FAU Institutional Repository

http://purl.fcla.edu/fau/fauir

This paper was submitted by the faculty of FAU's Harbor Branch Oceanographic Institute

Notice: © 2001 Kluwer Academic Publishers. This manuscript is a version of an article with the final publication found online at http://www.springerlink.com and may be cited as: Cook, Clayton B. and Simon K. Davy (2001) Are free amino acids responsible for the 'host factor' effects on symbiotic zooxanthellae in extracts of host tissue?, *Marine Biology* 461:71–78 DOI: 10.1023/A:1012785725378

Are free amino acids responsible for the 'host factor' effects on symbiotic zooxanthellae in extracts of host tissue?

Clayton B. Cook & Simon K. Davy[1]
Division of Marine Science, Harbor Branch Oceanographic Institution, 5600 U.S. 1 North, Fort Pierce, FL 34946, U.S.A.
E-mail :ccook@hboi.edu
[1]*Present address: Institute of Marine Studies, University of Plymouth, Drake Circus, Plymouth, Devon PL4 8AA, U.K.*

Key words: sea anemone, zooxanthellae, free amino acids, MAA, coral

Abstract

Symbiotic dinoflagellates ('zooxanthellae') typically release short-term photosynthetic products and have enhanced photosynthesis when exposed to extracts of host tissue. Published evidence has indicated that free amino acids (FAA) at concentrations exceeding 40 mM are responsible for these 'host factor' effects on zooxanthellae from sea anemones and corals. We have compared the ninhydrin-positive FAA concentrations of extracts of the symbiotic sea anemone *Aiptasia pallida* with their efficacy in eliciting these responses and found little effect on carbon release by freshly isolated *A. pallida* symbionts at concentrations up to 0.6 mM, the highest concentration of our samples. Extracts of the coral *Montastraea annularis* induced release from these algae at less than 0.1 mM FAA, but there was no correlation between total ninhydrin-positive FAA concentration and 'host factor' activity. However, all of these preparations stimulated photosynthesis. We tested a range of concentrations ($\leq$50 mM) of glycine, alanine and glutamic acid with the isolated *A. pallida* symbionts. There was a significant increase in the release of fixed carbon with increasing alanine concentrations, but not with the other two amino acids. There were no effects on photosynthesis. Our observations support other reports indicating that other compounds , or specific amino acids such as taurine and the mycosporine-like amino acids, are responsible for 'host factor' effects.

Introduction

Many species of marine invertebrate harbour endosymbiotic dinoflagellates ('zooxanthellae') within their cells. These symbionts release photosynthetically-fixed carbon to their animal hosts, largely in the form of low molecular weight compounds such as glycerol (Cook, 1983; Trench, 1993). The mechanism of this release has been the subject of much debate (Hinde, 1988; Falkowski et al., 1993), with the presence of a stimulatory factor (a 'host release factor'; HRF) in the host's tissues receiving considerable attention. These algae exhibit enhanced release of short-term photosynthetic products when incubated in extracts of tissue from a variety of hosts (Muscatine, 1967; Trench, 1971; Muscatine et al., 1972; Trench et al., 1981; Sutton & Hoegh-Guldberg, 1990; Masuda et al., 1994). Typically, these host tissue extracts also stimulate photosynthesis by the algae. However, it is still unknown whether HRF operates in the intact association, and its identity is a matter of continued controversy.

Gates et al. (1995, 1999) found that synthetic mixtures of free amino acids (FAA), synthesized to represent the FAA pool of active coral extracts, stimulated both release and photosynthesis by zooxanthellae at concentrations of 45 and 75 mM. They also found that mycosporine-like amino acids (MAA) that commonly occur in coral tissue also had these effects, and proposed that HRF consisted of a suite of free amino acids. Subsequent papers (Wang & Douglas, 1997; Withers et al., 1998) have raised questions about these experiments, and have presented evidence that taurine, a non-protein free amino acid (Wang & Douglas, 1997) or other compounds (Withers et al., 1998) are involved. In this paper, we present the results of experiments with zooxanthellae from the sea anemone

Aiptasia pallida (Verrill) that also indicate that the total FAA pool of tissue extracts per se may not be responsible for these 'host factor' effects. A preliminary abstract of the size-fractionation data in this paper has been previously published (Cook & Orlandini, 1992).

