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Sensitivity of zooxanthellae and non-symbiotic microalgae to stimulation of photosynthate excretion by giant clam tissue homogenate

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Abstract. Stimulation of photosynthate excretion from zooxanthellae and free-living algae by tissue homogenate of several bivalves was studied. Mantle tissue homogenate of *Tridacna derasa* enhanced 10- to 15-fold excretion of photosynthetically fixed carbon from freshly isolated zooxanthellae within 2 h incubation. Maximum carbon excretion was 35 to 45% of the total carbon fixed. This excretion stimulating activity was detected in the homogenates of the mantle, adductor muscle, gill, and kidney. However, no excretion stimulating activity was detected in the haemolymph. The excretion stimulation activity of mantle homogenate, directed against freshly isolated zooxanthellae from *T. derasa*, was higher in bivalves belonging to the Tridacnidae (*T. derasa*, *T. crocea*, *T. maxima*, *T. squamosa*, *Hippopus hippopus*) than in the Cardiidae (*Fragum fragum*, *F. mundum*, *F. unedo*), non-symbiotic bivalves (*Mytilus edulis*, *Meretrix lusoria*, *Ruditapes philippinarum*) or gastropods (*Umbonium giganteum*, *Turbo argyrostoma*). The mantle homogenate of *T. derasa* enhanced photosynthate excretion by free-living algae belonging to the Dinophyceae (*Prorocentrum micans*, *Amphidinium carterae*, and *Heterocapsa triquetra*) but did not enhance its excretion by free-living algae belonging to the Chlorophyceae, Cyanophyceae, Rhodophyceae, Prasinophyceae, and Haptophyceae. *T. derase* used in this study originated from Belau (Palau). *T. crocea, T. squamosa*, *T. maxima*, *H. hippopus* and *F. unedo* were collected at Ishigaki Island in Okinawa in 1992. *F. mundum* and *F. fragm* were collected at Okinawa Island in 1992.

Introduction

In zooxanthellae-invertebrates symbioses, 20 to 95% of daily photosynthetically fixed carbon was reported to be translocated to the host animal tissues in vivo (Cook 1983, Muscatine 1990). Host tissue homogenates stimulate release of photosynthetically fixed carbon from zooxanthellae in hermatypic corals (Muscatine 1967, Muscatine and Cernichiari 1969, Muscatine et al. 1972, Sutton and Hoegh-Guldberg 1990), sea anemones (Trench 1971, Sutton and Hoegh-Guldberg 1990) and giant clams (Muscatine 1967, Trench et al. 1981, Streamer et al. 1988). Tissue homogenate of aposymbiotic (free of zooxanthellae) sea anemone, however, does not enhance the excretion from zooxanthellae unless the anemone is infected with zooxanthellae (Trench 1971). These observations have led to the idea that animal tissues contain a "host factor" (HF) which stimulates excretion of photosynthetically fixed carbon from their symbiotic algae (Hinde 1988, Sutton and Hoegh-Guldberg 1990). However, the chemical nature of the HF is not clear, and the mechanism of the stimulation is not known.

Muscatine (1967) reported that the tissue homogenate of a giant clam (*Tridacna crocea*) and a coral (*Pocillopora damicornis*) equally promoted the excretion of fixed carbon from *T. crocea* zooxanthellae and from zooxanthellae of a coral (*Pocillopora damicornis*), respectively. While the tissue homogenate of a coral (*Plesiastrea versipora*) stimulates the carbon excretion from both its own and zoanthid (*Zoanthus robustus*) zooxanthellae but has no effect on nudibranch (*Pteraeolidia ianthina*) zooxanthellae, the tissue homogenate of *Z. robustus* and *P. ianthina* had little effect on the carbon excretion from both their own and other zooxanthellae (Sutton and Hoegh-Guldberg 1990). These cross-reactivity experiments have shown that HF is not ubiquitous in symbiotic animals, and the specificity of the HF is rather low. In these studies, the cross-reactivity of excretion stimulating activity (ESA) has been tested only in the host animals and their zooxanthellae. ESA of non-symbiotic animals and that against free-living microalgae has not been studied.

