BioMetals (2005) 18:537–540
DOI 10.1007/s10534-005-0836-0


# Evidence for the $Cd^{2+}$ activation of the aryl sulfatase from *helix pomatia*

Abigail M. Tokheim[1], Donna J. Spannaus-Martin[2] & Bruce L. Martin[1,3,*]
[1]*Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware Street, S.E., Minneapolis, MN 55455, USA;* [2]*Division of Medical Technology, University of Minnesota, 420 Delaware Street, S.E., Minneapolis, MN 55455, USA;* [3]*Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, 321 Church Street, SE, Minneapolis, MN 55455, USA; *Author for correspondence (E-mail: marti285@umn.edu; Tel: +1-612-625-5988)*

Received 15 March 2005; accepted 13 July 2005

*Key words:* Aryl sulfatase, activation, *Helix pomatia*, Metal ions, enzyme activation

**Abstract**

Often used to remove sulfate groups from carbohydrates, the regulatory properties of the aryl sulfatase from *Helix pomatia* remain little characterized. As many hydrolytic enzymes utilize exogenous metal ions in catalysis, the effect of various divalent metal ions on the sulfatase was investigated. Evidence for metal ion activation was collected, with $Cd^{2+}$ being notable for effective activation. The enzyme was inhibited by $Cu^{2+}$. The response of other common hydrolases to divalent metal ions was characterized. Activation by $Cd^{2+}$ was not observed for chymotrypsin, rabbit liver esterase, or β-galactosidase. Instead, Cd was found to inhibit both the esterase and the galactosidase. Inhibition by $Cu^{2+}$ and $Zn^{2+}$ was also observed for some of these hydrolases.

## Introduction

Bioconjugation of small molecules is an ubiquitous enzyme-catalyzed reaction involved in numerous pathways including the processing of nutrients and the detoxification of xenobiotics. One such reaction is the sulfation of molecules for the removal of sulfate and phenolic compounds (such as neurotransmitters) through the action of phenol sulfotransferases. Sulfation catalyzed by sulfotransferases can be either O-directed or N-directed and specific enzymes are known for these reactions (Chapman et al. 2004). Less clearly understood is the role of aryl sulfatases which catalyze the hydrolytic removal of the sulfate group from substrates (Parenti et al. 1997). Minimally, the sulfatases provide maintenance of sulfate levels for subsequent conjugation reactions and for the recovery of sulfated molecules.

Multiple strategies have been adopted by enzymes to catalyze biomolecular transformations, including the use of metal ions as cofactors in the reaction. Multiple roles for metals have been ascribed in these enzymes, including (1) orientation of substrate and enzymic functional groups; (2) activation of the nucleophilicity of a reactive group, notably water or hydroxide; and (3) electrophilic participation such as activation of a proton transfer. Hydrolytic enzymes commonly use metal ions in catalysis, but little has been reported regarding the action of the sulfatase class of enzymes. The effect of divalent metal ions on the activity of an aryl sulfatase has been studied. For comparison, the effects of metal ions on other selected hydrolases have been characterized.

## Experimental Procedures

### *Materials*

The aryl sulfatase from *Helix pomatia* was purchased from Sigma-Aldrich as was its substrate,

*para*-nitrophenylsulfate (pNPS) and the buffer (MOPS) used in the assays. The other hydrolases studied, α-chymotrypsin (bovine pancreas; C-4129), aryl esterase (rabbit liver; E-0887), and β-galactosidase (bovine liver; G-1875) were purchased from Sigma-Aldrich as well as were the substrates used, *para*-nitrophenyl acetate, *para*-nitrophenyl butyrate, and *ortho*-nitrophenyl β-D-galactopyranoside. Other reagents, notably the metal salts, used were from Fisher Scientific. All solutions were prepared using Chelex-100 treated water or buffers. Protein concentrations were determined by the Bradford assay (Bradford 1976).