Materials and methods

Experimental organisms

Clonal individuals of the subtropical sea anemone *Aiptasia pallida* (Bermuda strain) were maintained as described previously (Cook et al., 1988), in an incubator at 25 °C and 55 μmol photons m^{-2} s^{-1} on a 12 h light: 12 h dark cycle. The animals were either fed *Artemia* nauplii daily, or starved for various periods in 0.22 μm-filtered seawater (FSW) until use. Some experiments involved tissue obtained from the corals in the genus *Montastraea*. One colony of *Montastraea franksi* (Gregory) was collected at 3 m depth from a patch reef in Bailey's Bay in Bermuda (Cook et al., 1994). This coral was originally designated as *M. annularis* (Ellis and Solander) by Cook & Orlandini (1992), but is now considered to be *M. franksi* based on the criteria of Weil & Knowlton (1994). Five colonies of *Montastraea annularis* were collected from 2 m depth at Admiral's Reef, Key Largo, Florida. These corals were transported to Ft. Pierce and used for tissue fractionation within 24 h.

Assay of HRF activity of tissue extracts

The activity of host release factor (HRF) was assayed in preparations of both sea anemone and coral tissue, using freshly isolated zooxanthellae (FIZ) from fed *A. pallida*. For each preparation of symbiotic *A. pallida*, 3–5 anemones (pedal disc diameter: 7–10 mm) were homogenized in FSW at room temperature with a motorized tissue grinder. The homogenate was centrifuged at 1000 ***g*** for 4 min in an IEC clinical centrifuge, and the symbiont- free supernatant was used as crude homogenate. FIZ for these assays were obtained from the sedimented pellet, which was washed with three successive centrifugations in FSW. Final cell suspensions were adjusted to 0.5–1 $\times$ 10^6 cells mL^{-1}, as determined from haemocytometer counts. Coral tissue was removed from the skeletons of five colonies of *M. annularis* with ca 40 mL FSW, using a recycling Water-Pik (Johannes & Wiebe, 1970). After allowing skeletal pieces to settle, the slurry was decanted, centrifuged once as above, and the supernatant used as the source of host tissue.

HRF of anemone and coral tissue fractions was assayed using ^{14}C. Triplicate 300 μl aliquots each of algal suspension and test solution (FSW, tissue extracts) were added to 1.6 mL microcentrifuge tubes together with 1.0 μCi of $NaH^{14}CO_3$. Thus, cell concentrations in the incubations ranged from 2.5 to 5.0 $\times$ 10^5 mL^{-1}.The cells were incubated in the light (250 μmol photons m^{-2} s^{-1}; 24.5 $\pm$ 0.5 °C) for 30 min, with resuspension every 10 min. At the end of the incubations samples were taken of the cell suspensions. The suspensions were then centrifuged (14 000 ***g*** for 2 min) and samples taken of the cell-free supernatant. To correct for non-acid-volatile ^{14}C in the isotope stock solution, control vials were prepared by replacing the test solution with 300 μl of 20% borate-saturated formalin, incubated as above. These background values were subtracted from all samples. Medium samples were taken from these formalin vials to determine specific activity for photosynthetic rates. All samples were acidified with shaking for 60 min prior to counting by liquid scintillation, and corrected for formalin background values. HRF activity was measured as % release, or (supernatant dpm/total dpm) $*100$. Photosynthetic rates were calculated on a per cell basis using the value for total fixed C.

Size fractionation of host tissue extracts

Symbiont- free supernatants of sea anemone and coral tissue were used as crude homogenates. The homogenates were fractionated by molecular weight using Centri-Prep concentrators (3 kDa–100 kDa pore size; Amicon, Inc.) in a Sorvall high-speed centrifuge. Prior to use, the concentrators were rinsed overnight with deionized water as recommended by the manufacturer to remove traces of glycerol. The 3 kDa filtrates of *M. annularis* tissue were stored at -17 °C for 18 months before use; these samples lost less than 10% of HRF activity during this period.

Dose-dependency of HRF and [FAA] of tissue extracts

To determine if the HRF response exhibited dose-response characteristics, we employed a dilution series of a coral extract. We used a series of aliquots of the 3 kDa filtrate from *M. annularis*, and diluted the filtrate with FSW to a final volume of 600 μl. Six hundred μl of a suspension of FIZ from *A. pallida* was then added,

and HRF activity and photosynthesis was assayed as above.

