Pergamon

Marine Pollution Bulletin Vol. 42, No. 9, pp. 742–744, 2001
Published by Elsevier Science Ltd.
Printed in Great Britain
0025-326X/01 $ - see front matter

PII: S0025-326X(00)00210-1

Bioindication Potential of Carbonic Anhydrase Activity in Anemones and Corals

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Activity levels of carbonic anhydrase (CA) were assessed in anemones *Condylactis gigantea* and *Stichodactyla helianthus* with laboratory exposures to copper, nickel, lead, and vanadium, and also in animals collected from polluted vs pristine field sites. CA activity was found to be decreased with increase in metal concentration and also in animals collected from the polluted field site. Preliminary assessments to adapt the CA assay for use in the widespread coral *Montastraea cavernosa* show decreased CA activity in specimens from the polluted field site and provide an avenue for future research aimed at more thoroughly describing coral CA activity for potential application in bioindication. Published by Elsevier Science Ltd.

Keywords: carbonic anhydrase; metal pollution; anemones; coral reefs; Panama.

The activity of the zinc metalloenzyme carbonic anhydrase (CA), ubiquitous in bacteria, plant, and animal kingdoms, plays an important role in respiration by catalysing the interconversion of CO_2 and HCO_3^- and has been widely utilized in bivalve molluscs for bioindication purposes (Bundy, 1977; Henry, 1996). Comparatively much less study has been done on CA activity in anemones and corals, two potential candidates for coral reef bioindication. While oysters and many other bivalves are predisposed to accumulate heavy metal pollutants, anemones and corals have demonstrated considerable ability to regulate metal concentrations and show significantly lower levels of metal accumulation compared to other exposed invertebrates (Scott *et al.*, 1984; Weis, 1991). Proposed regulation mechanisms for the observed disequilibria with environmental metal concentrations include mucus secretion and expulsion of symbiotic zooxanthellae (Brown and Howard, 1985). Weis (1991) described the activity and nature of CA in the anemone *Aiptasia pulchella,* documenting CA activity in both symbiotic and aposymbiotic animals but also supporting the hypothesis that CA activity is enhanced by presence of symbiotic algae. In laboratory exposures both aposymbiotic and symbiotic anemones have only shown marked metal uptake of copper and zinc at concentrations above 0.2 mg l^{-1} (Brown and Howard, 1985).

This research examines the adaptation of an *in vitro* CA assay for use with anemones and corals. If effective in these organisms the CA assay may provide a more direct measure of metal-induced stress quantifiable by monitoring fluctuation in CA activity even as it may be associated with other regulation mechanisms. Harland and Nganro (1990) assess the implications of the proposed metal regulation processes on the use of coelenterates as bioindicators of environmental metal levels and conclude that for a bioassay to be effective, an organism must reflect a quantitative or otherwise predictable relationship with its environment. Through attempts to adapt a CA assay to anemones and corals, this preliminary research seeks to determine if such a relationship, elusive in previous physiological studies quantifying coelenterate metal uptake, may manifest itself on a protein level.

Materials and Methods

Anemone laboratory metal exposures

Anemones *Condylactis gigantea* (Weinland) and *Stichodactyla helianthus* were collected from pristine field sites in Bocas del Toro on the Caribbean coast of Panama. These anemones were acclimated for a one week period in a recirculating, aerated, filtered tank system maintained at approximately 30.5 ppt salinity and 28.5°C. Throughout acclimation the anemones were fed *Artemia nauplii* three times weekly. Three animals of approximately equal size of each species (25–35 mm pedal disc diameter) were then sequestered for 48 h in aerated, filtered tanks for each of the following treatments: Copper: 0, 10, 20, and 40 μg l^{-1}; Nickel: 0 and 40 μg l^{-1}, and 25 mg l^{-1}, Lead: 0 and 40 μg l^{-1}, and 20 mg l^{-1}, and Vanadium: 0, 10, 20, and 40 μg l^{-1}. Following exposure, sections of tentacle tissue were

