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The effects of HEBP, an inhibitor of mineral deposition, upon photosynthesis and calcification in the scleractinian coral, *Stylophora pistillata*

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Abstract

In reef-building corals, photosynthesis by endosymbiotic algae enhances calcification; this rate of calcification is reduced by shading and by inhibitors of photosynthesis. Calcification can also be reduced by compounds which prevent mineral deposition rather than affecting the metabolic processes. The effects of such compounds upon the photosynthesis of endosymbiotic algae has not yet been tested. In the present study, photosynthesis and calcification in branches of *Stylophora pistillata* were measured in the presence of 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP), a specific inhibitor of mineral deposition. HEBP strongly inhibited calcification of the coral with 36% inhibition at 0.01 mM, 90% at 0.1 mM and 99% at more than 0.5 mM. However, the level of photosynthetically fixed organic carbon was almost constant in concentrations of HEBP up to 2 mM. Rate of photosynthesis was not affected when calcification was inhibited using 0.5 mM HEBP. It is suggested that coral calcification does not enhance algal photosynthesis.

Keywords: Calcification; Coral; HEBP; Photosynthesis; *Stylophora pistillata*

1. Introduction

The formation of $CaCO_3$ skeletons of hermatypic corals is facilitated in the light by photosynthesis of endosymbiotic unicellular algae called "zooxanthellae" (Kawaguti & Sakumoto, 1948; Goreau, 1959; Goreau & Goreau, 1959). It has been suggested that zooxanthellae supply oxygen and photosynthetic products such as glycerol or lipids to the host coral (Muscatine, 1967; Battey & Patton, 1984). Light-enhanced calcification is strongly inhibited by shading or by treatment with DCMU (dichlorophenyl dimethyl urea), a specific inhibitor of photosystem II in photosynthesis (Barnes, 1985). Although there are many studies of

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the inhibitory effect of reduced photosynthesis on light-enhanced calcification, there have been non on the effects on photosynthesis of inhibiting mineral deposition in hermatypic corals.

Certain chelating reagents are used to prevent the precipitation of minerals and are the active constituents in anti-scaling reagents and certain industrial cleaners, corrosion inhibitors and household detergents (Nancollas & Sawada, 1982; Fischer, 1993). Bisphosphonates, a group of synthetic chelating reagents containing a P-C-P bond, retard both formation (Francis et al., 1969) and dissolution (Fleisch et al., 1969) of calcium phosphates in vivo and in vitro. It is suggested that 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP) inhibits crystal growth of both calcite and aragonite by adsorption at the growth surface (Nancollas & Sawada, 1982). Bisphosphonates have been used in studies of calcification in calcareous algae (Okazaki et al., 1993) and mammals (Shinoda et al., 1983; Ohya et al., 1991), where they strongly inhibited mineral deposition.

The present study reports on the effect of 1-hydroxyethylidene-1, 1-bisphosphonic acid on coral calcification and on zooxanthella photosynthesis in a short-term laboratory experiment designed to evaluate the role of calcification in photosynthesis in the coral-algae symbiotic system.

2. Materials and methods

A colony of *Stylophora pistillata* was collected on 30 October, 1993 from the reef flat in front of Sesoko Station of the University of the Ryukyus (127° 52E′, 26° 39′N), and transferred to an open circuit aquarium exposed to sunlight through a glass roof and windows. Branch tips less than 1 cm long were cut and glued, in groups of four, to 2×2 cm tiles with waterproof epoxy resin. Eight tiles were kept for 4 wk in the aquarium to acclimatize the corals before experiment. Natural water temperatures during the acclimation period ranged from 24 to 28°C. A tracer experiment was conducted on 3 December at the University of the Ryukyus Radioisotope Laboratory. Tiles with four coral tips each were incubated in a beaker containing 50 ml of freshly filtered seawater (0.45 μm, Millipore)to which various concentrations of Na_2HEBP (1-hydroxyethylidene-1, 1-bisphosphonic acid) were added; this resulted in final concentrations ranging from 0 to 2 mM. At more than 2 mM, seawater became turbid. About 2 min after the addition of HEBP, $NaH^{14}CO_3$ was also added to give a final concentration of 7.4 kBq · ml^{-1} seawater. All incubations were carried out at pH 8.2 under halogen lighting at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 2 h. The water was periodically stirred and remained at 25 ± 1°C. At the termination of incubation, coral samples were washed 3 times with filtered seawater. Coral soft tissue was digested in 3 ml of boiling 1 N NaOH. One ml of 6 N HCl was added to the resulting solution of digested soft tissues, to displace the remaining $H^{14}CO_3^-$ as $^{14}CO_2$, leaving photosynthetically fixed organic carbon. After removal of the tissue, each coral skeleton was washed with flowing tap water for 30 min and put in a chamber to which 6N HCl was slowly injected. Generated CO_2 was passed to a series of vials containing the CO_2 absorber,

