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A sponge/dinoflagellate association in the haplosclerid sponge *Haliclona* sp.: cellular origin of cytotoxic alkaloids by Percoll density gradient fractionation

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Abstract Light-microscopic and electron-microscopic studies of the tropical marine sponge *Haliclona* sp. (Order: Haplosclerida; Family: Haliclonidae) from Heron Island, Great Barrier Reef, have revealed that this sponge is characterized by the presence of dinoflagellates and by nematocysts. The dinoflagellates are 7–10 μm in size, intracellular, and contain a pyrenoid with a single stalk, whereas the single chloroplast is branched, curved, and lacks grana. Mitochondria are present, and the nucleus is oval and has distinct chromosomal structure. The dinoflagellates are morphologically similar to *Symbiodinium microadriaticum,* the common intracellular symbiont of corals, although more detailed biochemical and molecular studies are required to provide a precise taxonomic assignment. The major sponge cell types found in *Haliclona* sp. are spongocytes, choanocytes, and archaeocytes; groups of dinoflagellates are enclosed within large vacuoles in the archaeocytes. The occurrence of dinoflagellates in marine sponges has previously been thought to be restricted to a small group of sponges including the excavating hadromerid sponges; the dinoflagellates in these sponges are usually referred to as symbionts. The role of the dinoflagellates present in *Haliclona* sp. as a genuine symbiotic partner requires experimental investigation. The sponge grows on coral substrates, from which it may acquire the nematocysts, and shows features, such as mucus production, which are typical of some excavating sponges. The cytotoxic alkaloids, haliclonacyclamines A and B, associated with *Haliclona* sp. are shown by Percoll density gradient fractionation to be localized within the sponge cells rather than the dinoflagellates. The ability to synthesize bioactive compounds such as the haliclonacyclamines may help *Haliclona* sp. to preserve its remarkable ecological niche.

Key words Sponges · Alkaloids · Nematocysts · Percoll density gradient fractionation · Secondary metabolites · Dinoflagellates, *Symbiodinium microadriaticum* · Sponge, *Haliclona* sp. (Porifera)

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Introduction

Sponges (Porifera) are a major component of benthic fauna that play an important role in removing detritus and organic nutrients from ocean waters. In the nutrient-depleted waters of tropical reefs, sponges have to filter large volumes of water to acquire nutrients. Yet, despite the considerable energy demand of the filtration process, sponges represent the second largest biomass on tropical reefs after corals (Wilkinson 1983b; Corredor et al. 1988). Clearly, additional mechanisms for obtaining nutrients are an important contributing factor to the ecological competitiveness of tropical sponges. Some coral reef sponges use phototrophic metabolism, as do the majority of corals, to acquire nutrients. In corals, photosynthesis is performed uniquely by dinoflagellate (zooxanthellae) symbionts, which supply the host with nutrients by translocation (Muscatine and Cernichiari 1969). Marine sponges, in contrast, contain a range of microbial symbionts, some of which have a photosynthetic function in the host (Wilkinson 1987b; Rützler 1990). Unicellular or filamentous cyanobacteria (Wilkinson 1980a, 1983a; Vacelet 1982; Simpson 1984; Rützler 1990) are the most common photosynthetic symbionts, and probably provide products of carbon and nitrogen fixation to the sponge under normal environmental conditions (Wilkinson 1983a, 1987a,b; Arillo et al. 1993). The presence of cyanobacte-

rial symbionts is therefore highly beneficial to the sponge host. Bacterial symbionts, although ubiquitous in sponges and clearly implicated in their overall metabolic activity, are not generally autotrophic (Vacelet and Donadey 1977; Wilkinson 1978, 1987b), except in sponges that contain phototrophic sulfur bacteria and also sulfate-reducing bacteria (Imhoff and Trüper 1976). Eukaryotic algal symbionts are found in sponges (Vacelet 1982; Wilkinson 1987b; Rützler 1990) and include dinoflagellates (Sarà and Liaci 1964; Pang 1973; Vacelet 1982; Rützler 1990; Rosell and Uriz 1992; Hill 1996), zoochlorellae (Williamson 1979; Simpson 1984; Saller 1989), cryptophytes (Duclaux 1973), diatoms (Cox and Larkum 1983), and macroscopic algae (Price et al. 1984; Rützler 1990). Dinoflagellate symbionts, which are found in the majority of coral species, have a restricted distribution in marine sponges; they are associated with sponges from the order Hadromerida, in particular with excavating sponges from the genus *Cliona* (Sarà and Liaci 1964; Pang 1973; Pomponi 1976; Vacelet 1982; Rützler 1990; Hill 1996). The role of algae in the provision of nutrients to host sponges has only been investigated in freshwater sponges (Muscatine et al. 1967; Wilkinson 1980b).

