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Nutrients, Signals, and Photosynthate Release by Symbiotic Algae[1]

The Impact of Taurine on the Dinoflagellate Alga *Symbiodinium* from the Sea Anemone *Aiptasia pulchella*

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Exogenous concentrations of 10 μM to 1 mM of the nonprotein amino acid taurine stimulated photosynthate release from the dinoflagellate alga *Symbiodinium*, which had been freshly isolated from the sea anemone *Aiptasia pulchella*. Photosynthate release, as induced by taurine and animal extract, was metabolically equivalent at both concentrations in that they (a) stimulated photosynthate release to the same extent and (b) induced the selective release of photosynthetically derived organic acids. A complex mixture of amino acids at 75 mM also promoted photosynthate release, but the release rate was reduced by 34% after the omission of taurine (3 mM) from the mixture, suggesting that much of the effect of amino acids was largely attributable to taurine. Exogenous ^{14}C-labeled taurine was taken up by the cells, and more than 95% of the internalized ^{14}C was recovered as taurine, indicating that taurine-induced photosynthate release was not dependent on taurine metabolism. Both taurine uptake and taurine-induced photosynthate release by *Symbiodinium* exhibited saturation kinetics, but with significantly different K_m values of 68 and 21 μM, respectively. The difference in K_m values is compatible with the hypothesis that *Symbiodinium* has a taurine signal transducer that is responsible for photosynthate release and is distinct from the taurine transporter.

It is well established that the dinoflagellate alga *Symbiodinium* in symbiosis with marine Cnidaria (e.g. sea anemones, corals, and jellyfish) exhibits high rates of photosynthesis, releasing much of the photosynthetic carbon to the animal cells (Muscatine and Cernichiari, 1969; Schmitz and Kremer, 1977; Sutton and Hoegh-Guldberg, 1990), which provides part or all of the animal's carbon and energy requirements (Muscatine, 1990; Gattuso et al., 1993; Dubinsky and Jokiel, 1994). However, little is known about the processes underlying photosynthate release by *Symbiodinium*. Over the last 2 to 3 decades, most research on this topic has been shaped by the findings of Muscatine and Cernichiari (1969) and Trench (1971a, 1971b) that algae isolated from the symbiosis release little photosynthetic carbon unless a homogenate from the animal host tissues is added to the incubation medium. These observations have led to a widely held view in the literature that: (a) specific component(s) of host homogenate (often known as "host factor") induce photosynthate release; (b) the host factor is a signal that results in the diversion of photosynthetic carbon from intracellular metabolism to release; and (c) the host factor mediates photosynthate release in the intact symbiosis.

Progress in the identification of the putative host factor and elucidation of its mode of action has been slow (for review, see Hinde [1988]; Trench [1993]). In part, this reflects the small volumes and biochemical complexity of host homogenates. A more fundamental difficulty with the studies of isolated algal cells is how the host-factor effect relates to the conditions experienced by *Symbiodinium* in the symbiosis. *Symbiodinium* cells are located in the cytoplasm of the animal cells, but are separated from the cytoplasmic contents by a membrane of animal origin called the symbiosome membrane (Roth et al., 1988). This membrane is believed to control aspects of both the conditions experienced by the algae in the symbiosome (e.g. pH and ionic composition) and the flux of nutrients between algal and animal cells (Douglas, 1994). Although little is known about the conditions in the symbiosome (for review, see Rands et al. [1993]), they are not likely to resemble the composition of crude animal homogenates. Even so, there is a strong supposition in the literature that the host factor is an animal signal that results in the diversion of photosynthetic carbon from intracellular metabolism to release.

The recent paper of Gates et al. (1995) may provide the basis for a significant advance in our understanding of photosynthate release by *Symbiodinium*. These researchers demonstrated that *Symbiodinium* release a high percentage of photosynthetically fixed carbon when incubated with amino acids. The effect was not specific to amino acids of particular chemical properties (e.g. acidic and nonpolar) or a biosynthetic family, and was obtained for *Symbiodinium* derived from both a coral, *Pocillopora damicornis*, and a sea anemone, *Aiptasia pulchella*. This paper raises the possibility that the host factor may not be a signal, but instead may alter the photosynthetic metabolism of the algal cells

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Abbreviation: SW, artificial seawater.

through nutritional or other metabolic effects. Two unresolved aspects of the study are potentially important to the interpretation of these findings. First, there is no information on the variations in amino acid-induced photosynthate release with amino acid concentration. The experiments of Gates et al. (1995) were conducted with individual amino acids at the very high concentrations of 50 mM. Second, the impacts of amino acids and host homogenate on the photosynthetic metabolism of the algal cells were not compared beyond the demonstration that both induced the release of glycerol from the algae isolated from *P. damicornis*. It is, therefore, unclear whether the metabolic bases of photosynthate release induced by host homogenate and amino acids are strictly equivalent.

