Symbiosis, 11 (1991) 31–45
Balaban, Philadelphia/Rehovot

The Enzymatic Response of the Symbiotic Dinoflagellate *Symbiodinium microadriaticum* (Freudenthal) to Growth *in vitro* Under Varied Oxygen Tensions

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Received August 6, 1990; Accepted November 2, 1990

Abstract

Symbiotic dinoflagellates colonize microhabitats characterized by large fluctuations in oxygen tension. To test the hypothesis that oxygen detoxifying enzymes are activated in response to elevated oxygen tension (pO_2), the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal was grown *in vitro* under experimentally manipulated oxygen tensions representing hyperoxic, normoxic and hypoxic conditions. The specific activities, normalized to protein and to cell numbers, of three key enzymes believed to be involved in oxygen detoxification; superoxide dismutase (SOD), catalase and ascorbate peroxidase, were assayed in cell-free extracts. Activities of all three enzymes were found in varying amounts in supernatant and pelleted fractions after aqueous extraction. The pellet fraction has been overlooked in the past. We found a direct relation between O_2 tension and SOD activity per mg protein; SOD activities expressed per cell were similar in hyperoxic and normoxic treatments, and both were higher than hypoxic treatments. Although catalase activities (normalized to either protein or cell numbers) were proportional to O_2 tension in cells grown under hyperoxic and normoxic conditions, cells grown under hypoxic conditions demonstrated higher activities than cells grown under normoxic conditions. The specific activities per protein of ascorbate peroxidase generally decreased as a function of decreasing O_2, but the specific activities per cell showed the reverse relation.

Keywords: superoxide dismutase, catalase, ascorbate peroxidase, hyperoxia, *Symbiodinium*

1. Introduction

In all aerobic organisms, oxygen-derived free radicals are commonly produced as a by-product of several metabolic pathways (Asada and Takahashi, 1987). In chloroplasts, the superoxide radical (O_2^-) can be produced from the reduction of O_2 by electron acceptors associated with photosystem I (Asada et al., 1974; Asada and Takahashi, 1987; Nakano and Asada, 1980; Halliwell, 1984), and substantial amounts of H_2O_2 and the highly reactive hydroxyl radical ($OH^{\cdot}$) can arise during photosynthesis (Asada et al., 1974; Nakano and Asada, 1980; Asada and Takahashi, 1987; Rabinowitch and Fridovich, 1983).

In plants and algae several protective mechanisms against oxygen-derived free radicals have evolved; the main ones being enzymes, which catalytically deactivate oxygen-derived free radicals, and antioxidants, such as ascorbic acid, reduced glutathione and α-tocopherol (see Asada and Takahashi, 1987; Elstner, 1982; Halliwell, 1984). Carotenoid pigments decrease the formation of O_2^- by absorbing excess excitation energy from chlorophyll and also quench singlet oxygen (Foote et al., 1970; Siefermann-Harms, 1987). The enzymes superoxide dismutase (SOD), catalase, and various peroxidases function together to inactivate O_2^- and H_2O_2, thereby preventing the accumulation of $OH^{\cdot}$ and singlet oxygen and the subsequent cellular damage which would result from these reactive oxygen species (Fridovich, 1986; Asada and Takahashi, 1987; Bannister et al., 1987). All of these potential problems are further exacerbated when cells are exposed to hyperoxia.

The hypothesized high reactivity of the superoxide radical, and hence the essential role of SOD in oxygen detoxification, has been questioned by other investigators (Fee, 1981, 1982), but there appears to be a consensus on the role of H_2O_2 in producing the highly reactive $OH^{\cdot}$ by reacting with the superoxide radical (Haber and Weiss, 1934; Halliwell, 1984; Afanas'ev, 1989) or by Fenton type reactions in the presence of metal ions/complexes such as Fe^{++}.

