CRASSULACEAN ACID METABOLISM IN SUBMERGED AQUATIC PLANTS

JON E. KEELEY

1. INTRODUCTION

CO_2-fixation in the dark is known to occur in various organs of many plants. However, only in species possessing crassulacean acid metabolism (CAM) does dark CO_2-fixation contribute substantially to the carbon economy of the plant. Until very recently CAM was known only from terrestrial species, largely drought adapted succulents. The discovery of CAM in the submerged aquatic fern ally Isoetes howellii (Isoetaceae)(Keeley 1981) adds a new dimension to our understanding of crassulacean acid metabolism. In this paper I will summarize 1) the evidence of CAM in Isoetes howellii, 2) the data on the distribution of CAM in aquatic species, and 3) the work to date on the functional significance of CAM in aquatic species.

2. EVIDENCE OF CRASSULACEAN ACID METABOLISM IN ISOETES HOWELLII

The definition of CAM is changing as our understanding of the various conditions under which CAM may play a selective role increases. Teeri (1983) proposes that the minimum characteristics necessary to consider a plant CAM are the "photosynthetic cells have the ability to fix CO_2 in the dark via PEP carboxylase, forming malic acid which accumulates in the vacuole. During the following light period the malic acid is decarboxylated, and the CO_2 enters the PCR cycle in the same cell". Unlike previous definitions this minimal definition is entirely biochemical and presupposes nothing about other biochemical, physiological, structural or ecological characteristics which may be associated with CAM.

Such a definition is required for this discussion due to inherent properties of aquatic plants and their environment. For example, a diurnal pattern of high night/low daytime stomatal conductance has been used as one criterion for CAM but many aquatic plants lack stomata although some of them clearly possess CAM as defined above (Keeley 1982). Another example is the $\sigma^{13}C$ isotope ratio which, in terrestrial succulents, indicates nighttime CO_2 uptake via CAM when in the range of $\sim$-14 °/oo. However, in aquatic plants $\sigma^{13}C$ level is governed as much by the isotope ratio of the source carbon, diffusional resistances of the water and extent of HCO_3^- usage, as it is by the biochemistry of carboxylation events. Isoetes howellii is capable of substantial nighttime CO_2 uptake via CAM yet $\sigma^{13}C$ ratios are typically $\sim$-28 to -30 °/oo. Other species in these same pools which are not CAM (nor C_4) have ratios $\sim$ -20 °/oo. Thus, the stable carbon isotope ratio is not a clear indicator of photosynthetic pathway in these aquatic plants. The fact that I. howellii assimilates HCO_3^- very poorly whereas other species in these pools are HCO_3^- users may explain some of these differences. However, other complicating factors include the fact that inorganic carbon levels fluctuate diurnally and thus species taking carbon up at night are not using the same carbon source as daytime uptakers. Since the elevated nighttime CO_2 levels in these pools arises from respiration of the total pool flora and fauna then the carbon isotope ratios for the nighttime CO_2 source may reflect previous fractionation events.

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In Isoetes howelii CAM is suggested by the following characteristics (Keeley 1981; Keeley, Bowes 1982; Keeley, unpublished): 1) Dark CO_2-fixation occurs in photosynthetic tissues but not in corms, 2) malic acid accumulates in these tissues overnight, 3) there is a diurnal cycle of nighttime acidification and daytime deacidification involving a fluctuation of 100-300 μequevalents g^{-1} fresh weight, 4) PEP carboxylase activities are sufficient to account for observed rates of acid accumulation and 5) carbon released from malic acid is incorporated into PGA and other early products of C_3 photosynthesis.

3. DISTRIBUTION OF CAM IN AQUATIC PLANTS

Evidence of CAM based on the presence of substantial diurnal malic acid fluctuations indicates that it is widespread in Isoetes. Eighteen species of Isoetes representing the range of variability in the genus, have been tested and 15 are clearly CAM whereas three apparently are not (Keeley 1981, unpublished). The CAM species are all aquatics and the non-CAM species are strictly terrestrial. Two other aquatic species known to be CAM are Crassula aquatica (Crassulaceae) and Littorella uniflora (Plantaginaceae) (Keeley, Morton 1982). There is evidence of a weakly developed CAM pathway in Hydrilla verticillata (Hydrocharitaceae) and two species of Scirpus (Cyperaceae) (Keeley, Morton 1982). In general, aquatic species with a well developed CAM pathway are found in one of two habitats; either shallow seasonal pools or permanent bodies of water at high elevations and/or high latitudes which are intensely oligotrophic.

4. FUNCTIONAL SIGNIFICANCE OF CAM IN AQUATIC PLANTS

In all CAM plants the primary physiological significance of crassulacean acid metabolism is that it generates an internal source of CO_2 during the day. In many xeric adapted succulents CAM was undoubtedly selected for because it allows daytime C_3 photosynthesis to proceed with closed stomata. These plants have much higher stomatal conductances at night when water vapor pressure deficits are lower thus they have very high water-use efficiencies. However in terrestrial plants there is a great deal of variation with respect to the contribution of dark vs light CO_2 uptake and some CAM species show no uptake of carbon in the dark though they have a very active crassulacean acid metabolism. These plants refix respiratory CO_2 and, dependent upon the season, may or may not take up CO_2 in the daytime. In all of these cases CAM plays a significant role in the carbon economy of the plant.

Xeric adapted succulents represent only one situation where evolution has selected for the ability to generate an internal CO_2 source during the day (via CAM). I suggest there may be other situations where CAM could play an important role in internal CO_2 generation; e.g., certain aquatic habitats where intense ambient CO_2 deficiencies occur during the day. Aquatic CAM plant habitats represent daytime carbon deficient environments; shallow pools have large diurnal fluctuations in available carbon whereas oligotrophic lakes are deficient in total carbon.