The initial purpose of the study described here was to explore the two unresolved issues considered above using *Symbiodinium* isolated from *A. pulchella*. The results of the first experiments indicated that one amino acid, taurine, is a particularly effective inducer of photosynthate release. The greater part of this study was therefore devoted to the impact of this amino acid on carbon fixation and release by *Symbiodinium*. Our data have implications for the processes underlying photosynthate release by symbiotic algae.

MATERIALS AND METHODS

Maintenance of Experimental Organisms

All experiments were conducted on a clonal culture of *Aiptasia pulchella*, obtained from a seawater dike at Tongkang (22° N), Taiwan. The sea anemones were maintained in aerated, artificial seawater (Instant Ocean salts, Aquarium System Inc., Sarrebourg, France) at 25°C under a 12-h/12-h light/dark regime with a light intensity of 30 μE m^{-2} s^{-1} PAR, and fed with 1-d-old *Artemia nauplii* twice a week.

Preparation of Freshly Isolated *Symbiodinium* and Host Extract

To isolate the *Symbiodinium*, excised *A. pulchella* tentacles were homogenized in a hand-held glass tissue grinder with SW buffer (420 mM NaCl, 26 mM $MgSO_4$, 23 mM $MgCl_2$, 9 mM KCl, 9 mM $CaCl_2$, 2 mM $NaHCO_3$, and 10 mM Hepes, pH 8.2). The homogenate was passed through a 53-μm nylon mesh, and the filtrate was centrifuged at 274*g* for 1 min. The supernatant (host extract) was decanted and passed through a 0.8-/0.2-μm membrane filter (Acrodisc PF, Gelman, Northampton, UK) to remove tissue debris and algal contamination. The pellet of *Symbiodinium* was washed three times by suspension in SW buffer and centrifugation, and finally filtered through a 15-μm nylon mesh to eliminate nematocysts. The host extract was used within 30 min of preparation. All experiments on isolated *Symbiodinium* cells were conducted at 25°C, with a light intensity of 60 μE m^{-2} s^{-1}.

Photosynthate Release by *Symbiodinium*

The experimental design was based on preliminary studies that showed, first, that the rates of both photosynthetic carbon fixation and photosynthate release by *Symbiodinium* were linear over 1 to several h, and, second, that in pulse-chase experiments *Symbiodinium* released only the carbon fixed in the period of pulse (see also Trench [1971a]; Sutton and Hoegh-Guldberg [1990]).

Each sample of 5×10^5 *Symbiodinium* cells was incubated with 2 μCi sodium [^{14}C] bicarbonate (Amersham) in 0.8 mL of SW buffer (the control) or SW buffer supplemented with host extract or amino acids (see Table I). The various supplements had no detectable effect on the pH of the incubation medium, reflecting the high buffering capacity of SW buffer. Then, 20 μL of the suspension and 50 μL of the supernatant (obtained by centrifugation of the sample at 11,600*g* for 2 min) were acidified with 4 M acetic acid and shaken vigorously for 1 h to remove unfixed ^{14}C. Four milliliters of scintillation cocktail (Ultima Gold XR, Packard Instrument Co., Meriden, CT) was added, and the radioactivity in the sample was quantified in a β-counter (Tri-Carb, Packard) with preset ^{14}C windows and a quench curve.

The total carbon fixation during incubation was calculated from ^{14}C in a suspension of samples, and expressed as dpm $cell^{-1}$. The percentage of photosynthate release by *Symbiodinium* for each sample is the proportion of dpm in the supernatant to that in the total. The experiments included a "dark control," i.e. samples incubated as above but in darkness; ^{14}C fixation by the dark control was consistently 5% of the experimental samples.

