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Effect of Ammonium-supplemented Seawater on Glutamine Synthetase and Glutamate Dehydrogenase Activities in Host Tissue and Zooxanthellae of *Pocillopora damicornis* and on Ammonium Uptake Rates of the Zooxanthellae[1]

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ABSTRACT: Host glutamine synthetase activity decreases in *Pocillopora damicornis* (Linnaeus) following exposure of the coral to seawater containing elevated ammonium (20 μM). Zooxanthellae isolated from these corals exhibited lower ammonium uptake capacity and glutamine synthetase activity compared with those from the control corals. Ammonium concentration of the surrounding seawater had no effect on the NADPH-dependent glutamate dehydrogenase activity in the host.

INTRACELLULAR AMMONIUM in corals can be derived both from normal catabolic processes within coral tissue and by uptake of ammonium from seawater. The latter was first observed by Kawaguti (1953). Subsequently, Muscatine and D'Elia (1978) demonstrated that only corals symbiotic with zooxanthellae can take up ammonium and also retain it. They assumed that ammonium can be assimilated within the symbiotic association and that zooxanthellae are essential to this process.

Few enzymes directly utilize ammonium as a substrate. Apart from carbamoyl phosphate synthetase I, which is present in organisms that possess the urea cycle, glutamine synthetase (GS) is the principal enzyme used in ammonium utilization, although NADPH-dependent glutamate dehydrogenase (GDH) has also been implicated in ammonium assimilation despite its low affinity for ammonium. Both GS and NADPH-GDH have been detected in corals and other zooxanthellate symbioses. GS in *Acropora formosa* (Dana) (D.Y., unpubl. data) could not be detected in host tissue, although its presence is assumed because GS is the sole enzyme capable of synthesizing glutamine. Its unlikely absence would indicate either that zooxanthellae must act as the source of glutamine or that it is acquired from heterotrophic feeding. High NADPH-GDH activity has been detected in host tissue of *A. formosa* (Catmull et al. 1987) and *Stylophora pistillata* Esper (Rahav et al. 1989).

GS has been found in zooxanthellae (Wilkerson and Muscatine 1984, Anderson and Burris 1987), although no activity measurements were presented. The presence of GS and GOGAT (glutamate synthetase) in freshly isolated zooxanthellae from corals can also be concluded from the data presented by Summons et al. (1986). GOGAT catalyzes the reductive transfer of the amide-amino group of glutamine to α-ketoglutarate to produce two glutamates. NADPH-GDH is present in freshly isolated zooxanthellae from corals, although its specific activity is an order of magnitude higher after induction with ammonium (Dudler and Miller 1988).

This report examines two of the central aspects of ammonium assimilation in marine alga-invertebrate associations: the relation-

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ship of GS and GDH from both *Pocillopora damicornis* (Linnaeus) and its freshly isolated zooxanthellae to the seawater ammonium concentration; and whether the uptake of ammonium by the zooxanthellae is influenced by the environmental ammonium concentration.

MATERIALS AND METHODS

Corals

Coral branches of *P. damicornis* were obtained as previously described in this issue (Stambler et al. 1994). Samples of control (ambient) corals and corals in seawater supplemented with 20-μM and 50-μM concentrations of ammonium incubated for 2 weeks and 8 weeks were used. Animal tissue was removed from small coral branches with a jet of compressed air from a scuba tank. The tank was connected to a plastic tube and the air delivered through a wide-bore plastic pipette tip inserted in the end of the plastic tube. During this procedure (air piking), the coral branches were wetted with extraction buffer (50 mM Tris-HCl buffer, pH 8.0, containing 1 mM reduced glutathione, 1 mM DTT, 20 mM mercaptoethanol, 1 mM EDTA, and 10 mM $MgSO_4$) as required. Zooxanthellae were removed from air-piked samples by centrifugation for 5 min at room temperature in a microfuge (Beckman). Animal tissue was used for assay of both GDH and GS.