We also examined the relationship between host factor activity and the ninhydrin-positive free amino acid (FAA) content of various tissue extracts. In addition to the 3 kDa filtrates from the five colonies of *M. annularis,* we used crude supernatants of anemones starved for different periods to obtain extracts with varying FAA. Host factor activity of each sample was assayed as described above. At the beginning and end of each ^{14}C incubation, aliquots were taken of the extracts and stored in 80% ethanol at 4 °C for FAA analyses. Since [FAA] of the crude extracts tended to increase by 10–20% during the 30 min photosynthesis experiments, we used the mean of the initial and final values as the tissue concentration for a particular incubation. For FAA analysis, the samples in 80% ethanol were centrifuged (4 °C, 14 000 ***g*** for 30 min), and total FAA concentrations of the supernatants were determined spectrophotometrically with the ninhydrin method of Wylie & Johnson (1962). Glycine standards and blanks were made up in 80% ethanol in FSW. Results were expressed as total FAA concentrations (μM) of the incubation media

Effect of concentration of specific FAA on the host factor response

To further determine how the host factor response of *A. pallida* FIZ was related to FAA, we tested three amino acids (glycine, alanine and glutamic acid) over a concentration range of 0.04–50 mM. The experiments were run in two series, low (0.04–0.6 mM) and high (2.9 and 50 mM) concentrations in FSW. The lower concentrations corresponded to those of our sea anemone and coral extracts, while the higher concentrations corresponded to those reported in coral tissue preparations reported by Gates et al. (1995). To avoid any complications arising from lowered pH, we buffered the 100 mM stock solutions of glycine and alanine in FSW to seawater pH (8.1) with 6 N NaOH before preparing the incubation media for the two higher free amino acid concentrations. The suspensions of FIZ in FAA were assayed for HRF response as above.

Statistical procedures

All percentage data were transformed by the angular (arcsine) transformation prior to statistical analysis. Differences between cell suspensions in FSW alone and either host extract or FAA solutions were examined with paired *t*-tests for two groups; multiple group comparisons were done using ANOVA followed by Tukey's HSD post-hoc procedure. Simple correlations (Pearson *r*) were calculated using linear regression.

Results

Size fractionation of host homogenates

Figure 1 summarizes the results of fractionation of *A. pallida* 'host factor' by ultrafiltration (Fig. 1A). The activity of 3 kDa ultrafiltrates was similar to that of the crude homogenate, indicating that the active molecules were less than 3 kDa. However, due the variation in the FSW and crude homogenate samples, these two groups were not statistically different. The 3 kDa fraction would contain free amino acids (including taurine) and any mycosporine-like amino acids (MAA). Similar results were obtained for fractionation of tissue from *M. franksi*. Neither the 100 kDa nor the 3 kDa filtrates had significantly less HRF activity than did the crude homogenate (Fig. 1B), but both of these fractions had significantly enhanced activity compared to that of FSW. All of these filtrates and crude homogenates stimulated photosynthesis ($P < 0.05$ for all, Tukey HSD), and there were no differences in stimulation between the filtrates and crude homogenates.

Dose dependency and [FAA] of extracts

Figure 2A clearly indicates that there was a linear response of HRF to a range of dilutions of a 3 kDa filtrate from *M. annularis* ($P < 0.001$), with HRF activity doubling over this range. Photosynthesis by the algae also increased with the amount of extract added (Fig. 2B; $P < 0.01$), but in contrast to HRF the highest concentration of extract had only a 15% increase over the seawater rate.

In contrast to the linear HRF response of FIZ to increasing amounts of coral filtrate, we did not find a relationship between HRF and the total ninhydrin-positive free amino acid content of tissue extracts. Neither the responses to 3 kDa filtrates of *M. annularis* ($r = 0.121$) nor to crude homogenates of *A. pallida* ($r = 0.399$) were correlated with [FAA] (Fig. 3A; $P > 0.05$ for both). All of the 3 kDa coral extracts had relatively high activity (3–4 times the percentage release in seawater alone) at low [FAA], less than 0.3