To identify the ^{14}C-labeled compounds each algal and supernatant fraction was freeze-dried and extracted with methanol:chloroform:water:formic acid (12:5:2:1, v/v) by the procedure of Redgwell (1980), and the radioactivity was quantified (as above) in the chloroform (lipid), aqueous, and particulate fractions.

The aqueous layer from the supernatant fraction was further separated into neutral, acidic, and basic fractions by Sephadex SP and QAE ion-exchange chromatography (Redgwell, 1980). The eluted fractions from ion-exchange columns were dried in a rotary evaporator and resuspended in 1 mL of distilled water. The radioactivity of each

Table I. *Effect of host extract and amino acids on photosynthate release by Symbiodinium*

The "75 mM FAA" supplement is the amino acid mixture of Gates et al. (1995), which includes 3 mM taurine. FAA (no taurine) is the amino acid mixture of Gates et al. (1995) from which taurine has been omitted. Means followed by the same letter are not significantly different at P = 0.05 (Fisher's least significance difference test). The data displayed are the combined results of multiple experiments. Data are means ± SE.

Supplements to SW Buffer	*n*	Total Carbon Fixation	^{14}C in Medium
		dpm cell n^{-1}	%
None	36	0.482 ± 0.018a	8.3 ± 0.4a
Host extract	14	0.818 ± 0.058b	22.6 ± 1.0c
75 mM FAA	13	0.604 ± 0.032a	22.9 ± 0.6c
1 mM Taurine	5	0.566 ± 0.100a	24.9 ± 0.7c
FAA (no taurine)	3	0.622 ± 0.013a	15.3 ± 1.1b
25 mM Glutamate	3	0.446 ± 0.014a	6.7 ± 0.1a
25 mM Ser	3	0.480 ± 0.009a	9.3 ± 0.1a
25 mM Lys	3	0.474 ± 0.017a	8.5 ± 0.6a

sample from the concentrated eluate was quantified and standardized with cell numbers. The organic acid fractions from the ion-exchange column were separated by thin-layer electrophoresis and TLC on cellulose plates (Merck, Darmstadt, Germany) by the method of Schürmann (1969) and modified as follows: A 2-μL sample of the organic acid fraction containing about 5000 dpm of radioactivity was spotted onto the left corner of a cellulose plate and electrophoresed from cathode to anode at 450 V in pyridine: acetic acid:water (10:35:955, v/v) buffer, pH 3.9, for 35 min. The cellulose plate was dried and developed by isopropanol:formic acid:water (6:2:1, v/v) in the second dimension. The radioactive spots were visualized by exposing to radiographic film (Kodak) for 1 week, and identified by comparison with the authentic organic acids e.g., aspartate, citrate, fumarate, glutamate, glycolate, lactate, malate, oxaloacetate, pyruvate, and succinate.

Uptake of Exogenous Taurine by *Symbiodinium*

Isolated cells of *Symbiodinium* were incubated in 0.5 mL of SW buffer supplemented with 5 μM to 10 mM taurine and [1,2-^{14}C]taurine (DuPont). To terminate taurine uptake, the algal suspension was applied to a filter (GF/C, Whatman) using a 12-place filtration manifold (Hoefer, San Francisco, CA), and the filter was washed twice with 1 mL of SW buffer. The filter was added to 4 mL of scintillation cocktail for radioactivity determination. The metabolism of taurine taken up by *Symbiodinium* was detected by chromatography of the 70% ethanol extract on a cellulose plate with chloroform:methanol:17.5% NH_3 (2:2:1, v/v) as a developing solvent. *Symbiodinium* cells killed by incubation at 100°C for 10 min were used as a control for nonspecific binding of taurine to the algal surface.

Kinetic Study of Taurine Uptake and Taurine-Stimulated Photosynthate Release

Preliminary experiments with time courses of 5 s to 2 h indicated that taurine uptake was linear over at least 1 h from a range of external concentrations. For the parallel study of the kinetics of taurine uptake and taurine-induced photosynthate release, the *Symbiodinium* cells were incubated in SW buffer supplemented with 10 to 1000 μM taurine and either ^{14}C-taurine (0.01–0.2 μCi) or sodium [^{14}C]bicarbonate (2 μCi). Taurine uptake and photosynthate release were assayed as above. The V_{max} and kinetic constant of the half-maximum rate of taurine uptake or photosynthate release (K_m) were determined from Eadie-Hofstee plots.