The production of bioactive chemicals by marine sponges is another factor that enhances their competitiveness on coral reefs (Porter and Targett 1988). The processes of secondary metabolism that lead to these natural products are not yet well understood in marine systems (Garson 1989, 1993), and there has been much speculation about whether symbionts are involved in the production of chemicals, thereby enhancing the ecological competitiveness of the sponge-symbiont assemblage (Faulkner et al. 1993, 1994; Garson 1989, 1993, 1994). The Great Barrier Reef is dominated by cyanobacteria-containing sponges (Wilkinson 1983a), a number of which produce bioactive secondary metabolites (Faulkner 1996). The chemical and biological aspects of sponge-cyanobacteria associations have been well characterized in a number of sponges (Wilkinson 1978; Larkum et al. 1987; Faulkner et al. 1994; Hinde et al. 1994; Flowers et al. 1998). Cyanobacteria have been implicated in the synthesis of the biologically active secondary metabolites present in sponge tissue (Faulkner et al. 1994; Garson 1994). There are very few documented examples of Barrier Reef sponges containing other phototrophic symbionts (Price et al. 1984); the chemistry or biology of such associations has rarely been studied in detail. McCaffrey (1988) has described a haplosclerid sponge *Haliclona* sp. that is found at Heron Island and that is characterized by the presence of dinoflagellates within its tissue. She has found that the sponge contains antimicrobial components known to be toxic to hydroids, coral, crustaceans, and fish but has not identified the chemicals involved. Subsequently, our own research has shown that organic extracts of the sponge contain complex alkaloids, the haliclonacyclamines, which possess potent cytotoxic and antifungal activity (Charan et al. 1996; R.J. Clark et al., unpublished material). The ecological roles of the haliclonacyclamines are therefore of interest to us, but, before detailed ecological studies can be undertaken, it is important to establish whether the sponge or its dinoflagellate partner contains the haliclonacyclamines and is responsible for the observed biological activity. We have therefore applied our procedure of Percoll density gradient separation of glutaraldehyde-fixed cells (Flowers et al. 1998) to determine the cellular origin of the haliclonacyclamines.

Materials and methods

Chemicals and biochemicals

All chemicals used in the production of artificial seawater were purchased from Sigma (Mo., USA). One litre of Ca-Mg-free artifical seawater (CMF-ASW) was made up from NaCl (27.0 g), Na_2SO_4 (1.0 g), KCl (0.8 g), and $NaHCO_3$ (0.18 g) diluted to 1 litre with MilliQ-H_2O; the resulting solution was filtered through 0.45-µm pore filters, autoclaved for 20 min, and then adjusted to pH 7.4 before use. Solvents used in the extraction of compounds from cell separation experiments were glass-distilled. Percoll was purchased from Pharmacia (Uppsala, Sweden).

Instruments

^{1}H NMR experiments were recorded for solutions in deuteriochloroform at room temperature on a Bruker DRX-500 NMR spectrometer calibrated against deuteriochloroform.

Biological materials

Samples of *Haliclona* sp. were collected by hand by SCUBA at Coral Gardens (–10 to –15 m), Heron Island (23°27' S, 151°55' E) in May 1994 and March 1995 on the Great Barrier Reef, Australia under permit numbers G94/318 and G95/016 issued jointly by the Great Barrier Reef Marine Park Authority and the Queensland National Parks and Wildlife Service. Sponge samples used in biological experiments were maintained in running seawater at ambient temperature and light conditions prior to use. A voucher specimen of the sponge, G304086, is held at the Queensland Museum.

