Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2883–2887, April 1991
Botany

The role of phospho*enol*pyruvate carboxykinase in a marine macroalga with C_4-like photosynthetic characteristics

(3-mercaptopicolinic acid/photorespiration/carboxylase/*Udotea*/*Codium*)

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Communicated by William L. Ogren, January 3, 1991 (received for review October 25, 1990)

ABSTRACT ***Udotea flabellum*** **is a marine, macroscopic green alga with C_4-like photosynthetic characteristics, including little O_2 inhibition of photosynthesis, a low CO_2 compensation point, and minimal photorespiration; but it lacks anatomical features analogous to the Kranz compartmentation of C_4 plants, and phospho*enol*pyruvate carboxylase [PEPC; orthophosphate:oxaloacetate carboxy-lyase (phosphorylating), EC 4.1.1.31] activity is negligible. Phospho*enol*pyruvate carboxykinase (PEPCK) activity (carboxylating) in *Udotea* extracts was equivalent to that of ribulose-bisphosphate carboxylase [Rubisco; 3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39]. When PEPCK activity was inhibited *in vivo* with 3-mercaptopicolinic acid (MPA), thallus photosynthesis decreased by 70% and became sensitive to O_2. *Codium decorticatum*, a related species that lacks C_4-like photosynthetic features and PEPCK activity, showed no increase in O_2 inhibition upon exposure to MPA. Rubisco and PEPC activities in *Udotea* were not inhibited by MPA. Labeling of the early photosynthetic products malate and aspartate was reduced 66% by MPA, while intermediates of the photorespiratory carbon oxidation cycle showed a 3-fold increase. *Udotea* evolved O_2 in the light in the absence of inorganic carbon, suggesting it had an endogenous carbon source for photosynthesis. Exogenous malate stimulated this process, while MPA inhibited it. PEPCK was not involved in Crassulacean acid metabolism or dark CO_2 fixation. These MPA studies establish a direct link between PEPCK activity and the low O_2 inhibition of photosynthesis and low photorespiration in *Udotea*. The data are consistent with carboxylation by a cytosolic PEPCK providing a C_4 acid, such as malate, to the chloroplast for decarboxylation to elevate the CO_2 concentration at the Rubisco fixation site. *Udotea* is to date the most primitive plant with a C_4-like form of photosynthesis.**

Marine algae assimilate CO_2 predominately via the photosynthetic carbon reduction cycle, with ribulose-bisphosphate carboxylase [Rubisco; 3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39] as the carboxylation enzyme (1, 2). Such CO_2 fixation should be competitively inhibited by O_2, with the oxygenase activity of Rubisco initiating the photorespiratory carbon oxidation cycle. However, many marine algae show no O_2 inhibition of photosynthesis (1–3), but why this is so is uncertain.

Terrestrial C_4 and Crassulacean acid metabolism species circumvent O_2 inhibition of photosynthesis by the initial use of phospho*enol*pyruvate carboxylase [PEPC; orthophosphate:oxaloacetate carboxy-lyase (phosphorylating), EC 4.1.1.31]. The subsequent decarboxylation of C_4 acids delivers CO_2 to Rubisco at higher concentrations than occurs by diffusion from the air. Kranz anatomy in C_4 species prevents futile recycling of CO_2 by segregating the initial carboxylation and decarboxylation reactions in different cells, while in Crassulacean acid metabolism the two events are separated temporally rather than spatially. Another mechanism eliminates the O_2 inhibition of photosynthesis in unicellular algae and cyanobacteria. It also concentrates CO_2 internally, but this is mediated by inorganic carbon (C_i) transporters at the plasma membrane or chloroplast envelope and carbonic anhydrase (4).

For some terrestrial C_4 and Crassulacean acid metabolism plants, the decarboxylase providing CO_2 for refixation by Rubisco is phospho*enol*pyruvate carboxykinase (PEPCK) (5). In a wide variety of organisms PEPCK catalyzes the GD(T)P-dependent [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] or AD(T)P-dependent [ATP: oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.49] carboxylation of phospho*enol*pyruvate or decarboxylation of oxaloacetate. Thus, in parasitic helminths PEPCK acts as a carboxylase in carbohydrate catabolism (6), while cytosolic and mitochondrial isozymes, acting as GTP-requiring decarboxylases, regulate mammalian gluconeogenesis (7, 8). In the algal protist *Euglena*, two forms operate: one as a gluconeogenic decarboxylase and one in CO_2 fixation for the methylmalonyl CoA pathway (9).

