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Net uptake of dissolved free amino acids by the giant clam, *Tridacna maxima*: Alternative sources of energy and nitrogen?

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Abstract The role of dissolved free amino acids (DFAA) in nitrogen and energy budgets was investigated for the giant clam, *Tridacna maxima*, growing under field conditions at One Tree Island, at the southern end of the Great Barrier Reef, Australia. Giant clams (121.5–143.7 mm in shell length) took up neutral, acidic and basic amino acids. The rates of net uptake of DFAA did not differ between light and dark, nor for clams growing under normal or slightly enriched ammonium concentrations. Calculations based on the net uptake concentrations typical of the maximum concentrations of DFAA found in coral reef waters ($\sim 1 \mu\text{M}$) revealed that DFAA could only contribute 0.1% and 1% of the energy and nitrogen demands of giant clams, respectively. These results suggest that DFAA does not supply significant amounts of energy or nitrogen for giant clams or their symbionts.

Introduction

Many coral reefs are renowned for their low concentrations of both particulate and non-particulate nutrients (Odum and Odum 1955). The high productivity of these diverse communities has been paradoxical to workers who have sought to explain how the nutrient-impooverished waters of the tropics can sustain their high rates of productivity (Muscatine and Porter 1977). Partial resolution of this “paradox” has come from the

idea that symbioses, like those between corals and clams and dinoflagellates of the genus *Symbiodinium* (Freudenthal 1962); recycle nutrients within the tissues of the association.

Utilisation of dissolved organic nutrients has also been suggested as a solution to the general problem of the scarcity of nutrients in tropical waters (e.g. Lucas 1982). Uptake of dissolved free amino acids (DFAA) has been reported for a wide range of marine invertebrates including brittle stars (*Amphipholis squamuta* and *Ophiopholis aculeata*; Lesser and Walker 1992), mussels (*Mytilus californianus* and *M. edulis*; Wright et al. 1984; Silva and Wright 1992), oysters (*Crassostrea gigas*; Manahan 1983a, b, 1989), abalone (*Haliotis rufescens*; Jaeckle and Manahan 1989a), and echiuran worms (*Urechis caupo*; Jaeckle and Manahan 1989b) and may, therefore, play an important role in providing metabolic energy, especially when particulate food is scarce. Most of these organisms are from temperate oceans, however, and the relevance of DFAA to the nutrition of animals typical of tropical oceans has not been assessed to any great extent.

The concentrations of dissolved free amino acids in coral reef waters are similar to those of temperate oceans, and range from the limit of detection to about $1 \mu\text{M}$ ($0.05\text{--}0.22 \mu\text{M}$: Bermuda, Ferrier 1991; $0.86\text{--}1.08 \mu\text{M}$: Mombasa, Schlichter and Liebezeit 1991; and $0.05\text{--}0.17 \mu\text{M}$: Great Barrier Reef, Hoegh-Guldberg and Welborn 1992; Hoegh-Guldberg et al. 1997). Dissolved molecules like DFAA may play a role in the nutrition of many tropical marine organisms (*Acanthaster planci*, Hoegh-Guldberg 1994a; *Tridacna gigas*, Klumpp and Griffiths 1994; see also Stephens 1981), but experimental evidence is restricted to a few studies. The role of DFAA as a source of energy or nitrogen for corals and tridacnid clams has not been assessed experimentally.

This study explores the role of DFAA in the nutrition of the giant clam, *Tridacna maxima*, and its symbiotic zooxanthellae. Giant clams take up dissolved

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amino acids across their exposed surfaces. Uptake in this case is not affected by light or the presence of zooxanthellae (leucine; Goreau et al. 1973). In the present study, the role of DFAA as a source of energy and nitrogen atoms is examined. The results are unequivocal. Although clams can take up a variety of amino acids from the DFAA pool of seawater, DFAA do not appear to play a significant role in either the energy or nitrogen budgets of giant clams under ambient conditions.

Materials and methods

Collection and maintenance of giant clams

Eight giant clams, *Tridacna maxima* Roding (121.5–143.7 mm in shell length) were haphazardly selected from nitrogen-treated and control microatolls after 13 months of nitrogen addition (NH_4Cl) during the the ENCORE (Enrichment of Nutrient in Coral Reefs) experiment (see Steven and Larkum 1993; Ambariyanto and Hoegh-Guldberg 1996). The clams were cleaned of debris and epibionts using plastic brushes, scalpels and engravers. The clams were kept in plastic tanks with flowing seawater and used in the experiment within 12 h of collection.