Symbiotic associations between dinoflagellates and invertebrates are characteristic of shallow water tropical marine environments. The high insolation to which these associations are exposed is essential for the high primary productivity of such ecosystems (Muscatine, 1980), but the inter- or intracellular location of the algae in animals' tissues creates potentially severe problems with regard to hyperoxia (Dykens and Shick, 1982). For example, Dykens and Shick (1982) showed that when *Symbiodinium* sp. in the sea anemone *Anthopleura elegantissima* was exposed to 315 μmol·m^{-2}s^{-1} for less than 5 min, a pO_2 of 328 mm Hg. (2 atm) was measured in the animals' tissues. Consequently, both the animal hosts and the algal symbionts must be able to acclimate to periodic episodes of hyperoxia. Oxygen toxicity and the biochemical response to it

may represent in symbiotic associations, a significant physiological stress and ultimately, a cost to both the algae and their hosts (Lesser and Shick, 1989a).

Research on oxygen detoxification in algal-invertebrate symbioses during the last decade has focused primarily on the enzymatic defenses present in animal hosts (Dykens and Shick, 1982; Dykens, 1984; Tytler and Trench, 1988). More recently, studies of the enzymatic defenses of the algal symbionts have been initiated. Tytler and Trench (1986) examined the catalase activity in *Symbiodinium* spp. isolated from the sea anemones *Aiptasia pulchella* and *A. elegantissima*, the coral *Montipora verrucosa* and the clam *Tridacna maxima*. In those instances where levels of catalase activity could be compared between cultured and freshly isolated algae, higher activities per mg protein were found in cultured algae.

Lesser and Shick (1989a,b) showed that activities of SOD and catalase increased with increasing photosynthetically active radiation (PAR) as well as ultraviolet (UV) radiation in cultured and freshly isolated *Symbiodinium* sp. from acclimated *Aiptasia pulchella*. These investigators also reported that cultured and freshly isolated cells receiving equivalent levels of PAR along with "environmentally relevant UV" show a 30–40% increase in SOD activities compared with algae shielded from UV. Cultured cells consistently showed higher activities of both SOD and catalase than freshly isolated cells. No significant trends were found in terms of specific activity of ascorbate peroxidase except activities were higher in cultured than in freshly isolated cells.

All the previously cited studies of oxygen-detoxifying enzymes in cultured *Symbiodinium* spp. were either performed with cultures maintained at ambient (normoxic) oxygen tensions or with intact associations. In this study, we have experimentally manipulated oxygen tensions in cultures of *S. microadriaticum* (Trench and Blank, 1987) and the resulting changes in the specific activity of SOD, catalase and ascorbate peroxidase were assessed. The results show for the first time that there is enzyme activity associated with the pellet fractions after aqueous extraction (a component that has been overlooked in the past). We found a direct relation between O_2 tension and SOD activity per mg protein; SOD activities expressed per cell were similar in hyperoxic and normoxic treatments, and both were higher than hypoxic treatments. Although catalase activities (normalized to either protein or cell) were proportional to O_2 tension in cells grown under hyperoxic and normoxic conditions, cells grown under hypoxic conditions demonstrated higher activities than cells grown under normoxic conditions. The activities per mg protein of ascorbate peroxidase generally decreased as a function of decreasing O_2, but the activities per cell showed the reverse relation.

2. Materials and Methods

Culturing of algae

Symbiodinium microadriaticum Freudenthal (Trench and Blank, 1987) was inoculated into 100 ml of ASP-8A (Ahles, 1967) in 250 ml Erlenmeyer flasks. Ampicillin (100 μg/ml) and amphotericin B (10 μg/ml) were used as bactericide and fungicide, respectively, to maintain axenic cultures. Cultures were grown under a 14:10 hr (L:D) photoperiod at 26°C and illuminated at 65 μmol $m^{-2}s^{-1}$ by cool white fluorescent lamps (Sylvania, 40W). Irradiance was measured with a Licor quantum sensor (LI-192S) and photometer (LI-85). After 3–4 weeks, cultures were transferred to 2.8 l Fernbach flasks containing 1 l of the same growth medium and harvested after 6–8 weeks.