Substantial C_4 acid production by the β-carboxylation of phospho*enol*pyruvate occurs in some marine algae (1, 2). In brown macroalgae (phylum Phaeophyta) the enzyme responsible is not PEPC, but an AD(T)P-dependent PEPCK whose function has been variously ascribed to light-independent CO_2 fixation for anapleurosis (10), a component of mannitol degradation (11), a carboxylase for Crassulacean acid metabolism (2), and a decarboxylase for intermediary pathways (11). For marine diatoms and dinoflagellates PEPCK may supplement ATP production in low light (12). Thus, PEPCK is a versatile enzyme with diverse functions, even within the same organism. However, the evidence that PEPCK and C_4 acids have a photosynthetic role in marine macroalgae is circumstantial, and a direct correlation between C_4 acid formation and the absence of O_2 inhibition of photosynthesis is lacking.

A potent inhibitor, 3-mercaptopicolinic acid (MPA), has proved useful in unraveling the *in vivo* functioning of PEPCK. In animal studies, MPA inhibits carboxylation competitively with respect to phospho*enol*pyruvate, and it inhibits its decarboxylation noncompetitively with respect to oxaloacetate (7, 13). For plants in the C_4 PEPCK subgroup, photosynthesis and oxaloacetate decarboxylation are inhibited by MPA, as is $^{14}CO_2$ fixation and malate decarboxylation by isolated bundlesheath strands; also, the CO_2 compensation point is increased (14, 15).

We have shown that in the marine macroalga *Udotea* (phylum Chlorophyta), PEPCK activity equals that of

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Abbreviations: Chl, chlorophyll; C_i, inorganic carbon; MPA, 3-mercaptopicolinic acid; PEPC, phospho*enol*pyruvate carboxylase; PEPCK, phospho*enol*pyruvate carboxykinase; Rubisco, ribulose-bisphosphate carboxylase.

Rubisco and exceeds *in vivo* photosynthetic rates (16). In addition, *Udotea* has substantial activity of some enzymes associated with C_4 acid metabolism and phospho*enol*pyruvate regeneration and a rapid turnover of C_4 acids in the light (16). Concomitantly, gas exchange responses are C_4-like in regard to a lack of apparent photorespiration (16).

In this report on the effects of MPA, we provide further evidence for the operation of a C_4-like system in the macroalga *Udotea*. This PEPCK-mediated system results in reduced O_2 inhibition of photosynthesis without the aid of Kranz anatomy. Data from similar analyses of *Codium*, a macroalga closely related to *Udotea* but exhibiting O_2 inhibition and lacking PEPCK activity, demonstrate the diversity of photosynthetic mechanisms in the Chlorophyta.

MATERIALS AND METHODS

Plant Material. *Udotea flabellum* (Ellis and Solander) Lamouroux and *Codium decorticatum* (Woodward) Howe were collected and maintained as described (16). For light microscopic examination, 2.5- to 5-mm-long pieces were cut from the thallus tips of *Udotea*, fixed in 2.5% glutaraldehyde for 2 hr, and postfixed for 1.5 hr in 1% OsO_4. They were rinsed and dehydrated in an ethanol/acetone series, embedded in Spurr's plastic, and 1-μm-thick sections were cut with an LKB Ultratome III and stained with toluidine blue.