Isoetes howellii is being studied intensely as a system for evaluating the role of CAM in the carbon economy of a submerged aquatic.

4.1. Carbon uptake in the light

Isoetes howellii is widely distributed throughout western North America in shallow seasonal pools. Table 1 shows the physical and chemical changes in one of these pools during a 24 hr cycle. Overnight, due to an increase in pool respiration as well as a drop in temperature, free-CO_2 levels increase causing the pH to fall. During this time there is an increase in acidity of >350 μeq mg^{-1} Chl (∿170 μeq g^{-1} FW). Between 6 and 9 in the morning the changes in pool chemistry are slight relative to the following 3 hr period when there is a marked depletion of free-CO_2 in the water. Earlier it was hypothesized that such low CO_2 levels would limit daytime carbon uptake. Several lines of evidence support this hypothesis. Laboratory studies of carbon uptake in the light (Keeley 1983) demonstrated an order of magnitude drop between pH 6 and 8 (probably due to an inability to assimilate HCO_3^-). Field studies also support this in that CO_2 uptake rates throughout the day are significantly correlated ($P<0.02$) with free-CO_2 levels (Fig. 1).

Table 1. Characteristics of *Isoetes howellii* pool 23-24 May, 1983 (methods as in Keeley 1983). Chlorophyll levels were 0.50 ± 0.04 (submerged) and 1.00 ± 0.03 (emergent) mg g^{-1} fresh weight.

	1800	2100	2400	0600	0900	1200	1500	1800
QSR (μE $m^{-2}s^{-1}$)	300	0	0	210	1320	1920	1590	310
°C	28	22	20	15	21	29	34	31
O_2	101	69	31	23	79	134	149	122
pH	8.2	6.8	6.4	6.2	6.8	8.0	8.3	7.0
CO_2 (mg L^{-1})	0.2	5.8	15.9	22.4	5.9	0.4	0.1	3.2
Alk. (mg L^{-1})	16.7	16.8	18.3	18.4	17.5	16.7	14.5	15.5
SUBMERGED LEAVES								
Acidity[a]	24±1	87±9	261±18	370±66	388±27	163±38	32±7	24±13
Malic acid[b]	57±13	88±4	144±10	180±36	178±27	115±14	58±2	51±3
EMERGENT LEAVES								
Acidity[a]	12±3	-	-	26±5	-	-	-	5±5
Malic acid[b]	42±4	-	-	45±6	-	-	-	40±6

[a] μeq mg^{-1} Chl. [b] μmol mg^{-1} Chl. ($\bar{X}$ ± S.D., N=3).

4.2 Carbon uptake in the dark.

Laboratory studies have shown that dark, as well as light, CO_2 uptake are functions of pH and carbon level, though under similar conditions dark uptake is always less than light uptake (Keeley, Bowes 1982; Keeley 1983). The carbon gain from dark vs light uptake has been estimated for plants in the field by measuring C^{14} incorporation rates every 3 hrs through the 24 hr cycle shown in the Table 1. Over this period total carbon gain was ∿125 μmol mg^{-1} Chl. Dark uptake represented 47% of this total. Due to the rapid drop in free CO_2 the bulk of the CO_2 uptake in the light was restricted to a narrow window between 6 and 9 am. In fact the overnight carbon uptake was double the daytime carbon assimilated between 9 am and 6 pm. Thus dark uptake contributes substantially to the total carbon gain. Studies are underway to determine respiratory losses in order to calculate the contribution of dark CO_2 uptake to the net carbon gain.

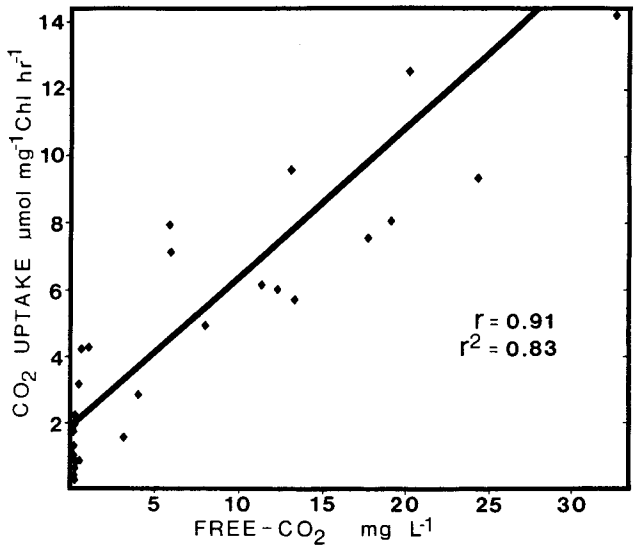

Fig. 1. CO_2-uptake rates in the light in I. howellii leaves vs free CO_2 levels in the pool throughout the 1983 season. Methods for C^{14} incorporation were similar to those described in Keeley 1983.

Also apparent from the dark uptake experiments is the fact that the total nighttime carbon assimilation accounted for only 48% of the malic acid accumulation. Thus, apparently CAM also plays a role in recycling respiratory CO_2.

4.3. Photosynthetic characteristics upon emergence

As shown in Table 1 CAM is lost upon emergence (even though chlorophyll levels increase) and dark CO_2 uptake represents ∿6% of the total carbon gain. Such changes would be predicted by the hypotheses that CAM was selected for under carbon limited aquatic conditions.

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Authors address: Dr. Jon E. Keeley, Department of Biology
Occidental College, Los Angeles, CA 90041 USA