Measurement of DFAA uptake

The clams were rinsed prior to being placed in filtered seawater (0.2 μm membrane filter; 47 mm COSTAR) in l-l acid-washed-glass beakers. Seawater used in this experiment was taken from a well-flushed tidal channel (depth > 4 m), called “Shark Alley”, in One Tree Island lagoon. Four 50 W halogen lights (Wotan HaloStar Klr 51) were used to illuminate the clams in each of these beakers. A clear perspex sheet was placed between the beakers and the light sources in order to eliminate heat produced by the lights. The clams were acclimatised to the new conditions (indicated by valve opening and mantle extension). A mixture of 16 different amino acids (Sigma LLA-21) was added to each beaker so that the initial concentration of each was approximately 0.4 μM (mean total [DFAA] = 6.1 μM).

A total of eight clams (four clams each from the nitrogen enrichment and control treatments of the ENCORE experiment) were used in the experiment. These experiments were completed over two days. On the first day, four clams (two from the N-enrichment and two from controls) were selected for incubation in light conditions and the other four for incubation under in the dark. All clams were left overnight in the dark, and subsequently incubated in the light or dark during the next day. Light-treated clams were placed in the beaker facing the light source. Light intensities ranged between 1000–1300 $\mu\text{E m}^{-2} \text{s}^{-1}$, and were measured inside the beaker with a photometer (LICOR LI-189) equipped with a quantum sensor (LI-190SA). Beakers in the dark incubation were covered with a thick black cloth to exclude light. The net uptake by empty shells ($n = 3$) from freshly killed clams of similar sizes were also measured to investigate non-clam related uptake.

Amino acid analysis

Water samples were collected 0, 5, 15, 30 and 60 min after substrate addition and filtered (0.4 μm NALGENE) into 1.5 ml micro-centrifuge tubes (Eppendorf). Gloves were used to prevent human-derived DFAA from contaminating the samples. Controls (seawater without any clams) were sampled 0, 15, and 60 min after substrate

addition. Samples were stored at -20°C until analysed. The samples were thawed and filtered through 0.2 μm syringe filters (NALGENE) and analysed using reverse-phase high performance liquid chromatography (HPLC) with pre-column OPA (orthophthalaldehyde) derivatization (Lindroth and Mopper 1979). OPA [(10 μl of 37 mM OPA (Pierce) in 0.5 M potassium borate buffer (pH = 10) with 4 ml l^{-1} of 2-mercaptoethanol (Aldrich)] and sample (400 μl) were mixed in an autosampler (Waters 717, Millepore) precisely two minutes prior to analysis. The fluorescently-labelled amino acids were pumped (Waters 510 pumps, Millepore) in a solvent gradient through a C_{18} column (NOVA-PAKTM, 3.9 mm x 150 mm ID) equipped with a guard column at a flow rate of 1.0 ml min^{-1} . The amino acids were detected at 420 nm with a fluorescence detector (Waters Model 420). Concentrations of amino acids were calculated by measuring the areas under each peak and comparing them to the area under standard peaks (Pierce H) using Maxima 820 (version 3.3) chromatography software (Waters, Millepore, Milford, USA).

A two factor analysis of variance [treatment (N-treated or untreated clams) and incubation (light or dark)] was done on the total net DFAA uptake per clam or per wet tissue weight of the clam. Homogeneity of variance was tested using Cochran's test prior to ANOVA. The slope of the depletion of DFAA over time was calculated for the clams (N-treated and untreated clams), empty shells and of controls (seawater only) using regression analysis. Analysis of covariance was also carried out on the DFAA depletion rates by the clams.

The contribution of transported DFAAs to nitrogen and energy budgets

The contribution of the energy gained from transported DFAA was compared to the energy required by the clams (for respiration and growth). Data on the growth and respiratory rates of clams from the same experiment were derived from Ambariyanto (1996). The energy requirement for growth was determined by calculating daily growth rate (as buoyant weight, BW), ash-free dry weight of the tissue (g) and the average energy content of lipid, carbohydrate, and protein (27 kJ g^{-1} ; Gnaiger 1983). The energy required for maintenance was calculated from the respiration rate ($\mu\text{M oxygen g}^{-1} \text{BW h}^{-1}$) and the conversion of oxygen into energy units (470 kJ mol O_2^{-1} , Gnaiger 1983). The supply of energy from DFAA was determined by converting the net rate of DFAA uptake ($\mu\text{mol DFAA g}^{-1} \text{BW h}^{-1}$) into energy based on 470 kJ mol O_2^{-1} , and 1 oxygen atom burned per C atom in each substrate molecule used during aerobic respiration (Hoegh-Guldberg 1994b). The contribution of DFAA uptake at concentrations of 0.4 μM per amino acid (concentration used in this experiment) was estimated in relation to the energy and nitrogen requirements of the clams. The contribution of DFAA at the more likely concentration of 0.1 μM (ambient concentration of individual amino acids; Hoegh-Guldberg et al. 1997) was also calculated. This was done using the assumption that DFAA net uptake by the clams is linear up to 1 μM , which is approximately true for Michaelis-Menten functions like those of Class A amino acids transporters (most bivalve uptake systems are linear at least up to 1–2 μM , Preston and Stevens 1982). In any case, estimates obtained using this method are likely to slightly underestimate the net uptake rate at the lower concentrations (Hoegh-Guldberg 1994a), and therefore are likely to be conservative estimates of the impact of DFAA transport in supplying the energy and nitrogen needs of the giant clams.