Statistical Analysis

Parametric statistical tests were used to explore the effects of SW buffer, amino acids, and host extract on (a) the total carbon fixation of *Symbiodinium* and (b) the percentage of photosynthate released (after arc-sin square root transformation of the data). Comparisons between pairs of treatments were made using Fisher's least significance difference test with a significance level of 0.05. The impact of incubation conditions on the distribution of ^{14}C content between biochemical factors (e.g. lipid and aqueous) in the algal cells and medium was analyzed by multivariate analysis of variance and profile analysis (Morrison, 1976; Seber, 1984; Douglas, 1996). Univariate statistical analysis was not appropriate because the incorporation of ^{14}C into one fraction could not be assumed to be independent of the level of ^{14}C incorporated into other fractions. To compare K_m for taurine uptake and photosynthate release the difference between the slopes of linear regression lines from Eadie-Hofstee plots was analyzed by a general linear model.

RESULTS

Photosynthetic ^{14}C Fixation and Release

In the first experiments *Symbiodinium* cells were isolated from *A. pulchella* and incubated in SW buffer or SW buffer supplemented with host extract or amino acids, as indicated in Table I. The photosynthetic ^{14}C fixation rates varied significantly between the treatments ($F_{7, 72} = 9.31$, $P < 0.0001$), and the Fisher's least significance difference test revealed that the fixation rate was significantly elevated by host extract alone but not by any of the amino acid supplements tested.

In all treatments fixed ^{14}C was recovered from the incubation medium. Consistent with previous studies, the amount of ^{14}C released into the medium was <10% of the total for cells in SW-buffer, and between 20 and 30% for cells incubated with host extract and with the 75 mM amino acid mixture of Gates et al. (1995). In the experiment displayed in Table I the percentage of photosynthate released varied significantly between treatments ($F_{7, 72} = 74.69$, $P < 0.0001$), and 1 mM taurine was found to be as effective as the host extract and 75 mM amino acid mixture in promoting release. The impact of three other amino acids, glutamate, Ser, and Lys, on photosynthate release was tested. At the concentration of 25 mM, none promoted ^{14}C release. Omission of taurine from the 75 mM amino acid mixture reduced ^{14}C release by 34%.

These results suggest that taurine contributes substantially to the effect of amino acids on photosynthetic metabolism by *Symbiodinium*. Subsequent experiments compared the impacts of taurine and host homogenates on photosynthate release.

The Metabolic Fate of Photosynthetically Fixed ^{14}C

The effect of host extract and taurine on the metabolism of photosynthetically fixed ^{14}C by the isolated *Symbiodinium* cells is shown in Figure 1. ^{14}C was recovered from the aqueous, lipid, and particulate fractions of the cells, as well as from the medium. The data displayed in Table I shows that algal cells fixed more ^{14}C when incubated with host extract than with taurine and SW buffer. Furthermore, the distribution of photosynthetically fixed ^{14}C between the fractions varied significantly with treatments (multivariate analysis of variance, $F_{6, 26} = 32.97$, $P < 0.0001$), and profile analysis revealed that the cells in the taurine and host-extract treatments released more ^{14}C into the medium