CarboSorb (Packard). Radioactivity trapped in CarboSorb was measured using a liquid scintillation counter. Quenching was corrected by the external standards method. The rate of ^{14}C uptake was expressed as dpm · cm^{-2} coral surface · h^{-1}. Coral surface area was calculated from measurements of the length, width and height of each coral tip using the technique of Rinkevich & Loya (1984). To determine the effect of calcification on photosynthesis, four coral branches on each of 6 tiles (3 tiles incubated with 0.5 mM HEBP and 3 without) were incubated at 10, 40 and 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 2 h on April, 1994 and treated as before. To test whether or not HEBP alter the rate of isotopic exchange of coral skeleton, only the branches of skeletons were incubated for 2 h with 0.5 mM HEBP and without as control.

3. Results

In incubations in the light, increasing concentrations of 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP) increasingly diminished incorporation of ^{14}C into the skeleton of *S. pistillata* without affecting photosynthetic fixation of ^{14}C into tissues (Fig. 1). With no HEBP, incorporation of ^{14}C into the tissues was 3 times higher than incorporation into the skeleton. Inhibition of calcification relative to controls was 36% with 0.01 mM HEBP. Inhibition was 80 and 90% with 0.05 and 0.1 mM HEBP, respectively, and was 99% at HEBP concentrations of 0.5 mM or greater.

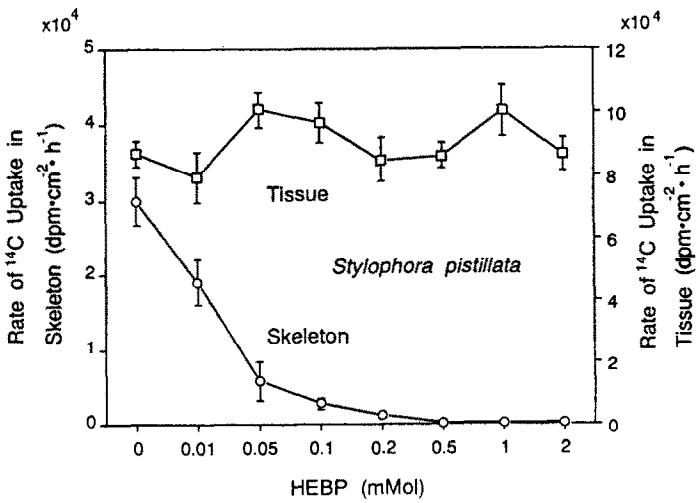

Fig. 1. Effects of 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP) on the rates of calcification and photosynthesis in branches of the coral *S. pistillata*. This reagent strongly inhibits calcium carbonate deposition but does not affect photosynthetic activity of the symbiotic algae. Mean ± SD, $n = 4$.

The rate of isotopic exchange using branches of skeleton was not affected by the addition of 0.5 mM HEBP (not shown), therefore, 1% of ^{14}C uptake in skeletons of live coral branches at more than 0.5 mM of HEBP is caused by ordinary isotopic exchange. The ratio of photosynthesis/calcification in different light conditions ranged from 0.5 (10) to 2.9 (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) in control corals (Fig. 2A). On the other hand, the rates of photosynthetically fixed ^{14}C, where calcification was perfectly inhibited with 0.5 mM HEBP, did not change in comparison to those in the control corals (Fig. 2B).

