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Catalase Activity in Cell-Free Preparations of Some Symbiotic and Nonsymbiotic Marine Invertebrates

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Abstract

Activity of catalase (E.C. 1.11.1.6) was detected in the blood and in cell-free extracts of the hypertrophied siphon (mantle) of *Tridacna maxima* which harbours symbiotic dinoflagellates (*Symbiodinium* spp.). Catalase activity was also detected in cell-free extracts from symbiotic, aposymbiotic and nonsymbiotic sea anemones and a symbiotic zoanthid. There was no relation between levels of catalase activity in animal tissues and symbiosis with dinoflagellates.

Keywords: catalase, hydrogen peroxide, symbiosis, *Tridacna, Aiptasia, Anthopleura, Metridium, Palythoa*

1. Introduction

Hydrogen peroxide (H_2O_2) is a reactive oxygen species which can be produced in photosynthesizing and non-photosynthesizing organisms by several processes including oxygen reduction by photosystem I under conditions of high irradiance and limiting CO_2 (Patterson and Myers, 1973), glycollate oxidation in the photorespiratory pathway (Raven and Beardall, 1981), dismutation of the superoxide radical (O_2^-) by superoxide dismutase (SOD) (Halliwell, 1977), oxidative reactions, e.g. glucose oxidase, D-amino oxidase,

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fatty acyl-CoA oxidase and by reactions with reduced flavoproteins (Chance et al., 1979). High intracellular concentrations of H_2O_2 can cause enzyme inhibition (Codd and Stewart, 1980; Tytler et al., 1984) and oxidation of cellular components. This condition can lead to photoinhibition, photo-oxidation and ultimately death of photosynthesizing organisms (Abeliovich and Shilo, 1972). H_2O_2 can also react with O_2^- which is formed as a respiratory product in almost all aerobic cells, and is another important radical in the mechanism of oxygen toxicity (Fridovich, 1975, 1978; Chance et al., 1979). O_2^- is itself not a strong oxidizing agent (Sawyer and Gibian, 1979), but in the presence of H_2O_2, more reactive oxygen species are produced, including the perhydroxyl radical (HO_2) (Gebicki and Bielski, 1981), singlet oxygen ($^1O_2$), (Khan, 1981) and the hydroxyl radical (OH·) (Fridovich, 1975, 1978). In living cells, the superoxide radical is deactivated by SOD, and H_2O_2 can be deactivated by catalase and peroxidases.

Symbiotic dinoflagellates (*Symbiodinium* spp.) are found in association with a range of marine invertebrates including corals, tridacnid bivalves, zoanthids and sea anemones (Blank and Trench, 1985; Trench and Blank, 1987). It has been shown that the sea anemone *Anthopleura elegantissima* was exposed to hyperoxic conditions when the symbiotic algae were actively photosynthesizing (Dykens and Shick, 1982). It is likely that other animals that harbour symbiotic algae are also exposed to hyperoxic conditions which promote the formation of reactive oxygen species such as H_2O_2 and O_2^-. It has been demonstrated that *A. elegantissima* and some corals are tolerant of hyperoxia (Dykens and Shick, 1982; D'Aoust et al., 1976; Falkowski and Dubinsky, 1981). These observations suggested that symbiotic associations involving invertebrates and algae have effective mechanisms for deactivating reactive oxygen species. SOD has been detected in invertebrate hosts (Dykens and Shick, 1982; Dykens, 1984; Shick and Dykens, 1985) and in *Symbiodinium* spp. (Schoenberg and Trench, 1980; Trench, 1981). Activity of catalase has recently been demonstrated in *Symbiodinium* spp. (Tytler and Trench, 1986) and in crude extracts from hosts harbouring *Symbiodinium* spp. including the clam *Tridacna maxima*, alcyonarian and scleractinian corals, the zoanthid *Palythoa tuberculosa*, the sea anemones *Heteractis magnifica* (Shick and Dykens, 1985) and *A. elegantissima* and the scyphomedusa *Cassiopeia xamachana* (Dykens, 1984). Shick and Dykens (1985) indicated a positive correlation between chlorophyll content of symbiotic systems and the levels of activity of SOD and catalase.

Activity of catalase in uncontaminated, cell-free extracts of animal host tissue has yet to be confirmed and quantified. In this paper we report quantitative measurement of catalase activity in cell-free extracts from *Tridacna maxima* and from several symbiotic, aposymbiotic and non-symbiotic coelenterates.

