Bioavailability of Hg in Aquatic Systems Using a Whole-Cell Bacterial Biosensor

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Introduction

Mercury (Hg) has long been considered as one of the most hazardous contaminants in aquatic environments, resulting in more fresh water fish advisories in the U.S. than any other anthropogenically-released pollutants [1]. Most of the Hg released from anthropogenic activities is derived from the combustion of fossil fuels (i.e., coal burning power plants), making Hg emissions coupled to those of CO₂ [2]. Therefore, as the concentration of CO₂ in atmosphere continues to increase, the problem of Hg contamination is likely to be concomitantly aggravated. Among the various Hg species, methylmercury (CH₃Hg⁺, MeHg) is the most toxic due to its high bioaccumulation potential and neurotoxicity. The U.S. Environmental Protection Agency (U.S. EPA) has stated that consumption of fish and shellfish contaminated with this toxin is the primary route of human exposure to Hg [3]. Over 95% of the MeHg found in aquatic environments is converted from inorganic Hg by sulfate-reducing bacteria in anoxic sediments [4], and it is well accepted that Hg methylation occurs only after Hg has passed through the cell membrane and entered the cell [5, 6]. Accordingly, it is essential to understand the factors influencing the bacterial uptake of Hg, in order to ensure the success of future efforts on MeHg production control and to protect public health.

The chemical speciation of metals affects their bioavailability [7]. However, knowledge is still lacking on the mechanisms underlying Hg uptake by bacteria, partially due to the difficulty of directly measuring intracellular Hg. Already established models, such as the free-ion activity model (FIAM) [7] and the biotic ligand model (BLM) [8], suggest that formation of metal-ligand complexes reduce metal bioavailability. But its validity on Hg has been challenged by recent studies that Hg uptake by bacteria was enhanced by some organic ligands [9, 10]. Fortunately, recent developments in biosensor technology led to mercury-sensitive bioreporters that could be utilized to monitor intracellular mercury [11-13], providing us with a powerful alternative to identify the compounds that facilitate the Hg across the bacterial cell membrane.

In this research, a whole-cell biosensor based on a genetically engineered strain of *E. coli* was applied to probe the effects of different organic ligands on Hg uptake by bacteria. Two groups of ligands, aminopolyacrylic and thiol-containing ligands, were tested due to their potential presence in natural aquatic environment as well as strong affinity to Hg. Through developing a dose-dependent effect of these ligands on Hg biouptake, our results emphasize the importance of organic ligands on Hg bioavailability and methylation, but invalidates the sole use of models based on Hg chemical speciation to predict Hg bioavailability. Our study provides new insights into the possible mechanisms that control Hg bioavailability in aquatic systems, which would be beneficial to scientific community and environmental regulating agencies.
Results and discussion

1. Whole-cell biosensor assay

Our whole-cell biosensor, named *E. coli* ARL 1, contains a merR::luxCDABE fusion inserted into the chromosome of *E. coli* and produces bioluminescence in the presence of intracellular mercury. The detailed gene information and principle of our biosensor system are shown in Figure 1.

![Figure 1](image1.png)

(A) Diagram of plasmid pUTK80 containing mer::luxCDABE construct that was inserted into chromosome of *E. coli* ARL 1 [14]; (B) Schematic of biosensor principle [15].

Standard solutions of 10~100 nM mercury chloride (HgCl₂) were used to build a dose-response relationship of the biosensor system (Figure 2). Generally, a lag phase was observed within the first 20 minutes of *E. coli* exposure to Hg, after which the bioluminescence increased exponentially. Then the biosensor signal reached a relatively stable value and started to slightly decrease (Figure 2A). A dose-response curve with 95% confidence interval was created from the highest signal produced by the biosensor (Figure 2B). Within the range of 10~50 nM Hg, the bioluminescence signal is linearly proportional to the Hg concentration in the sample, indicating that our biosensor is a reliable indicator quantitatively measuring bioavailable Hg.