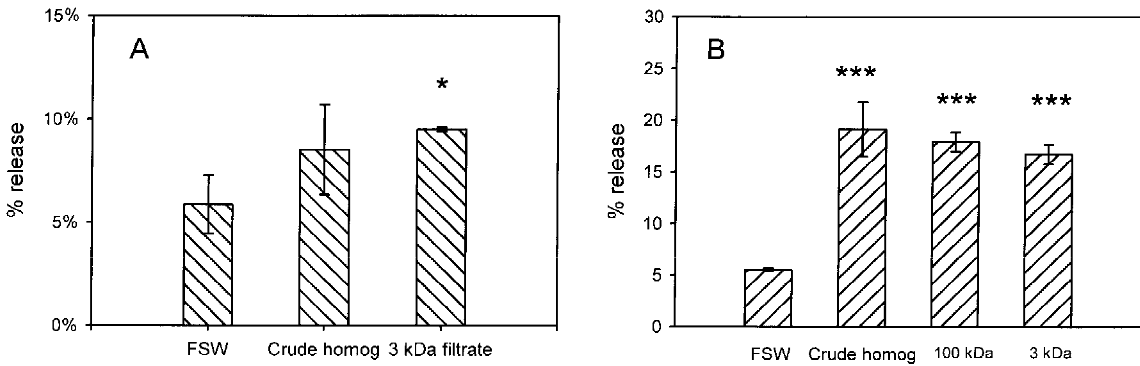

Figure 1. Size fractionation of HRF activity in *Aiptasia pallida* and *M. franksi.* Data expressed as the percent of fixed carbon released by cell suspensions during 30 min of photosynthesis. (A) CentriPrep ultrafiltration, 3 kDa exclusion of *A. pallida* tissue. (B) CentriPrep ultrafiltration of *M. franksi* tissue. FSW, filtered seawater. $N = 3$ for all; means ± sd. Asterisks indicate significant difference from FSW: *, $P < 0.05$; ***, $P < 0.001$ (Tukey's HSD).

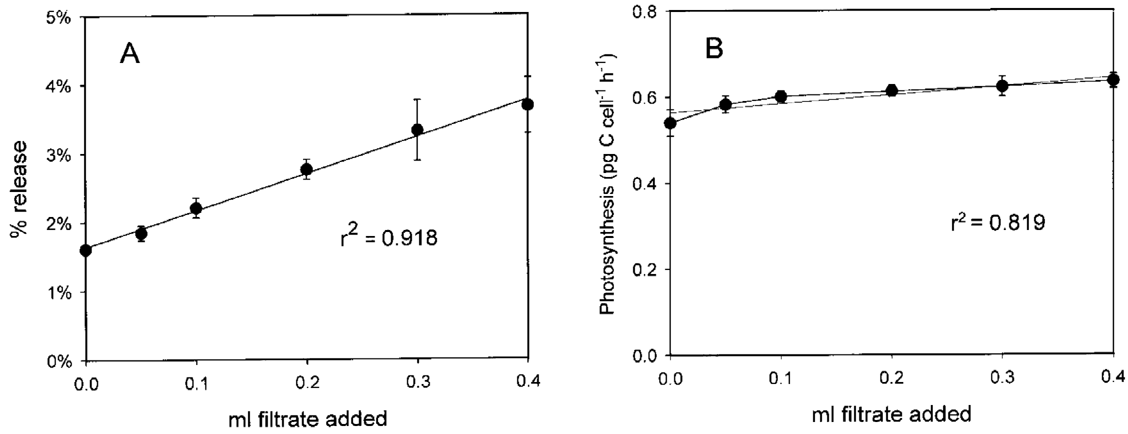

Figure 2. Dose dependency of HRF activity and effect on photosynthesis of a 3 kDa filtrate of *Montastraea annularis* tissue on zooxanthellae of *A. pallida*. Statistical parameters as in Figure 1; least-squares regression lines are shown.

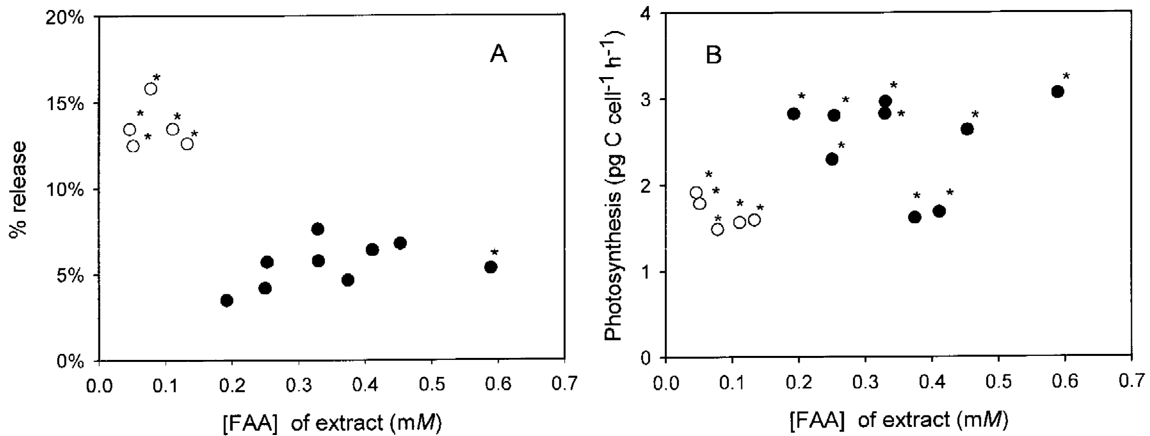

Figure 3. Relationship between ninhydrin-positive FAA concentration of tissue extracts and (A) per cent release of fixed carbon and (B) photosynthesis by isolated symbionts of *A. pallida* . (O), 3 kDa filtrate of *M. annularis*; (•), crude supernatant of *A. pallida* tissue. Corresponding % release rates and photosynthetic in FSW are plotted as pinpoints with error bars. Asterisks indicate extracts with significantly more release or greater photosynthetic rates than in FSW alone ($P < 0.05$);. $N = 3$ for all samples.