Gas Exchange Analyses. Net photosynthetic rates at 21% and 1% O_2 (220 and 11 μM dissolved O_2, respectively) and dark respiration were determined by O_2 evolution or uptake, respectively, in a Hansatech O_2 electrode system on 1-cm^2 sections of *Udotea* thalli and 2-cm-long *Codium* segments. Rates were measured in buffered (20 mM Hepes·NaOH) artificial seawater with 2 mM $NaHCO_3$ at pH 8.0 (16). Prior to measurement, the segments were incubated for 60 min at 23°C at a quantum irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm) in artificial seawater with 2 mM $NaHCO_3$ and from 0 to 5 mM MPA. Measurements were made at the predetermined optimal temperature (23°C for *Udotea* and 20°C for *Codium*) and a saturating quantum irradiance of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm). The percentage O_2 inhibition of the photosynthetic rate was calculated from the formula 100 × [1 − (rate with 220 μM O_2)/(rate with 11 μM O_2)]. A similar formula was used to determine the degree of inhibition by MPA. In experiments to determine whether 5 mM malate or aspartate supported photosynthetic O_2 evolution in the absence of C_i, the artificial seawater with or without 3 mM MPA, was sparged at low pH with CO_2-free air. Buffer and *Udotea* sections were then added and incubated in the C_i-free, closed system in the light for 60 min to deplete endogenous C_i sources before addition of malate or aspartate. All experiments were performed in triplicate.

Enzyme Extraction and Assays. The extraction and assay procedures used for Rubisco, PEPC, and PEPCK were as described by Reiskind *et al.* (16). To directly correlate the effects of MPA on thallus photosynthesis and enzyme activities, some enzyme assays were performed on extracts of washed thalli that had been used for photosynthetic measurements in the presence of MPA. All assays were performed in triplicate.

Fixation of ^{14}C. Sections of *Udotea* thalli (1 cm^2) were incubated, with or without 3 mM MPA, in artificial seawater containing 2 mM $NaHCO_3$ buffered at pH 8.0 for 60 min at 23°C with a quantum irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm). They were then exposed under similar conditions for 10 s to a fresh solution containing 2 mM $NaH^{14}CO_3$ (57.8 $Ci \cdot mol^{-1}$; 1 Ci = 37 GBq) with or without MPA, after which they were rapidly rinsed and frozen in liquid N_2.

Analysis of ^{14}C Fixation Products. The frozen samples were extracted, separated, and analyzed by ion-exchange and thin-layer chromatography (16, 17).

Malate and Chlorophyll (Chl) Analyses. Malate concentrations during the day and night (3), and Chl (16), were measured as described.

RESULTS

Udotea is a siphonaceous macroalga with a 10- to 15-cm-diameter blade-like thallus attached to a stipe (Fig. 1*A*). Transverse sections showed the thallus was constructed of anatomically similar filaments, and was six to eight filaments wide. Each filament was ≈24 μm in diameter and had a bilayered wall, a small central vacuole, and a dense population of chloroplasts adjacent to the plasmalemma (Fig. 1*B*). Micrographs and microdissection indicated the chloroplasts occupied ≈16% of the filament volume. No Kranz-like anatomical differences were detected among the filaments.

Considerable PEPCK (carboxylating) activity was found in *Udotea* extracts (16), and the direct addition of MPA to the extracts caused inhibition of activity *in vitro* (Fig. 2). The presence of 0.5 mM MPA resulted in 50% inhibition of PEPCK activity, while complete inhibition occurred at 2 mM. Photosynthetic O_2 evolution by the *Udotea* thallus was also inhibited by MPA; up to 5 mM MPA caused substantial, but never total, inhibition of photosynthesis (Fig. 2). At 3 mM