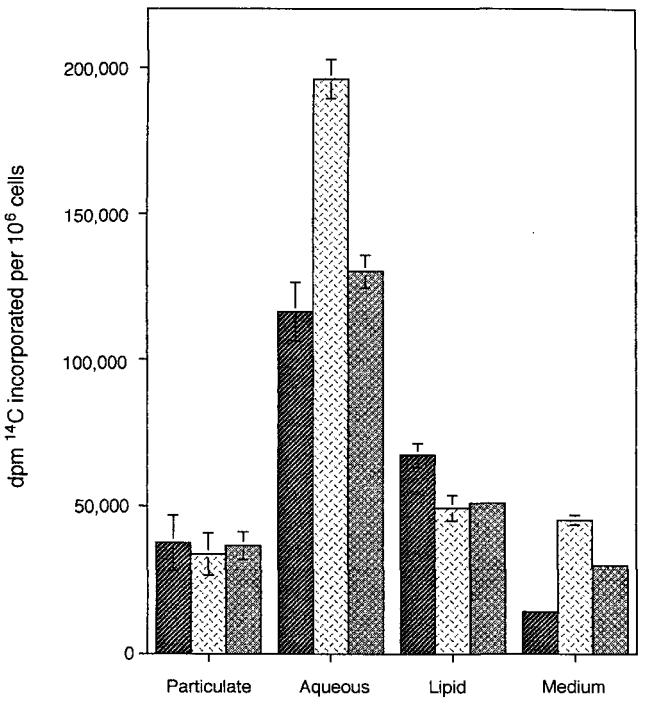

Figure 1. Incorporation of photosynthetically fixed ^{14}C by freshly isolated *Symbiodinium* incubated in SW buffer (▨) or in SW buffer supplemented with either host extract (▩) or 1 mM taurine (▦). Results are expressed as means ± SD (n = 6).

relative to the aqueous, lipid, and particulate fractions of the cells than did the cells in SW buffer. No significant differences in the distribution of ^{14}C between host extract and taurine treatments ($P > 0.05$) were revealed by this analysis.

The ^{14}C-labeled compounds in the medium were studied further. As Figure 2 shows, most of the ^{14}C was recovered from the water-soluble fraction, including neutral compounds (probably predominantly sugars), amino acids, and organic acids, whereas the lipid fraction accounted for less than 5% of the radioactivity for SW-buffer incubation and less than 1% for both host extract and taurine incubations. The higher ^{14}C content in the medium of cells incubated with host extract and taurine than in SW buffer could be attributed primarily to an increase in organic acids. Analysis of the released ^{14}C-organic acids by thin-layer electrophoresis/TLC revealed a single spot (0.85, 6.5) for the taurine incubations (R_F on TLC; motility on thin-layer electrophoresis in centimeters), and two spots (0.77, 2.7 and 0.85, 6.2) for host-extract incubation. These were identified as fumarate for taurine treatment, and fumarate and succinate for the host-extract treatment. Gates et al. (1995) identified lactate and glycolate as ^{14}C-release products of *Symbiodinium* from *A. pulchella*, but these compounds (0.92, 3.8 for lactate and 0.76, 4.2 for glycolate) were not detected in this study.

In summary, these results indicate that host extract and taurine have similar, but not identical, effects on photosynthate metabolism and release by *Symbiodinium* cells.

Kinetics of Taurine Uptake and Taurine-Stimulated Photosynthate Release

The first experiments showed that isolated cells of *Symbiodinium* took up ^{14}C-taurine from SW buffer at linear rates for over 1 h. More than 95% of radioactivity was recovered from the ethanol fraction of the cells, and the sole ^{14}C product in this fraction was identified by TLC as taurine, indicating that this amino acid was not extensively metabolized. Incorporation of ^{14}C from exogenous taurine was reduced by 90% in heat-killed cells.

The variation in initial taurine uptake rates with exogenous taurine concentration between 10 μM and 1 mM revealed saturation kinetics (Fig. 3A), with a K_m of 68 μM and a V_{max} of 271 pmol (10^6 cells)$^{-1}$ min^{-1}. Cells incubated with exogenous taurine at concentrations greater than 1 mM exhibited a further increase in taurine uptake rates, perhaps indicative of a low-affinity uptake system, but this was not investigated further.

The effect of exogenous taurine concentration on ^{14}C-photosynthate release was also studied (Fig. 3B) and also exhibited saturation kinetics, with a K_m of 21 μM and a V_{max} of 260 dpm (10^6 cells)$^{-1}$ min^{-1}. The K_m for taurine-stimulated photosynthate release was significantly lower than that for taurine uptake ($F_{1,25} = 10.84$, $P < 0.005$).

Separate experiments have established that the concentration of taurine in *Symbiodinium* cells freshly isolated from *A. pulchella* is 4 to 5 mM (J.-T. Wang and A.E. Douglas, unpublished results). The implication is that taurine is

