Nitric Oxide and Cnidarian-Dinoflagellate Symbioses: Pieces of a Puzzle

Henry G. Trapido-Rosenthal

Katherine H. Sharp

Roger Williams University, ksharp@rwu.edu

Follow this and additional works at: https://docs.rwu.edu/fcas_fp

Part of the Biology Commons, Marine Biology Commons, and the Microbiology Commons

Recommended Citation

Nitric Oxide and Cnidarian-Dinoflagellate Symbioses: Pieces of a Puzzle

HENRY G. TRAPIDO-ROSENTHAL, KATHERINE H. SHARP, TAMARA S. GALLOWAY, AND CLARE E. MORRALL

Bermuda Biological Station for Research, Inc., Ferry Reach, St. George’s GE-01, Bermuda

SYNOPSIS. The presence of nitric oxide synthase (NOS) activity is demonstrated in the tropical marine cnidarian Aiptasia pallida and in its symbiotic dinoflagellate algae, Symbiodinium bermudense. Enzyme activity was assayed by measuring the conversion of arginine to citrulline. Biochemical characterization of NOS from Aiptasia was characterized with respect to cellular localization, substrate and co-factor requirements, inhibitors, and kinetics. In response to acute temperature shock, anemones retracted their tentacles. Animals subjected to such stress had lower NOS activities than did controls. Treatment with NOS inhibitors caused tentacular retraction, while treatment with the NOS substrate L-arginine inhibited this response to stress, as did treatment with NO donors. These results provide a preliminary biochemical characterization of, and suggest a functional significance for, NOS activity in anthozoan-algal symbiotic assemblages.

INTRODUCTION

Nitric oxide (NO)

Over the past several years, NO has been recognized as a molecule of fundamental significance in mediating a variety of interactions between cells. It plays important roles as a weapon in the cellular defenses of metazoans (Nathan and Hibbs, 1991), as a modulatory molecule in the chemosensory modality of olfaction (Breer and Shepherd, 1993), as a mediator of a variety of secretory processes, including those for the export of molecules such as amino acids and peptides, and ions such as bicarbonate (Schmidt and Walter, 1994), and, most famously if not most importantly, as a regulator of vascular processes (Burnett et al., 1992). Although the role of NO in these phenomena has been best characterized in vertebrates, and usually in mammals, NO is implicated in the chemosensory responsiveness of aquatic invertebrates from taxa as diverse as cnidarians (Colasanti et al., 1995), molluscs (Elphick et al., 1995a) and arthropods (Elphick et al., 1995b). Furthermore, each of the listed functions can be seen as being of potential fundamental importance in the establishment, maintenance, and termination of endosymbiotic relationships such as those that exist between anthozoans and photosynthetic dinoflagellates.

NO differs from many molecules used in cellular signaling in several fundamental aspects. Because it passes through cellular membranes, it cannot be stored in vesicles and exocytosed upon demand. Rather than being made in advance, NO must be generated on an as-needed basis. Furthermore, due to the transparency of biological membranes to this molecule, its many effects are not mediated by non-covalent, spatially complementary interactions with cell surface receptors. Instead, NO exerts its effects by covalently interacting with molecular oxygen, the superoxide anion, transition metals, and thiol groups (Trapp, 1994). Proteins that complex with metal ions or that contain thiol groups are thus candidates for the sites at which NO can exert a physiologically meaningful function.
Nitric oxide synthase (NOS)

The fact that NO must be produced on an as-needed basis has led to extensive studies of NOS, the enzyme(s) responsible for the generation of this molecule. Although it is now known that there are multiple isoforms of NOS, they are all currently grouped under the single Enzyme Commission number 1.14.13.39. The first NOS to be isolated (Bredt and Snyder, 1990) and cloned, sequenced, and functionally expressed (Bredt et al., 1991) came from a mammalian neural source (rat cerebellum). To perform its function of converting L-arginine to citrulline and NO, this NOS required as cofactors calmodulin, NADPH, and flavin mononucleotides. Analysis of cDNA and deduced amino acid sequences demonstrated that this enzyme had a high degree of resemblance to cytochrome P-450 reductase, an enzyme involved in electron transfer reactions that also requires flavins and NADPH as cofactors.