![Figure 2](image2.png)

(A) Time-dependent biosensor signal from different concentrations of HgCl₂; (B) Hg dose-response curve with 95% confidence interval.
2. Biosensor response and predicted mercury speciation in the presence of aminopolycarboxylic ligands

The four aminopolycarboxylic ligands analyzed in this study include ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-disuccinic acid (EDDS), diethylenetriaminepentaacetic acid (DTPA), and nitrilotriacetic acid (NTA). They are anthropogenically produced and potential to be released into the aquatic environments. Figure 3 illustrates the dose-effects of these aminopolycarboxylic ligands on biosensor signal. Except for NTA, all the ligands exhibited a bell-shape dose-effect. Specifically, the greatest Hg biouptake was observed at the moderate concentration of ligands (10μM, 1μM and 10μM for EDTA, EDDS and DTPA, respectively), enhanced by a factor of at least two compared to control (only Hg). For NTA, the Hg uptake increased as more ligands were added. In order to evaluate whether Hg bioavailability was correlated to the Hg species in the presence of organic ligands, chemical speciation of Hg was determined for each experimental condition using the ChemEQL software (developed by EAWAG/ETH, Switzerland). However, no relationship was observed between Hg bioavailability and chemical speciation. For example, the percentage of Hg-EDTA complexes in total Hg increased from 0% to 100% as EDTA concentrations increased from 0.1μM to 1000μM, which is unable to explain the appearance of highest Hg bioavailability at 10μM. In addition, the increase of Hg biouptake in the presence of EDTA was unexpected because formation of Hg-EDTA complex was supposed to decrease Hg bioavailability according to the FIAM model. Similar inability of chemical speciation to predict Hg bioavailability was also observed for the other ligands.

![Figure 3](image)

Figure 3 Biosensor signal in the presence of increasing concentrations of EDTA (A), EDDS (B), DTPA (C) and NTA (D) normalized to 30nM Hg control. Asterisk indicates the biosensor signal reached the upper detection limit.
3. Biosensor response and predicted mercury speciation in presence of thiol-containing ligands

The three thiol-containing ligands analyzed in this study include cysteine, penicillamine (PEN), and glutathione (GSH). They are naturally present in the aquatic environments and participate in important cell metabolic activities such as protein synthesis and anti-oxidation. Figure 4 shows the dose-effects of these ligands on Hg bioavailability. Cysteine was found to enhance Hg biouptake as its concentration increased, consistent with a recent study by Schaefer and Morel that cysteine enhanced Hg methylation rate by sulfur-reducing bacteria [9]. But calculated chemical speciation reveals that all the Hg formed Hg(cysteine)$_2$ complex even at the lowest concentration of cysteine (0.1μM), therefore no change of Hg speciation occurred as concentration of more cysteine was added. PEN and GSH inhibited Hg biouptake dramatically when increasing their concentration. This phenomenon may be due to their larger structures than cysteine, which lead to Hg-ligand complexes that are too big to enter the bacterial cells.

![Figure 4](image)

**Figure 4** Biosensor signal in the presence of increasing concentrations of cysteine (A), PEN (B), and GSH (C) normalized to 30nM Hg control. Asterisk indicates the biosensor signal reached the upper detection limit.

**Conclusion and broader impacts**

With the help of a whole-cell biosensor, we show that different organic ligands exhibited various behaviors in changing Hg bioavailability to bacteria, indicating the importance of organic ligands in influencing Hg bioavailability and methylation in the aquatic environments. Neither established bioavailability model nor calculated chemical speciation was able to predict Hg bioavailability. Therefore, it is inappropriate to assess environmental risks posed by Hg solely based on these methods. Our research not only deepens our understanding of Hg bioavailability, but also provides new data supporting environmental regulating agencies to make reasonable regulatory framework on Hg. Two oral presentations have been given at the International Materials Institute for Solar Energy and Environment workshop (September 2011, China) and 2012 22nd V.M Goldschmidt Conference (July 2012, Canada). One manuscript is in preparation to be submitted to *Environmental Chemistry* this October.
References