Cell separation experiments

The wet sponge (73 g) was cleaned of any debris, rinsed twice with chilled CMF-ASW, pH 7.4, to remove natural seawater, then roughly chopped by using a scalpel blade, and transferred directly to 3% glutaraldehyde in CMF-ASW. The tissue was gently squeezed with tweezers to aid cell dissociation and then left for 2 h at 4°C for fixation to proceed; the cells were then filtered through a clean nylon mesh (50 µm) to give a dissociated cell preparation. The filtered cells were spun at 600 *g* for 5 min at 4°C, and the supernatant decanted to leave behind a dissociated cell pellet, which was stored in glutaraldehyde fixative. Two days later, the dissociated cell pellet was resuspended in fresh CMF-ASW and washed three times with CMF-ASW to remove excess fixative. A small sample was taken for microscopy, and half of the remaining cells were fractionated according to density by centrifugation (600 *g*) across discontinuous Percoll gradients (20%, 40%, 60%, and 80% in CMF-ASW) with methods modified after Garson et al. (1992) and Flowers et al. (1998). The bands of cells that accumulated at the density interfaces were isolated individually by pipette, rinsed twice with CMF-ASW, and pelleted by centrifugation; small samples of all the pellets were fixed with modified Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.066 M cacodylate buffer, pH 7.2.) for microscopy. The remaining cells were transferred to sample vials and frozen for later chemical analysis as described below. Cell fractions A_1–A_5, were identified as in Table 1. Fraction A_4 was resuspended

Table 1 Fractionation of *Haliclona* cell types using Percoll gradients

Cell separation fraction	Position	LM and EM analysis	Alkaloids detected by NMR and TLC
A1	20% layer and above	Small sponge cells and some dinoflagellates	++++
A2	Interface 20–40%	Sponge cells, dinoflagellates and occ. nematocyst	+++
A3	Interface 40–60%	Dinoflagellates and archaeocytes	++
A4	Interface 60–80%	Dinoflagellates, nematocysts and some archaeocytes	+
A5	Pellet	Nematocysts, dinoflagellates and spicules	–
A6	Interface 20–40%	Dinoflagellates and occ. archaeocytes	+
A7	Interface 40–60%	Dinoflagellates	–
A8	Interface 60–80%	Nematocysts	–
A9	Pellet	Nematocysts	–

in CMF-ASW and placed on a second Percoll gradient (20%, 40%, 60%, 80% in CMF-ASW) and spun at 600 *g* for 10 min at 4°C. Bands of cells separated by the Percoll gradients were worked up as before to give fractions A_6–A_9.

Chemical analysis

The fractions were extracted with dichloromethane/methanol (1/1, 5 ml, 1 h) followed by two further extractions with the same solvent (5 ml) for 30 min. The combined extracts were dried over anhydrous magnesium sulfate, the solvent removed under a stream of nitrogen, and the residual sample dried in vacuo to yield a crude extract that was subsequently analyzed by silica thin-layer chromatography (TLC) with dichloromethane/methanol (1/1) and by proton NMR spectroscopy.

Electron-microscopic studies

Tissue samples were fixed in a modified Karnovsky's fixative, whereas dissociated cell preparations were fixed in 3% glutaraldehyde in CMF-ASW. Specimens were stored in these fixatives at 4°C until further processing could be carried out. After being washed in 0.2 M cacodylate buffer containing 0.4 M sucrose, samples were postfixed in 1% osmium tetroxide in cacodylate buffer. The samples were subsequently washed in buffer, dehydrated through a graded methanol series, and embedded in Spurr's resin. Ultrathin sections cut on a Reichert-Jung Ultracut E microtome were mounted on copper grids and stained with 5% uranyl acetate in 50% methanol and Reynold's lead citrate. Sections were viewed by using Hitachi H-800 and Jeol 1010 transmission electron microscopes. Light-microscopic observations of dissociated cells and cell fractions were made on an Olympus BH-2 microscope by Nomarski interference optics.

Results

Sponge description

Haliclona sp. is one of the dominant sponges of the channel zone at Heron Island where it commonly occurs at depths of 10–15 m at the base of the reef slope. The sponge grows on coral substrate and, when damaged or collected, exudes mucus. Its preferred substrate is *Acropora nobilis*; however, specimens have also been observed to grow on *Stylopora pistillata*, *Pocillopora* sp., *Seriatopora hytrix*, and sand-covered coral rock (McCaffrey 1988). Although the sponge does not burrow into the coral substrate, it kills the coral substrate on which it grows. The sponge growth form resembles erect fingers emerging from an encrusting base restricted to a single coral branch. Coral tissue within approximately 1 cm distance of the point of attachment appears bleached with signs of necrosis. Some fingers of the sponge coalese to form a fan. The ectosome is olive-brown, and the endosome is orange in live tissue. Exhalent oscules of mean diameter 2.6±1.2 mm are flush with the sponge surface on one side of the fingers only. The major spicule type is an oxea, slightly recurved in the mid-line, whereas the spongin fibers present are neither laminated nor cored. The sponge tissue is soft and easily torn, although feeding scars to indicate predation by fish or other scavengers are not apparent. The sponge is also free of epibiotic fouling, suggesting the presence of inhibitory chemicals.