Subsequently, NOSs from other tissue types and other organisms have been functionally and molecularly characterized. As enough data have accumulated from mammalian sources, comparisons have shown that the functional, antigenic, and sequential similarities between the various NOSs are more striking than the differences (Nathan and Xie, 1994). Enough differences exist, however, to group the NOSs into two categories. One category, which includes the neuronal NOS first characterized by Bredt and colleagues, is constitutively expressed, and is calmodulin dependent. The other category is inducible (or, more accurately, perhaps, subject to dramatic up-regulation) and is not as dependent on calmodulin (Marietta, 1994). Sequential homologies are stronger within constitutive and inducible categories than between them, but the isoforms are still clearly structurally similar. The striking functional and structural conservatism of the NOSs that have been characterized to date make it plausible to hypothesize that many of the NOSs from non-mammalian sources may be similar to them. Studies of NOSs from lower vertebrates (Liepe et al., 1994), molluscs (Elphick et al., 1995b) so far seem to corroborate this hypothesis.

Chemical mediation of cnidarian-algal symbioses

From the earliest recognition of the existence of cnidarian-algal symbioses, it was hypothesized that the partners in the relationship exchanged chemicals (reviewed by Shick, 1991). Throughout this century, there have been numerous demonstrations of such metabolic interchanges, which have been summarized in a number of recent reviews (Trench, 1971a, b, c; Muscatine and Porter, 1977; Cook, 1983; Shick, 1991). Much of this work has focussed on the transfer of photosynthetically-generated carbohydrate from algal symbiont to cnidian host. Movement of nitrogen from host to symbiont, usually in the form of ammonium, and the transfer of amino acids between the partners have also been studied. Of particular interest are studies by McCauley (1986, 1987, 1988, 1991) demonstrating that Chlorella, the genus of algae that live in symbiosis with Hydra viridissima, can internalize exogenous amino acids, and that amino acid transport rates are higher in algae that have been freshly isolated from a symbiotic host than they are in algae that have been maintained in culture. In a related line of investigation, Ferrier (1992) showed that the free amino acid pool of symbiotic dinoflagellate algae (Symbiodinium) freshly isolated from the sea anemone host Aiptasia pallida was dominated by the basic amino acid arginine, while this amino acid was present in only very low concentrations in conspecific algae that had been maintained in culture.

Compared to studies of nutritional fluxes, the study of chemical interactions between host and symbiont that are fundamentally informational in nature has a shorter and more recent history. Fitt (1984, 1985) has demonstrated that amino acids, presumably host-derived, can serve as chemoattractants for free-living cells of Symbiodinium microadiaticum (as all strains of this dinoflagellate were then called); his results can be interpreted as a demonstration of host-to-symbiont chemical communication. A number of investigators have sought to identify
and characterize a host-derived chemical or mixture of chemicals, known as host factor (HF), that can induce symbionts to export photosynthetically-generated carbohydrate. Their studies have produced a body of contradictory results, with HF being described as being both heat stable and heat labile (Muscatine, 1967; Muscatine et al., 1972; Sutton and Hoegh-Guldberg, 1990), as being both a large and a small molecule (Cook and Orlandini, 1992), as being both present and absent in dinoflagellate-free anemones (Cook and Orlandini, 1992), and as both enhancing and not enhancing carbon fixation (Trench, 1971c; Masuda et al., 1994). Gates et al. (1995) demonstrated that a crude aqueous extract of tissue from the coral Pocillopora damicornis both enhances the fixation of carbon by, and induces the export of fixed carbon from, Symbiodinium isolated either from P. damicornis or from the sea anemone Aiptasia pulchella. They characterized the amino acid composition of this crude extract, and demonstrated that a synthetic free amino acid mixture based upon this composition was capable of mimicking the carbon fixation and exporting effects of the crude extract. In similar experiments, Bester et al. (1997) demonstrated that an amino acid mixture qualitatively and quantitatively representative of the free amino acid composition of A. pallida is capable of converting the low-capacity, high-affinity amino acid transport of free-living, cultured Symbiodinium bermudense cells to the high-capacity, low-affinity transport characteristic of cells of S. bermudense that have been freshly isolated from a symbiotic relationship. Experiments such as these have clearly demonstrated that identified chemical signals from the host can induce changes in the cellular physiology of photosynthetic symbionts. The ways in which these signals are transduced by the algae remain to be elucidated. Also unknown is whether chemicals that function as signals, rather than as nutrients, pass from the symbionts to the host organism.

Research goal

The goal of our research is to advance our understanding of the chemical communication between the members of cnidian-dinoflagellate symbioses. In the following sections, we describe work done to date to test the hypothesis that NO may be an important signal molecule in these assemblages.