*Enzyme assays*

The aryl sulfatase from *Helix pomatia* was assayed at 30 °C in the following reaction mixture: 20 mM MOPS, pH 7.0; and 1.0 mM pNPS. The reaction was monitored by the release of pNP at 410 nm. The standard assay mixture contained 10 μg/ml sulfatase. To measure the effects of various metal ions on the activity of sulfatase, metal ions typically were added to a final concentration of 1.0 mM. Because of previous experience with $Mg^{2+}$, experiments were done with this metal ion at 20.0 mM for comparison to transition metal ions. The assays were run in triplicate over a ten minute time course using a Beckman model 650 Spectrophotometer. Additional experiments were done with $CdCl_2$ and $CuCl_2$ to further characterize their effects on the sulfatase. The concentration of the chosen metal ion was varied from 10 μM to 1 mM in these experiments. Chymotrypsin, aryl esterase, and galactosidase were all assayed in 20 mM Mops, pH 7.5 at 30 °C. Chymotrypsin was assayed using 20 mM *para*-nitrophenyl acetate; aryl esterase was assayed using 0.1 mM *para*-nitrophenyl butyrate; and galactosidase was assayed using *ortho*-nitrophenyl-β-D-galactopyranoside. The reactions were monitored by the release of nitrophenol as measured spectrophotometrically at 410 nm using a Beckman model DU7400 Spectrophotometer. As with the aryl sulfatase, assays were done in the presence of various divalent metal ions. Select metal ions were further characterized as inhibitors when supported by the data. Data fitting and numerical estimates ($IC_{50}$ values) were done using the program Deltagraph (SPSS, Inc.).

## Results and discussion

*Effect of divalent metal ions on an aryl sulfatase*

The aryl sulfatase from *Helix pomatia* was assayed using *para*-nitrophenyl sulfate as substrate with the addition of divalent metal ions to 1.0 mM, except for $Mg^{2+}$ which was screened at 20.0 mM. The metal ions screened are indicated

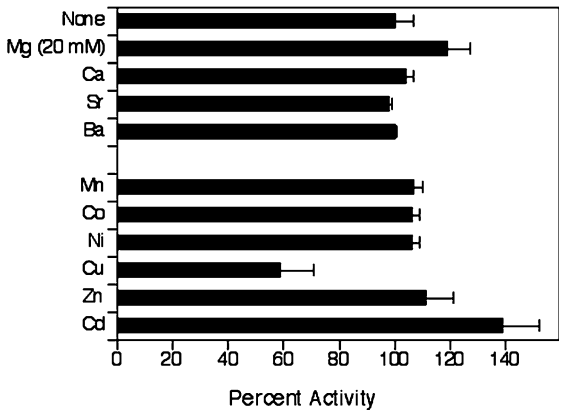


*Figure 1.* Effect of metal ions on aryl sulfatase activity. Sulfatase activity was assayed by measuring the release of pNP from pNPS as described in the text. Each data point is the average of 3 determinations with the error bars showing the standard deviation of the measurement.

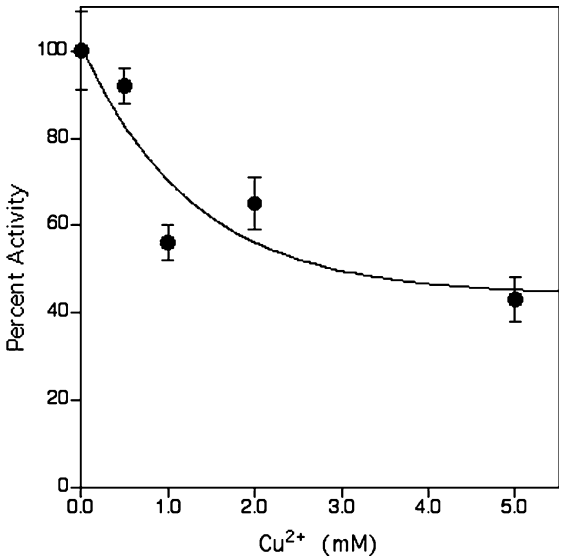


*Figure 2.* Inhibition of aryl sulfatase by $Cu^{2+}$. Activity was measured by monitoring the release of pNP from pNPS as described in the text. Each data point is the average of 3 determinations with the error bars showing the standard deviation of the measurement.

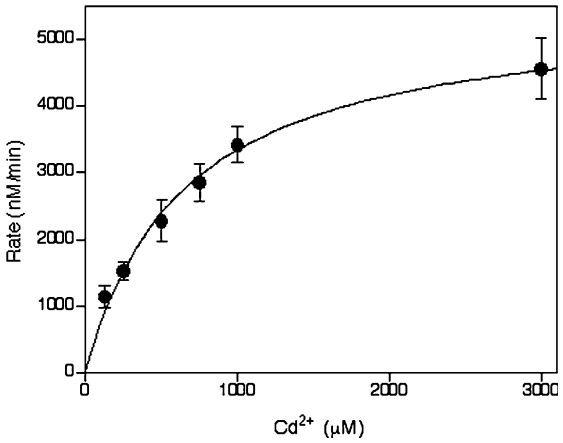


*Figure 3*. Activation of aryl sulfatase by $Cd^{2+}$. Activity was measured by monitoring the release of pNP from pNPS as described in the text. The activity measurements shown were corrected for activity in the absence of metal ion. Each data point is the average of three determinations with the error bars showing the standard deviation of the measurement.

