ESRF	**Experiment title:** HERFD of Hg(II) coordination in bacterial samples	**Experiment number**: 30-02-1118
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Report:

Increased anthropogenic mercury (Hg) release into the environment directly leads to higher methylmercury (MeHg) concentrations in fish.[1] Certain bacteria (*e.g.*, sulfate and iron reducing) as well as archaea (*e.g.*, methanogens) found in sediment primarily produce MeHg, requiring inorganic Hg(II) to first pass through the cell's membrane layers.[2, 3] However, a pathway for Hg(II) biouptake has yet to be discovered. To gain insight into bacterial Hg(II) uptake, our experiment (proposal reference number 52529) involved using high energy resolution fluoresence detection (HERFD) to determine the coordination environment of Hg(II) in bacteria exposed to low concentrations of total Hg. Due to the simplicity of modifying the genome, we tested mutant and wild-type strains of *Escherichia coli* (*E. coli*) as a preliminary experiment to specifically explore the role of single genes suspected to influence Hg(II)-cell coordination.

Experiment: *E. coli* strains were harvested in exponential growth phase and exposed to varying concentrations of total Hg(II) and cystiene – a ligand with a high Hg(II) affinity – for 3 hours. Afterwards, the cells were washed 2 times in 0.1 M $NaClO_4$, collected on 0.2 μm cellulose nitrate filter paper, and sandwiched between 2 pieces of Kapton tape. All samples were plunged in liquid nitrogen and remained frozen throughout analysis. All Hg standards and samples were measured in HERFD mode with spherically bent Si crystal analyzers (radius = 0.5 m, diameter = 0.1 m). The Hg $L_{\alpha 1}$ fluoresence line was selected using the 555 reflection, and the diffracted fluorescence was measured with a silicon drift detector (SDD, Vortex EX-90). Data normalization and linear combination fits of the XANES derivative to determine Hg speciation in bacterial samples were performed with Athena.[4]

Results: Our previous XAS results collected at the APS with conventional XANES and EXAFS showed that bacteria will coordinate Hg(II) with 4 sulfur atoms, but the nature of the sulfur atoms was difficult to determine (i.e., organic $Hg(SR)_4$ or inorganic β-$HgS_{(s)}$).[5] In this preliminary experiment, we show that the HR-XANES of $Hg(Cys)_4$ and β-$HgS_{(s)}$ standards are easily distinguished (Figure 1). We did not detect the $Hg(Cys)_4$ signature in any of the bacterial samples that we tested; however, we found the β-$HgS_{(s)}$ signature in all samples (Table 1). We have also previously shown that cysteine is degraded by exponentially growing cells into sulfide,[5] likely by cysteine desulfhydrase enzymes.[6] Cysteine degradation into sulfide may be the sulfide source for the formation of cell-assosciated β-$HgS_{(s)}$, explaining why exogenous cysteine addition to the wild-type strain leads to more cell-associated β-$HgS_{(s)}$ (Table 1).

Table 1: Cell-associated Hg speciation from HR-XANES

Sample	$Hg(Cys)_2$	α-HgS	β-HgS
50 nM Hg + 100 µM Cys (wt)	42.2	0	57.8
500 nM Hg (wt)	28.6	17.0	54.4
500 nM Hg + 1 mM Cys (wt)	22.1	0	77.9
500 nM Hg (Δ*ybaO*)	9.6	19.1	71.3
500 nM Hg + 1 mM Cys (Δ*ybaO*)	6.8	85.1	8.1

*wt = wild-type, Δ*ybaO* = mutant lacking cysteine desulfhydrase enzyme

Interestingly, cell-associated β-$HgS_{(s)}$ is also forming in the absence of exogenous cysteine (sample 500 nM Hg (wt)). To test the effect of cysteine degradation by cysteine desulfhydrase enzymes on Hg(II)-cell coordination, we also probed a mutant strain of *E. coli* (Δ*ybaO*). To our surprise, the removal of one pathway for cysteine degradation to sulfide in the cell actually increased the presence of cell-associated β-$HgS_{(s)}$ compared the the wild-type (Table 1). Additionally, the Hg speciation assocaited with the mutant strain that was exposed to 500 nM Hg and 1 mM cysteine was primarily α-$HgS_{(s)}$, unlike the wild-type where cell-associated Hg was primarily β-$HgS_{(s)}$. From our HR-XANES results, it is clear that the cysteine desulfhydrase mutant still has the ability to produce sulfide via another pathway. Although biogenic sulfide production was not completely inhibited with the cysteine desulfhydrase mutant, we demonstrate that altering the sulfide chemistry in the cells does change the Hg(II)-cell coordination in the presence and absence of exogenous cysteine. The role of biosynthesized sulfide in Hg(II) coordination and biouptake should be explored further, as we find that sulfide is responsible for coordinating Hg(II) in *E. coli*.

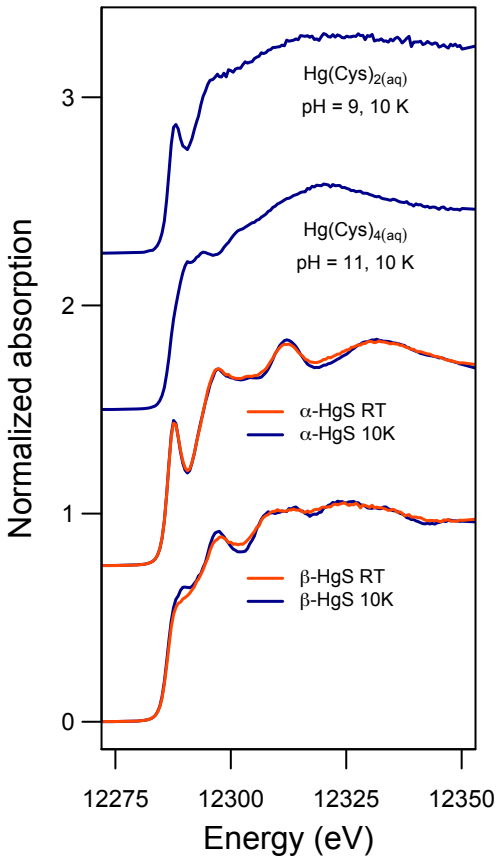

Figure 1: HR-XANES of Hg standards measured at the Hg L_{III}-edge.

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