Zooxanthellae

Zooxanthellae were prepared by removing the coral tissue from the skeleton of *P. damicornis* colonies with filtered seawater using the Water Pik technique (Johannes and Wiebe 1970). The zooxanthellae were washed three times with filtered seawater and then resuspended in extraction buffer (containing 5 mM mercaptoethanol) before lysing the cells in a precooled (4°C) Yeda Press at 9000 kPa (1200 psi). The lysate was centrifuged in a microfuge for 5 min to remove unbroken cells and cell debris. The supernatant was used for assay of GDH and GS.

Enzyme Assays

GDH was assayed in 50 mM HEPES-KOH buffer (pH 7.4) containing 100 mM $(NH_4)_2SO_4$, 0.2 mM NAD(P)H, 10 mM α-ketoglutarate, and enzyme extract (total volume = 1.05 ml). The reaction was monitored at 340 nm in a diode array spectrophotometer (Hewlett Packard 8452) at 30°C. Any NAD(P)H oxidase activity was measured for 2 min before initiating the GDH reaction with α-ketoglutarate. The GDH activity was adjusted for any NAD(P)H oxidase activity present. The oxidase activity was always $<20\%$ of the GDH activity.

The transferase and synthetase activities of GS were determined as described by Rhodes et al. (1975) and Guiz et al. (1979), respectively. Protein was measured by the Bradford (1976) method using reagents supplied by BioRad. Bovine serum albumin was used as a calibration standard.

Ammonium Uptake

Zooxanthellae ($0.5–1 \times 10^6$ cells ml^{-1}), prepared by the Water Pik technique and washing three times in filtered seawater, were incubated in seawater containing 20 μM NH_4Cl. The samples were incubated at 30°C under a fluorescent light source (200 $\mu E\ m^{-2}\ sec^{-1}$) and mixed regularly. Aliquots (5 ml) were removed at regular intervals, vacuum-filtered on GF/C filters (Whatman) and the ammonium content of the sample measured (Liddicoat et al. 1975).

RESULTS AND DISCUSSION

Zooxanthellae, freshly isolated from corals exposed to either 20- or 50-μM concentrations of ammonium, incubated in an elevated ammonium concentration took up ammonium ions at a slower rate than zooxanthellae isolated from control corals (Figure 1). The uptake rate for zooxanthellae from control corals (0.042 pmol $NH_4\ hr^{-1}\ cell^{-1}$) was roughly equivalent to that for freshly isolated zooxanthellae from *Acropora formosa* (Gunnersen et al. 1988). However, a five-fold

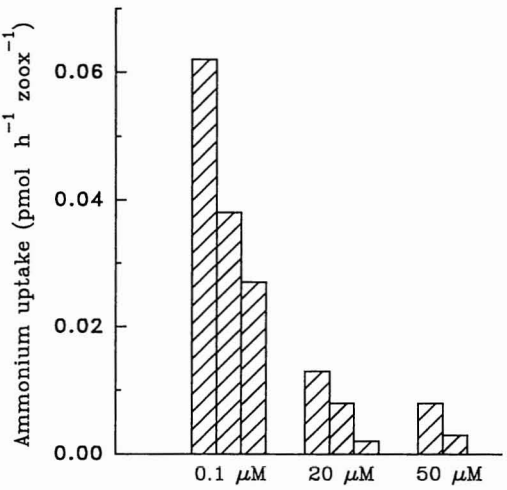

FIGURE 1. The depletion of ammonium from seawater by freshly isolated zooxanthellae from *Pocillopora damicornis*. The corals were maintained in seawater containing either 20-μM ($n = 3$) or 50-μM ($n = 2$) concentrations of ammonium. The ambient ammonium concentration of seawater used for the control corals ($n = 3$) was 0.1 μM.

decrease in the mean uptake rate was observed in zooxanthellae isolated from *P. damicornis* grown in 20-μM-supplemented seawater (8 fmol NH_4 hr^{-1} cell^{-1}). The uptake rate observed with zooxanthellae isolated from corals grown in 50-μM ammonium-supplemented seawater was not significantly different from that of corals grown in 20-μM-supplemented seawater. This indicates that the N status of the zooxanthellae was influenced by the seawater concentration; the ammonium uptake system was repressed with increased concentrations of ammonium. This response has not been previously reported in zooxanthellate symbioses, but Rees (1990) observed a similar result in symbiotic *Chlorella* and used this to argue for a low ambient concentration of ammonium in the perialgal space.