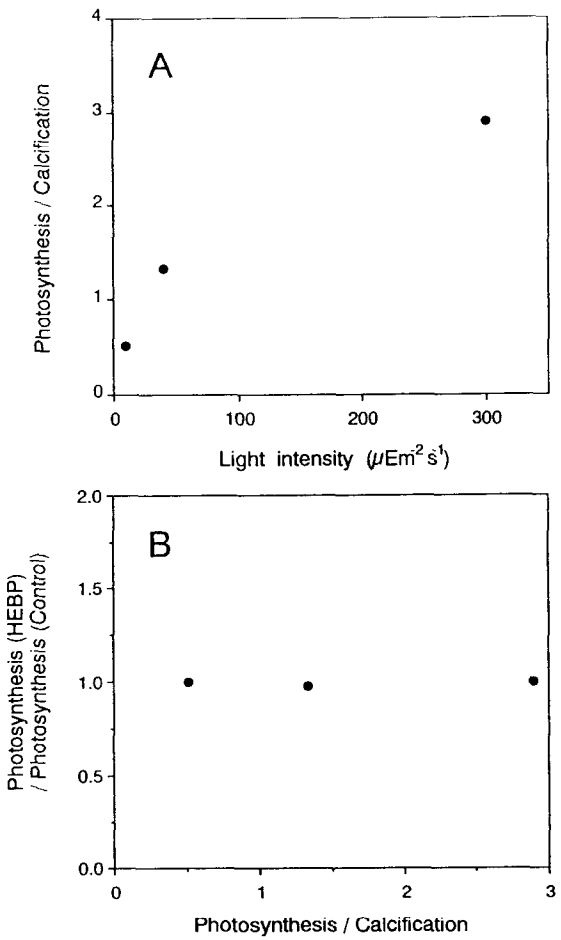

Fig. 2. *Stylophora pistillata*. (A) Rates of calcification and photosynthesis in different light conditions (control). (B) Rates of photosynthesis treated with 0.5 mM of 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP) were compared to the control in the same light conditions.

4. Discussion

Coral calcification is reduced by shading and by treatment with photosynthetic inhibitors such as DCMU and Diamox and by some uncouplers of oxidative phosphorylation (Kawaguti & Sakumoto, 1948; Goreau, 1959; Goreau & Goreau, 1959; Yamazato, 1966; Pearse and Muscatine, 1971; Chalker & Taylor, 1975; Barnes, 1985). These works show that the light-enhanced calcification can be reduced by inhibition of metabolic processes as a reduced supply of oxygen and organic materials which could enhance the calcification of the host coral. Goreau (1959) suggested that the calcification process generates protons which decrease pH and convert HCO_3^- to CO_2 which may be fixed by photosynthesizing zooxanthellae. A functional linkage between calcification and photosynthesis has been discussed, and it has been suggested that these two reactions offset each other (McConnaughey, 1994; Suzuki et al., 1995). If calcification enhances algal photosynthesis by supplying CO_2 as substrate for algal photosynthesis, the rate of photosynthesis could be reduced if calcification is blocked. However, such reverse effect has not been demonstrated by direct measurement of carbon translocation.

The results presented here show that the inhibition of coral calcification with 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP), which blocks mineral deposition at the site of mineralization, did not alter photosynthesis, in comparison to that in control corals in different photosynthesis/calcification conditions. Therefore, it was suggested that coral calcification does not enhance algal photosynthesis in the present experiment. Furthermore, in the unicellular coccolithophorid alga *Emiliania huxleyi*, photosynthetic fixation of CO_2 is enhanced when calcification is completely suppressed by HEBP (Sekino and Shiraiwa, 1994). This enhancement is explained by the allocation of internal carbon pool. These two results are not consistent with those deduced from the carbonate equibilium model which counterbalances photosynthesis and calcification.

In order to elucidate the carbon budget between photosynthesis and calcification in scleractinian corals, the following estimations caused by biological activities will be required: translocation and allocation of carbon species between host and algae, localized pH values at different sites of coral and active transports of Ca^{2+}, H^+, OH^- and carbon species. Treatment by 1-hydroxyethylidene-1, 1-bisphosphonic acid (HEBP) is able to block only calcification without a reduction of photosynthetically produced oxygen and organic materials supplied by the symbiotic algae. A reagent, HEBP would be useful in understanding the carbon budget of calcifying plants and animals where calcification is controlled by symbionts.

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