Symbiotic bivalves containing zooxanthellae belong to the family Tridacnidae (Kawaguti 1966, Fankboner 1971, Goreau et al. 1973, Trench et al. 1981) and the family Cardiidae (Kawaguti 1950, Kawaguti 1968, Kawaguti 1983, Umeshita and Yamasu 1985). In the present study, ESA of non-symbiotic clam tissue, directed against *Tridacna derasa* zooxanthellae, was compared with that of symbiotic bivalves belonging to the genera *Tridacna* (Tridacnidae), *Hippopus* (Tridacnidae) and *Fragum* (Cardiidae). Furthermore, the sensitivities of free-living microalgae and zoox-

anthellae to the mantle homogenate of *T. derasa* were also compared. Our data suggest that ESA directed against *T. derasa* zooxanthellae was higher in the tissues of *Tridacna* (Tridacnidae) and *Hippopus* (Tridacnidae) than in those of *Fragum* (Cardiidae) and non-symbiotic clams. It is also suggested that some species of free-living dinoflagellates are sensitive to the mantle homogenate of *T. derasa*.

Materials and methods

Host animals

Tridacna derasa, originating from the Micronesian Mariculture Demonstration Center in Belau (Palau), were obtained from the Kuruma Shrimp Company, Okinawa (Japan) in 1992. *T. crocea*, *T. squamosa*, *T. maxima*, *Hippopus hippopus* and *Fragum unedo*, collected at Ishigaki Island in Okinawa in 1992, were kindly provided by Mr. Ohshiro of Okinawa Prefectural Fisheries Experimental Station, Yaeyama Branch Station. The shell length of the giant clams was 8.0 to 10.0 cm. *F. fragum* and *F. mundum*, which had been collected in 1992 at Okinawa Island, were kind gifts from Dr. Ohno of Kyoto University. The shell lengths of *F. fragum*, *F. mundum*, and *F. unedo* were ca. 2.5, 0.6, and 5.5 cm, respectively. These clams were maintained in an aquarium at 25 °C under fluorescent light (85 μE m^{-2} s^{-1}) with a 14 h light : 10 h dark regime. Water in the aquarium (51 liters) was replenished with 50% fresh seawater during each circulation cycle (135 min).

Isolation of zooxanthellae from *Tridacna derasa*

Mantle tissue of *Tridacna derasa* was homogenized using a polytron type homogenizer with a tip of 20 mm diameter (Ystral GmbH, Dottingen) at 50 V for 5 to 8 s in 10 ml Artificial Pacific Seawater (Borowitzka and Larkum 1976) without $NaHCO_3$ ($APSW^-$). The $APSW^-$ was adjusted to pH 8.0 with 0.1 *N* NaOH. The homogenate was filtered through 20-μm mesh nylon cloth to remove animal tissue debris. The filtrate was centrifuged at 450 × *g* for 5 min, and the pellet of zooxanthellae was washed with 50 ml of $APSW^-$. This centrifugation-rinsing procedure was repeated three to four times. The freshly isolated zooxanthellae were resuspended in $APSW^-$, giving a final concentration of 1.0 to 1.5×10^6 cells ml^{-1}. The number of zooxanthellae was counted with a Tohma haemocytometer. Microscopic observations of the prepared zooxanthellae indicated that this procedure did not disrupt zooxanthellae.

In order to see the effect of pH on the excretion of fixed carbon, 10 m*M* N,N-Bis(2-hyroxyethyl)glycine and 3-(N-Morpholino) propanesulfonic acid (Wako Pure Chemical Industries, LTD., Japan) was added to $APSW^-$. The pH was adjusted to 6.5–9.0 with 0.1 *N* NaOH.

Preparation of host homogenates

The mantles of *Tridacna derasa*, *T. crocea*, *T. maxima*, *T. squamosa*, and *Hippopus hippopus* were dissected with a pair of scissors and then homogenized by a polytron type homogenizer at 90 V in 5 to 10 ml $APSW^-$ for 10 to 15 s on ice. The homogenates were centrifuged (4000 × *g*) for 10 min at 5 °C. The supernatants were used as mantle homogenates. The homogenates of the gill, kidney and adductor muscle from *T. derasa* were prepared in the same way.

Homogenates containing both mantle and adductor muscle of *Fragum fragum* and *F. unedo* were also prepared as described above. Because of the small size of *F. mundum*, whole soft tissues were homogenized.

Mantle and adductor muscles from non-symbiotic bivalves (*Meretrix lusoria*, *Ruditapes philippinarum*, *Mytilus edulis*) were cut out and homogenized in 5 to 10 ml $APSW^-$ for 10 to 15 s on ice. Tissue homogenates of gastropods (*Umbonium giganteum*, *Turbo argyrostoma*) were also prepared. After removing the digestive organs, the remaining tissues were homogenized as described above.

To compare the ESA in tissue homogenates, the concentration of host tissue homogenate in the reaction mixture was adjusted based on the protein concentration. Protein concentration was determined by the method of Bradford (1976) using bovine gamma globulin as a standard.