Normoxic cultures were grown at ambient O_2 tensions. Hyperoxic treatments were generated by bubbling the growth medium with O_2:N_2:CO_2 gas mixtures of 63%, 36%, 0.03% respectively. Hypoxic treatment consisted of N_2:CO_2 of 99% and 0.03% respectively. This latter gas mixture was always bubbled in the morning to prevent anoxia at night. The liquid cultures were maintained in equilibrium with the gas atmosphere by stirring. Cultures were maintained in rubber stoppered 2.8 l Fernbach flasks with tygon tubing and three-way stopcocks and were bubbled with appropriate gas mixtures 3 times a week, except the week prior to harvesting when bubbling was done daily. The O_2 and CO_2 tensions were monitored regularly throughout 24 hr periods by drawing gas samples from gas space above the cultures, and these were analyzed with an O_2/CO_2 analyzer (Applied Electrochemistry, Model S-3A/1) equipped with O_2 (Model N-22) and CO_2 (Model P-61B) sensors. These analyses showed that the pO_2 during a 24 hr period in hyperoxic cultures was 399 $\pm$ 63 mm Hg (53% $\pm$ 8%), and 48 $\pm$ 16 mm Hg (6% $\pm$ 2%) in hypoxic cultures.

Cell harvesting and preparation of cell-free extracts

Cells were harvested by centrifugation at 8,000×g for 10 min and resuspended in ice-cold extraction buffer (50 mM sodium phosphate (pH 7.5), 20% (w/v) sorbitol, 0.1 mM Na_4EDTA,1 mM L-ascorbic acid, 5 mM phenylmethylsulfonylfluoride (PMSF), 40 mg ml^{-1} 1,10-phenanthroline, 400 mM benzamidine, 500 mg ml^{-1} bovine serum albumin). Cells were passed three times through a French pressure cell at 83 MPa. The suspensions were centrifuged at 20,000×g for 1 hr at 4°C. The supernatant solutions were assayed immediately for enzyme activity whereas the pellet fraction was resuspended in 4 ml of extraction buffer containing 0.1% (v/v) Triton X-100, extracted in the dark at 4°C for 1 hr and centrifuged using the same procedure described above.

This supernatant solution (hereafter referred to as pellet fraction) was assayed immediately for enzyme activity.

Enzyme assays

Activity of SOD (EC 1.15.1.1) was assayed using a polarographic oxygen electrode (YSI Model 53) and the O_2 evolution method of Tyler (1975) at 25°C. The SOD assay buffer consisted of 50 mM sodium phosphate (pH 7.4), 16 mM $NaSO_3^-$, 50 μM xanthine, 0.1 mM Na_4EDTA. The reaction was started by adding 5 μl of xanthine oxidase (Calbiochem, 1.32 U/mg protein). Catalase (EC 1.11 1.6) was assayed at 25°C using the polarographic method and the buffer system used by Tytler and Trench (1986). In both instances a final reaction volume of 3 ml was used including 100 μl of cell-free extract.

Ascorbate peroxidase was assayed by a modification of the spectrophotometric method of Nakano and Asada (1981) based on the oxidation of ascorbate. This assay was performed as three consecutive parts in 1 ml final volume. First (i) 100 μl of cell-free extract was added to 50 mM sodium phosphate (pH 7.5) containing 20% sorbitol (w/v), and ΔA_{290}nm was monitored for 3–5 min. Second (ii), 2 mM H_2O_2 was added and the decrease in absorbance was again measured for 3–5 min. Third (iii), 0.1 mM ascorbate was added and the same procedure repeated. The rate of ascorbate oxidation was corrected by subtracting ascorbate oxidase activity of reactions (i) and (ii), representing non-enzymatic oxidation of ascorbate by H_2O_2, from the final rate of ascorbate oxidation (iii). This procedure is considered to provide a conservative estimate of ascorbate peroxidase activity as during step (ii) there will be some enzyme activity due to the presence of cellular ascorbate in the cell-free extract, which can act as an electron donor.

Sorbitol (20% w/v) was found to be an essential component of the extraction and assay buffers required to maintain ascorbate peroxidase activity which is consistent with the conclusions of Nakano and Asada (1987).