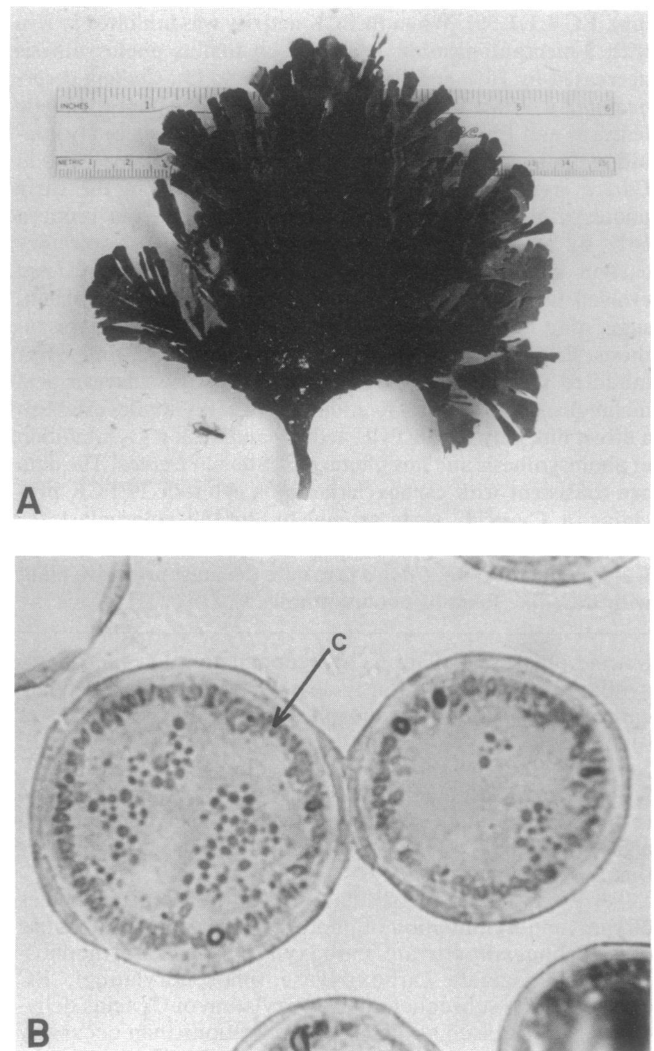

FIG. 1. The morphology and anatomy of *Udotea*. (*A*) Mature thallus and stipe. (*B*) Transection of *Udotea* thallus. At 2 mm from the tip it is constructed of anatomically similar, chloroplastic filaments in rows of six to eight. C, peripheral ring of chloroplasts within the cell. (×1550.)

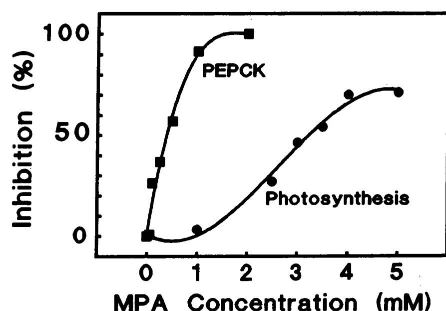

FIG. 2. The inhibitory effects of MPA on PEPCK activity and net photosynthetic rate in extracts and thalli, respectively, of *Udotea*. For PEPCK determinations, the MPA was added directly to the assay medium. Photosynthetic rates were determined as O_2 evolution by thalli after incubation with MPA for 60 min. The PEPCK activity and photosynthetic rate in the absence of MPA were 41.2 ± 1.9 and 23.6 ± 2.9 μmol per mg of Chl per hr, respectively.

MPA, ≈50% inhibition of thallus photosynthesis occurred, and this concentration was used in subsequent experiments.

The effect of MPA on photosynthetic gas exchange characteristics is shown in Table 1. Similar net photosynthetic rates at 220 and 11 μM O_2 demonstrated that O_2 had little inhibitory effect on *Udotea* photosynthesis at a C_i concentration typical of seawater (2 mM). However, when MPA was present, O_2 inhibition increased to the degree observed in C_3 plants. In low O_2, the effect of MPA on photosynthesis was minor. Unlike *Udotea*, photosynthesis in *Codium* was substantially inhibited by O_2; this was not further increased by MPA, although MPA did cause some O_2-independent inhibition (Table 1).

The dark respiration rate for *Udotea* was ≈12% of the photosynthetic rate, and it was not inhibited by MPA (2.8 ± 0.2 and 2.0 ± 0.1 μmol of O_2 uptake per mg of Chl per hr with and without 3 mM MPA, respectively).

Immediately after measurement of the photosynthetic rates (Table 1), the thalli were extracted and Rubisco and PEPCK (carboxylating) activities were determined (Table 2). The carboxylating activity of PEPCK in *Udotea* thalli not exposed to MPA was equivalent to the Rubisco activity. Treatment of the thalli with MPA did not inhibit Rubisco but did substantially inhibit PEPCK activity. In *Codium*, PEPCK activity was below detectable levels (Table 2). The activity of *Codium* Rubisco was slightly lower after MPA treatment, which was consistent with the small, O_2-insensitive decrease in the intact thallus photosynthetic rate in the presence of MPA (Table 1). The activity of PEPC in both *Udotea* and *Codium* was very low (1.0 and 5.4 μmol per mg of Chl per hr, respectively), and was unaffected by the presence of MPA.