Collection of haemolymph from *Tridacna derasa*

After all seawater was drained from the inlet siphon, the adductor muscle was severed with a scalpel and the haemolymph of *Tridacna derasa* was allowed to drain into a 50-ml beaker.

Culture of zooxanthellae and microalgae

Table 1 shows the algal species used in ESA assay and their culture media. All free-living algae were cultured under fluorescent light at 30 μE m^{-2} s^{-1} with a 12 h light : 12 h dark regime at 25 °C for 7 to 12 d before experiments. *Symbiodinium microadriaticum* (zooxanthellae) which had been isolated from *Tridacna derasa* in Palau were also cultured for 16 d before experiments as described above.

Measurement of carbon fixation and excretion

Zooxanthellae or free-living microalgae were suspended in 2.0 ml $APSW^-$ containing tissue homogenate in a 15-ml glass vial. At the beginning of each experiment, 40 μl of $NaH^{14}CO_3$ (Amersham, Japan) was added to give a final radioactivity of 1.85×10^5 Bq ml^{-1} (specific activity, 92.5 MBq $mmol^{-1}$). Initial concentration of $NaHCO_3$ in the medium was 2 m*M*. The vial with algal suspension was laid on a rotator (MIX-ROTOR, UMR-5, Iuchi, Japan) and was

Table 1. Algae used in excretion-stimulating activity assay and their culture media

Family Species of microalgae	Culture medium
Chlorophyceae	
Nannochloris atomus (CCAP 251/4B)	ESM[a]
Nannochloropsis salina (CCAP 849/2)	ESM
Chlorella marina (CCAP 211/27)	ESM
Cyanophyceae	
Synechococcus sp. (PCC 7002)	MN[b]
Rhodopyceae	
Porphyridium cruentum (IAM-R1)	Koch[c]
Prasinophyceae	
Tetraselmis chui (CS-26)	K[d]
Haptophyceae	
Pavlova lutheri (CS-23)	K
Dinophyceae	
Prorocentrum micans (NIES-12)	f/2[e]
Amphidinium carterae (NIES-331)	ESM
Heterocapsa triquetra (NIES-7)	f/2
Symbiodinium microadriaticum (CS-161)	K

[a] Okaichi et al. (1982)
[b] Waterbury and Stanier (1981)
[c] Koch (1953)
[d] Keller et al. (1987)
[e] Guillard and Ryther (1962)

rotated (40 rpm) to ensure complete mixing. Each sample was incubated at 25 °C under fluorescent light (180 to 200 μE m^{-2} s^{-1}). The light intensity was measured with an LI-COR Quantum Sensor and an LI-1000 Data Logger (LI-COR, Inc./LI-COR, Ltd., USA).

Total fixed carbon and excreted organic carbon were measured using the silicone layer centrifugation method (Abe et al. 1987). After 1 h incubation, duplicate 200 μl samples were removed from each vial and were layered on top of 60 μl of a silicon oil mixture comprising SH 550 and SH 556 (Toray Silicone Co., Japan) in a 3:2 v/v ratio which was layered over 20 μl of 1 *M* glycine (pH 10) containing 0.75% SDS in a 400 μl microtube. The microtube was centrifuged (17 000×*g*) for 20 s with a microcentrifuge (Sakuma M-15–3S, Japan). The tubes were then frozen in liquid nitrogen immediately after centrifugation. The bottom layer (algae) and the top layer ($APSW^-$) were separated at the silicon layer with a wire cutter and each separated part was put in 430 μl of 0.1 *M* NaOH. An aliquot (170 μl) was transferred to a scintillation vial containing 200 μl of 0.1 *M* NaOH. Another aliquot (170 μl) was transferred to a scintillation vial containing 200 μl of 0.5 *M* HCl and was dried to remove acid-labile inorganic carbon. Excreted organic carbon was estimated from the radioactivities in the acidified samples of the top layer, while total photosynthetically fixed carbon was calculated from the sum of the radioactivities of acidified samples of bottom layer (algae) and the top layer (excreted organic carbon). The radioactivity was measured following the addition of 3 ml of ACS II scintillation cocktail (Amersham, Japan) using a Tri-Carb Liquid Scintillation Analyzer 1900CA (Packard, USA). Excretion was expressed as percentage of excreted carbon in total fixed carbon (i.e., fixed carbon in algal cells+excreted organic carbon).

Measurement of Chlorophyll

Suspensions of microalgae were filtered on glass fiber filters (Toyo Roshi Kaisha, Ltd., Japan). The filters were ground in 90% acetone with a glass mortar and a pestle. After removing glass fibers by centrifugation (1100×*g*, 10 min), absorbances at 630, 647 and 664 nm were measured. The chlorophyll *a* (chl *a*) content was calculated from the standard equations of Jeffrey and Humphrey (1975).

