Photosynthesis in *Elodea canadensis* Michx.

FOUR-CARBON ACID SYNTHESIS

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**ABSTRACT**

Experiments to determine the early labeled photosynthetic products in *Elodea canadensis* show that after 2 seconds of exposure to NaH¹⁴CO₃, 45% of the ¹⁴C incorporated is located in malate and aspartate. Phosphoglyceric acid and sugars account for 27% of the label during similar exposures. Equivalent amounts of organic acids and C₄ cycle products are present after 8 seconds. Four-carbon acids remain relatively unchanged throughout the first 45 seconds of exposure, while sugars increase in a linear fashion. Enzyme assays indicate that ribulose diphosphate and phosphoenolpyruvate carboxylase enzymes are present in a ratio of approximately 2:1. It appears that *E. canadensis* is able to synthesize significant amounts of four-carbon acids via β-carboxylation and this may play a role in maintaining a pH favorable for carboxylation in aquatic plants.

Recently, several studies have shown that aquatic plants can synthesize and metabolize four-carbon acids. Döhler (11) found that aspartate was the earliest labeled photosynthetic product in *Anacystis nidulans* after a few seconds of exposure to ¹⁴C, and three blue-green algal species, including *Anacystis*, were later observed to have up to five times more PEP carboxylase than RuDP carboxylase (9). Most recently, Benedict and Scott (5) reported that C₄ acids were the earliest labeled photosynthetic products in *Thalassia*. In still other studies, Brown and associates (6) investigated primary photosynthetic products in two flowering plants, *Egeria densa* and *Lagarosiphon major*, which were growing in fresh water lakes in New Zealand. They noted that C₄ acid production never exceeded 30% of the total label incorporated in either species, and that photorespiration rates were similar to those of C₃ plants. In contrast, another aquatic angiosperm, *Elodea*, had a compensation point of zero under low O₂ concentrations. *Elodea* also differed from the other two species with respect to light saturation point. It did not photosaturate up to 3,000 ft·c while *Egeria* and *Lagarosiphon* saturated at approximately 1,000 ft·c.

Along with the aquatic species just described, other plant tissues or organs have been shown to utilize PEP carboxylase to synthesize C₄ acids (13, 25). It is clear from these studies that certain aspects of the C₄ pathway, notably the synthesis and metabolism of four-carbon acids, relatively high PEP carboxylase levels and the resultant low ¹⁴C/¹²C ratios, are present in more than just C₄ plants. The purpose of the present investigation was to examine the mechanism and pattern of CO₂ assimilation in an aquatic plant species and to relate that information to the pathway of carbon assimilation in C₄ plants.

**MATERIALS AND METHODS**

*Elodea canadensis* Michx. was grown in a greenhouse pond at 20 to 25 °C with a pH of 8.5 to 9.2. Prior to use for incorporation studies, *Elodea* tissue was collected and placed in a two-liter flask containing 20 μM NaH¹⁴CO₃ (pH 6.5) and illuminated at 1,200 μeinstein sec⁻¹ (2800 ft·c) at 20 °C. This preincubation period was generally 30 to 45 min and experiments were initiated when a high rate of O₂ evolution was observed from the cut end of the spriis. Incorporations were carried out in a water-jacketed chamber containing 20 μM NaH¹⁴CO₃ (pH 6.5) with a light intensity of 1,200 μeinstein sec⁻¹. Assimilation experiments were terminated by removing the leaves and immediately plunging them into liquid N₂. For pulse-chase experiments, tissue was removed from the incorporation chamber after exposure to NaH¹⁴CO₃ and placed in a flow through beaker. Unlabeled bicarbonate at the same concentration was then passed over the spriis at a rate of 1 liter/min and the tissue was killed as described above. All subsequent extraction, separation, and identification procedures have been reported previously (17). Chlorophyll was determined according to Arnon (1) and total protein as given by Lowry et al. (19).