Microscopy

The individual cell types and other cell constituents found in *Haliclona* sp. were characterized by light and electron microscopy. In intact tissue samples, the major cell types were sponge cells and dinoflagellates, and there were frequent spicules and nematocysts throughout the preparation. The dinoflagellates (Fig. 1A) were approximately 7–10 μm in diameter and showed morphological features including a curved and branched chloroplast (located at the periphery of the cell and lacking grana), a pyrenoid with a single stalk, and an oval nucleus displaying chromosomes and occupying about 10% of the cellular volume; the cell wall was sloughed, and there was no evidence of flagellar structures. This description matches that of the dinoflagellate strain reported in other marine sponges (Vacelet 1982; Rützler 1990) and closely resembles the description of *Symbiodinium microadriaticum* (Trench and Blank 1987). Algal cells were grouped together in clumps of 6–10 cells and were enclosed by sponge cells, rather than being randomly distributed throughout the mesohyl (Fig. 1B). The presence of a nucleolus in the host cell nucleus suggested that it was an archaeocyte (Fig. 1C). There was no evidence of algal cell digestion by the sponge cells; a region of ill-defined fibrillar or granular material was typically observed be-

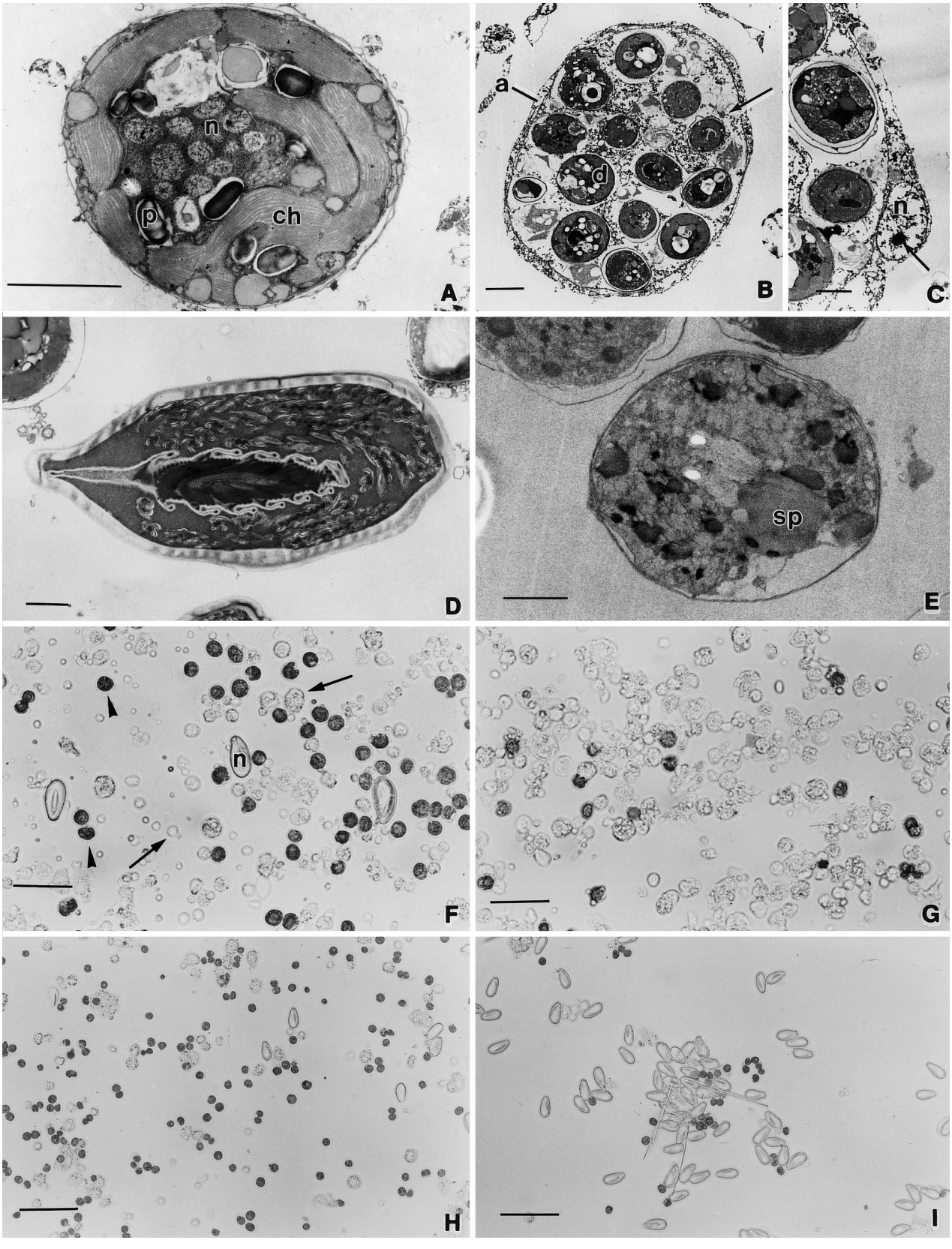
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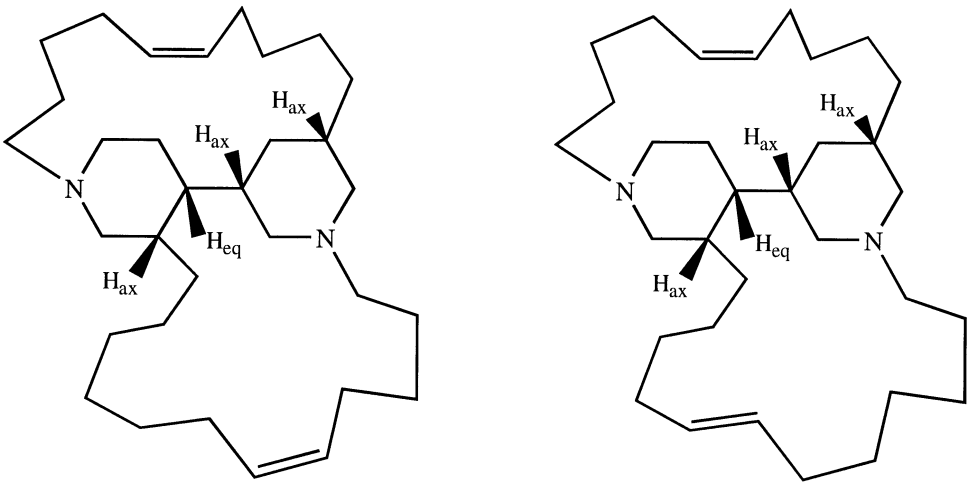
Fig. 2 Structures of alkaloids isolated from *Haliclona* sp. collected at Heron Island



tween the algal cells and the surrounding sponge membrane (Fig. 1B). The algae were considered to be intracellular. The nematocyst constituents of *Haliclona* sp. (Fig. 1D) were approximately 15–20 μm in length and were composed of a spine-bearing butt and a long tightly coiled thread. They were randomly distributed