Results

The uptake of dissolved free amino acids

The rates of DFAA depletion by *Tridacna maxima* were significantly greater than zero. Fresh empty shells did

Table 1 Net rate of uptake of total dissolved free amino acids (DFAA = sum of the uptake rates of all individual amino acids) by giant clams, *Tridacna maxima*, from two different treatments (N-enriched and control clams) during light (1000-1 300 $\mu\text{E m}^{-2} \text{s}^{-1}$) and dark incubations. Rates are presented on the basis of individual clams ($\mu\text{mol clam}^{-1} \text{h}^{-1}$), tissue wet weight (TWT; $\mu\text{mol g}^{-1} \text{h}^{-1}$) and buoyant weight (BW; $\mu\text{mol g}^{-1} \text{h}^{-1}$). n = 4 in all cases

Light	Dark					
	Per clam	Per TWT	Per BW	Per clam	Per TWT	Per BW
N-enriched clams				N-enriched clams		
Mean	5.00	0.087	0.033	Mean	5.23	0.087
SD	0.217	0.017	0.005	SD	0.426	0.006
Control clams				Control clams		
Mean	4.52	0.083	0.031	Mean	4.74	0.087
SD	0.316	0.005	0.001	SD	0.197	0.002

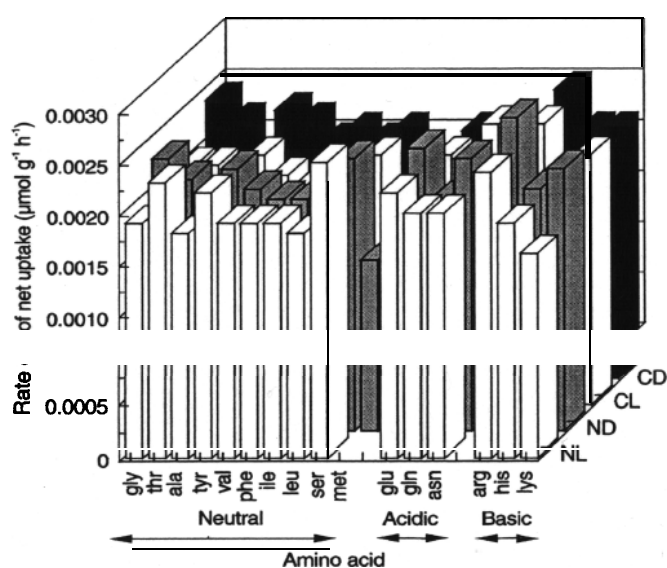


Fig. 1 Mean rates of net uptake of 16 amino acids by N-treated (N) and untreated (C) giant clams, *Tridacna maxima*, in light (L) and dark (D) incubations, standardised on a buoyant weight (g) basis

not deplete DFAA during dark incubation, (mean \pm SE: $0.28 \pm 0.03 \mu\text{mol clam}^{-1} \text{h}^{-1}$), but did to a small extent incubation in the light (mean \pm SE: $1.05 \pm 0.13 \mu\text{mol clam}^{-1} \text{h}^{-1}$, $P = 0.015$). The mean net uptake of DFAA by N-treated and control clams in light and dark incubations were the same (ANCOVA, $F_{3,12} = 0.194$, $P > 0.05$). Giant clams took up DFAA at rates between 3.9 and $6.05 \mu\text{mol clam}^{-1} \text{h}^{-1}$, 0.07 and $0.11 \mu\text{mol g}^{-1}$ wet tissue weight h^{-1} , and at 0.02 and $0.04 \mu\text{mol g}^{-1}$ buoyant weight h^{-1} (Table 1). Rates of DFAA net uptake were slightly lower in the light than in the dark, although this difference was not significant. There was no effect of nitrogen enrichment on DFAA net uptake by the clams ($P > 0.05$).