Table 3 shows the incorporation of ^{14}C into the initial photosynthetic products of *Udotea* after treatment with MPA. The percentage of label in the C_4 acids malate and aspartate was reduced by two-thirds after exposure to MPA, while ^{14}C incorporation into 3-phosphoglycerate, sugar phosphates, neutral compounds, and insoluble compounds was unchanged. Concomitant with the MPA-induced decrease in C_4 acid labeling, there was a 3-fold increase in labeling of glycolate, glycine, and serine, which are intermediates of the photorespiratory carbon oxidation cycle. The lipid fraction also showed some increase.

Because substantial ^{14}C was incorporated into C_4 acids, the possibility of Crassulacean acid metabolism-like malic acid fluctuations was investigated. However, pool sizes were small and showed no diel fluctuation (1.1 ± 0.1 and 0.9 ± 0.2 μmol of malate per g fresh weight, day and night, respectively).

The data in Table 4 show the effect of adding 5 mM malate or 5 mM aspartate to *Udotea* thalli, after incubation in C_i-free seawater at low irradiance. The thalli in C_i-free conditions exhibited a light-dependent O_2 evolution rate that declined during the incubation period. In the absence of C_i, the addition of 5 mM malate stimulated photosynthetic O_2 evolution in the light to a rate similar to that supported by 2 mM C_i, but the presence of 3 mM MPA prevented this stimulation (Table 4). Aspartate was far less effective in supporting O_2 evolution, but the small stimulation observed was reduced by MPA treatment.

Table 1. Effect of O_2 and MPA on net photosynthetic rates of *Udotea* and *Codium* thalli

Macroalga	O_2, μM	MPA, mM	Photosynthetic rate,* μmol of O_2 per mg of Chl per hr	Inhibition, %	
				O_2	MPA
Udotea	220	0	16.5 ± 1.2	11	—
	220	3	9.2 ± 1.4	49	44
	11	0	18.5 ± 1.2	—	—
	11	3	17.9 ± 1.3	—	3
Codium	220	0	37.2 ± 3.1	40	—
	220	3	30.3 ± 2.8	39	19
	11	0	62.3 ± 2.0	—	—
	11	3	49.6 ± 1.2	—	20

*Mean of three replicate determinations ± SE.

Table 2. Activities of Rubisco and PEPCK (carboxylating) enzymes in extracts of *Udotea* and *Codium* thalli that had been incubated with or without 3 mM MPA

Macroalgal enzyme	Exogenous MPA, mM	Enzyme activity,* μmol per mg of Chl per hr	MPA inhibition, %
Udotea			
Rubisco	0	76.1 ± 1.3	—
	3	88.7 ± 1.0	0
PEPCK	0	84.7 ± 3.4	—
	3	22.4 ± 0.3	74
Codium			
Rubisco	0	64.9 ± 1.6	—
	3	54.4 ± 0.6	16
PEPCK	0	ND	—
	3	ND	—

The thalli used for the photosynthetic rate measurements in Table 1 were extracted for these enzyme activity determinations. ND, not detected.
*Mean of three replicate determinations ± SE.

DISCUSSION

We have shown that *Udotea* has characteristics indicative of low photorespiration (1, 16). They include little O_2 inhibition of photosynthesis; a low, O_2-insensitive CO_2 compensation point; negligible photorespiratory CO_2 evolution; a low apparent K_m (CO_2) for photosynthesis; and minimal ^{14}C incor-

Table 3. Incorporation of ^{14}C into photosynthetic products of *Udotea* thalli incubated with and without 3 mM MPA

Labeled compound	^{14}C incorporated, %	
	Without MPA	With MPA
Malate	11.1	2.3
Aspartate	14.1	6.4
3-Phosphoglycerate	22.5	21.6
Sugar phosphates	36.8	34.3
Glycolate + glycine + serine	4.3	13.8
Neutral + insoluble	14.1	14.0
Lipid	2.2	7.4
Total ^{14}C recovered	105.1	99.8