between the ectosome and endosome of the sponge and were usually undischarged in intact tissue. Sponge samples collected from acroporid substrates always contained nematocysts. Infrequently, samples contained low populations of nematocysts, usually when collected from sand-covered coral rock.

The sponge cell types in *Haliclona* sp.that were routinely encountered in electron-microscopic preparations of intact tissue included spongocytes, choanocytes, and archaeocytes. The cells that were identified as spongocytes (approximate diameter 10 μm) were usually grouped in clumps, had a uniformly stained nucleus, contained dark inclusions and rough endoplasmic reticulum in their cytoplasm, and frequently partially enveloped a spicule as in Fig. 1E (Garrone and Pottu 1973; Bergquist 1978; Simpson 1984). There were two other sponge cell types represented; although the cell contents, including nuclei, were intact, short sections of the surrounding cell membrane were always missing. One of these two sponge cell types was small in size (approximate diameter 7–9 μm) and took on a rounded shape after dissociation and fixation, thus making identification difficult; the small size together with the large spherical nucleus and absence of a nucleolus suggested that these were choanocytes. Microvilli were sometimes present. The other sponge cell type, which was larger in size and of variable shape, was identified as an archaeocyte because of the presence of a vesicular and nucleolated nucleus (Bergquist 1978). Other features, such as rough endoplasmic reticulum and phagosomes, were also consistent with this identification. These cells frequently engulfed clumps of dinoflagellates. Although *Haliclona* spp. are frequently reported to contain spherulous cells (Simpson 1984), we have found no specialized secretory cell types in intact tissue preparations of this sponge.