GS activity in host tissue was measured using both the transferase and synthetase assay; however, the measurement of synthetase activity proved unreliable and only the results for transferase are reported in Table 1. The transferase/synthetase ratios for GS in plants (15–100) and giant clam (*Tridacna gigas*) animal tissue (36 [Rees et al. 1994]) indicate that at the transferase levels observed, synthetase activity measurements would have been low. A decrease (>50%) in GS was observed after incubation of corals in seawater supplemented with 20 μM ammonium ions for 2 weeks. Only one sample was measured after 8 weeks exposure, and the GS level in that was further reduced. Although the presence of GS has been assumed, this paper represents the first report of GS activity in coral tissue.

In contrast, no evidence was obtained for changes in GDH in host tissue, indicating that any intracellular change in ammonium concentration resulting from an increase in seawater concentration did not influence the level of GDH. In all instances, >95% of GDH activity measured was NADP-dependent, and there was no evidence for any change in the coenzyme ratio

TABLE 1

GLUTAMINE SYNTHETASE AND GLUTAMATE DEHYDROGENASE ACTIVITIES IN THE CORAL *Pocillopora damicornis* AND ITS ZOOXANTHELLAE

	GLUTAMINE SYNTHETASE		GLUTAMATE DEHYDROGENASE	
TREATMENT	HOST	FRESHLY ISOLATED ZOOXANTHELLAE	HOST	FRESHLY ISOLATED ZOOXANTHELLAE
Control	186 (±23)	15	34 (±3)	ND
2 weeks	77 (±5)	7	31 (±4)	ND
8 weeks	55	—	29	—

NOTE: Units are expressed as nmoles min^{-1} (mg protein)$^{-1}$. Each assay was repeated and the mean taken. The results presented are the means of separate experiments ($n = 3$) with their standard errors. ND denotes not detected.

of dependence. Deaminating activity of GDH was not measured. Aminating activity was, however, 12-fold greater than that reported for *Stylophora pistillata* (Rahav et al. 1989), but six-fold less than that of *Acropora formosa* (Catmull et al. 1987).

Assuming that the concentration of ammonium in seawater influences the intracellular concentration, the results indicate that GS activity must be regulated either directly or indirectly by cytoplasmic ammonium ion concentration. However, without knowing the intracellular concentration of ammonium ions or metabolites derived from its assimilation, it is not possible to speculate further on the control of GS levels in host tissues.

The specific activity of both GS and GDH in zooxanthellae was always $< 10\%$ of that in the host tissue. Indeed, in the case of GDH it was impossible to reliably detect any oxidation of either NADPH or NADH above background, indicating that GDH levels were very low. However, GS was detected in zooxanthellae using the transferase assay, indicating that it must be the major means of ammonium assimilation in zooxanthellae.

From these results, we propose that the host has a role in the assimilation of ammonium ions in the symbiosis. It has been argued that the zooxanthellae are the driving force behind the acquisition and retention of ammonium in the symbiosis (Muscatine and D'Elia 1978). However, uptake of ammonium by zooxanthellae relies on diffusion of ammonium through the host cell and into the perialgal space. With relatively high concentrations of ammonium-assimilating enzymes in the cytoplasm of the control hosts, there is a likelihood that much of the ammonium when it is present at natural seawater concentrations will be assimilated before it can diffuse to the perialgal membrane. The repression of the ammonium uptake rate of zooxanthellae in corals exposed to elevated ammonium ions supports this conclusion.

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