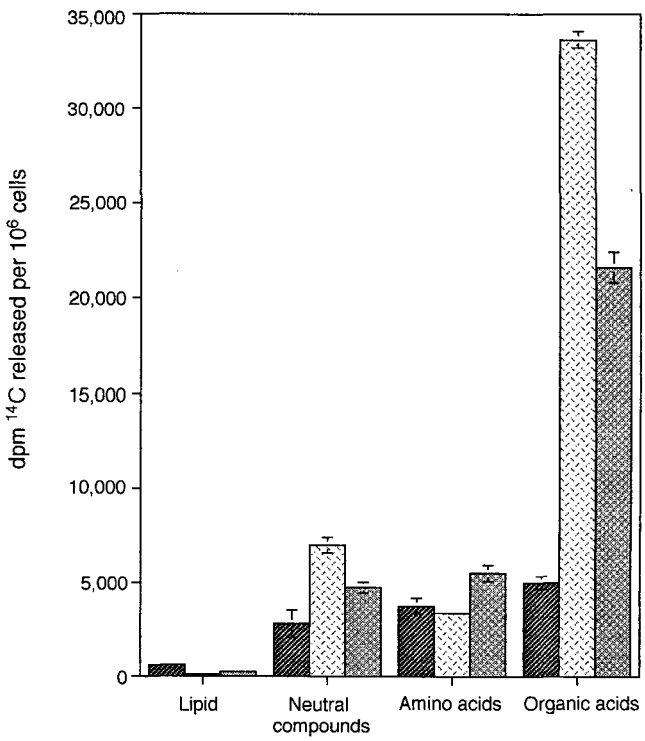

Figure 2. Distribution of ^{14}C-labeled compounds in photosynthate released from *Symbiodinium* into SW buffer (▨) or SW buffer supplemented with host extract (▩) or 1 mM taurine (▦). Results are expressed as means ± SD (n = 6).

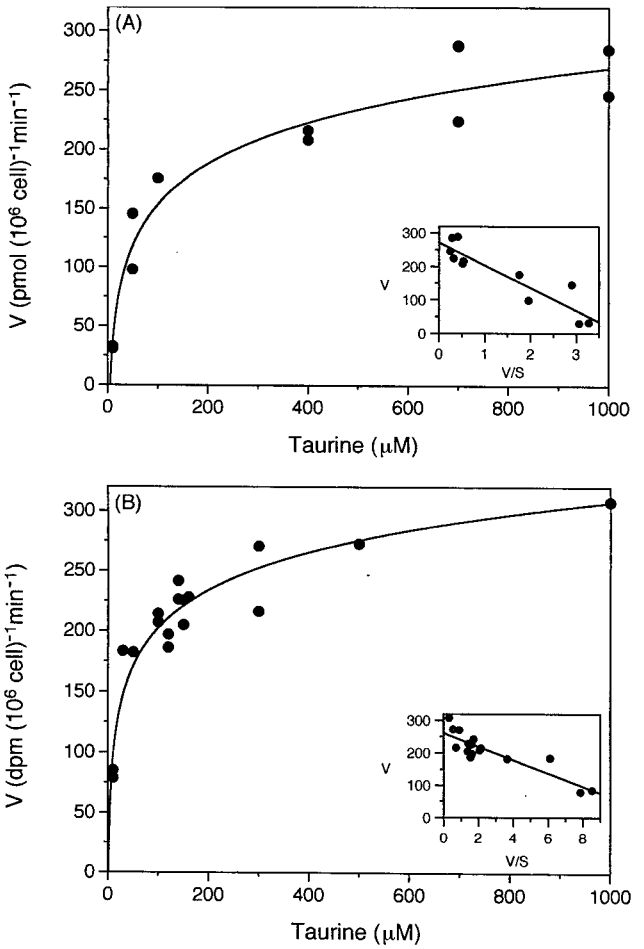

Figure 3. Kinetics of taurine uptake (A) and taurine-stimulated photosynthate release (B) by *Symbiodinium*. The rate of photosynthate release was calculated by subtracting the amount of radioactivity in the SW-buffer control from that in the taurine treatment. The regression equations for the Eadie-Hofstee plots are: $y = 271 - 68x$; $r^2 = 0.84$ for taurine uptake and $y = 260 - 21x$; and $r^2 = 0.78$ for taurine-stimulated photosynthate release.

taken up by cells against a concentration gradient, which is consistent with the conclusion of Deane and O'Brien (1981) that taurine uptake by *Symbiodinium* is energy dependent.