Results

Effect of mantle homogenate of *Tridacna derasa*

Fig. 1 shows that the carbon fixation by freshly isolated zooxanthellae of *Tridacna derasa* increased almost linearly for 50 min then reached a plateau after 60 to 70 min of incubation with or without mantle homogenate of *T. derasa*. In the absence of mantle homogenate, the excretion of organic carbon from zooxanthellae was approximately 3% of the total fixed carbon after 2 h incubation (Fig. 1 a). Excretion of organic carbon was enhanced (40%) by mantle homogenate. The excretion increased linearly for 1 h, then the excretion rate decreased slightly (Fig. 1 b). After incubation with mantle homogenate, zooxanthellae were microscopically indistinguishable from freshly isolated zooxanthellae. The incubation period was fixed to 60 min in the following experiments.

Dose dependency of organic carbon excretion by mantle homogenate of *Tridacna derasa*

The mantle homogenate stimulated photosynthesis of freshly isolated zooxanthellae of *Tridacna derasa* with a maximum value of 230 μmol C mg^{-1} chl *a* h^{-1} (1.4-fold of that in the absence of the homogenate) at 0.31 mg protein ml^{-1} (Fig. 2 a). Higher concentrations of the homogenate suppressed the carbon fixation. The amount of fixed carbon excretion increased with increasing concentrations of mantle homogenate from 0 to 0.6 mg protein ml^{-1} and decreased slightly at higher concentrations. The percentage of the excretion increased from 3.47±0.58 (mean ± SD) without mantle homogenate to 42.3±5.2 in the presence of mantle homogenate at 5.0 mg protein ml^{-1} (Fig. 2 b). The photosynthetic rate of zooxanthellae which had been suppressed by mantle homogenate (4.0 mg protein ml^{-1}) for 1 h recovered after washing with filtered seawater (data not shown).

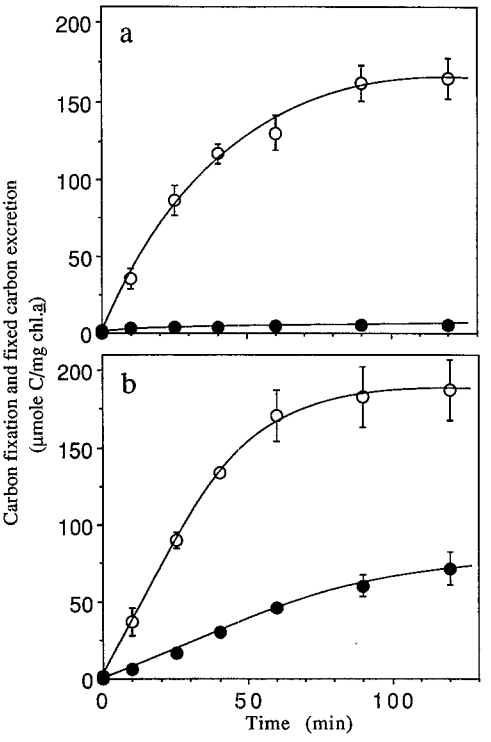

Fig. 1. Time-courses of carbon fixation (○) and excretion (●) by freshly isolated zooxanthellae with or without mantle homogenate of *Tridacna derasa*. (a) reaction without mantle homogenate; (b) reaction with mantle homogenate. Protein concentration of the mantle homogenate in the medium was 1.0 mg ml^{-1}. Bars represent standard deviations. $n = 3$

Effects of pH

Carbon fixation was almost constant, 150 to 180 μmol C mg^{-1} chl *a* h^{-1}, at pH 6.5 to 8.5, but decreased to 90–100 μmol C mg^{-1} chl *a* h^{-1} at pH 9.0 (Fig. 3). Without the mantle homogenate, percentage of excreted organic carbon to the total fixed carbon was 13 to 14 at pH 6.5 to 7.0 but it lowered to 9–3 at pH 7.5 to 9.0. When the mantle homogenate was added at 1.0 mg protein ml^{-1} in the zooxanthellae suspension, carbon excretion was increased 2- to 5-fold (16 to 30%), and it decreased with increasing pH. In the experiments to follow, pH of the $APSW^-$ changed from 7.6 to 8.5 during 1-h incubation unless otherwise stated.

