EFFECTS OF HEAVY METALS ON CARBONIC ANHYDRASE AND OSMOREGULATION IN THE BLUE CRAB (*CALLINECTES SAPIDUS*) AND THE GREEN CRAB (*CARCINUS MAENAS*)

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The enzyme carbonic anhydrase (CA), which catalyzes the reversible hydration/dehydration of CO_2 and H_2O, serves a variety of functions in a number of different tissues. In the euryhaline crabs *Callinectes sapidus* and *Carcinus maenas*, CA plays a role in both CO_2 excretion and ion-uptake. In the ion-uptake mechanism, H^+ and HCO_3^- are generated from the catalyzed hydration of CO_2 and water. These ions are then used as counterions for Na^+ and Cl^- uptake, respectively, across the gill.

Inhibition of CA by heavy metals has been documented in lower vertebrates (e.g., channel catfish, *Ictalurus punctatus*) (Christiansen, G. and Tucker, J. *Chem.-Biol. Interactions* 13:181-192, 1976) and euryhaline crustaceans (Rodriguez, E. et al., *Comp Biochem Physiol* 122C: 121-129, 1999). If CA is a site of heavy metal action, multiple transport processes could be affected, and the ability of the animal to invade the dilute waters of the estuary could be compromised. The initial studies in this project involved titrating branchial CA activity from two species of crabs against four metals (Ag, Cd, Zn, and Cu). Cytoplasmic and membrane-associated CA was isolated by differential centrifugation from the gills of crabs acclimated to low salinity (10 ppt). CA activity was monitored by an electrometric delta pH method, and the inhibition data were calculated as double reciprocal plots (Easson, L.H., and E. Stedman, *Proc. Royal Soc. Lond. Ser. B* 121:142-164, 1937) in order to obtain values for Ki, the inhibition constant for each metal.

Branchial CA from *C. sapidus* is much more sensitive to heavy metal inhibition than that from *C. maenas*. Ki values are in the range of 40 nM to 5 uM (Table 1), and while these are higher than Ki values for the powerful sulfonamide inhibitors (e.g., acetazolamide), they still indicate a relatively high affinity between the metal and the enzyme. For CA from *C. maenas*, however, all metals showed weak inhibition, with Ki values in the range of 1 mM. For *C. sapidus*, the similarity between Ki values for Ag, C, and Cu, determined at 1.0 and 2.5 mM CO_2 concentration, indicate that the slopes of the double reciprocal inhibitor plots would be nearly parallel. This, in turn, suggests that the metal is not inhibiting directly at the active site, but rather it is binding to the proton shuttle and preventing the removal of H^+ that result from the first step in the hydration reaction mechanism (Tu et al., *J. Biol. Chem.* 256:9446-94470). Zn does not appear to inhibit in this manner, however.

Table 1. Ki values (uM) for branchial cytoplasmic CA from *Callinectes sapidus* and *Carcinus maenas* titrated against increasing concentrations of heavy metals. Titrations were performed in the presence of two concentrations of substrate (1.0 and 2.5 mM CO_2) and represent the means of triplicate assays.

	C. sapidus		*C. maenas*	
	CO_2 concentration (mM)			
	1.0	2.5	1.0	2.5
Metal				
Ag	0.0583	0.0803	1,208	1,358
Cd	0.5969	0.4656	1,726	1,766
Cu	0.3728	0.4282	960	622
Zn	5.9548	2,1800	1,296	844

Inhibitor titrations and Ki values are less reliable for weak inhibitors, as seen for metal inhibition of CA from *C. maenas*, so the concentration of metal needed to effect 95% inhibition of CA activity was also determined for both cytoplasmic and membrane-associated CA from both species.

Table 2. Concentration of heavy metals (uM) needed for 95% inhibition of branchial cytoplasmic (S3) and membrane-associated (P3) CA activity from *Callinectes sapidus* and *Carcinus maenas*. Values are the means of triplicate assays.

	C. sapidus		*C. maenas*	
	S3	P3	S3	P3
Metal				
Ag	1.67	2,490	2,500	5,810
Cd	16.7	1,992	1,660	3,320
Cu	16.7	1,328	3,330	4,980
Zn	166.7	1,000	1,660	4,150

Once again, there are large differences in metal sensitivity between cytoplasmic CA of *C. sapidus* vs *C. maenas*, with the former being more sensitive to metal inhibition by a factor of about one thousand (Table 2). In addition, there is an equally large difference in sensitivity between the cytoplasmic and membrane-associated fractions within the gill of *C. sapidus*. The difference is much less pronounced in *C. maenas*.

These results suggest a number of interesting conclusions. First, it appears that *C. maenas* possesses a more metal-resistant CA isoform in its branchial cytoplasm than does *C. sapidus*. Because cytoplasmic CA supplies multiple ion transport processes in the gill, the systemic mechanism of osmoregulation could be more resistant to heavy metal toxicity in *C. maenas*. Unless heavy metals are accumulated to a great extent within the gill, it is doubtful that branchial CA in *C. maenas* would ever be significantly inhibited, as metal concentrations in the water would never approach the Ki values for the enzyme. Further

evidence supporting this idea came from whole-organism experiments, in which neither Cd nor Zn (0.1 to 10 mM) had any effect on mortality or hemolymph osmotic and ionic concentrations in crabs acclimated to 33 ppt and transferred to 10 ppt (data not shown). This may allow *C. maenas* to invade dilute estuarine waters and exploit habitats that are impacted by heavy metals to a greater degree than other crustacean species.

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