Fig. 1 **A** A TEM micrograph showing the common dinoflagellate symbiont from *Haliclona* sp. (*ch* chloroplast, *n* nucleus showing chromosomes, *p* pyrenoid). ×9000. *Bar* 2.2 μm. **B** Section (TEM) of an archaeocyte engulfing a group of dinoflagellates, showing fibrillar material between algae and archaeocyte (*arrowed*). (*d* Dinoflagellate, *a* archaeocyte). ×1400. *Bar* 5 μm. **C** TEM of a portion of another archaeocyte engulfing dinoflagellates and illustrating the archaeocyte nucleus (*n* nucleus with nucleolus) (*arrowed*). ×2400. *Bar* 2 μm. **D** TEM of a undischarged nematocyst. ×4500. *Bar* 2 μm. **E** Section (TEM) of a spongocyte engulfing a spicule (*sp* spicule). ×7500. *Bar* 1.4 μm. **F** Dissociated cell preparation from *Haliclona* sp. showing (LM): sponge cells (*arrowed*), mainly choanocytes, archaeocytes and spongocytes, and dinoflagellates (*arrowheads*) (*n* nematocysts). ×400. *Bar* 25 μm. **G** Fraction A_1 from Percoll density gradient fractionation showing small sponge cells (mainly choanocytes, spongocytes, and archaeocytes) and dinoflagellates. ×400. *Bar* 25 μm. **H** Fraction A_4 from Percoll density gradient fractionation showing archaeocytes, dinoflagellates and nematocysts. ×200. *Bar* 50 μm. **I** Fraction A_5 from Percoll density gradient fractionation showing dinoflagellates and nematocysts. ×200. *Bar* 50 μm

Cell separation

Since preliminary experiments indicated that the fragile sponge cells were easily damaged during cell dissociation in CMF-ASW, cell preparations were routinely prepared by mincing tissue directly into glutaraldehyde fixative, so that cells were fixed immediately on dissociation. After a short period of fixation, the cells were filtered to remove tissue debris and then pelleted, which helped to remove small pieces of broken cells. The cells were stored in fixative until needed and washed carefully to remove fixative just prior to cell separation. Based on earlier light-microscopic characterization, the resulting dissociated cell preparation contained dinoflagellates, sponge cells of

varying sizes, and nematocysts (Fig. 1F). Although the separation of cell types was complicated by the similar size and shape of some cells types, it proved possible to partition the dissociated cell preparation into five distinct fractions, A_1–A_5, by Percoll density gradient centrifugation.

Light-microscopic investigations demonstrated that the main constituents of fraction A_1 were small sponge cells (choanocytes, spongocytes) with occasional large sponge cells (probably archaeocytes), and dinoflagellates (Fig. 1G), whereas fraction A_2 contained a mixture of all types of sponge cells together with dinoflagellates. Dinoflagellates and archaeocytes were the major components of fractions A_3 and A_4, the dinoflagellates being most concentrated in fraction A_4 (Fig. 1H). Although nematocysts were distributed throughout fractions A_2–A_5, their abundance increased toward the bottom of the Percoll gradient. As expected, spicules were also abundant in fraction A_5 (Fig. 1I), having dropped straight through the Percoll layers on centrifugation. Since a clean separation of the archaeocyte/dinoflagellate fraction had not been obtained, fraction A_4 was repartitioned on a second discontinuous Percoll density gradient by centrifugation to give fractions A_6–A_9. Archaeocytes were found only in fraction A_6, whereas dinoflagellates were distributed throughout fractions A_6 and A_7. Nematocysts were present in fractions A_8 and A_9.