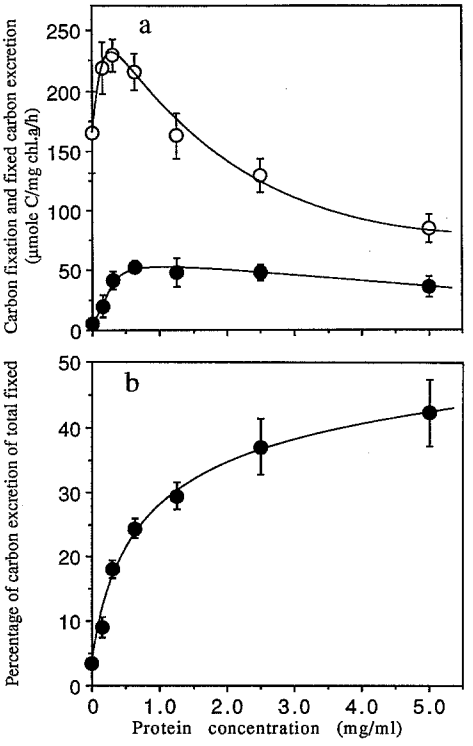

Fig. 2. Dose dependency of photosynthesis and carbon excretion from zooxanthellae on mantle homogenate of *Tridacna derasa*. (a) carbon fixation (○) and excretion (●) after 1 h; (b) percentage of total fixed carbon excreted. Bars represent standard deviations. $n = 3$

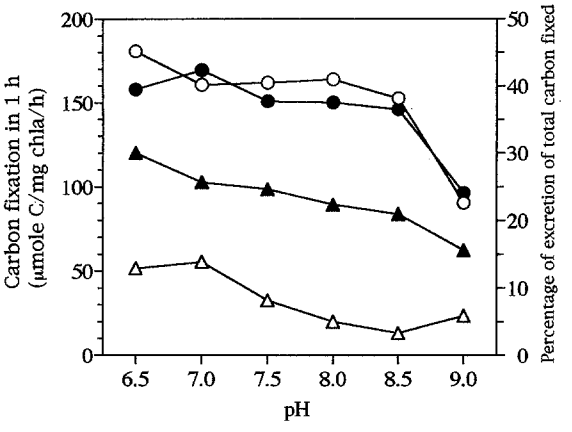

Fig. 3. Effects of pH on photosynthesis and excretion of fixed carbon in freshly isolated zooxanthellae of *Tridacna derasa* with or without mantle homogenate. Protein concentration of mantle homogenate in zooxanthellae suspension was 1.0 mg ml^{-1}. (○): carbon fixation without mantle homogenate; (●): carbon fixation with mantle homogenate; (△): percentage of fixed carbon excretion without mantle homogenate; (▲): percentage of fixed carbon excretion with mantle homogenate

Localization of ESA

The homogenates of the mantle, adductor muscle, gill, and kidney stimulated fixed carbon excretion (22 to 52% of total carbon fixed) by zooxanthellae (Table 2). However, ESA was not detected in haemolymph even when the zooxanthellae were suspended in haemolymph instead of $APSW^-$.

ESA in other molluscs

Fig. 4 shows the difference in ESA of 11 bivalves and two gastropods directed against freshly isolated zooxanthellae of *Tridacna derasa*. The mantle homogenates of *T. derasa*, *T. maxima*, *T. crocea*, *T. squamosa* and *Hippopus hippopus* stimulated the fixed carbon excretion of zooxanthellae to 14–23% of the total carbon fixed ($p < 0.05$). Tissue homogenates of *Fragum unedo* and *F. fragum* stimulated the carbon excretion from *T. derasa* zooxanthellae to 9 and 8% of the total, respectively. Because the final protein concentration in the test medium was 0.2 mg protein ml^{-1}, the percentage of excretion (6%) by the tissue homogenate of *F. mundum* might be an underestimate. Tissue homogenates of non-symbiotic bivalves and gastropods, *Meretrix lusoria*, *Mytilus edulis*, and *Turbo argyrostoma*, slightly increased fixed carbon excretion from zooxanthellae ($p < 0.05$). The homogenate of *Ruditapes philippinarum* and *Umbonium giganteum* did not enhance the excretion significantly.