2. Materials and Methods

Populations of the sea anemones *Aiptasia pulchella* and *A. tagetes* were cloned from individuals originally obtained from Kaneohe Bay, Hawaii and Discovery Bay, Jamaica, respectively. *Anthopleura elegantissima* and *Metridium senile* were collected from the coast of Santa Barbara, CA. The zoanthid *Palythoa* sp. was collected from Kaneohe Bay, Hawaii and the clam *Tridacna maxima* from the Phillipines. The sea anemones *A. elegantissima* and *A. pulchella* were rendered aposymbiotic by maintenance in darkness. Cultured *Symbiodinium* species have been maintained in the laboratory as described elsewhere (see Trench and Blank, 1987).

Extracts from *A. pulchella* and *A. tagetes* were prepared by homogenizing in cold TBS buffer pH 7.8 (Tytler and Spencer-Davies, 1983) with a glass-glass Potter homogenizer. Extracs from *A. elegantissima* and *M. senile* were prepared by first anaesthetizing the anemones in 50% (v/v) sea water, 0.17 M $MgCl_2$ and then blending in cold TBS buffer with a Virtis "45" blender. An extract from *Palythoa* sp. was prepared by detaching a colony from its coral substratum and blending in cold Millipore-filtered (porosity 0.22 μm) sea water in a Sears Counter Craft blender. After cutting the aductor muscle of *T. maxima*, a sample of blood from the pedal sinus was taken by syringe and subsequently the mantle tissue was excised and blended in cold TBS buffer. Large pieces of debris were removed by filtration through muslin or nylon mesh. The homogenates were centrifuged at 20,000×g for 30 min. The sample from *Palythoa* sp. was desalted by passage through a column of Sephadex G25 equilibrated with 25 mM Tris-HCl, pH 8.0, 1 mM Na_2EDTA.

Catalase (E.C. 1.11.1.6) activity was measured as H_2O_2-dependent O_2 evolution at 20°C by adding 10–50 μl samples of the cell-free extracts to 3 ml, 25 mM Tris-HCl pH 8.0, 1 mM Na_2EDTA, using a polarographic O_2 electrode as previously described (Chua, 1971; Tytler et al., 1984; Tytler and Trench, 1986). Replicate samples were taken from different animals. Protein content was determined by the method of Bradford (1976) using ovalbumin as standard.

Table 1. Catalase activity in blood and mantle of *Tridacna maxima* and cell-free extracts from sea anemones with and without symbiotic algae. (S, symbiotic, Ap, aposymbiotic, N, nonsymbiotic).

	Catalase activity (mean ± 1 S.D.) (μmol $O_2 \cdot mg^{-1}$ protein$\cdot h^{-1}$)	n
A. *Tridacna maxima* (S)		
Mantle	97.4 ± 7.7	3
Blood	19.6 ± 1.9	2
B. Sea anemones		
Aiptasia pulchella (S)	480 ± 30	3
A. pulchella (Ap)	5923 ± 775	3
A. tagetes (S)	2574 ± 295	3
Anthopleura elegantissima (S)	260.8 ± 3.8	2
A. elegantissima (Ap)	170.9 ± 29.9	2
Metridium senile (N)	116.1 ± 7.9	3
Palythoa sp. (S)	40.3 ± 4.0	3

3. Results

Activity of catalase was detected in all extracts (Table 1). There is no relation between the level of catalase activity and the presence of algal symbionts. Non-symbiotic *M. senile* had lower catalase activity than the symbiotic actinians, but had higher activity than the symbiotic zoanthid *Palythoa* sp., and the symbiotic clam *T. maxima*. In *A. elegantissima*, catalase activity was higher in symbiotic anemones than in aposymbiotic ones, but in *A. pulchella*, catalase activity was twelve times higher inaposymbiotic anemones than in symbiotic ones. Symbiotic *A. tagetes* had higher levels of catalase activity than symbiotic *A. pulchella*.

In the clam *T. maxima*, catalase activity was five times higher in the mantle tissue than in the blood. Catalase activity in the mantle was higher than that in cell-free extracts of cultured or freshly isolated *Symbiodinium* spp. from *T. maxima* (Tytler and Trench, 1986). Similarly in *A. pulchella*, catalase activity was higher in anemone tissue than in cultured or freshly isolated symbionts. The level of catalase activity in the tissues of *A. elegantissima* was similar to that observed in the algae separated from that host (Tytler and Trench, 1986) but it was not possible to obtain preparations of algae entirely free of animal contamination.