Chemistry

Whole tissue samples of the sponge, when extracted, contained the haliclonacyclamines A (1) and B (2; Fig. 2) with pronounced biological activity (Charan et al. 1996). Organic extracts of the individual Percoll fractions, A_1–A_9, were analyzed by TLC and by proton NMR. The results revealed that the alkaloids were mainly present in fractions A_1–A_4 and A_6, which contained small sponge cells such as spongocytes and choanocytes together with the larger archaeocytes, and completely absent from fractions A_5 and A_7–A_9, which were predominantly composed of dinoflagellates and nematocysts.

Discussion

Sponges and corals compete for space in the nutrient-depleted environment of a tropical coral reef. Both of these invertebrates can make use of phototrophic mechanisms to acquire nutrients. Although the vast majority of corals contain intracellular dinoflagellate symbionts that contribute photosynthetic products, sponges contain a range of microbial symbionts, not all of which are photosynthetic. Sponge symbionts can also be either intercellular or intracellular. The association between sponges and symbiotic microorganisms is metabolically profitable for both partners and may also significantly influence the secondary metabolism shown by the host sponges. A major factor contributing to the ecological success of sponges is their ability to produce biologically active metabolites; these chemicals have been implicated in allelochemical interactions with corals (Porter and Targett 1988). The natural-products chemistry of the Porifera is well documented (Faulkner 1996), and the diverse range of sponge metabolites undergoing clinical development as potential pharmaceuticals (Carté 1996) is an interesting, but indirect, consequence of their ecological competitiveness in situ. The natural-products literature contains innumerable hypotheses about the sponge or symbiont source of sponge secondary metabolites; firm experimental evidence has only recently been obtained on the role of symbionts in sponge chemistry (Faulkner et al. 1993, 1994). Whereas the symbionts are frequently associated with potent biological activity (Faulkner et al. 1994; Bewley et al. 1996), the bioactive chemicals may also be localized in the sponge cells (Thompson et al. 1984; Garson et al. 1992; Uriz et al. 1996a,b). A variety of experimental methods are now available to separate sponge and symbiont cells so that their individual metabolite compositions can be evaluated (Faulkner et al. 1993; Unson et al. 1994; Bewley et al. 1996; Flowers et al. 1998). When novel sponge/microbial associations are encountered, assumptions need no longer be made about the possible cellular location of any bioactive chemicals produced by the assemblage.

Our cytochemical work on *Haliclona* sp., which was prompted by the finding of dinoflagellates in the tissue of this sponge (McCaffrey 1988), has been designed to identify the cellular location and hence the probable site of synthesis of the cytotoxic biologically active alkaloids present. *Haliclona* sp. is a soft fragile sponge that does not survive well under aquarium conditions. The rapid tissue deterioration, together with the presence of numerous spicules, complicates both the transmission electron-microscopic investigation of intact tissue and cell separation studies. After many trials, we have found that the best procedure for cell dissociation is to shred sponge tissue directly in glutaraldehyde fixative; this has enabled us to preserve the major sponge cell type, the spongocyte, intact. The choanocytes and archaeocytes are preserved whole, although their cell membranes are not always found to be completely intact when examined by electron microscopy. The symbiont cells are protected by their thick cell wall and survive fixation intact. We have found that small cell fragments can be removed by centrifugation and do not therefore interfere with the subsequent cell separation. The fixed cell preparation, when washed free of fixative, can be fractionated according to density on Percoll as the support medium according to our published protocol (Flowers et al. 1998); as expected, smaller sponge cells (mainly spongocytes and choanocytes) are present in the top fractions, whereas larger archaeocytes are present throughout the gradients. The large size range of archeocytes may explain the presence of archaeocytes throughout the gradient, although this may also be because lysed cells have different densities from intact cells. The denser dinoflagellates generally partition toward the bottom

of the gradients, as do the nematocysts. We have determined that the haliclonacyclamines are localized in fractions rich in sponge cells rather than in fractions rich in dinoflagellates and are therefore products of sponge rather than algal metabolism. Because of the similarities in the cell dimensions of the spongocytes and choanocytes, we have been unable to determine which of these two cell types is responsible for the synthesis of the haliclonacyclamines. Archaeocytes may also contain these compounds. A more precise cellular localization of the haliclonacyclamines requires the complementary technique of flow cytometry (Unson et al. 1994; Pomponi and Willoughby 1994).