The highest mean rate of individual amino acid net uptake by the clams was $0.44 \mu\text{mol clam}^{-1} \text{h}^{-1}$ or $0.01 \mu\text{mol g}$ wet tissue weight h^{-1} (arginine, N-treated clams in dark incubation), and the lowest was $0.26 \mu\text{mol clam}^{-1} \text{h}^{-1}$ or $0.005 \mu\text{mol g}$ wet tissue

weight h^{-1} (histidine, untreated clams in light incubation). There was no difference among the average net uptake rates of neutral, acidic and basic amino acids (Fig. 1). The net uptake rates of glutamate (glu) by N-treated clams in both light and dark incubations were slightly higher than those of untreated clams. This difference was not statistically significant.

Contribution of DFAA to clam energy and N-budgets

Estimates of the potential contribution of DFAA uptake to the energy budget of *Tridacna maxima* revealed that DFAA can contribute very little to the energy requirements (maintenance and growth) of clams. At laboratory concentrations of $0.4 \mu\text{M}$, contributions from the DFAA pool are only 0.5% and 0.6% of the energy demand of N-treated and untreated clams, respectively (Table 2). Under more natural conditions, when concentrations of amino acids in the water of the lagoon of One Tree island are approximately $0.1 \mu\text{M}$ (Hoegh-Guldberg et al. 1997), contributions from this source are even less (0.1% and 0.2% respectively, Table 2). Estimates of the contribution of uptake of DFAA to nitrogen budgets at $0.4 \mu\text{M}$ were slightly higher (4.3% and 3.9% for N-treated and untreated clams respectively; Table 2) when compared to the contribution of the same uptake rates to the energy budgets of the clams.

Discussion

The results of the present study reveal that the giant clam, *Tridacna maxima*, can absorb 16 different amino acids during both light and dark conditions. The average net transport rate was $0.09 \pm 0.006 \mu\text{mol g}^{-1}$ wet tissue weight h^{-1} , slightly higher than those of brittle stars $\text{CO.035} \mu\text{mol g}^{-1}$ wet tissue weight h^{-1} by *Ophioneris annulata*, (Lesser and Walker 1992), and $0.029 \mu\text{mol g}^{-1}$ wet tissue weight h^{-1} by *O. aculeata* (Davis et al. 1985)]. There was no preference by the

Table 2 Potential contribution of dissolved free amino acids (DFAA) taken up by the clam *Tridacna maxima*. A. Daily energy and B. Nitrogen requirements of N-enriched (N) and control (C) clams
A. Daily energy demand

	N	C	Unit
1. Growth rate [as buoyant weight (BW); Ambariyanto (1996)]	0.31	0.28	% d ⁻¹
2. AFDW per g BW (Ambariyanto 1996)	45.5	58.9	mg g ⁻¹
3. Growth in AFDW per g BW = [(1) (2)]	0.141	0.165	mg g ⁻¹ d ⁻¹
4. Average energy (kJ) per AFDW (Gnaiger 1983)	27	27	kJ g ⁻¹
5. Growth in energy per g BW [(3) x (4)]	3.807	4.455	J g ⁻¹ d ⁻¹
6. Maintenance (respiratory rate per g BW; Ambariyanto 1996)	1.99	1.55	μmol O ₂ g ⁻¹ h ⁻¹
7. Oxycaloric equivalent (Gnaiger 1983)	470	470	kJ mol O ₂ ⁻¹
8. Maintenance per day, per g BW [(6) x (7) x 24 h]	22.440	17.472	J g ⁻¹ d ⁻¹
9. Total energy needs per g BW [(8) + (9)]	26.247	21.927	J g ⁻¹ d ⁻¹
Supply of energy from DFAA uptake			
10. Average net uptake of individual amino acids per g BW per h (see Fig. 1)	0.002	0.002	μmol g ⁻¹ h ⁻¹
11. Net uptake of AA per g BW, per day [(10) x 24 h]	0.048	0.048	μmol g ⁻¹ d ⁻¹
12. Energy imported as C-6 AA (based on 47 kJ/mole l-oxygen and 1 oxygen burned per C during aerobic respiration) per BW per day = [(11) x 6 x 470]	0.135	0.135	J g ⁻¹ d ⁻¹
Contribution of DFAA to daily energy demand (maintenance and growth)			
13. Contribution at 0.4 μM	0.514	0.615	%
14. Contribution at 0.1 μM	0.128	0.153	%
15. Concentration needed to supply 100% of daily energy demand	78.1	65.4	μM
B. Daily nitrogen demand			
16. Percent N of mass	11.02	10.48	%
17. Demand of N mg per g BW = [(1) x (2) x (16)]	15.54	17.28	μg N g ⁻¹ d ⁻¹
N supply through DFAA			
18. Average N atom mass uptake per day (assumes a single N per amino acid molecule)	0.672	0.672	μg N g ⁻¹ d ⁻¹
Contribution of DFAA to the organic nitrogen needs of the clams			
19. Contribution at 0.4 μM	4.324	3.889	%
20. Contribution at 0.1 μM	1.081	0.972	%
21. Concentration needed to supply 100% of daily nitrogen demand	9.250	10.288	μM