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dissected from each animal, weighed (wet weights 0.5 ± 0.03 g), and homogenized in 20 mM HEPES buffer pH 8.5 with 100 µl l^{-1} 2-Mercaptoethanol and 100 mM PMSF. (This buffer was used for all anemone experimentation and will be referred to as HEPES solution.) The CA activity in tissue homogenates was determined by rate of decrease in pH resulting from the generation of H^+ associated with the hydration of CO_2 to HCO_3^- following substrate addition. Distilled water saturated with CO_2 served as substrate and was prepared as described by Weis (1991). Procedures were completed as described for the *in vitro* CA assay by Weis *et al.* (1989) with the following adjustments. Three milliliters of the HEPES solution was added to 0.5 ml tissue homogenate supernatant in a small plastic test tube. The probe of a Denver Instruments basic pH meter was immersed in the solution and decrease in pH was recorded for 60 s at 5 s intervals upon reaction catalysis by injection of 0.5 ml substrate. A standard baseline was run using serial dilutions of bovine CA (Worthington Biochemical Corporation Catalog Number: 39M3352) to generate the function: $y = 0.0023x + 0.021 (R^2 = 0.9843)$ where y is the reaction rate as ΔpH units s^{-1} and $x = [CA]$ (µg ml^{-1}). Non-specific change in pH was monitored by control assay with 0.5 ml buffer in place of supernatant.

Field experiments and polluted site collection

Anemones *C. gigantea* and *S. helianthus* and several pieces of coral *Montastraea cavernosa* were collected from a site contaminated with petroleum hydrocarbons from two oil spills in 1968 and 1986 and by the presence of a petroleum refinery near the Smithsonian Galeta field station in Colon on the Caribbean coast of Panama. Several pieces of *M. cavernosa* were also collected from a relatively pristine site near the field station. The animals were kept overnight in the above-described recirculating system and then assayed the following morning for CA activity. The anemones were assayed as described above.

Adaptation of CA assay for use with corals

The corals were assayed similarly to the anemones but with the following adjustments. Approximately 1 ml composites of tissue, mucus, and small pieces of skeleton were scraped from the coral pieces using a chisel and placed in 2 ml eppendorf tubes with 0.5 ml HEPES solution. The mixture was homogenized and then spun at 14000 rpm for 10 min. Mucus and supernatant were separated from skeleton by decantating to a new eppendorf tube. An additional 0.5 ml of HEPES solution was added to this new tube and the sample was spun again at 14000 rpm for 30 min. Supernatant was then cautiously removed and used for assay with procedures described for anemone supernatant.

Regressions of reaction rates were qualified by a minimum of $R^2 = 0.9$ or three data points. Units of CA activity were calculated using a standard generated with bovine CA.

Results

Anemone metal exposures

With the exception of the highly concentrated 25 mg l^{-1} nickel and 20 mg l^{-1} lead treatments which resulted in immediate tentacle withdrawal and death of both species of anemones within 24 h, no bleaching (zooxanthellae expulsion) was evident. CA activity decreased significantly in both *S. helianthus* and *C. gigantea* tissue samples with the other listed exposures to copper, nickel, and lead, and with all exposures over 10 µg l^{-1} vanadium (Fig. 1). Regressions generated for copper and vanadium exposures exhibited polynomial and exponential relationships, respectively (Fig. 2).

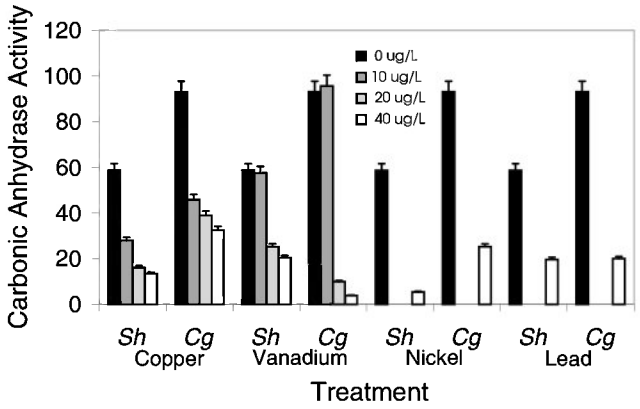

Fig. 1 Carbon anhydrase activity (µg CA/g wet tissue) in *S. helianthus* (Sh) and *C. gigantea* (Cg) with heavy metal exposures. CA activity decreased significantly in both *S. helianthus* and *C. gigantea* tissue samples with exposure to copper, nickel, and lead, and with all exposures over 10 µg l^{-1} vanadium.