Dinoflagellates (zooxanthellae) represent the best-documented example of a non-cyanobacterial algal association in sponges. Their occurrence in sponges has primarily been reported in excavating sponges, of the order Hadromerida, which colonize coral or shell substrates in the Mediterranean and the Caribbean. The dinoflagellates are concentrated in the surface tissue and are normally intracellular, being contained in archaeocyte vacuoles (Vacelet 1982; Rützler 1990). Occasionally, they are intercellular but surrounded by pseudopodia (Rützler 1990). Morphologically, the dinoflagellate in these hadromerid sponges conforms to the description for the species termed *Symbiodinium microadriaticum*, except that the chloroplast structure is denser (Vacelet 1982; Rützler 1990). The occurrence of zooxanthellae in excavating hadromerid sponges, in contrast to their ubiquitous presence in reef-building corals, presents an interesting dichotomy. Some workers contend that the photosynthetic activity of the dinoflagellates increases the boring activity of the hadromerid sponge, thus enhancing the fitness of the sponge host (Vacelet 1982; Hill 1996). Sullivan et al. (1983) have shown that the boring sponge *Siphonodictyon* (*Aka*) sp. (order Haplosclerida, family Niphatidae) contains toxic chemicals, the siphonodictines, which inhibit the growth of coral polyps. A suggested factor in the boring mechanism of this sponge is the use of mucus to maintain a dead zone in the coral skeleton around the base of the sponge. Boring sponges of the family Clionidae are also reported to contain secondary chemicals; unfortunately, the chemical instability of these metabolites complicates an evaluation of their biological activities (Andersen and Stonard 1979; Stonard and Andersen 1980). It is not yet known whether the dinoflagellate partner provides nutrients to the host tissue of hadromerid sponges by translocation, as is the case for corals and freshwater sponges (Muscatine et al. 1967; Muscatine and Cernichiari 1969; Wilkinson 1980b). The role of dinoflagellates in nitrogen metabolism in their boring sponge host is also considered to be valuable (Corredor et al. 1988).

Although *Haliclona* sp. does not excavate calcium carbonate substrates, it shares some characteristics in common with the successful group of boring sponges. First, a species of dinoflagellate is present whose taxonomic features resemble *S. microadriaticum* and that is found in close association with archaeocytes. A feature in common with the burrowing sponge *Siphonodictyon* (*Aka*) sp. is the production of mucus that causes the necrosis of surrounding coral tissue (Sullivan et al. 1983). The precise taxonomic identification of dinoflagellates is based on detailed ultrastructural examination (Trench and Blank 1987; Trench 1992; Banaszak et al. 1993) complemented by molecular studies (Rowan and Powers 1991, 1992). Since the host specified for *S. microadriaticum* is the jellyfish *Cassiopeia* spp. (Trench and Blank 1987), the dinoflagellate found in *Haliclona* sp. and in other hadromerid sponges may eventually be better described as a new species. As far as we can determine, this is the first report of a non-excavating sponge containing a dinoflagellate.

The preferred habitat for the *Haliclona*/dinoflagellate association is similar to that of hadromerid sponges, usually an exposed location where the light intensity is optimal for the zooxanthellae (Pang 1973). The sponge represents an interesting candidate in which to study carbon fixation and nutrient translocation from the dinoflagellate partner to the host cells. A feature of *Haliclona* sp., to our knowledge not shown by any other sponge, is that its tissue contains nematocysts. Although detailed studies of the origin of the nematocysts are beyond the scope of the current investigation, sponge tissue collected from sand-covered coral rock does not contain as many nematocysts, suggesting they may therefore be taken up from the living coral substrate on which the sponge grows. Detailed molecular and biochemical studies will be needed to determine whether or not the associated dinoflagellate is a genuine symbiont, or whether it is also taken up from coral substrate.

The sponge also produces bioactive components that are toxic to corals and function as feeding deterrents (McCaffrey 1988). Although not established, it seems probable that the bioactive components detected by McCaffrey must have included the haliclonacyclamines, since these metabolites are known to have potent cytotoxic and antifungal activities (Charan et al. 1996), and our own recent studies have shown the haliclonacyclamines have biological effects in situ. Ecological studies to determine explicitly the role of the haliclonacyclamines in coral tissue necrosis and as feeding deterrents are currently in progress in our laboratory.

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