Sensitivity of various microalgae to mantle homogenate of *Tridacna derasa*

The mantle homogenate of *Tridacna derasa* (1 mg protein ml^{-1}) suppressed the fixed carbon excretion from *Synechococcus* sp., *Porphyridium cruentum* and *Pavlova lutheri* to 1/10–2/3, and stimulated those from *Nannochloris atomus* and *Nannochloropisis salina* by ca. 2- to 3-fold (Fig. 5). *Chlorella marina* and *Tetraselmis chui* were not sensitive to the mantle homogenate. The mantle homogenate did not affect the carbon fixation of these algae. On the other hand, excretion by *Prorocentrum micans*, *Amphidinium carterae* and *Heterocapsa triquetra* (Dinophyceae) was stimulated 6- to 10-fold by mantle homogenate ($p < 0.05$), and it was 20 to 25% of the total carbon fixed. The photosynthetic carbon fixation of these free-living dinoflagellates were reduced to one-half by mantle homogenate. After 1 h incubation with mantle homogenate, these dinoflagellats were not disrupted and they were swimming. When these dinoflagellate cells were resuspended in seawater without mantle homogenate, their photosynthetic rates were restored to those observed before the treatment.

Sensitivity of *Tridacna crocea* zooxanthellae to mantle homogenate of *T. derasa* seemed to be lower than those of zooxanthellae from *T. derasa* and *T. squamosa*. Zooxanthellae from *Fragum unedo* was also stimulated by mantle homogenate of *T. derasa*. No morphological change was observed in these microalgae as the result of incubation with mantle homogenate.

Effect of mantle homogenate of *Tridacna derasa* on cultured zooxanthellae

The carbon fixation of cultured zooxanthellae isolated from *Tridacna derasa* (*Symbiodinium microadriaticum*, CS-161) was suppressed by mantle homogenate from 200 to 100 μmol C mg^{-1} chl *a* h^{-1} ($p < 0.05$) (Table 3). The

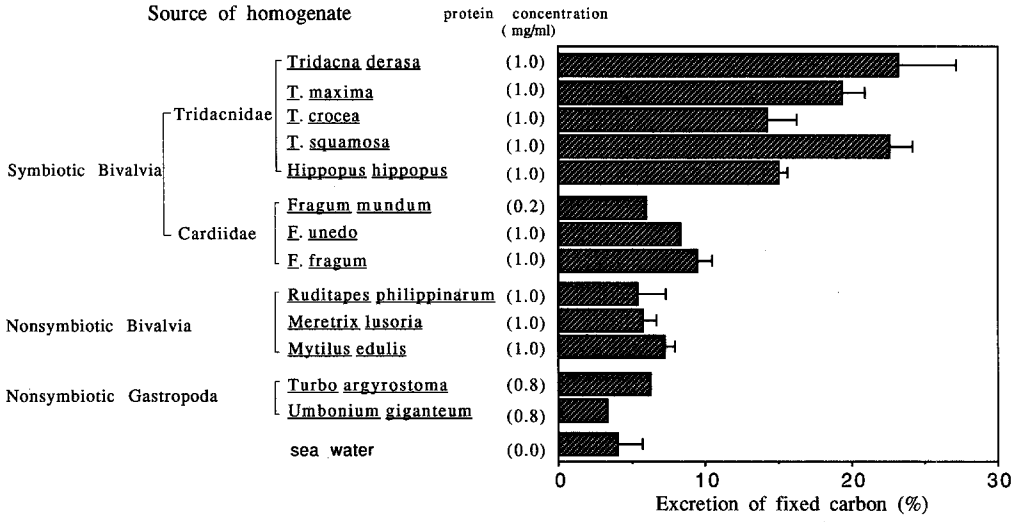

Fig. 4. Percentage of total fixed carbon excreted by *Tridacna derasa* zooxanthellae incubated with tissue homogenate of symbiotic or non-symbiotic clams