MATERIALS AND METHODS

Chemicals

Radiolabeled L-arginine (3H, 40Ci/mm) was obtained from New England Nuclear, and the liquid scintillation cocktail UltimaGold was from Packard Instrument Corporation. All other reagents were from Sigma Chemical Company.

Organisms

Samples of A. pallida were collected from Walsingham Pond, Bermuda. Anemones were maintained at 25°C in 100 ml glass petri dishes in low nutrient seawater (collected from a depth of 10 m in the Sargasso Sea, 175 km southeast of Bermuda) with a 12 hr light/dark photoperiod; they were acclimatized for at least 48 hr before use. Individuals of 5 mm oral disc diameter or above were used for experiments. Symbiotic specimens of Symbiodinium bermudense were isolated from A. pallida by mechanical homogenization of the symbiotic assemblage, with subsequent removal of the algae from the homogenate by repeated centrifugation and filtration. Algae were ultimately suspended in sterile F/2 medium (Guillard, 1975). Cultured specimens of S. bermudense were obtained from the Provasoli-Guillard National Collection of Marine Phytoplankton, and maintained at a concentration of 250,000 cell/ml in sterile F/2 medium at 26°C with a 12 hr photoperiod. These cultures were originated from algae isolated from specimens of A. pallida that had been collected from Walsingham Pond, Bermuda.

Determination of NOS activity

Individual anemones were rinsed with 2 ml and homogenized in an equal volume of homogenization buffer (HB: 50 mM HE-PES, 1 mM EDTA, pH 7.4) using a 15-ml glass Potter homogenizer. The homogenate was centrifuged for 10 min at 16,000 RCF and the resulting supernatant collected. NOS activity was determined by monitor-
ing conversion of $^3$H-arginine to $^3$H-citrul-line using a modification of the method of Bredt and Snyder (1989), as described by Morrall et al. (2000). Briefly, 100 µl of homogenate supernatant was brought to 200 µl with HB. The incubation was initiated by adding 100 µl of HB containing cofactors and radiolabel. Final concentrations of cofactors in the incubation were: 1 mM NADPH, 1.25 mM CaCl$_2$, 1 mM dithiothreitol, 10 µM tetrahydrobiopterin, 10 units/ml of calmodulin, and 20 µM $^{3}$H-L-arginine. Following incubation at 25°C for 30 min, reactions were terminated by addition of 900 µl ice-cold stop solution (100 mM HEPES, 10 mM EDTA, pH 5.5). Entire incubation volumes were applied to a 1-ml column of Dowex AG50WX–8 (Na$^+$ form) and $^3$H-citrulline was eluted with 3 ml ice-cold H$_2$O with pulse centrifugation at 1,310 × g. A 1-ml aliquot of eluant was mixed with 5 ml Ultima Gold, and $^3$H-citrulline quantified by liquid scintillation spectrophotometry. Enzyme activity is expressed as pmol of $^{3}$H-citrulline formed per µg protein per minute incubation, with protein being quantified by means of the dye-binding assay of Bradford (1976), using bovine serum albumin as standard. The results obtained are expressed as means ± SD of determinations on multiple anemones; typically, each data point represents NOS activity from three specimens. Kinetics of the conversion of arginine to citrulline were investigated with substrate concentrations in the range 0.01–1,000 µM. $V_{max}$ and $K_m$ values were derived by Lineweaver-Burke analysis. Potential inhibitors of NOS activity were included in incubation media at concentrations indicated in legends to figures.

To determine NOS activity in Symbiodinium, algae, either isolated from anemones or taken from culture, were first rinsed with ice-cold HB. They were then sonicated in 1 ml of HB, in an ice-jacketed 1.5-ml microcentrifuge tube with a Heat Systems sonicator. The sonicate was centrifuged for 10 min at 16,000 RCF and the resulting supernatant collected. NOS activity was then determined as described above.

**Effects of temperature stress on sea anemone behavior and NOS activity**

The short-term effects of the acute thermal stressing of animals on NOS activity was investigated by incubating anemones at either 17°C for 120 min, or 33°C for either 30 or 120 min, prior to homogenization and assay; results were compared with those obtained from control animals maintained at 25°C. Long-term effects were investigated by incubating anemones in water that was slowly warmed over a period of several days, prior to homogenization and assay. In both experiments, anemone behavior was observed during these incubations.