in Figure 1 showing the level of activity supported. Essentially no effect was observed with most of the metal ions tested. Inhibition was observed with $Cu^{2+}$. The inhibition by $Cu^{2+}$ was evaluated more fully as shown in Figure 2 yielding an $IC_{50}$ value of 2.9 $\mu$M for $Cu^{2+}$. More interesting, 39% activation was observed with $Cd^{2+}$ at 1.0 mM compared to activity without added metal ion. This was an unexpected event considering the reputation of $Cd^{2+}$ as a toxic heavy metal. Characterization of the activation curve was done and is shown in Figure 3. A value of 652 $\mu$M was calculated for $K_{act}$ from these data. These data may account for the differential hepatotoxicity caused by cadmium and copper in *Helix pomatia* (Manzl et al. 2004). Although both metal ions were found to be toxic, copper was more toxic. *Helix aspersa aspersa* and *H. aspersa maxima* are snails distinct from *Helix pomatia* and both respond negatively to $Cd^{2+}$. $Cd^{2+}$ caused decreased fecundity and growth, but no mortality difference over four months (Laskowski & Hopkin 1996; Gomot 1997). It is intriguing to question whether the biological effects of $Cd^{2+}$ on these snails include the activation of the aryl sulfatase.

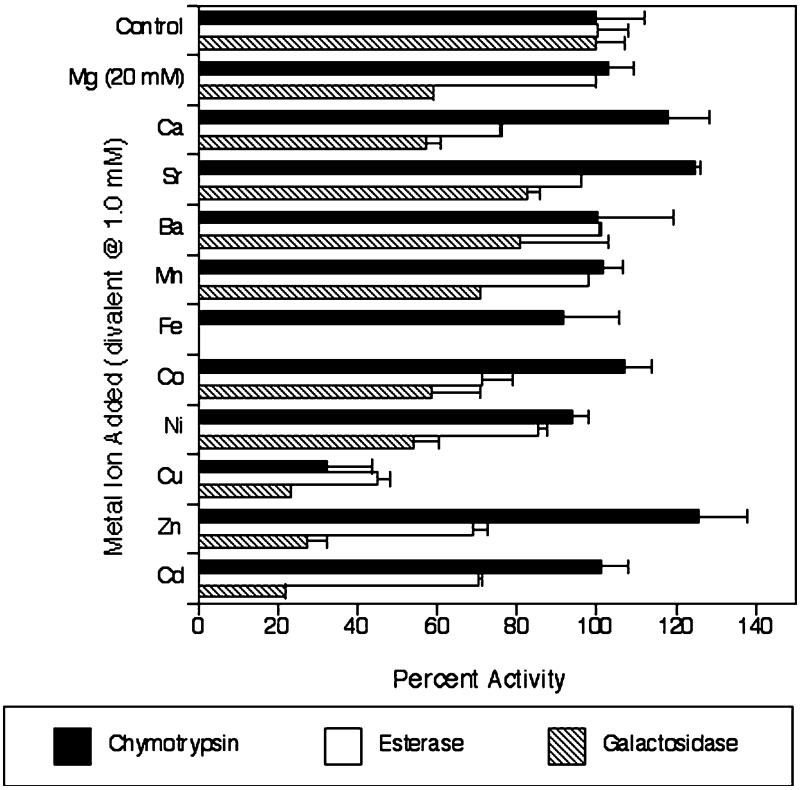


*Figure 4*. Effect of metal ions on the activity of hydrolytic enzymes. Each enzyme was assayed with an appropriate ester of *para*-nitrophenol and their activities were measuring by monitoring the release of pNP from the substrates as described in the text. Each data point is the average of 3 determinations with the error bars showing the standard deviation of the measurement.