Table 4. Ability of exogenous C_4 acids (5 mM malate or aspartate) to support net photosynthetic O_2 evolution in C_i-free seawater by *Udotea* thalli incubated with or without 3 mM MPA

C_4 acid	C_i, mM	Photosynthetic rate,* μmol of O_2 per mg of Chl per hr	
		Without MPA	With MPA
None	2	16.5 ± 1.2	9.2 ± 1.4
None	0	5.2 ± 0.5	3.8 ± 0.3
Malate	0	16.8 ± 1.8	5.2 ± 0.7
Aspartate	0	7.3 ± 0.3	4.3 ± 0.1

*Mean of three replicate determinations ± SE.

poration into the photorespiratory carbon oxidation cycle. These attributes are consistent with a CO_2 concentrating system. Concomitantly, *Udotea* has considerable C_4 acid metabolism in the light, as evidenced by substantial activities of certain C_4 enzymes, especially PEPCK and pyruvate, P_i dikinase; the photosynthetic production of malate and aspartate; and the rapid turnover of C_4 acids in the light, with ^{14}C incorporation into carbohydrates (16). These data are circumstantial evidence for C_4-like photosynthesis but do not establish a direct correlation between the low photorespiration condition and the presence of PEPCK activity with its light-dependent metabolism of C_4 acids. The present results with MPA establish such a correlation.

The addition of 2.0 mM MPA to *Udotea* extracts completely inhibited *in vitro* PEPCK activity. A similar concentration inhibits phospho*enol*pyruvate carboxylation by a brown macroalgal extract (10), although in terrestrial plants the amount required is an order of magnitude lower (14, 15). It is possible that the macroalgal enzyme differs kinetically from that in terrestrial C_4 PEPCK species.

The importance of PEPCK in *Udotea* might be inferred from its high activity, but a more conclusive demonstration of its role in CO_2 fixation is the 50% reduction in thallus photosynthesis upon exposure to MPA concentrations that inhibited *in vivo* PEPCK activity. Also, the large reduction in initial C_4 acid labeling in the light when PEPCK was inhibited *in vivo* by MPA is indicative of a carboxylating role. Photosynthesis of terrestrial C_4 PEPCK, but not C_3, species also is decreased by MPA (14). The decreases in CO_2 fixation and C_4 acid formation by *Udotea* are not due to MPA effects on PEPC or Rubisco, as PEPC activity was <3% of the PEPCK value and, like Rubisco, was unaffected by MPA. Instead, they are consistent with the postulate that in *Udotea* a substantial amount of C_i is initially fixed by PEPCK before entering the photosynthetic carbon reduction cycle via Rubisco (16). However, the labeling data do not exclude some direct (parallel) fixation of CO_2 by Rubisco, especially if the algal chloroplast can take up HCO_3^-.

Dark CO_2 fixation by *Udotea* was only 6% of the light fixation rate (16), and malate pools were small and did not fluctuate between day and night. Also, dark respiration was not inhibited by MPA. Thus, for *Udotea*, any major involvement of PEPCK in dark CO_2 fixation, respiration, or Crassulacean acid metabolism is unlikely. This differs from the brown macroalgae, in which PEPCK acts as a light-independent carboxylase to provide organic acids for anapleurotic reactions (2, 11) or a limited form of Crassulacean acid metabolism (18).

The PEPCK in *Udotea* might simply recycle internal photorespiratory CO_2 before it escapes in a system reminiscent of some C_3–C_4 intermediate plants (19). However, a MPA-facilitated escape of photorespiratory CO_2, given a photorespiration/photosynthesis ratio of ≈0.25 (2), could not cause the large inhibition of photosynthetic O_2 evolution that was observed. Moreover, MPA raised the O_2 sensitivity of *Udotea* photosynthesis to that of C_3 plants and increased ^{14}C incorporation into products of the photorespiratory carbon oxidation cycle. These observations indicate that MPA indirectly stimulated the O_2 inhibition and oxygenase activity of Rubisco, as well as the release of photorespiratory CO_2. The implication is that PEPCK is a crucial component in a system that concentrates CO_2 at the site of fixation by Rubisco.