mM. The lowest FAA concentration of these samples was 0.07 mM, and this sample had three times the per cent release of FSW. All of the anemone homogenates had higher [FAA] than the coral filtrates (maximum: 0.6 mM), yet only one of the nine samples – at the highest [FAA] – produced a significant increase in the per cent release of fixed carbon over that of seawater alone. These data suggest that the FIZ of *A. pallida* had responded to compounds in the coral extracts that were not present in the sea anemone extracts, and that these compounds probably were not free amino acids.

All of the samples produced significantly higher photosynthetic rates compared to FSW alone (Fig. 3B). The enhancement of photosynthesis was particularly evident in the crude homogenates of sea anemone tissue. The mean increase was 65.4% (range: 31.4–107.5%), while the coral extracts had a mean increase of 35.6% (range: 29.8–45.7%). However, there was no correlation of photosynthetic rate with [FAA] for either the anemone ($r = 0.01$) or coral ($r = 0.70$) extracts, indicating that the effect of these extracts on photosynthesis was not due to total free amino acid content.

Effect of increasing concentrations of FAA on HRF activity

We tested three amino acids (glycine, alanine, glutamate) over a range of concentrations up to 50 mM for their effects in eliciting HRF activity and on photosynthesis. The lower range of concentrations corresponded to those of the extracts in Figure 3. There was no consistent pattern between the concentration of FAA and the release of short-term photosynthetic products (Fig. 4A, B). There was a significant effect with alanine: the per cent release increased with concentration over the entire range ($P < 0.001$; ANOVA). At the highest concentration (50 mM) the HRF response was 2.5 greater than in FSW alone. However, there was no effect with either glycine or glutamate. There was no effect of any amino acid concentration on photosynthesis over the range tested (Fig. 4C, D).

Discussion

Since the initial observation that extracts of marine invertebrate hosts stimulated the release of short-term photosynthetic products from isolated symbiotic zooxanthellae (Muscatine, 1967), the nature of these 'host release factors' has been a matter of much interest. The stimulation of photosynthesis by these extracts has also been considered a 'host factor' effect. Two issues have attracted attention: the compounds that are responsible for these effects, and whether these compounds act in intact symbioses to effect the translocation of photosynthetic products to the host.

In general, these compounds appear to be small molecules, one report notwithstanding (Sutton & Hoegh-Guldberg, 1990, but see discussion in Gates et al., 1995). Our data show that both the HRF and photosynthesis-stimulating factors of *A, pallida* and *M. franksi* tissue are less than 3 kDa in size, while dialysis indicated molecular weights less than 500 Da (Cook & Orlandini, 1992). Grant et al. (1998) found that both HRF activity and photosynthetic enhancement of tissue from the scleractinian *Plesiastrea versipora* (Lamarck) was contained in a fraction <1 kDa. Gates et al. (1995) found that the HRF of *A. pulchella* eluted with the void volume of a Sephadex G-25 column, indicating a molecular weight <4 kDa. Separation of this material with HPLC yielded active fractions containing FAA and mycosporine-like amino acids (MAA). Synthetic mixtures of FAA at concentrations found in extracts (total [FAA] of 76 and 45 mM) mimicked the HRF and photosynthetic effects of host homogenate, and Gates et al. (1995) proposed that FAA operate as these host factors. Subsequent work with 200 mM mixtures of FAA revealed a variety of physiological effects that were consistent with this idea (Gates et al., 1999).

In contrast to this view, other workers have found that FAA may not be responsible for the host factor effects seen in tissue extracts; this view is supported by our results. We found no relationship between HRF and the concentration of ninhydrin-positive free amino acids of active extracts from the coral *M. annularis*, even though these extracts exhibited a dose-response curve (Figs 2 and 3), and there was little effect of concentrations of individual amino acids up to 50 mM (Fig. 4). Similarly, Withers et al. (1998) found no relationship between carbon release and [FAA] in active fractions from *P. versipora*. They also tested an FAA mixture representative of the FAA pool of active extracts of *P. versipora*: in some cases, this stimulated the release of photosynthetic products from zooxanthellae, but at substantially lower rates than the extracts themselves. They suggested that other molecules – including taurine, which was not included in the FAA mixture – were primarily responsible for the HRF activity. Their extract was effective at a total [FAA] of 0.47 mM, while some of our coral extracts

