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Academic Year URG Final Report

The effect of anthropogenically and naturally produced organic ligands on the bioavailability of mercury in aquatic systems

During my winter independent study, I was able to analyze the bacterial biouptake of mercury in the presence of four anthropogenically produced ligands (EDTA, EDDS, DTPA, and NTA) and three ligands that are present in aquatic environments naturally (cysteine, penicillamine, and glutathione). I quantified the bioavailability of mercury in the presence of these ligands using a genetically modified E. coli biosensor that emits light at intensities proportional to the amount of mercury that enters the bacterial cell. The speciation of mercury in the presence of the organic ligands was determined using ChemEQL, and I looked for correlations between mercury species and mercury bioavailability. According to the Free Ion Activity Model, the bioavailability of a trace metal depends on the concentration of the free metal ion. Increasing concentrations of organic ligands that have a high binding affinity for metals decreases the concentration of the free metal ion; thus, the bioavailability of that metal is expected to decrease as well. When I compared my bioavailability results to the mercury speciation, I found five out of the seven ligands I tested greatly disobeyed the Free Ion Activity Model.

As representative examples of this observed phenomenon, graphs of the normalized luminescence signal emitted by the biosensor in the presence of 30nM Hg and various concentrations of EDTA and cysteine is presented in Figure 1. The speciation of mercury in the presence of the specified concentrations of EDTA and cysteine is included in Table 1. From the figure, it is clear that the introduction of greater concentrations of EDTA in the presence of 30nM Hg did not decrease the bioavailability, but in fact enhanced it. Looking at the mercury speciation in the presence of EDTA, no mercury is bound to EDTA at 0.1µM EDTA. An increasing fraction of the total mercury is bound to EDTA as the concentration of EDTA increases until all the mercury is bound to EDTA at 1000µM. From the speciation, I would expect the bioavailability or luminescence signal to decrease as more mercury is bound to EDTA. Similar trends were found for the other three organic

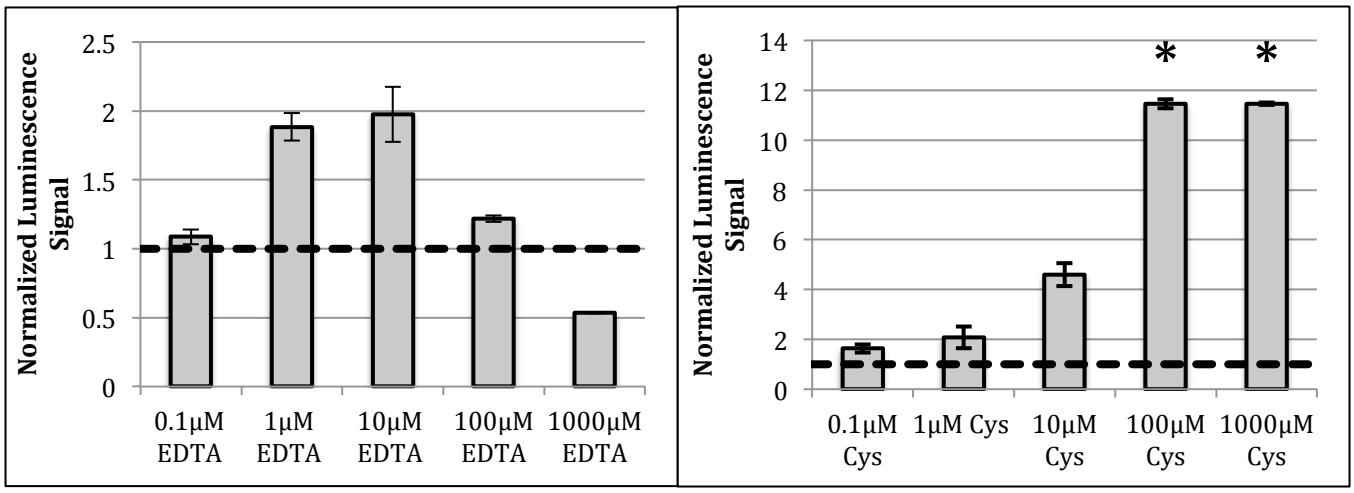

Figure 1: The normalized luminescence signal emitted by the biosensor for increasing concentrations of EDTA and cysteine in the presence of 30nM Hg. The dashed line indicates the signal of the control, where no organic ligand was added.

Table 1: The percentage of mercury bound to different concentrations of the organic ligand of interest

Species	**Percentage of Total Mercury**				
	.1μM	**1μM**	**10μM**	**100μM**	**1000μM**
EDTA					
$HgEDTA^{2-}$	0%	2%	15%	68%	93%
$HgOHEDTA^{3-}$	0%	0%	1%	4%	7%
Cysteine					
$Hg(Cysteine)_2^{2-}$	100%	100%	100%	100%	100%

ligands with structures related to EDTA. The luminescence of the biosensor does not only depend on the presence of Hg but requires substrate to provide energy as well. The biosensor is situated in nutrient limited media in order to ensure Hg complexation with the introduced organic ligands; therefore, it is possible that the addition of EDTA is providing the biosensor with an essential trace metal or nutrient that is limiting the production of luminescence proteins. Another possible explanation for the increase in luminescence signal in the presence of EDTA could be attributed to EDTA's ability to disorganize the outer membrane of gram negative bacteria, which includes the E. coli biosensor. It is possible that Hg-EDTA complexes are able to pass through disorganized cell membranes, which would explain an increase in Hg bioavailability corresponding to higher Hg-EDTA complex concentrations.

To test my hypothesis that EDTA and related ligands were capable of disorganizing and permeating the outer membrane of the biosensor allowing Hg-EDTA complexes to be taken up, I examined the membrane integrity of the biosensor using a viability probe. I did not observe a significant difference in membrane integrity in the presence of the concentrations of EDTA used in the biosensor experiment. However, I attribute my results to the method chosen; another method should be used to confirm that EDTA does not disorganize the outer membrane to the point where an Hg-EDTA complex can pass through.

In the case of cysteine, it is clear from Figure 1 that the bioavailability of mercury in the presence of 100µM and 1000µM cysteine was greatly enhanced by a factor greater than 10, but 100% of the mercury was present as $Hg(Cysteine)_2^{2-}$ for all cysteine concentrations. These results correspond to another study examining the bioavailability of mercury in the presence of cysteine through the measurement of mercury methylation rates. One possible explanation for the enhancement in bioavailability could be the resemblance of $Hg(Cysteine)_2^{2-}$ to cystine, an amino acid. It is possible that the biosensor has a transporter on the outer membrane to uptake cystine, and the cell is mistakenly taking up $Hg(Cysteine)_2^{2-}$ instead.

Because I was able to demonstrate that the bacterial biouptake of Hg cannot be predicted by mercury speciation alone, I feel my research will benefit the scientific community. This summer I plan to publish a paper in a scientific journal, and I will present my results at the 22nd V.M. Goldschmidt Conference in Montreal as well.