The kinetics of the various enzyme-substrate reactions was monitored by means of software installed in a personal computer. Voltage outputs were transformed into μmoles O_2 using the O_2 solubility tables of Grasshoff et al. (1983). The rate of product formation or substrate utilization was calculated for every assay from V_{max} values. These were obtained by means of linear regression analyses. In all cases, initial rates of reactions were employed. Control slopes obtained in the absence of cell-free extracts were subtracted from the experimental rates in the SOD and catalase activity assays.

Because like catalase, bromoperoxidases also use H_2O_2 as substrate and evolve O_2, bromoperoxidase was assayed in extracts of cells grown in ASP-8A

supplemented with 1 mM Br^- and 1 μm I^- according to the method of Everett et al. (1990), and in polyacrylamide gels according to Weaver et al. (1985).

Protein concentrations were estimated by the method of Bradford (1976), using a commercial protein assay kit (Bio-Rad), and bovine serum albumin as standard. Estimates of cell numbers were made at 160× magnification by means of a compound microscope equipped with an improved Neubauer grid; calculations were made according to Guillard (1978).

3. Results

The protein and cell-specific activities of SOD, catalase and ascorbate peroxidase were determined in cell-free extracts after exposure of *S. microadriaticum* to hyperoxic and hypoxic growth conditions. Normoxic conditions served as control. The three enzymes were detected in both supernatant and pellet fractions in all treatments. None of the assays for bromoperoxidase yielded positive results.

SOD activity

The SOD activity per mg protein varied directly as a function of pO_2. The highest combined (supernatant plus pellet fraction) SOD specific activity was evident in hyperoxic cultures, and this value decreased with decreasing oxygen tension (Table 1). In all treatments there was more protein in the supernatant than in the pellet fraction. Thus when SOD activity is expressed per mg protein, there appears to be more enzyme activity in the pellet fraction than in the supernatant. As the data in Table 1 indicate, normalizing enzyme activity per mg protein may be misleading. Similar activities for SOD were observed in the supernatant and pellet fractions of cells grown under hyperoxic conditions, but because the protein content of the pellet fraction was an order of magnitude lower than that in the supernatant the resulting activity per mg protein was higher in the pellet fraction.

SOD activities per cell, were approximately the same in cells grown under hyperoxic and normoxic conditions. Both of these values were higher than found in cells grown under hypoxic conditions.

The enzyme activity of hyperoxic cultures was found to be strongly influenced by the frequency of bubbling. The maximum specific activities of SOD reported in this study were obtained after bubbling cultures daily with hyperoxic gas for 1 week prior to harvesting.

Table 1. SOD activities in supernatant (S) and pellet fractions (P) from cell-free preparations of *S. microadriaticum* grown under hyperoxic, normoxic and hypoxic conditions in culture. Values normalized to protein, number (No.) of cells used in assay and expressed as mean ±1 S.E.; N = number of replicates; 1 Unit = 1 μmol $O_2 \cdot min^{-1}$.

	Enzyme activity (U)	Protein (mg)	Specific activity per protein (U mg protein^{-1})	No. of cells ($\times 10^4$)	Specific activity per cell (U·cell·10^{-6})	N
Hyperoxic						
supernatant	0.61±0.001	0.158	3.83±0.008	4.73	12.80±0.03	6
pellet fraction	0.59±0.002	0.018	33.40±0.099		12.57±0.04	6
S+P			37.23		25.37	
S:P			0.11		1.02	
Normoxic						
supernatant	0.83±0.008	0.076	10.98±0.105	5.00	16.59±0.16	4
pellet fraction	0.53±0.003	0.040	13.30±0.066		10.59±0.05	4
S+P			24.28		27.18	
S:P			0.83		1.57	
Hypoxic						
supernatant	0.34±0.006	0.111	3.09±0.052	5.10	6.74±0.11	5
pellet fraction	0.26±0.003	0.045	5.75±0.073		5.10±0.07	5
S+P			8.84		11.84	
S:P			0.54		1.32	

Catalase activity

On the basis of either mg protein or per cell, catalase activity was found to be highest in cells grown under hyperoxic conditions (Table 2). However, cells grown under hypoxic conditions had higher catalase activity than cells grown under normoxic conditions. In this case, the supernatant fractions consistently had higher activities than the pellet fractions.