**Effects of NOS substrate, NOS inhibitors, and NO donors on sea anemone behavior**

Effects of NOS inhibitors and NO donors on anemone behavior were examined by observing anemones that were incubated in seawater containing 1 mM concentrations of either the NOS substrate L-arginine, the NOS inhibitor N$^G$-monomethyl-L-arginine (NMMA), or the NO donor S-nitrosoglutathione (SNOG), at both 25°C and 28°C. Control incubations contained no additions.

**RESULTS**

**Preliminary observations: NOS activity in anemones**

In preliminary time-course experiments, the generation of $^3$H-citrulline was linear with time for up to 40 min using standard reaction mixtures containing 20 µM arginine (data not shown). In subsequent experiments, incubations were standardized at 30 min. The relationship between protein concentration and citrulline generation was linear up to 25 µg protein per assay (Fig. 1; Morrall et al., 2000). Activity was completely abolished by prior heating of the homogenate to 60°C for 2 min (data not shown). The NOS activity of whole and cytosolic homogenate fractions were essentially equal, indicating a predominantly cytosolic location for the enzyme activity (Fig. 2). The kinetics of this enzymatic activity are characterized by a $K_m$ of 19.04 µM and a $V_{max}$ of 2.96 pmol/min/µg protein (Fig. 3; Morrall et al., 2000).

The arginine analogues N$^G$-monomethyl-
L-arginine acetate (L-NMMA), D-NMMA, N\(^6\)-nitro-L-arginine methyl ester (L-NAME), and N\(^6\)-nitro-L-arginine (L-NNA), and the arginase inhibitors L-valine and L-ornithine were pre-incubated with anemone cytosol for 10 min at up to 1 mM concentrations, after which NOS activity was determined. The effects of treatment with these potential inhibitors are shown in Figure 4 (Morrall et al., 2000). Formation of \(^3\)H-citrulline decreased with increasing concentrations of L-NMMA (Fig. 5; Morrall et al., 2000). Concentrations of L-NMMA of 500 and 1,000 \(\mu\)M reduced citrulline activity by 70% and 75% respectively, while at inhibitor concentrations below 500 \(\mu\)M, little inhibition was evident.

NOS activity levels were optimized by the addition of exogenous NADPH, were unaffected by addition of exogenous tetra-
hydrobiopterin, dithiothreitol, or calmodulin, and were reduced by the addition of exogenous CaCl₂ (Morrall et al., 2000).

**NOS activity in zooxanthellae**

Algal cells that had been freshly isolated from *A. pallida* possessed NOS activity, whereas cells that had been maintained in non-symbiotic conditions (in culture) had little or no ability to convert arginine to citrulline (Fig. 6). However, if cultured zooxanthellae were preincubated for 48 hr in an amino acid mixture that mimicked the free amino acid concentration of *A. pallida* (Bester et al., 1997), when assayed they had NOS activity comparable to cells that had just been removed from a symbiotic relationship (Fig. 6).

**Effect of temperature stress on NOS activity**

Transferring anemones directly from 25°C water to 33°C resulted in a pronounced retraction of the animals’ tentacles. Upon homogenization and assay, either 30 min or 120 min after temperature change, NOS activities were significantly lower in temperature-stressed animals than they were in unstressed controls (Fig. 7). The same phenomena of tentacular retraction and lowered NOS activities were seen when animals were subjected to cooler, 17°C water (Morrall et al., 1998).

**References**

Morrall et al., 2000, with permission from Elsevier Science.

Guillard, 1975.

Bester et al., 1997.

Morrall et al., 1998.
Effects of NOS substrate, NOS inhibitors, and NO donors on anemone behavior

The effects of incubation in the NOS substrate L-arginine, in the NOS inhibitor L-NMMA, and the NO donor SNOG are reported in Table 1. At the ambient temperature of 25°C, organisms incubated in L-NMMA were more retracted than either control anemones or anemones incubated in L-arginine or SNOG. Under conditions of elevated temperature, control anemones retracted their tentacles, whereas animals incubated with either L-arginine or SNOG appeared as fully extended as did animals at ambient temperature.