*Effect of divalent metal ions on other hydrolases*

Because of the apparent anomalous results with the aryl sulfatase, the responses of other common hydrolytic enzymes to the divalent metal ions were evaluated for comparison to the sulfatase. Surprisingly, no report was found in available literature regarding the effect of a panel of metal ions on these enzymes. Chymotrypsin has been shown to bind $Cd^{2+}$ in place of $Ca^{2+}$ (Abedodun & Jordan 1989) and an aryl esterase from gastropods was found to be indirectly down regulated by $Cd^{2+}$ (Mazon et al. 1998). Figure 4 shows the collected data for the screening of metal ions with the enzymes. Chymotrypsin was little affected by the metal ions, except for inhibition by $Cu^{2+}$. There was evidence of precipitation with $Cu^{2+}$ and the inhibition of chymotrypsin was not characterized further. No other example of $Cd^{2+}$ activation was observed, but both the aryl esterase and the β-galactosidase were inhibited by $Cd^{2+}$ consistent with its toxic effect. Each of these enzymes also was inhibited by $Cu^{2+}$ and $Zn^{2+}$, again consistent with the expected effects. The galactosidase was more sensitive to inhibition than was the esterase, particularly to $Cd^{2+}$, for which there was an approximately 10-fold difference in $IC_{50}$ values. Table 1 collects the inhibition data for all the enzyme and metal ions characterized. For some metal ions, particularly $Fe^{2+}$, precipitation problems prevented the characterization of any activity effect.

*Significance of cadmium activation of aryl sulfatase*

These data showed $Cu^{2+}$ having a broad set of targets and causing inhibition and consistent with its toxic effects being manifested by the inhibition of enzymes. The data for $Cd^{2+}$ was not the same for all enzymes. For two of the hydrolytic enzymes, $Cd^{2+}$ did inhibit activity consistent with a toxic effect but activation was observed with the aryl sulfatase. This result has been interpreted with the perspective that the sulfatase may be responding to $Cd^{2+}$ as a means to recover conjugated molecules for the subsequent processing of $Cd^{2+}$. One possible pathway is the recovery of sulfate for conversion to sulfide and the formation and excretion of cadmium sulfide. In this manner, activation of sulfatase by $Cd^{2+}$ provides a means for the organism to remove the toxin. Because cadmium deposited in the kidney has a long duration, its removal is critical before it can be reabsorbed by the intestine. Cadmium activation may also result in novel substrates being hydrolyzed. Attempts to demonstrate hydrolysis of sulfo-cholecystokinin by the aryl sulfatase have not been successful.

*Table 1.* Inhibition by metal ions.

| Enzyme | Values of $IC_{50}$ (mM) | | |
|---|---|---|---|
| | $Cu^{2+}$ | $Zn^{2+}$ | $Cd^{2+}$ |
| Sulfatase | 2.90 | | Activation |
| Esterase | 0.32 | 0.94 | 1.37 |
| Galactosidase | ppt | 0.15 | 0.14 |

$Zn^{2+}$ did not demonstrate any effect on the aryl sulfatase. In the assays of the galactosidase, there was some precipitation with $Cu^{2+}$, particularly at higher concentrations making measurement of the $IC_{50}$ value problematic.

## References


Adebodun F, Jordan F. 1989 Multinuclear magnetic resonance studies on the calcium (II) binding site in trypsin, chymotrypsin, and subtilisin. *Biochemistry* **28**, 7524–7531.

Bradford MM. 1976 A rapid and sensitive method for the quantitation for microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.

Chapman E, Best MD, Hanson SR, Wong CH. 2004 Sulfotransferases: structure, mechanism, biological activity, inhibition, and synthetic utility. *Angew Chem Int Ed Engl.* **43**, 3526–3548.

Gomot A. 1997 Dose-dependent effects of cadmium on the growth of snails in toxicity bioassays. *Arch Environ Contam Toxicol* **33**, 209–216.

Laskowski R, Hopkin SP. 1996 Effect of Zn, Cu, Pb, and Cd on fitness in snails (*Helix aspersa*). *Ecotoxicol Environ Saf* **34**, 59–69.

Manzl C, Krumschnabel G, Schwarzbaum PJ, Dallinger R. 2004 Acute toxicity of cadmium and copper in hepatopancreas cells from the Roman snail (*Helix pomatia*). *Comp Biochem Physiol C Toxicol Pharmacol* **138**, 45–52.

Mazon LI, Gonzalez G, Vicario A, Estomba A, Aguirre A. 1998 Inhibition of esterases in the marine gastropod *Littorina littorea* exposed to cadmium. *Ecotoxicol Environ Saf* **41**, 284–287.

Parenti G, Meroni G, Ballabio A. 1997 The sulfatase gene family. *Curr Opin Genet Dev* **7**, 386–391.