The results presented in Table 1, in the context of the data presented by Tytler and Trench (1986), suggested that the animal hosts are capable of deactivating a larger proportion of H_2O_2 produced by the associations

than their symbiotic algae, and raised the possibility that H_2O_2 produced and released directly by the algae could be deactivated by the hosts. To test this latter possibility, light-dependent H_2O_2 evolution from cultured *Symbiodinium* was assayed by the method of Patterson and Myers (1973). No light-dependent H_2O_2 evolution could be detected in *Symbiodinium* spp. from *A. pulchella* and *T. maxima* or *S. kawagutii* from the coral *Montipora verrucosa* by this method. Freshly isolated algae were not assayed.

4. Discussion

Consistent with the observations of Dykens (1984) and Shick and Dykens (1985), our analyses demonstrated marked differences in the levels of catalase activity in cell-free extracts derived from different animals. For example, catalase activity in aposymbiotic *A. pulchella* was two orders of magnitude higher than that in the symbiotic *Palythoa* sp., and twenty-two times higher than in symbiotic *A. elegantissima*. Similarly Dykens (1984) found that catalase activity in *A. elegantissima* was eight times higher than that in *Cassiopeia xamachana*.

The levels of catalase activity (Table 1) found in animal tissues (on a protein-specific basis) was consistently higher than those found in the algae either freshly isolated from the animals or isolated and maintained in culture (Tytler and Trench, 1986). Taken together, the evidence available indicates that both algal symbionts (Schoenberg and Trench, 1980; Trench, 1981) and animal hosts (Shick and Dykens, 1985; Tytler and Trench, 1986; Table 1) possess SOD and catalase, (Dykens and Shick, 1982). which may well function independently of each other.

We could observe no clear relation between the level of catalase activity in cell-free extracts of animal tissues and the presence of symbiotic algae. Dykens (1984) and Shick and Dykens (1985) found a significant positive correlation between the levels of animal catalase activities and algal biomass (measured as chlorophyll content) in cnidaria and *Tridacna*; in *A. elegantissima*, the correlation was less convincing. Our findings, based on studies with the symbiotic species *A. pulchella*, *T. maxima* and *Palythoa* sp., indicated low levels of animal catalase; only in *A. elegantissima* was there higher catalase activity in animals with symbiotic algae than without. Recent unpublished studies (M. Shick, pers. commun.) with *A. pulchella* are consistent with our observations. Nonetheless, we did observe higher levels of catalase activity in the mantle tissue of *T. maxima* (where the symbiotic algae reside, see Trench et al., 1981), than in the samples of blood taken from the pedal

sinus (where there are no algae).

Release of H_2O_2 by cultured *Symbiodinium* spp. could not demonstrated; the possibility of H_2O_2 release *in hospite* could not be experimentally asessed. Patterson and Myers (1973) concluded that H_2O_2 release by the cyanobacterium *Anacystis (= Synechococcus) nidulans* was due to metabolic defect, and did not expect to observe this as a general phenomenon.

From our observations, it is not clear how significant a role animal catalase plays in combination with SOD in copying with the potentially hyperoxic conditions that may occur during periods of high photosynthesis by symbiotic algae in the tissues or cells of their hosts. It is not known whether *de novo* H_2O_2 production by a host is increased by elevated O_2 tensions experienced during intense photosynthesis by symbiotic algae in their tissues or cells. The role of peroxidases in the deactivation of H_2O_2 in invertebrate hosts with symbiotic algae has still to be assessed.

The correlation between the levels of activity of SOD and catalase and photosynthetic O_2 production by symbiotic algae indicated by Dykens and Shick (1982), Dykens (1984) and Shick and Dykens (1985) is not generally corroborated by our study. Studies of the photoadaptive response of *Symbiodinium* spp. (e.g. Chang et al., 1983) indicate that high chlorophyll content does not (necessarily indicate high photosynthesis, and in turn does not necessarily correlate with a hyperbaric condition resulting directly in elevated SOD and catalase activities. The positive correlation between the elevated levels of catalase activity and the photosynthetic activity of algal endosymbionts found by Dykens (1984) in *C. xamachana*, does not appear to be a universal phenomenon in symbiotic associations. The levels of activity of these enzymes do not always parallel ambient oygen tensions. For example, the activities of SOD, catalase and glutathione peroxidase were elevated in Chinese hamster ovary cells adapted to 99% O_2 (van der Valk et al., 1985), while activities and levels of these enzymes were not elevated in Hela cells adapted to 80% O_2 (Joenje et al., 1985).

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