In contrast to *Udotea*, the related macroalga *Codium* exhibits gas exchange characteristics resembling terrestrial C_3 plants, and neither C_4 acids nor PEPCK play a part in photosynthesis (1, 16). Concordantly, MPA did not affect the substantial O_2 inhibition. Thus, *Codium* seems to lack an effective CO_2 concentrating mechanism. In an analogous terrestrial experiment, MPA increased the photorespiration of a C_4 PEPCK, but not a C_3, plant (14).

The MPA results, together with the data on C_4 enzymes and C_4 acid production and turnover (16), suggest some parallel between the function of PEPC in C_4 plants and PEPCK in *Udotea*. However, the lack of Kranz or analogous anatomy in *Udotea* poses a problem as to how the organism segregates primary CO_2 fixation from decarboxylation/refixation to avoid futile cycling. Our working hypothesis invokes an organellar, rather than cellular, compartmentation, with fixation via PEPCK occurring in the cytosol, and with decarboxylation in the chloroplast where Rubisco is localized. Active transport of a C_4 acid such as malate into the chloroplast could provide the substrate for elevating the CO_2 concentration specifically inside this organelle.

Recent work on CO_2 accumulation by isolated algal chloroplasts (20) provides a precedent for this postulate and demonstrates that chloroplasts can retard the efflux of CO_2 while actively transporting ions. If chloroplasts, rather than the whole cell, concentrate CO_2 this may be difficult to detect by protoplast or thallus measurements (21), as chloroplasts comprise only 16% of the cell volume in *Udotea*.

Further evidence for a C_4-like system in *Udotea* comes from the observation that in seawater lacking C_i the thallus showed light-dependent O_2 evolution, suggesting that some internal carbon source temporarily sustained photosynthesis. Exogenous malate was able to drive thallus photosynthesis in the absence of C_i at a rate equal to that with 2 mM C_i.

These data, and C_4 acid turnover, indicate that rapid decarboxylation occurs, although a search for a decarboxylase revealed only trace amounts of NAD malic enzyme and no detectable NADP malic enzyme (16). However, a mitochondrion-based NAD malic enzyme would fuel futile CO_2 recycling through PEPCK carboxylation in the cytosol. Decarboxylation in the chloroplast would avoid this difficulty. It is possible that PEPCK performs two functions in *Udotea*: carboxylation in the cytosol and decarboxylation in the chloroplast. A decarboxylation role for PEPCK is supported by the fact that malate-driven photosynthesis is almost 70% lower in the presence of MPA. The PEPCK of higher plants is located in the cytoplasm (5, 22), although in other organisms it has more than one intracellular location (7–9). We are attempting to establish the location(s) of PEPCK in *Udotea* cells.

If PEPCK does function in dual carboxylation and decarboxylation roles then it poses interesting problems of regulation. We are investigating whether two PEPCK isozymes, with differing kinetic characteristics, exist in *Udotea*, as is the case for *Euglena* (9). The concentrations of C_4 acids and ATP at the enzyme location could also determine the reaction direction, as may occur in the brown macroalga *Laminaria* (11).

In some marine algae a HCO_3^- utilization system, in concert with carbonic anhydrase, is responsible for the reduced O_2 effect (2, 4). Such a system cannot account for the MPA effects on *Udotea*. Also, ethoxyzolamide, an inhibitor of carbonic anhydrase, does not cause *Udotea* photosynthe-

sis to become O_2 sensitive, and CO_2 is used more effectively than HCO_3^- (1, 16).

Seawater contains 2.0–2.5 mM C_i, but this is insufficient to suppress photorespiration, unless a CO_2 concentrating system is present. In a chloroplast containing 2.2 mM C_i, the same as seawater, the free CO_2 concentration would be only 12 μM, given a pK_1 for carbonic acid of 6.0 at 25°C (2) and a stromal pH of 8.2. This is borne out experimentally, as photosynthesis by some marine macroalgae is inhibited by O_2 at seawater pH and C_i values (1–3).