Discussion

The results presented here and in the work of Morrall et al. (2000) indicate that NOS activity is present in the tissue of the tropical anemone A. pallida. The activity of the enzyme when measured by the formation of 3H-citrulline from 3H-arginine indicated a basal level in whole body homogenate of 0.50–1.16 pmol/min/μg protein (Morrall et al., 2000), which is similar to levels previously reported in rat heart homogenate using similar methods of detection (Giraldez and Zweier, 1998). The possibility that formation of 3H-citrulline was occurring through the activity of arginase and ornithine transcarbamylase, which can indirectly synthesize L-citrulline from L-arginine (Blachier et al., 1991) was ruled out by the lack of inhibition seen with the arginase inhibitors L-valine and L-ornithine. This is an important consideration when working with a whole body homogenate in which multiple and related enzymatic pathways may be present. The $K_m$ of 19.05 μM calculated for the enzyme was higher than previously reported values of 2.9 μM for mammalian constitutive (Pollock et al., 1991) and 2.5 μM for invertebrate inducible (Colasanti et al., 1995) forms of the enzyme. In addition, the $V_{max}$ value obtained was lower than other reports at 2.96 pmol/min/μg protein. The differences in purification state of the enzyme preparations used in our studies and in those of other workers may account for some of these quantitative differences, and may in fact make comparisons of kinetic parameters from different studies of only marginal value.

The fact that enzyme activity was not affected by pre-treatment with bacterial lipopolysaccharide (Morrall et al., 2000), taken together with the cytosolic location, suggest that the enzyme activity bears some similarity to mammalian neuronal NOS (nNOS). The rank order of inhibition seen for competitive arginine analogues is in agreement with this, although the relative potency of L-NMMA and L-NNA as inhibitors, especially of constitutive nNOS, are reportedly highly dependent on experimental conditions (Klatt et al., 1994). Formation of 3H-citrulline was inhibited by L-NMMA in a dose-dependent manner, with residual activity remaining even in the presence of high concentrations of this inhibitor. This lack of complete inhibition even when L-NMMA is present at concentrations high-

---

**Table 1. The effect of L-NMMA, L-arginine and SNOG on Aiptasia behavior.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>25°C</th>
<th>28°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Fully expanded tentacles.</td>
<td>Tentacles and column contracted.</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>Tentacles retracted and more flaccid than control.</td>
<td>Some tentacles fully retracted into column. Oral disc very small.</td>
</tr>
<tr>
<td>L-arginine</td>
<td>Tentacles fully expanded. Wide oral disc—not visibly different from control.</td>
<td>Tentacles and column fully expanded. Oral disc widely expanded.</td>
</tr>
<tr>
<td>SNOG</td>
<td>Tentacles fully expanded. Wide oral disc—not visibly different from control.</td>
<td>Tentacles and column fully expanded. Oral disc widely expanded.</td>
</tr>
</tbody>
</table>

* Incubations were performed and observations were made as described in Materials and Methods. The indicated compounds were present at 1 mM concentrations.
er than the competing substrate arginine, has been noted by others (Griffiths and Gross, 1996).

Comparison of enzyme activity with and without cofactor addition (Morrall et al., 2000) indicates that 75% activity can be achieved without exogenous cofactor addition (assuming maximum activity is achieved when full cofactor mix is added). These findings suggest that endogenous cofactors are present in the cytosolic fraction in concentrations sufficient to support close to maximal activity. These findings are similar to those of Elphick et al. (1993), who found that NOS activity in locust brain extracts was reduced to 80% of the maximum when NADPH was omitted. The reduction in enzyme activity seen when CaCl₂ was added is a striking finding, and has been noted by other researchers; Radomski et al. (1991) showed an approximately 30% reduction in activity on addition of 200 μM Ca²⁺. This was interpreted as evidence of the presence of a calcium independent NOS enzyme. Alternatively, such observations are also consistent with an enzyme possessing a biphasic response to this divalent cation. Experiments designed to determine which of these interpretations is correct need to be conducted. In addition, the apparent lack of calmodulin dependency shown in this work warrants further investigation using specific calmodulin inhibitors.

The functional similarities between the invertebrate enzyme reported here and mammalian NOS enzymes is worth noting, separated as they are by 500 million years of evolution. However, a direct comparison to mammalian constitutive and inducible isoforms may be inappropriate. Recent phylogenetic evidence indicates that the gene duplication preceding the evolution of an immunological role for iNOS in mammalian species may have occurred after the divergence of vertebrates and invertebrates (Hughes, 1998). Diversity of function and regulation would then have arisen convergently in these groups.