Crude enzyme extracts were prepared by homogenizing *Elodea* tissue in a chilled mortar and pestle. The grinding buffer consisted of 195 mM K-phosphate (pH 7), 10 mM MgCl₂, 20 mM 2-mercaptoethanol, 0.2 mM EDTA, and 16 g/l PVP (3). The resulting homogenate was centrifuged at 10,000g at 12 °C and the supernatant was used for enzyme assays.

The ribulose diphosphate carboxylase (RuDP Case; EC 4.1.1.39) assay described by Chu and Bassham (2, 8) was used with some modifications. The reaction mixture contained 25 mM Tricine (pH 8), 10 mM MgCl₂, 5 mM 2-mercaptoethanol, 5.5 mM RuDP, 0.05 mM NADPH, 1 mM FDP, 1.25 mM NaH¹⁴CO₃, and 5 to 25 μl of enzyme extract. Final volume of the reaction mixture was 100 μl. A 5-min preincubation period was employed and reactions were initiated by the addition of RuDP. Assays were terminated after 1 or 2 min by the addition of 50 μl of glacial acetic acid. Aliquots of 50 μl were taken, dried, and counted by liquid scintillation spectroscopy (15). Enzyme activities were expressed on the amount of Chl/g leaf tissue, rather than that contained in the enzyme extract.

Phosphoenolpyruvate carboxylase (PEP Case; EC 4.1.1.32) activity was assayed using a reaction mixture that contained 25 mM Tricine (pH 8.3), 10 mM MgCl₂, 5 mM 2-mercaptoethanol, 15 mM PEP, 15 mM sodium L-glutamate, 1.25 μM NaH¹⁴CO₃, and 5 to 25 μl of tissue extract in a final volume of 100 μl. Assays were terminated as previously described.
RESULTS

Time course experiments of NaH\(^{14}\)CO\(_3\) incorporation in Elodea indicate that after 2 sec the most heavily labeled product is malate (Fig. 1). Total C\(_4\) acids, malate plus aspartate, account for 45\% of the label in this period, while PGA contains 15\% and sugars 12\%. Malate alone increased to 40\% of the total \(^1\)\(\text{C}\) fixed after 8 sec of exposure; PGA and sugars increase to 27 and 23\%, respectively. After 45 sec, the malate pool remains essentially unchanged indicating that the turnover rate of the four-carbon acid pool may be slow.

PGA and sugars show curves which are typical of C\(_4\) pathway products in the first 45 sec of exposure to \(^1\)\(\text{C}\). PGA drops sharply at first, while sugars continue to rise. There is a small increase in the PGA pool after 45 sec coinciding with a decrease in C\(_4\) acids. Sugars increase linearly in the period from 10 to 120 sec.

Pulse-chase experiments again illustrate the slow turnover rate of four-carbon acids (Fig. 2) (7, 16). The malate pool is almost completely retained after 60 sec in unlabeled bicarbonate. C\(_3\) products, on the other hand, show an expected increase in labeling during the chase phase.

Activities of PEP and RuDP carboxylase are shown in Table I. Spinach, a typical C\(_3\) species, has a RuDP to PEP ratio of 6; whereas corn, a C\(_4\) plant, has a ratio of 0.2. The RuDP to PEP carboxylase ratio in Elodea is intermediate between these two plants with a value of nearly 2.

DISCUSSION

In the present experiments, E. canadensis was found to have relatively higher levels of PEP carboxylase and more synthesis of four-carbon acids in short time periods than would normally be expected for a C\(_3\) plant. C\(_4\) acids were labeled twice as heavily as C\(_3\) products after 2 sec and there was 34\% more PEP carboxylase relative to RuDP carboxylase in Elodea than in spinach. In other studies, Elodea has been shown to have a low CO\(_2\) compensation point and high light saturation (6). Other characteristics of photosynthesis in this species illustrate distinct differences between it and C\(_4\) plants. After the shortest exposure times to \(^{14}\)CO\(_2\), labeled C\(_4\) acids contained less than 50\% of the total radioactivity; whereas in C\(_4\) plants this percentage is generally about 90\% (12, 17). Also, from pulse-chase experiments, turnover of C\(_4\) acids did not occur as rapidly in this study as occurs in C\(_4\) plants.