^aAFDW = ash free dry weight of the clam tissue (mantle)

clams for specific classes of amino acid (neutral, acidic or basic, see Fig. 1). Similar results have been reported for the uptake of DFAA by veliger larvae of the oyster, *Crassostrea gigas* (Manahan 1989), and red abalone, *Haliotis rufescens* (Jaekle and Manahan 1989a). This is in contrast to the coral *Galaxea fasciculuris*, which took up neutral amino acids faster than basic and acidic amino acids (Al-Moghrabi et al. 1992). Other marine invertebrates that have been reported to preferentially take up specific classes of amino acids include the larvae of the echiuran worm, *Urechis caupo* (Jaekle and Manahan 1989b) and three species of starfish from the genus *Patriella* (Moreno and Hoegh-Guldberg, unpublished data). These organisms also take up neutral amino acids at faster rates than charged amino acids.

Light influences the rate of DFAA uptake by the corals *Heliofungia actiniformis* (Baker 1994) and *Pocillopora damicornis* (Hoegh-Guldberg and Williamson 1999). These two studies proposed that the lower rates of DFAA uptake in the light are related to the higher

concentrations of amino acids in coral tissues during the day produced by photosynthetic algal symbionts. In the present study, however, no effect of light on the rate of DFAA uptake by clams was detected. This is in agreement with Goreau et al. (1973), who also reported that leucine uptake by *Tridacna maxima* was not influenced by light intensity.

DFAA have been suggested as a potentially important source of energy for marine organisms (review, Manahan 1990). Alanine (at a concentrations of 595 nM) for example, is theoretically capable of providing 51% of the metabolic energy for larvae of *Urechis caupo* (Jaekle and Manahan 1989b). In another study, 39 to 70% of the metabolic demand of larval abalone, *Haliotis rufescens*, could be satisfied by amino acid uptake from a concentration of 1.6 μM (Jaekle and Manahan 1989a). The results of the present study, however, suggest that DFAA do not have much potential to contribute to the energy requirements of adult giant clams. At the experimental concentrations of

0.4 μM , DFAA potentially contributes only 0.5% and 0.6% of the daily energy requirements of N-treated and untreated clams respectively. Furthermore, at naturally occurring concentrations of DFAA in coral reef waters (approximately 0.1 μM ; Hoegh-Guldberg et al. 1997), DFAA can contribute only 0.13% and 0.15% of the energy demands of N-treated and untreated clams, respectively. The concentrations of DFAA would have to be as high as 78.12 μM (N-treated clams) or 65.36 μM (untreated clams) in order to satisfy 100% of the daily energy requirements for growth and maintenance of these clams (Table 2). These results do reveal, however, that *T. maxima* can utilise DFAA should they become sporadically available at higher concentrations (e.g. DFAA resulting from the spillage of cytoplasm during fish feeding).

This study also assessed the potential role of DFAA as a source of nitrogen to the adult clams. Hoegh-Guldberg (1994b) showed that DFAA can potentially play a more significant, though still small, role as a source of N for several symbiotic organisms. In the present study, the uptake of DFAA also had a slightly greater potential to supply nitrogen to tridacnid clams. The uptake of DFAA at 0.4 μM could satisfy between 4.32% and 3.89% of the daily nitrogen demand of N-treated and untreated clams, respectively. But, at a typical Great Barrier Reef DFAA concentration of 0.1 μM (Hoegh-Guldberg and Welborn 1992), the estimated contribution of DFAA uptake is 1.08% for N-treated clams and 0.97% for untreated clams. This is almost ten times greater than the contribution of DFAA uptake to the energy demand of the clams. The contributions are still low, however, and suggest that DFAA does not play a major role as a source of N to adult giant clams under either the normal or nitrogen enriched conditions.

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