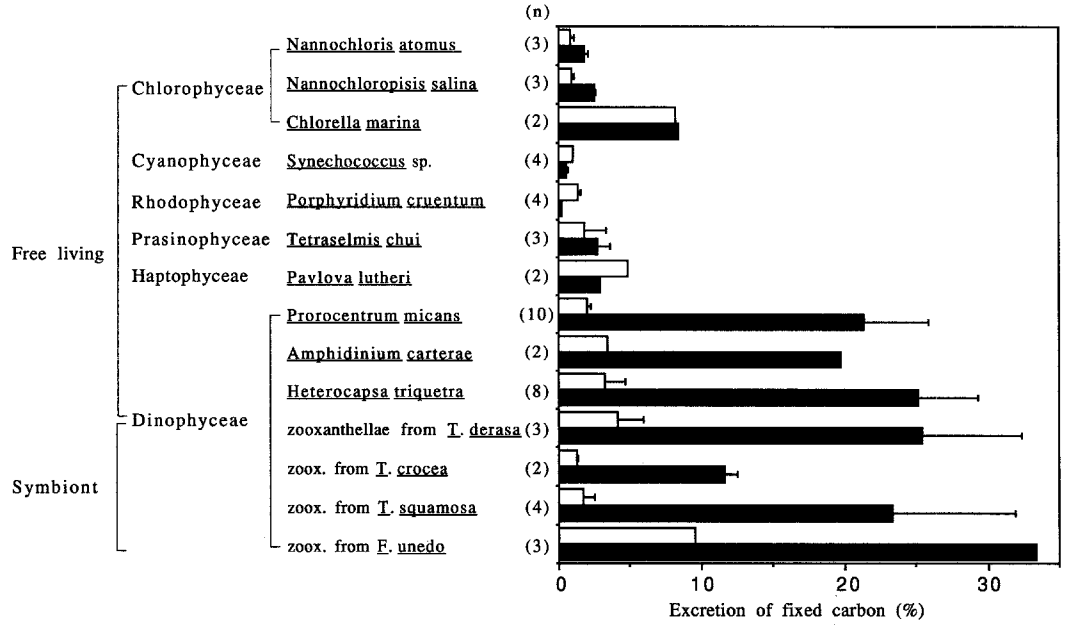

Fig. 5. Percentage of total fixed carbon excreted by various microalgae in the presence (filled bars) or absence (open bars) of *Tridacna derasa* mantle homogenate. Protein concentration of mantle homogenate in the medium was 1.0 mg ml^{-1}

Table 2. Carbon fixation and fixed carbon excretion by freshly isolated zooxanthellae in the presence or absence of various tissue homogenates from *Tridacna derasa*. Mean ± SD; $n = 3$

Source of homogenate	Protein concentration (mg ml^{-1})	Total fixed carbon (μmol C mg^{-1} chl *a* h^{-1})	Excreted carbon (μmol C mg^{-1} chl *a* h^{-1})	% excretion of total fixed carbon
Mantle	1.20	185.4 ± 24.8	51.4 ± 9.6	27.7 ± 1.7
Adductor muscle	1.20	239.8 ± 76.2	52.7 ± 9.6	21.9 ± 10.0
Gill	1.40	93.7 ± 30.6	48.8 ± 15.3	52.3 ± 2.4
Kidney	1.00	193.0 ± 91.8	42.3 ± 9.6	21.7 ± 4.3
Haemolymph[a]	0.13	139.7 ± 15.3	4.48 ± 1.92	3.21 ± 1.31
Seawater	0.00	139.1 ± 47.8	4.34 ± 1.92	3.12 ± 1.16

[a] Isolated zooxanthellae were suspended in 100% haemolymph instead of APSW$^-$

Table 3. Carbon fixation and fixed carbon excretion by cultured (*Symbiodinium microadriaticum*, CS-161) and freshly isolated zooxanthellae with or without mantle homogenate of *Tridacna derasa*. Protein concentration of mantle homogenate was 0.54 mg ml^{-1}. Mean ± SD; $n = 6$

Sample	Total fixed carbon (μmol C mg^{-1} chl *a* h^{-1})	Excreted carbon (μmol C mg^{-1} chl *a* h^{-1})	% excretion of total fixed carbon
Cultured zoox –TDMH	200.7 ± 57.4	5.74 ± 1.92	2.89 ± 1.69
Cultured zoox +TDMH	99.5 ± 28.6	13.4 ± 3.8	13.5 ± 2.5
Freshly isolated zoox –TDMH	179.1 ± 22.9	8.29 ± 3.82	4.63 ± 2.32
Freshly isolated zoox +TDMH	223.0 ± 47.8	44.4 ± 5.8	20.1 ± 0.6

percentage of total fixed carbon excreted by cultured and freshly isolated zooxanthellae was stimulated to 13 from 3 and to 20 from 5%, respectively, by the addition of mantle homogenate.

Discussion

Zooxanthellae retained more than 95% of the fixed carbon during 2 h of incubation in the absence of mantle homogenate of *Tridacna derasa* (Fig. 1). This indicates that the zooxanthellae was not damaged by mixing with the rotator.