DISCUSSION

The principal result of this study is that taurine and host extract have broadly comparable effects on the metabolism of photosynthetically fixed carbon by *Symbiodinium* from *A. pulchella*. The evidence is that taurine and the host extract both promote a 2-fold increase in photosynthate release (Table I), primarily in the form of organic acids (Fig. 2). Their effects on algal metabolism differ, however, in that (a) only the host extract enhances ^{14}C fixation rates, and (b) the dominant released compounds are succinate and fumarate in host-extract incubations and just fumarate in taurine incubations. Our interpretation is that these latter effects are possibly attributable to components of the host extract other than those responsible for the stimulation of photosynthate release; further experiments are needed to establish the basis of these differences. However, it is concluded that taurine can be used with confidence as a chemically defined alternative to host extracts in the study of photosynthate release by *Symbiodinium* from *A. pulchella*.

The value of experiments conducted with a chemically defined inducer of photosynthate release is illustrated by the advances in our understanding of the processes underlying release obtained from the studies of Gates et al. (1995) and from our studies. There are three broad issues:

First, the demonstration in this study that exogenous taurine stimulates photosynthate release without being metabolized by the algal cells indicates firmly that the effect of amino acids on the photosynthetic metabolism of *Symbiodinium* is not linked to their role as nitrogenous nutrients.

Second, the results of this study are consistent with the conclusion of Gates et al. (1995) that amino acids do not induce the release of a uniform array of photosynthetic compounds from *Symbiodinium*. In that study the principal compound released by algae from *Pocillopora damicornis* was glycerol, whereas the algae from *A. pulchella* released a mixture of glycerol, glycolate, lactate, and succinate. However, in the present study *Symbiodinium* from *A. pulchella* released fumarate, but no detectable amounts of any of the compounds identified by Gates et al., when incubated with taurine. (Fumarate was also identified by Trench [1971a] as the major release product of the algae from *A. pulchella*.) It is also known that only recently fixed photosynthetic carbon is released (Sutton and Hoegh-Guldberg, 1990; J.-T. Wang and A.E. Douglas, unpublished results). A reasonable inference is that photosynthetic carbon is diverted from intracellular metabolism to release at a point(s) in photosynthetic metabolism not far removed from carbon fixation, and that neither exogenous amino acids nor host extract determines the biochemistry of the subsequent metabolism of carbon destined for release. The identity of the compounds released may vary between lineages of *Symbiodinium* (as previously suggested by Gates et al. [1995]) and with the physiological condition of the algal cells in different symbioses.

Third, the experiments on the variation in photosynthate release rates with taurine concentration indicate that the effect of taurine on the process(es) regulating photosynthate release is saturable and has a high affinity (K_m of 21 μM) for taurine. These kinetic experiments also illustrate the experimental value of a defined compound over host extracts in experiments on photosynthate release. The results obtained in Figure 3 could not have been obtained with crude host extracts because the concentration of the putative host factor in a given volume of homogenate is unknown and likely to vary between preparations.

There remain the crucial, but still unresolved, issues of whether taurine is the host factor in host extracts of *A. pulchella* and whether photosynthate release by the algal cells in the symbiosis is induced by taurine. The involvement of taurine in the effects of host extract is very likely, given that the concentration of taurine in the *A. pulchella* homogenates used in this study was at least 0.1 mM (J.-T.

Wang and A.E. Douglas, unpublished results), but as yet there is no firm information on the role of taurine in the intact symbiosis. If taurine is the inducer of photosynthate release in the intact symbiosis, one would predict that the symbiosome membrane bears a taurine transporter and that the rate of photosynthate release from the algae is correlated with the flux of taurine across this membrane from the animal cytoplasm. This scenario provides a cellular basis for the possibility that taurine may act as a signaling molecule between the animal and algal cells. Following the classical paradigm for intercellular signaling, the binding of taurine to algal receptors may trigger intracellular signaling cascades that are directly responsible for the diversion of photosynthetic carbon from an intracellular to extracellular fate (as considered above). As a precedent for the putative role of taurine as a signal between the animal and *Symbiodinium* cells, taurine is implicated as an inhibitory neurotransmitter in some animals (Nistri and Constani, 1976; Hue et al., 1979; Giles and Usherwood, 1985; Huxtable, 1989) and a stimulator of the motor nerve net of jellyfish (Carlberg et al., 1995). All of the data obtained in this study, including the relatively low half-saturation constant for taurine-induced photosynthate release, are compatible with this interpretation. However, further study is required to establish the role of taurine in the intact *A. pulchella* symbiosis and to establish whether this effect is widely distributed among *Symbiodinium* symbioses.

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