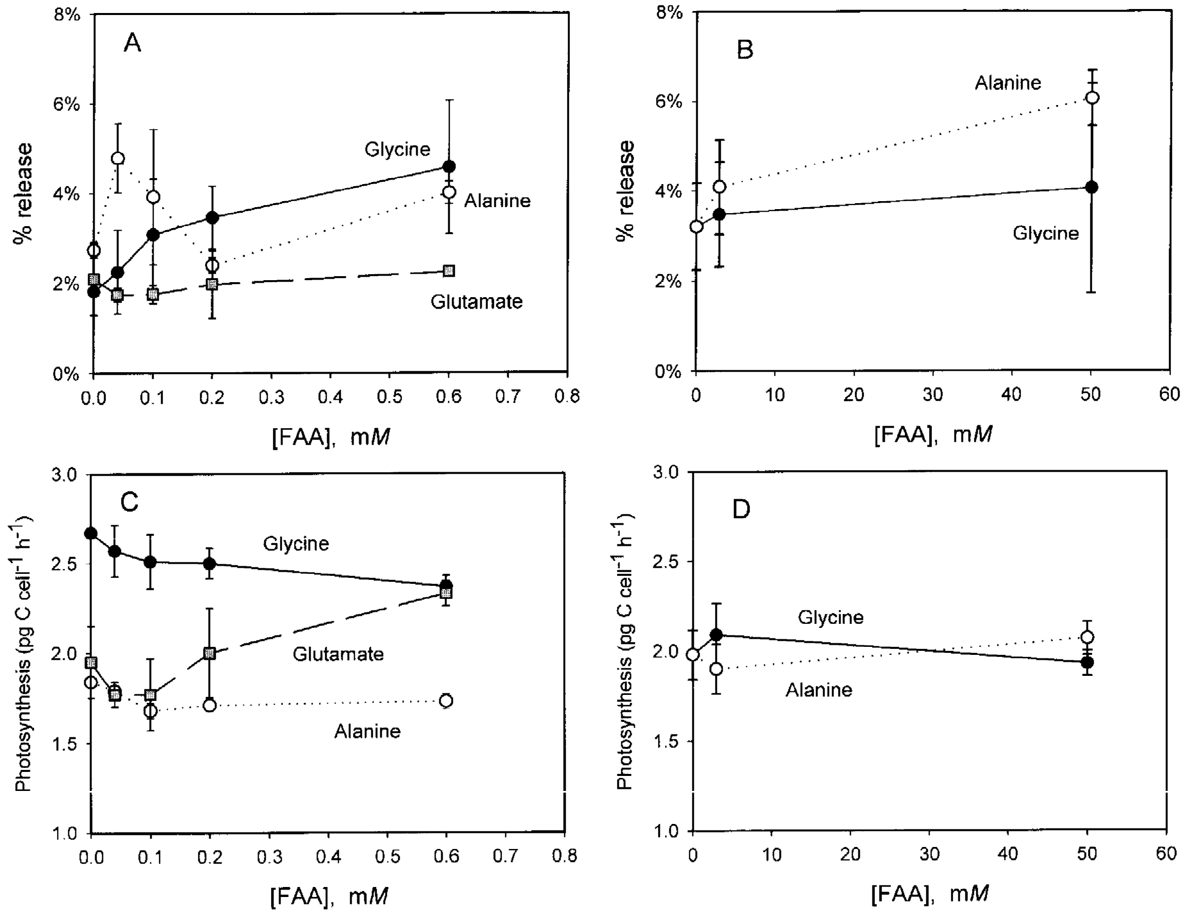

Figure 4. Effect of increasing concentrations of single amino acids on the HRF response and photosynthesis of *A. pallida* zooxanthellae. (A) The release of recently fixed carbon at low concentrations. (B) The release of recently fixed carbon at high concentrations. (C) Photosynthesis at low concentrations. (D) Photosynthesis at high concentrations. Low and high concentration data from separate experiments. Statistical parameters as in Figure 1.

exhibited HRF activity with total [FAA] <0.1 mM (Fig. 3). Individual FAA had no effect on HRF activity at these low concentrations (Fig. 4). Wang & Douglas (1997) found that 25 mM FAA had no effect on HRF of *A. pulchella* symbionts; however, 1 mM taurine (a free amino acid not used for protein synthesis) was as effective as host extract. The similarities between the kinetics of HRF activity and those of taurine uptake by isolated zooxanthellae provide additional support for a role for taurine in these extracts. In contrast, Withers et al. (1998) found that 1 mM taurine had little effect on HRF activity using the symbionts from *P. versipora*. We did not examine the effects of taurine in our experiments.

