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The effect of natural organic matter on the adsorption of mercury to bacterial cells

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Abstract

We investigated the ability of non-metabolizing *Bacillus subtilis*, *Shewanella oneidensis* MR-1, and *Geobacter sulfurreducens* bacterial species to adsorb mercury in the absence and presence of Suwanee River fulvic acid (FA). Bulk adsorption and X-ray absorption spectroscopy (XAS) experiments were conducted at three pH conditions, and the results indicate that the presence of FA decreases the extent of Hg adsorption to biomass under all of the pH conditions studied. Hg XAS results show that the presence of FA does not alter the binding environment of Hg adsorbed onto the biomass regardless of pH or FA concentration, indicating that ternary bacteria–Hg–FA complexes do not form to an appreciable extent under the experimental conditions, and that Hg binding on the bacteria is dominated by sulfhydryl binding. We used the experimental results to calculate apparent partition coefficients, K_d, for Hg under each experimental condition. The calculations yield similar coefficients for Hg onto each of the bacterial species studies, suggesting there is no significant difference in Hg partitioning between the three bacterial species. The calculations also indicate similar coefficients for Hg–bacteria and Hg–FA complexes. S XAS measurements confirm the presence of sulfhydryl sites on both the FA and bacterial cells, and demonstrate the presence of a wide range of S moieties on the FA in contrast to the bacterial biomass, whose S sites are dominated by thiols. Our results suggest that although FA can compete with bacterial binding sites for aqueous Hg, because of the relatively similar partition coefficients for the types of sorbents, the competition is not dominated by either bacteria or FA unless the concentration of one type of site greatly exceeds that of the other.

1. INTRODUCTION

Heavy metals, such as Hg, adsorb to proton-active functional groups on bacterial cell envelopes (e.g., Beveridge and Murray, 1976; Fortin and Beveridge, 1997; Daughney et al., 2002; Fein, 2006; Kenney and Fein, 2011), affecting the speciation and distribution of these metals in environmental systems. Recent studies (e.g., Guiné et al., 2006; Mishra et al., 2007, 2009, 2010, 2011; Joe-Wong et al., 2012; Pokrovsky et al., 2012; Song et al., 2012; Colombo et al., 2013; Yu et al., 2014) have shown that at least some bacterial cell envelopes contain proton-active sulfhydryl functional groups. Because Hg binds readily and strongly to sulfur compounds (Compeau and Bartha, 1987; Winfrey and Rudd, 1990; Benoit et al., 1999), bacterial adsorption of Hg may dramatically affect the distribution, transport and fate of Hg in geologic systems.

Natural organic matter (NOM) is present in nearly every near-surface geologic system, and complexation reactions between metals and NOM can dramatically change the behavior of the metals in the environment (McDowell,

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2003; Ravichandran, 2004). NOM molecules contain a range of functional group types, including carboxyl, phenol, amino, and sulfhydryl groups, that have the potential to create highly stable complexes with metal ions across the pH scale (Ephraim, 1992; Ravichandran et al., 1999; Drexel et al., 2002; Haitzer et al., 2002; Croué et al., 2003; Ravichandran, 2004). Hg binds strongly to the sulfhydryl groups present within the NOM structure (Dong et al., 2011; Muresan et al., 2011). The relative thermodynamic stabilities of Hg–NOM and Hg–bacteria complexes are not well known. Depending on these relative stabilities, the formation of metal–NOM complexes may decrease adsorption of Hg to bacteria cell envelopes due to a competitive ligand effect, or under certain conditions may increase adsorption of Hg to bacteria due to ternary complexation with NOM. For example, investigating Pb, Cu, and Ni separately, Borrok et al. (2007) found that ternary metal–FA–bacteria complexes form, and that the importance of the complexes is strongly affected by pH. Conversely, Wightman and Fein (2001) found that the presence of NOM decreases the amount of Cd adsorbed to bacteria under mid- and high-pH conditions, and that the presence of Cd does not affect the adsorption of NOM to bacteria, suggesting that ternary complexes do not occur. No studies have been conducted to date to determine the effects of NOM on Hg binding to bacteria. However, because Hg forms strong complexes both with cell envelopes (Daughney et al., 2002; Mishra et al., 2011; Dunham-Cheatham et al., 2014) and NOM (Loux, 1998; Ravichandran, 2004; Skyllberg et al., 2006), it is likely that significant changes to Hg adsorption behavior occur in the presence of NOM.