Table 2. Catalase activities in supernatant (S) and pellet fractions (P) from cell-free preparations of *S. microadriaticum* grown under hyperoxic, normoxic and hypoxic conditions in culture. Values normalized to protein, number (No.) of cells and expressed as mean ±1 S.E.; N = number of replicates; 1 Unit = 1 μmol $O_2 \cdot min^{-1}$.

	Enzyme activity (U)	Protein (mg)	Specific activity per protein (U mg protein^{-1})	No. of cells ($\times 10^4$)	Specific activity per cell (U·cell·10^{-6})	N
Hyperoxic						
supernatant	2.33±0.072	0.158	14.71±0.452	4.73	49.20±1.51	6
pellet fraction	0.32±0.012	0.057	5.54±0.201		6.68±0.24	6
S+P			20.25		55.88	
S:P			2.66		7.37	
Normoxic						
supernatant	0.19±0.003	0.076	2.49±0.045	5.00	3.76±0.07	4
pellet fraction	0.03±0.002	0.040	0.81±0.053		0.65±0.04	3
S+P			3.30		4.41	
S:P			3.07		5.78	
Hypoxic						
supernatant	1.22±0.067	0.111	10.99±0.605	5.10	23.99±1.32	5
pellet fraction	0.14±0.004	0.045	3.18±0.004		2.82±0.08	5
S+P			14.17		26.81	
S:P			3.46		8.51	

Ascorbate peroxidase activity

The combined specific activities (supernatant and pellet fraction; S+P) per mg protein of ascorbate peroxidase were highest in cells grown under hyperoxic conditions; cells grown under normoxic and hypoxic conditions demonstrated similar levels of activity (Table 3). The differences in the protein content of the supernatant and pellet fractions markedly influenced the specific activities per mg protein (Table 3); when the data were normalized to number of cells (Table 3), algae grown under hyperoxic conditions demonstrated lower

Table 3. Ascorbate peroxidase activities in supernatant (S) and pellet fractions (P) from cell-free preparations of *S. microadriaticum* grown under hyperoxic, normoxic and hypoxic conditions in culture. Values normalized to protein, number (No.) of cells and expressed as mean ±1 S.E.; N = number of replicates; 1 Unit = 1 nmol $O_2 \cdot min^{-1}$.

	Enzyme activity (U)	Protein (mg)	Specific activity per protein (U mg protein^{-1})	No. of cells ($\times 10^4$)	Specific activity per cell (U·cell·10^{-5})	N
Hyperoxic						
supernatant	0.44±0.37	0.230	1.91±1.61	4.73	0.93±0.78	3
pellet fraction	0.77±0.03	0.004	191.99±7.70		1.62±0.07	2
S+P			193.90		2.55	
S:P			0.01		0.57	
Normoxic						
supernatant	0.95±0.36	0.095	10.04±3.83	5.00	1.91±0.73	4
pellet fraction	1.17±0.17	0.022	53.15±7.58		2.34±0.33	3
S+P			63.19		4.25	
S:P			0.19		0.82	
Hypoxic						
supernatant	2.91±0.32	0.056	51.99±7.58	5.12	5.69±0.62	2
pellet fraction	0.50±0.16	0.034	14.64±4.71		0.97±0.31	3
S+P			66.63		6.66	
S:P			3.55		5.85	

combined activities (S + P) than cells grown under normoxic and hypoxic conditions.

