retain CAM, while aerial leaf tips largely lack CAM. The physiological mechanism behind this switch is unknown, but there are some clues as to the environmental stimuli responsible for the change from aquatic to aerial metabolism. Two possible cues for CAM loss have been tested: the aerial leaves could respond to the lower ratio of red–far-red light, or to the lower atmospheric water potential. Isoetes howellii plants were grown out of water in a high or low ratio of red–far-red light, combined with a high or low atmospheric humidity. Filtering light through water (which increases the ratio of red–far-red light) had no influence on overnight acid accumulation. However, when atmospheric humidity was maintained near saturation, CAM was lost despite the fact that the plants were growing in an aerial environment. A similar finding has been reported for another CAM plant, Littorella uniflora.

Shallow temporary pools occur worldwide and often contain species of Isoetes and Crassula. Species in both genera from pools in North America, South America, Europe and Australia have been shown to possess CAM (Ref. 3 and Keeley, unpublished data). These genera also occur in lacustrine environments. With rare exceptions, the only permanent bodies of water...
when the temperature is near the dew point, CAM plants are regularly inhabited by aquatic CAM plants. Lactucine environments are quite different from seasonal pools. Such habitats lack diurnal fluctuations in CO₂, however, carbon limitation is imposed by extremely low organic carbon levels in the water column. Under these conditions, CAM plants allow to maintain above-saturating intercellular CO₂ concentrations throughout the day and night. Depending upon the ambient carbon level, CAM plants may contribute from a third to over 90% of the total 24 h carbon uptake.

A number of freshwater species are capable of CO₂ fixation in the dark, but organic acids do not accumulate as do they in CAM plants, and it appears that these products may be metabolized in the dark. Although it seems doubtful that dark CO₂ fixation plays a role in the photosynthesis of these species, it is unknown which contribution such dark carbon uptake makes to the overall carbon economy of these plants.

Box 1. C₃, C₄, and CAM photosynthetic pathways

From terrestrial plant studies, three photosynthetic modes are recognized: C₃, C₄, and CAM. The C₃ pathway is characterized by the conversion of CO₂ into RuBP carboxylase, which is then converted to malate and aspartate, generating ATP and NADPH for the Calvin cycle. The C₄ pathway involves the conversion of CO₂ into PEP carboxylase, which is then converted to malate and aspartate, generating ATP and NADPH for the Calvin cycle. The CAM pathway involves the conversion of CO₂ into malate and aspartate, generating ATP and NADPH for the Calvin cycle. CAM plants are capable of CO₂ fixation at night, catalyzed by RuBP carboxylase, and organic acids are stored overnight in the cell vacuole. During the day, these acids are decarboxylated, in the same cells, and this carbon is assimilated through the C₃ cycle. CAM plants have the two carbon fixation events separated temporally, rather than spatially, as in C₄ plants. The typical or prototype CAM plant shows high CO₂ conductance at night and low CO₂ conductance, and low water loss, during the day.

**Fig. 1.** Cross-section of a floating leaf of *Oc t u t t i a c a l i f o r n i a*, illustrating the rings of chloroplast-laden bundle sheath cells surrounding the vascular system - a pattern known as Kranz anatomy.
acids, largely aspartate, and pulse-chase studies reveal a turnover of these products similar to that observed in terrestrial C₃ plants (Keely, unpublished). Later in development the submerged leaves are replaced with leaves that float on the water surface. Floating leaves also have C₃ fixation but, unlike the submerged leaves, they have well-developed Kranz anatomy (Fig. 1), this is also true of the third set of leaves, produced once the pools have dried down.

The tribe Orcuttieae comprises a dozen species, all of which are endemic to Californian seasonal pools and all of which are C₃ (Keely, unpublished data). The affinities of these grasses indicate that they are derived from terrestrial ancestors. The most primitive member of the tribe, Neostapfia colusana, produces submerged leaves with Kranz anatomy as well as stomata. The more advanced Orcuttia californica represents an evolutionary loss of Kranz anatomy, as well as loss of other structures such as stomata. Thus, we can surmise that in Orcuttia, and possibly other aquatic plants, there may be an advantage to combining C₃ photosynthesis with intracellular compartmentation of carboxylating enzymes, rather than intercellular compartmentation as in terrestrial C₃ plants.

Carbon isotopes

The ¹³C isotope accounts for 99% of carbon in nature. Most of the remaining carbon consists of atoms of the heavier isotope, ¹²C. Stable isotopes such as these are measured on a mass spectrometer and expressed as:

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\frac{¹³C/¹²C \text{ ratio of sample}}{¹³C/¹²C \text{ ratio of standard}} - 1
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This number is multiplied by 1000, expressed as ‘per mil’ (‰) and abbreviated as δ¹³C (the carbon isotope ratio). In plant matter, it is always negative; the more negative the ratio, the less ¹³C present.

In terrestrial plants, δ¹³C values of photosynthetic tissues vary from −8‰ to −15‰ in C₃ plants, and from −20‰ to −35‰ in C₄ plants. CAM plants may range from C₃-like to C₄-like in their carbon isotope ratio, depending upon the balance between nighttime and daytime stomatal conductance. Atmospheric CO₂ is −8‰, and during photosynthesis plants discriminate against ¹³C; this is most pronounced in C₃ plants. Discrimination comes about largely because the C₃ enzyme RuBP carboxylase shows a marked preference for the ¹³C isotope. Since ¹³C is discriminated against, it will accumulate, but readily diffuses away as it is mixed with atmospheric air passing through the stomatal pores. C₃ plants fix atmospheric carbon with PEP carboxylase, which has much less discrimination against ¹³C. Even though the products of C₄ fixation are broken down and the CO₂ is relaxed with RuBP carboxylase, discrimination at this stage is largely prevented because RuBP carboxylase is restricted to the interior of the leaf in the Kranz-type bundle sheath cells. Although the ¹³C isotope is discriminated against, it diffuses out of the bundle sheath cells very slowly; thus, it accumulates within these cells and eventually the enzyme is forced to fix this isotope.

In aquatic plants, the δ¹³C value is not indicative of the photosynthetic pathway. There are several reasons for this. As mentioned earlier, some aquatic plants can take up HCO₃⁻ in addition to CO₂. When inorganic carbon species are at equilibrium in solution, HCO₃⁻ will have a less negative δ¹³C than CO₂; depending upon pH and temperature, this may result in an 8–10‰ difference. Thus, theoretically, two aquatic plants with identical photosynthetic pathways may differ substantially if one utilizes HCO₃⁻ and the other is restricted solely to CO₂ uptake.

Another profound influence is created by the boundary-layer effects resulting from the aquatic milieu. In aquatic C₃ plants RuBP carboxylase discrimination against the ¹³C isotope will lead to the accumulation of this isotope in the boundary layer. As it accumulates, the higher concentration will overcome discrimination and, in effect, the enzyme will be forced to fix the ¹³C and thus discrimination will be minimal. In many aquatic environments, diffusion-limited resistance to CO₂ will be the dominant factor influencing δ¹³C values.

Another factor in aquatic environ-
It has been suggested that $\delta^{13}C$ measurements may have some potential for evaluating diffusional resistances to carbon uptake in aquatic plants. For aquatic plants that use CO$_2$ and rely exclusively on C$_3$-type RuBP carboxylase fixation, the $\delta^{13}C$ value of the biomass approaches that of the source carbon as diffusional resistances to carbon uptake increase. Plants from stagnant pools of water have less negative $\delta^{13}C$ values than plants from fast-moving streams, because the boundary-layer effect results in the accumulation of CO$_2$ and swamps out the discrimination by RuBP carboxylase.