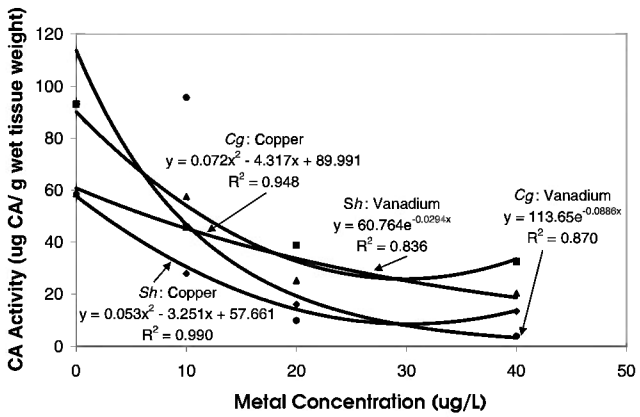

Fig. 2 Trends in carbon anhydrase activity (µg CA/g wet tissue weight) in *S. helianthus* (Sh) and *C. gigantea* (Cg) with heavy metal exposures. Displayed regressions were selected for maximum R^2. No regressions were attempted with nickel or lead exposure data due to lack of sufficient data points. (Highest concentration exposures for these metals resulted in animal death and did not allow for measurement of CA activity.)

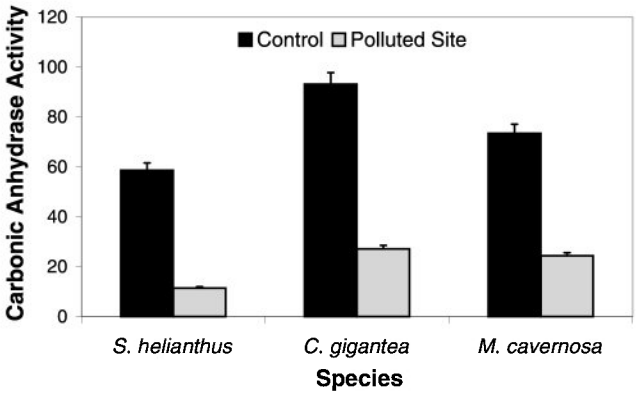

Fig. 3 Carbon anhydrase activity in anemones (μg CA/g wet tissue weight) and the coral *M. cavernosa* (μg CA/ml coral homogenate) from pristine vs polluted sites. CA activity was significantly higher in control anemones and corals than in those collected from the polluted site near a petroleum refinery.

Polluted site collection anemones

CA activity in both species of anemones was significantly lower in samples from the polluted collection site (Fig. 3). For *C. gigantea* and *S. helianthus*, respectively, control samples contained approximately 350% and 500% of the CA activity of samples from the polluted site.

Adaptation of CA assay for use with corals

The adapted assay was functional in measuring CA activity in *M. cavernosa*. When compared with samples from the polluted collection site, coral control samples were found to be significantly higher in CA activity by approximately 300% (Fig. 3).

Discussion and Conclusions

Harland and Nganro (1990) concluded that for a bioassay to be effective, an organism must reflect a quantitative or otherwise predictable relationship with its environment. It seems that while CA activity in *S. helianthus* and *C. gigantea* is affected by environmental stress, and certain polynomial and exponential regressions have been derived for copper and vanadium exposures, further studies are necessary to more completely identify and confirm the sought predictability to these relationships. As decrease in CA activity was observed for metal exposures in which zooxanthellae expulsion was not noted, the CA assay may be useful as a more sensitive measure of stress. It is still unclear, however, whether particularities of CA activity will in the future be attributable to specific causes of stress such as presence of heavy metals or petroleum hydrocarbons or even fluctuations in temperature and salinity. While a stress-specific bioindicator would be ideal, if CA is not found to be as such, there remains application for a wider-scale indicator of stress.

The polluted site study generated promising results for assigning bioindication potential to the studied anemone species. Perhaps with assay functionality in corals and encouraging results contrasting specimens from polluted vs pristine field sites, further research studying CA activity in these animals will be undertaken as well.

We would like to thank A. Domingo, I. Hernandez, and C. Guevara, for all of their help with general research and collection. We thank B. Birinyi at Mayo Clinic for helping to obtain the reagents. This research was partially sponsored by the Elektra Noreste Fellowship Program, Panama (to A.L.G.), the Smithsonian Institution Environmental Science Program, and the Smithsonian Tropical Research Institute.

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