4. Discussion

The studies of Dykens and Shick (1982), Dykens (1984), Shick and Dykens (1985), Lesser and Shick (1989b) and Lesser et al. (1990) have consistently demonstrated a positive correlation between the activities per mg protein of

the enzymes SOD, catalase and ascorbate peroxidase and increased levels of PAR and UV in marine invertebrates and their symbiotic dinoflagellates. The implication has been that high irradiance results in high photosynthesis by the endosymbiotic algae, thereby increasing the oxygen tension within the association, which in turn increases the levels of reactive forms of oxygen. Under these conditions, algae and their respective hosts increase the activities of the oxygen detoxifying enzymes.

Direct evidence of elevated oxygen tension in host's tissue during photosynthesis by symbiotic algae has been provided by D'Aoust et al. (1976) and Dykens and Shick (1982). However, two important points should be borne in mind, particularly in the context of comparative studies. Firstly, the light-saturated rates of photosynthesis (P_{max}) of symbiotic dinoflagellates inhabiting different hosts vary at the same level of illumination (Chang et al., 1983; Iglesias-Prieto, unpublished). Second, contrary to previous assumptions, the rates of oxygen consumption increase with increasing oxygen tension in many invertebrates (Edmunds and Spencer-Davies, 1988; Shick, 1989). Therefore, increased host respiration could reduce the interstitial oxygen tensions. Third, the positive correlation between the activities per mg protein of SOD and catalase in invertebrates harboring symbiotic dinoflagellates was questioned by Tytler and Trench (1988); they found higher levels of catalase in some non-symbiotic animals than in symbiotic ones. Fourth, although the superoxide radical (O_2^-) is regarded as deleterious by Lesser et al. (1990) citing Fridovich (1986), due to its alleged oxidative reactions with membrane lipids, proteins and nucleic acids, other investigators (Fee, 1981, 1982; Afanas'ev, 1989) have emphasized the unreactivity of the superoxide radical. However, there is a consensus that O_2^- reacts with H_2O_2 to produce the highly reactive $OH^{\cdot}$ which can cause a broad range of cellular damage (Haber and Weiss, 1934; Halliwell, 1984; Afanas'ev, 1989).

A significant finding in our study is that often more than 50% of the activity of the combined supernatant and pellet fractions of SOD and ascorbate peroxidase was detected in the pellet fraction. Studies in the past have overlooked this fraction. The high level of enzyme activity found in the pellet fraction after extraction with a non-ionic detergent is a significant new finding for two reasons. Firstly, it suggests that estimates of total cellular enzyme activities of *S. microadriaticum* can be obtained only when both the supernatant and pellet fractions are assayed. The estimates of enzyme activity of the pellet fractions presented in this work are conservative since it is possible that repeated extraction with detergent might have further increased the yield of the enzyme. Second, it is apparent that the supernatant and pellet fractions of the three enzymes assayed can be differentially expressed as a function of oxygen tension.

For example, with increasing O_2 tension more SOD and ascorbate peroxidase activity were found in the pellet fractions, whereas the reverse pattern was generally true for catalase.

Lesser et al. (1990) expressed reservations in normalizing their data on the concentration of UV absorbing substances in *Symbiodinium* sp. to variable protein, but considered it appropriate to express enzyme activities from the same organism on a protein basis. Although enzyme activities are frequently expressed per unit of protein, we found this approach to be untenable because of the variable quantities of protein in the various fractions (supernatant versus pellet). We therefore expressed enzyme activities per number of cells and found that whereas catalase activities showed the same trends whether expressed on a per protein or cell basis, SOD and ascorbate peroxidase gave different results.

Our results with *S. microadriaticum* grown in culture indicate a direct relation between O_2 tension and SOD activity per mg protein. However, the SOD activities per cell indicated that cultures grown under hyperoxic and normoxic conditions had similar levels of enzyme activity. Lesser and Shick (1989b) also found that the specific SOD activity per mg protein varied directly with irradiance (and presumably O_2 tension) in algae freshly isolated from several cnidarian hosts.

The estimates of SOD activity that we obtained with a polarographic oxygen electrode are in agreement with the SOD activity estimates of Lesser and Shick (1989a) obtained by means of a spectrophotometric method. The SOD activity per protein (10.98 U·mg protein^{-1}) of our normoxic (supernatant) cells is virtually the same value (10.0 U·mg protein^{-1}) as that reported by Lesser and Shick (1989a) for normoxic cultures (supernatant) of *Symbiodinium* sp. isolated from the anemone *Aiptasia pulchella* grown at 70 μmol m^{-2}s^{-1}.