The pattern of carbon metabolism in Elodea most closely resembles that found in Anacystis by Döhler (11). In that study, aspartate was the major labeled product of CO\(_2\) fixation containing approximately 50 to 60\% of the total \(^1\)\(\text{C}\) fixed in the first 1 to 2 min, while PGA had 15 to 25\% of the label. Quite similar labeling patterns were also reported for Thallasia, a marine grass (5). What all three of these organisms have in common is an aqueous environment. The absolute amounts of carbon in an aqueous system are about 50 times those in air at 20 C but the form of carbon available, CO\(_2\) versus HCO\(_3\)^\(-\), is very pH-dependent (6, 22, 24). Carbon availability is compounded by the fact that the diffusive pressure of CO\(_2\) in water is 100,000 times smaller than in air (0.16 cm\(^2\) sec\(^{-1}\) in air compared to 1.6 \(\times\) 10\(^{-6}\) cm\(^2\) sec\(^{-1}\) in water) (22, 23). If RuDP carboxylase uses CO\(_2\) (10) and PEP carboxylase uses bicarbonate (20), then carboxylation of PEP and four-carbon acid formation would be favored under conditions above a pH of 6.5. Since these pH levels are frequent in ponds (6, 14), C\(_4\) acid synthesis would result in plants which contained even moderate amounts of PEP carboxylase.

Fig. 1. Time course of NaH\(^{14}\)CO\(_3\) labeling of early photosynthetic products in E. canadensis expressed as a percentage of total \(^1\)\(\text{C}\) incorporated. The photosynthetic rate average 15 \(\mu\text{mol/mg Chl/hr.}\)

Fig. 2. Pulse-chase experiment in which Elodea leaves were exposed to NaH\(^{14}\)CO\(_3\) for 8 sec followed by a NaH\(^{14}\)CO\(_3\) chase for an additional 60 sec.

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Two of the three plants discussed above, *Elodea* (Table I) and *Anacystis* (9), have been shown to contain relatively high levels of this enzyme.

The capability to synthesize and metabolize four-carbon acids (4), and the resultant low $^{13}C/^{12}C$ ratios, does not mean that the three aquatic species discussed above possess other structural and functional features of C₄ plants (18). From our observations (unpublished results), *Elodea* leaves are simply two cells thick and the midrib is the only vasculature present. Chloroplasts show extensive grana with no observable peripheral reticulum. In all respects they appear similar to C₃ plant chloroplasts as earlier described by Muhlenthaler and Frey-Wyssling (21). Benedict and Scott (5) also found that C₄ acid synthesis in *Thalassia* did not correlate with Kranz anatomy.

The presence of both C₃ and C₄ cycle enzyme systems in some aquatic plants may mean that under various environmental conditions one, the other, or both enzymes may be actively fixing carbon. Functionally, this may be important in the maintenance of a cytoplasmic pH that is slightly acid. In the acidic condition, bicarbonate ions would be protonated to H₂CO₃ which in turn would increase the level of free CO₂ that diffuses into the cell, thus creating a gradient of CO₂ from the cytoplasm to the chloroplast that would facilitate Calvin cycle activity in that organelle. The slow turnover rate of four-carbon acids in the present experiments may also mean they represent a pool of CO₂ that could be drawn upon by decarboxylation. Regardless of the functional significance it appears that several aquatic plants, as well as other assimilating plant tissues and organs (13, 25), are able to synthesize four-carbon acids. While these examples at first seem anomalous with respect to strict C₃-C₄ classification, they do not possess additional features of C₄ plants and should not be confused with the latter.

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**LITERATURE CITED**


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Corrections

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DeGroote, Dave, and Robert A. Kennedy. Photosynthesis in
*Elodea canadensis* Michx. Four-Carbon Acid Synthesis.
Page 1133, column 2, paragraph 3, line 5, and paragraph 4, line 4
should be corrected to read: 50 mM NaH\(^{14}\)CO\(_3\).