The mantle homogenate stimulated the photosynthetic carbon fixation at a low concentration, but suppressed it at a high concentration (Fig. 2). This result agrees with that reported in zooxanthellae of scleractinian coral, *Agaricia agaricites* (Muscatine et al. 1972). Effects of the host tissue homogenates on zooxanthellae photosynthesis have been reported to be different in various host-algae symbioses. A stimulatory effect of tissue homogenate was reported in a sea anemone, *Anthopleura elegantissima* (Trench 1971), and in a coral, *Favia pallida* (Wafar and Qasim 1975). A suppressing effect was reported in a hydrocoral, *Millepora alcicornis* (Muscatine et al. 1972). No effect was reported in the zoanthids, *Zoanthus robustus*, nudibranch, *Pteraeolidia ianthina*, and a scleractinian coral, *Plesiastrea versipora* (Sutton and Hoegh-Guldberg 1990). The mantle homogenate of *Tridacna derasa* exhibits both stimulating and suppressing effects on carbon fixation. The carbon fixation of zooxanthellae, which had been suppressed during incubation with mantle homogenate, was restored after washing with seawater to the original level. This indicated that mantle homogenate did not disrupt or lyse the zooxanthellae. The maximum carbon excretion was 35 to 45% of total carbon fixed. This value in *T. derasa* agrees with those of *T. crocea* (Muscatine 1967), *T. maxima* (Trench et al. 1981), and *T. gigas* (Streamer et al. 1988).

Lower pH (pH 4.0) stimulates the excretion of organic carbon from zoochlorellae in Hydra (Muscatine 1965, Cernichiari et al. 1969). The carbon excretion from zooxanthellae of *Tridacna derasa* was also influenced by pH in the medium (Fig. 3). However, the mantle homogenate of *T. derasa* (1 mg protein ml^{-1}) stimulated the excretion by 2- to 5-fold at pH 6.5 to 9.0. Furthermore, the pH of the medium during the ESA experiment was 7.6 to 8.5. Therefore, we conclude that the excretion of fixed carbon by zooxanthellae was stimulated by mantle homogenate, but not by change in pH.

Our data indicate that ESA is present not only in the mantle tissue in which most of zooxanthellae reside but also in the adductor muscle, gill, and kidney. Because haemolymph did not have ESA (Table 2), the substance(s) which cause(s) ESA is not transported with haemolymph. In giant clams, zooxanthellae live within a zooxanthellal tube originates in the clam stomach and ends in the mantle tissue (Mansour 1946, Norton et al. 1992). The zooxanthellal tube system has no connection with the haemolymph (Norton et al. 1992). ESA in the zooxathellal tube remains to be studied.

ESA of mantle homogenates of Tridacnidae (*Tridacna derasa*, *T. maxima*, *T. crocea*, *T. squamosa*, and *H. hippopus*), directed against *T. derasa* zooxanthellae, was higher than to tissue homogenates of Cardiidae (*Fragum unedo*, *F. fragum*) or non-symbiotic clams (*Mytilus edulis*, *Meretrix lusoria*, *Ruditapes philippinarum*, *Turbo argyrostoma*, and *Umbonium giganteum*) (Fig. 4). This result suggests that the effect of the tissue homogenate on fixed carbon excretion from zooxanthellae varies with the origins of tissue homogenates. Sutton and Hoegh-Goldberg (1990) reported that the excretion of fixed carbon from coral zooxanthellae was stimulated by the homogenate of the original host animal more than by that of zoanthid or nudibranch. *T. derase* zooxanthellae show lower but distinct ESA response to tissue homogenates of non-symbiotic bivalves and gastropods, *M. edulis*, *M. lusoria*, *R. philippinarum*, *T. argyrostoma*. Therefore, a substance which has ESA may be common in molluscs.

Present data suggest that the sensitivity of some species of free-living dinoflagellate to the mantle homogenate of *Tridacna derasa* is comparable to that of zooxanthellae. The metabolism of carbon excretion by these free-living dinoflagellate in the presence of mantle homogenate remains to be studied. Sensitivities of zooxanthellae from different host species to the mantle homogenate of *T. derasa* is varied. It is not clear whether this variation in the sensitivity of zooxanthellae to the host tissue homogenate is due to the diversity of the species of zooxanthellae in different invertebrate hosts (Blank and Trench 1985). The variation of sensitivity to coral (*Pesiastrea versipora*) extract was reported in zooxanthellae which were isolated from different host animals, including coral (*P. versipora*), zoanthid (*Zoanthus robustus*), and nudibranch (*Pteraeolidia ianthina*) (Sutton and Hoegh-Goldberg 1990).

Mantle homogenate of *Tridacna derasa* (1.0 mg protein ml^{-1}) suppressed the carbon fixation of cultured zooxanthellae by ca. 50% (Table 3). The mantle homogenate seems to contain a carbon fixation suppressing substance as shown in Fig. 3. Cultured zooxanthellae may be more sensitive to the suppressing substance than freshly isolated zooxanthellae (Table 3). The carbon fixation of free-living dinoflagellates was also suppressed by the mantle homogenate at lower concentrations. The effects of mantle homogenate on cultured zooxanthellae are similar to those of mantle homogenate on free-living dinoflagellates.

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