Although the results presented here give no direct information on the functional role of NOS in A. pallida, it is possible to speculate on the likely role of the enzyme based on studies of other invertebrate species. The demonstration by Morrall et al. (2000) that NOS activity, as localized by NADPH diaphorase (NADPHd) histochemistry, is distributed throughout the outer epithelial layer as well as in cells of the gastrodermal layer is compatible with a broad involvement of NOS in both sensory and motor activities. Elofsson et al. (1993) found only non-selective staining in Aiptasia sp. and in another cnidarian, Hydra oligactis, and suggested that the distribution of NADPHd positive cells might be restricted to invertebrates having more developed nervous systems. However, in many invertebrate species in which NOS has been studied, the enzyme appears in the central and peripheral nervous system. In molluscs, NO is apparently involved in muscle cell physiology (Martinez, 1995). In addition, individual nigro-tropic neurons in both the buccal ganglion and cerebral ganglion of Lymnaea stagnalis are directly involved in that mollusc’s feeding behavior (Korneev et al., 1998, 1999; Park et al., 1998). The polyplacophorans show NADPHd staining only in buccal muscle cells. In other phyla, activity is apparent in the neurons innervating these muscles, which may indicate a switch in the site of NO-mediated control of muscle motility. NOS activity has been shown to be a controlling factor in the olfactory feeding response of the cnidarian Hydra vulgaris (Colasanti et al., 1997). The response consists of tentacular writhing and mouth opening and is similar to the feeding response seen in A. pallida.

Many of the effects of NO in invertebrates, including phenomena as diverse as control of smooth muscle activity (Elphick and Melarange, 1998), olfaction (Müller and Hildebrandt, 1995), feeding (Colasanti et al., 1995), and learning (Robertson et al., 1996), are thought to be brought about by its activation of soluble guanylyl cyclase (sGC), which, by generating the intracellular second messenger cyclic GMP (cGMP), can control a variety of cellular functions. The NO-sGC-cGMP pathway has been demonstrated in a number of animal taxa, including invertebrates such as cnidarians (Colasanti et al., 1995), annelids (Leake and Moroz, 1996), molluscs (Jacklet and
Koh, 1999), nematodes (Bascal et al., 1995), arthropods (Müller, 1997), and echinoderms (Elphick and Melarange, 1998), as well as vertebrates (Ishii et al., 1989). It remains to be seen if the NO-sGC-cGMP signaling pathway is involved in physiological responses of A. pallida to NO.

To our knowledge, the demonstration of NOS activity in the alga Symbiodinium bermudense is the first time that such activity has been described in symbiotic dinoflagellates. It is important to note that the activity induced in cultured zooxanthellae by incubation with a host-mimicking mixture of amino acids cannot be interpreted as activity emanating from residual anemone tissue that could possibly be present in incubations of zooxanthellae freshly isolated from a symbiotic relationship. It is clear that much more work is needed to fully characterize algal NOS activity before its functional significance can be understood with any confidence. If, however, as our bioassays indicate, NO is involved in the extension of this cnidarian’s tentacles, it is tempting to speculate that, since the symbiotic algae are present in the highest densities in the anemone’s tentacles, the NO that they generate may contribute to this behavior. Pearse (1974a, b) showed that zooxanthellate specimens of the anemone Anthopleura elegantissima expanded their tentacles in conditions of moderate light, and retracted them in bright light or in darkness, whereas azooxanthellate anemones were indifferent to light. Further experiments could test the hypothesis that symbiont-generated NO may be exerting a significant degree of control over the host’s behavioral responses to light, an environmental factor of extreme importance to this photosynthesizing symbiotic assemblage.

In conclusion, we offer this characterization of NOS activity in the tropical marine anemone A. pallida as an addition to our knowledge of NOSs in invertebrates. Furthermore, the demonstration of NOS activity in the animal’s symbionts, coupled with bioassays suggesting that NO may be involved in mediating behavioral aspects of the cnidarian-algal symbiotic assemblage, point towards a new role for the multifaceted signaling molecule NO. As we collect and put into place more pieces of this puzzle, we believe that an interesting and attractive picture of the ways in which NO is involved in cnidarian-algal symbioses will be assembled.

ACKNOWLEDGMENTS

The symposium Nitric Oxide in the Invertebrates: Comparative Physiology and Diverse Functions was supported by National Science Foundation grant IBN-9977863 and by the host organization, the Society for Integrative and Comparative Biology. This support is gratefully acknowledged. Research support from National Science Foundation grants OCE-9619718 and DEB-9615211 and from Diversa Corporation is likewise gratefully acknowledged. The authors would also like to express their thanks to anonymous reviewers whose suggestions resulted in an improved manuscript. This is Contribution Number 1591 from the Bermuda Biological Station for Research, Inc.

REFERENCES