Contrary to what was hypothesized, higher catalase activity was found in hypoxic cultures than in normoxic cultures, regardless of whether activity was normalized to protein or numbers of cells. However, catalase activities were proportional to O_2 tension for the combined supernatant and pellet fractions of hyperoxic and normoxic cultures.

Combined (S+P) ascorbate peroxidase activities, per mg protein, were higher in hyperoxic than normoxic and hypoxic-treated cells although activities per cell were the lowest in hyperoxic treatments. The values of ascorbate peroxidase activity per mg protein obtained for the normoxic supernatant were approximately 5-fold and 3-fold lower than the values reported by Lesser and Shick (1989a) and Lesser et al. (1990) respectively. However, caution should be exercised when comparing our results with those of Lesser et al. because of differences in light and temperature regimes to which algal cells were exposed. In addition the ascorbate peroxidase activity data presented in Table 3

are conservative estimates since values were corrected for ascorbate oxidase activity and the non-enzymatic oxidation of ascorbate by H_2O_2.

In general, the values that we obtained for the specific activities of SOD and catalase in the supernatant fraction of normoxic cultures of *S. microadriaticum* were similar to those reported by Lesser and Shick (1989a) for normoxic cultures of *Symbiodinium* sp. from the anemone *Aiptasia pulchella* grown under similar conditions of illumination.

The precise cellular location, and the specific function of ascorbate peroxidases in dinoflagellates are unknown. However, spinach chloroplasts contain various ascorbate peroxidases which are very effective in scavenging H_2O_2 (Asada and Takahashi, 1987; Groden and Beck, 1980; Nakano and Asada, 1980, 1981, 1987; Asada and Badger,1984; Hossain et al., 1984; Gerbling et al., 1984). Ascorbate peroxidase has a lower apparent K_m (higher affinity) value (300 μM) than catalase (K_m = 47–1100 μM; depending on source; Asada and Takahashi, 1987). In plant cells catalase is localized in peroxisomes but is absent from chloroplasts (Asada and Takahashi, 1987). Although there is one ultrastructural report (Thinh et al., 1986) of the detection of peroxisomes in a *Symbiodinium*-like alga symbiotic with a *Zoanthus* sp., additional cytochemical and confirmation is not available (Lesser and Shick, 1989a).

Other factors that can influence the activities of the three enzymes examined, besides the O_2 tension, should be considered in view of our findings. The activities of catalase and ascorbate peroxidase can also be influenced by the cellular levels of their substrate, H_2O_2, which can be produced by several processes including oxygen reduction by photosystem I under conditions of high irradiance and limiting CO_2 (Patterson and Myers, 1973), glycolate oxidation in the photorespiratory pathway (Raven and Beardall, 1981), oxidative reactions (e.g. glucose oxidase, D-amino oxidase, fatty acyl-Co A oxidase), by reactions with reduced flavoproteins (Chance et al., 1979), in cyanide-resistant electron transport, and ascorbate synthesis from glucuronic acid (Goodwin and Mercer, 1983).

SOD, catalase and ascorbate peroxidase may not be the main mechanism that protects *S. microadriaticum* during hyperoxia. We are currently testing the hypothesis that cellular concentrations of antioxidants, tocopherols, ascorbate and reduced glutathione, show a direct relation with oxygen tension.

Acknowledgements

Roberto Iglesias-Prieto contributed critical suggestions for the experimental design used. Dr. John Bedi provided access to the O_2-CO_2 analyzer. Patricia

Thorne assisted with culturing of algae. The suggestions made by two anonymous reviewers improved the quality of this manuscript.

This study was supported by the University of California President's Fellowship Program (to J.L.M.), and by the Office of Naval Research, Award No. NOOO14-88-K-0463 (to R.K.T.).

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