The second effect of host tissue extracts on isolated symbionts is the stimulation of photosynthesis. Tissue extracts which elicit HRF activity tend to increase photosynthetic rate, and Gates et al. (1995, 1999) suggest that both are characteristic of 'host factor' activity. Both our work and that of others indicate that these effects are likely due to different components of such preparations. Our coral extracts in Figure 3 stimulated both the release of photosynthetic products from FIZ and carbon fixation, while the anemone extracts were ineffective in promoting release, but all stimulated photosynthesis. Other studies have shown that extracts or solutions that have HRF activity may not affect photosynthesis (Masuda et al., 1994; Wang & Douglas, 1997).

The absence of an effect of MAA on photosynthesis led Gates et al. (1995) to consider other FAA as 'host factors', even though MAA elicited HRF activity. Ritchie et al. (1997) reported that clotrimazole, a mycosporin, mimicked the HRF activity

of *P. versipora* extracts. MAA are commonly found in reef corals, where they act as UV-protective sunscreens (Dunlap & Shick, 1998). MAA, particularly mycosporine-glycine, occurs in shallow-water colonies of corals in the *M. annularis* complex (Gleason & Wellington, 1993; Lesser, 2000). We found a UV absorbance peak in our *M. annularis* extracts at 310 nm, corresponding to the absorbance maximum of mycosporine-glycine. Thus, MAA were present these extracts, and we suggest that they could have been responsible in part for the HRF responses that we observed with them.

Whether zooxanthellae living in host tissue respond to 'host factors' as they do in *in vitro* experiments is an unresolved question, and the artifactual nature of these host extract experiments is generally recognized. One major issue is that intracellular zooxanthellae typically reside in membrane-limited vacuoles ('symbiosomes') that isolate them from the cytoplasmic milieu of host cells. We know little about the environment within these vacuoles, but it seems reasonable that the vacuole membrane partly controls fluxes of molecules between host and symbiont. Marine cnidarians such as sea anemones have relatively high internal concentrations of amino acids [ca. 100 mM; Shick (1991)], and this is the range used in some studies with FAA mixtures (Gates et al., 1995, 1999). Whether zooxanthellae in these vacuoles are actually exposed to these high concentrations is not clear. One point which argues against this is the increasing evidence that zooxanthellae in reef corals tend to be nitrogen-limited (Hoegh-Guldberg & Smith, 1989; Muscatine et al., 1989; Cook et al., 1994).

Conclusions

We concur with the position of others (Wang & Douglas, 1997; Withers et al., 1998) that, while free amino acids in host tissue extracts may have some effect in eliciting the 'host release factor' response of zooxanthellae, it is very likely that other compounds – or specific amino acids such as taurine and the mycosporine-like amino acids – are largely responsible. The genetic diversity of these algae (Rowan, 1998), and differences in responses of algae to host extract preparations (e.g. Sutton & Hoegh-Guldberg, 1990) serve as a *caveats* against generalizing results from particular experimental systems.

Acknowledgements

We thank Jana Orlandini, Dawn Olson and Lori Hrdlicka for assistance. SKD was supported by the Harbor Branch postdoctoral program. This work was supported by grants from the U.S. Office of Naval Research to CBC (N0014-91-J-1408, N00014-96-1-0602). This is contribution #1609 of the Bermuda Biological Station for Research, Inc. and #1420 of the Harbor Branch Oceanographic Institution.

References

Cook, C. B., 1983. Metabolic interchange in algae-invertebrate symbioses. Int. Rev. Cytol. Suppl. 14: 177–210.

Cook, C. B., C. F. D'Elia & G. Muller-Parker, 1988. Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida*. Mar. Biol. 98: 253–262.

Cook, C. B., G. Muller-Parker & C. D. Orlandini, 1994. Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and*Montastrea annularis*. Mar. Biol. 118: 157–165.

Cook, C. B. & C. O. Orlandini, 1992. Initial studies of host factors inducing photosynthate release in corals (*Montastrea annularis*) and sea anemones (*Aiptasia pallida*). Am. Zool. 32: 111A.

Dunlap, W. C. & J. M. Shick, 1998. Ultraviolet radiation-asborbing mycosporine-like amin o acids in coral reef organisms: a biochemical and environmental perspective. J. Phycol. 34: 418–430.

Falkowski, P. G., Z. Dubinsky, L. Muscatine & L. McCloskey, 1993. Population control in symbiotic corals. BioScience 43: 606–611.

Gates, R. D., K. Y. Bil & L. Muscatine, 1999. The influence of an anthozoan 'host factor' on the physiology of a symbiotic dinoflagellate. J. exp. mar. Biol. Ecol. 232: 241–259.