Any ecological significance to the C_4-based system in *Udotea*, and its absence in *Codium*, has yet to be established. In distribution, *Udotea* is tropical, whereas *Codium* is more temperate, with their ranges overlapping in Florida waters. *Codium* is attached to rocks in the intertidal zone, where wave action equilibrates the water with air CO_2 and O_2. In contrast, *Udotea* inhabits deeper water. In fresh water, photosynthesis can deplete CO_2 and cause O_2 supersaturation, conditions that favor plants with reduced photorespiration. In accord with this concept, an inducible C_4-like system that concentrates CO_2 internally without Kranz anatomy, and mitigates deleterious aquatic conditions, has been described in the submersed flowering plant *Hydrilla* (23).

Udotea is the most primitive plant demonstrated to have a C_4-based form of photosynthesis. It raises the intriguing evolutionary possibility that the biochemical components of C_4 photosynthesis were present in an aquatic environment before they appeared on land.

We thank Drs. W. Latshaw and N. DiTullio of Smith, Kline, and French Laboratories for supplying the MPA and Dr. T. Lucansky for the microscope facilities. This work was supported by the United States Department of Agriculture, Science and Education Administration, Competitive Research Grants Office, Photosynthesis Program Grants 88-37130-3703 and 90-37130-5576.

1. Bowes, G. (1985) in *Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms*, eds. Lucas, W. L. & Berry, J. A. (Am. Soc. Plant Physiol., Rockville, MD), pp. 187–210.
2. Kerby, N. W. & Raven, J. A. (1985) *Adv. Bot. Res.* **11**, 71–123.
3. Holbrook, G. P., Beer, S., Spencer, W. E., Reiskind, J. B., Davis, J. S. & Bowes, G. (1988) *Can. J. Bot.* **66**, 577–582.
4. Badger, M. R. (1987) in *The Biochemistry of Plants*, eds. Hatch, M. D. & Boardman, N. K. (Academic, San Diego), Vol. 10, pp. 219–274.
5. Edwards, G. E. & Huber, S. C. (1981) in *The Biochemistry of Plants*, eds. Hatch, M. D. & Boardman, N. K. (Academic, New York), Vol. 8, pp. 237–281.
6. Lloyd, G. M. & Barrett, J. (1983) *Exp. Parasitol.* **56**, 259–265.
7. Jomain-Baum, M., Schramm, V. L. & Hanson, R. W. (1976) *J. Biol. Chem.* **251**, 37–44.
8. Hod, Y., Cook, J. S., Weldon, S. L., Short, J. M., Wynshaw-Boris, A. & Hanson, R. W. (1982) *Ann. N.Y. Acad. Sci.* **478**, 31–44.
9. Pônsgen-Schmidt, E., Schneider, T., Hammer, U. & Betz, A. (1988) *Plant Physiol.* **86**, 457–462.
10. Kerby, N. W. & Evans, L. V. (1983) *J. Phycol.* **19**, 1–3.
11. Weidner, M. & Küppers, U. (1982) *Z. Pflanzenphysiol.* **108**, 353–364.
12. Holdsworth, E. S. & Bruck, K. (1977) *Arch. Biochem. Biophys.* **182**, 87–94.
13. Reynolds, C. H. (1980) *Comp. Biochem. Physiol. B* **65**, 481–487.
14. Ray, T. B. & Black, C. C. (1976) *J. Biol. Chem.* **251**, 5824–5826.
15. Rathnam, C. K. M. & Edwards, G. E. (1977) *Plant Cell Physiol.* **18**, 963–968.
16. Reiskind, J. B., Seamon, P. T. & Bowes, G. (1988) *Plant Physiol.* **87**, 686–692.
17. Salvucci, M. E. & Bowes, G. (1983) *Plant Physiol.* **73**, 488–496.
18. Johnston, A. M. & Raven, J. A. (1986) *J. Phycol.* **22**, 78–83.
19. Monson, R. K. & Moore, B. d. (1989) *Plant Cell Environ.* **12**, 689–699.
20. Moroney, J. V., Kitayama, M., Togasaki, R. K. & Tolbert, N. E. (1987) *Plant Physiol.* **83**, 460–463.
21. Smith, R. G. & Bidwell, R. G. S. (1989) *Mar. Biol.* **102**, 1–4.
22. Watanabe, M., Ohnishi, J. & Kanai, R. (1984) *Plant Cell Physiol.* **25**, 69–79.
23. Bowes, G. & Salvucci, M. E. (1989) *Aquat. Bot.* **34**, 233–266.