In this study, we used bulk adsorption and Hg X-ray absorption spectroscopy (XAS) experiments, conducted as a function of pH and FA concentration, using intact non-metabolizing bacterial cells to study Hg binding onto three different bacterial species and to compare the ability of bacteria to adsorb mercury in the presence and absence of a fulvic acid (FA). We used the experimental results to calculate apparent partition coefficients, K_d, for Hg–bacteria and Hg–FA complexes. This study examined both Gram-positive and Gram-negative bacterial species in order to determine if cell envelope structure affects the binding reactions, and one species was a Hg methylator, which we examined in order to determine if the extent or nature of Hg binding onto that species differed from that exhibited by the non-methylators.

2. METHODS

2.1. Experimental methods

2.1.1. Bacterial growth and washing procedure

Bacillus subtilis (a Gram-positive aerobic soil species) and *Shewanella oneidensis* MR-1 (a Gram-negative facultative anaerobic species) cells were cultured and prepared following the procedures outlined in Borrok et al. (2007). Briefly, cells were maintained on agar plates consisting of trypticase soy agar with 0.5% yeast extract added. Cells for all experiments were grown by first inoculating a test-tube containing 3 mL of trypticase soy broth with 0.5% yeast extract, and incubating it for 24 h at 32 °C. The 3 mL bacterial suspension was then transferred to 1 L of trypticase soy broth with 0.5% yeast extract for another 24 h on an incubator shaker table at 32 °C. Cells were pelleted by centrifugation at 8100 g for 5 min, and rinsed 5 times with 0.1 M $NaClO_4$.

Geobacter sulfurreducens (a Gram-negative species capable of Hg methylation) cells were cultured and prepared using a different procedure than detailed above. Cells were maintained in 50 mL of anaerobic freshwater basal media (ATCC 51573) at 32 °C (Lovely and Phillips, 1988). Cells for all experiments were grown by first inoculating an anaerobic serum bottle containing 50 mL of freshwater basal media, and incubating it for 5 days at 32 °C. Cells were pelleted by centrifugation at 8100*g* for 5 min, and rinsed 5 times with 0.1 M $NaClO_4$ stripped of dissolved oxygen by bubbling a 85%/5%/10% $N_2/H_2/CO_2$ gas mixture through it for 30 min. After washing, each of the three types of bacteria was then pelleted by centrifugation at 8100*g* for 60 min to remove excess water in order to determine the wet mass so that suspensions of known bacterial concentration could be created. All bacterial concentrations in this study are given in terms of gm wet biomass per liter. Bacterial cells were harvested during stationary phase, and all adsorption experiments were performed under oxic, non-metabolizing, electron donor-free conditions.

2.1.2. Adsorption experiments

To prepare experiments, aqueous Hg, NOM, and suspended bacteria stock solutions were mixed in different proportions to achieve the desired final concentrations for each experiment. The experiments were conducted in sets with constant pH (at pH 4.0 ± 0.1, 6.0 ± 0.1, or 8.0 ± 0.3) and constant bacterial concentration (0.2 g bacteria L^{-1} in all cases) at three different FA concentrations (0, 25, or 50 mg L^{-1}), with Hg log molalities ranging from −6.30 to −5.00 (0.1 to 2.0 mg L^{-1}).

FA stock solutions were prepared in Teflon bottles by dissolving dried, powdered International Humic Substances Society Suwannee River FA Standard I in a 0.1 M $NaClO_4$ buffer solution to achieve the desired final FA concentration for each experiment. A known mass of wet biomass was then suspended in the FA stock solution, and the pH of the FA-bacteria parent solution was immediately adjusted to the experimental pH using 0.2 M HNO_3 and/or NaOH. To prepare experimental solutions, aliquots of the FA-bacteria parent solution were added gravimetrically to Teflon reaction vessels, followed by a small aliquot of commercially-supplied 1000 mg L^{-1} Hg aqueous standard to achieve the desired final Hg concentration. The pH of each suspension was again adjusted immediately to the experimental pH. The vessels were placed on an end-over-end rotator to agitate the suspensions for the duration of the experiment (2 h for *B. subtilis* and *G. sulfurreducens* and 3 h for *S. oneidensis* MR-1, as determined by initial kinetics experiments (results not shown)). The pH of the suspensions was monitored and adjusted every 15 min throughout the duration of the experiment, except during the last 30 min, when the suspensions were undisturbed.