Gates, R. D., O. Hoegh-Guldberg, M. J. Mc-Fall-Ngai, K. Y. Bil & L. Muscatine, 1995. Free amino acids exhibit anthozoan 'host factor' activity: they induce the release of photosynthate from symbiotic dinoflagellates *in vitro.* Proc. natn. Acad. Sci. U.S.A. 92: 7430–7434.

Gleason, D. F. & G. M. Wellington,1993. Ultraviolet radiation and coral bleaching. Nature 365: 836–838.

Grant, A. J., M. Rémond & R. Hinde, 1998. Low molecular-weight factor from *Plesiastrea versipora* (Scleractinia) the modifies release and glycerol metabolism of isolated symbiotic algae. Mar. Biol. 130: 553–557.

Hinde, R., 1988. Factors produced by symbiotic marine invertebrates which affect translocation between the symbionts. In Scannerini, S., D. Smith, P. Bonfonte-Fasolo & V. Gianinazzi-Pearson (eds), Cell to Cell Signals in Plant, Animal and Microbial Symbioses, Vol. H17. NATO ASI Series. Springer-Verlag, New York: 311–324.

Hoegh-Guldberg, O. & G. J. Smith, 1989. Influence of the population density of zooxanthellae and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata*. Mar. Ecol. Prog. Ser. 57: 173–186.

Johannes, R. E. & W. J. Wiebe, 1970. A method for determination of coral tissue biomass and composition. Limnol. Oceanogr. 15: 822–824.

Lesser, M. P., 2000. Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. Mar Ecol. Prog. Ser. 192: 137–151.

Masuda, K., S. Miyachi & T. Maruyama,1994. Sensitivity of zooxanthellae and non-symbiotic micro-algae to stimulation of photosynthetic excretion by giant clam homogenate. Mar. Biol. 118: 687–693.

Muscatine, L., 1967. Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. Science 156: 516–519.

Muscatine, L., P. G. Falkowski, Z. Dubinsky, P. A. Cook & L. R. McCloskey, 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. r. Soc. Lond. B236: 311–324.

Muscatine, L., R. R. Pool & E. Cernichiari, 1972. Some factors influencing selective release of soluble organic material by zooxanthellae from reef corals. Mar. Biol. 13: 298–308.

Ritchie, R. J., A. J. Grant, K. Eltringham & R. Hinde, 1997. Clotrimazole, a model compound for the host release factor of the coral *Plesiastrea versipora*. Aust. J. Plant Physiol. 24: 283–290.

Rowan, R., 1998. Diversity and ecology of zooxanthellae on coral reefs. J. Phycol. 34: 407–417.

Shick, J. M., 1991. A Functional Biology of Sea Anemones, 1. Chapman & Hall, New York: 395 pp.

Sutton, D. C. & O. Hoegh-Guldberg, 1990. Host-zooxanthella interactions in four temperate marine invertebrate symbioses: assessment of effect of host extracts on symbionts. Biol. Bull. 178: 175–186.

Trench, R. K., 1971. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. III. The effect of homogenates of host tissues on the excretion of photosynthetic products *in vitro* by zooxanthellae from two marine coelenterates. Proc. r. Soc. Lond. B. 177: 251–264.

Trench, R. K., 1993. Microalgal-invertebrate symbioses: a review. Endocyt. Cell. Res. 9: 135–175.

Trench, R. K., D. S. Wethey & J. W. Porter, 1981. Observations on the symbiosis with zooxanthellae among the Tridacnidae (Mollusca, Bivalvia). Biol. Bull. 161: 180–198.

Wang, J.-T. & A. E. Douglas, 1997. Nutrients, signals and photosynthate release by symbiotic algae: the impact of taurine on the dinoflagellate alga *Symbiodinium* from the sea anemone *Aiptasia pulchella*. Plant Physiol. 114: 631–636.

Weil, E. & N. Knowlton, 1994. A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis & Solander, 1786) and its two sibling species, *M. faveolata* (Ellis & Solander, 1786) and *M. franksi* (Gregory, 1895). Bull. mar. Sci. 55: 151–175.

Withers, K. J. T., A. J. Grant & R. Hinde, 1998. Effects of amino acids on the isolated symbiotic algae of the coral *Plesiastrea versipora* (Lamarck): absence of a host release factor response. Comp. Biochem. Physiol. A 120: 599–607.

Wylie, E. B. & M. J. Johnson, 1962. Effect of penicillin on the cell wall of *Escherichia coli*. Biochim. Biophys. Acta 59: 450–457.