Probing changes in Hg(II) coordination during its bacterial uptake

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Abstract. We present XAFS data collected at the Hg LIII-edge for bacterial cells of Escherichia coli that have been exposed to 500 and 50 nano-molar Hg²⁺ in aqueous solution, which corresponds to ∼30 and ∼3 µg Hg per g cells (wet weight). These concentrations are respectively 1 and 2 orders of magnitude lower than what has been previously reported for Hg-bacteria XAFS experiments. The cells were metabolically active while exposed to Hg(II), providing coordination information that can be directly compared to Hg(II) biouptake experiments. At these amounts of total dissolved metal, Hg(II) binds primarily to thiol moieties that are either present at the cell membrane or localized in the cytoplasm. We show that in this case the Hg binding environment is a mixture of 2- and 4-fold coordination to thiols. This information can be inferred from XANES spectra but the EXAFS provides a more quantitative answer.

1. Introduction
Mercury (Hg) is a potent neurotoxin that is affecting human and ecological health worldwide [1]. In its gaseous phase, elemental mercury – Hg⁰ – can travel far distances through the atmosphere and enters aquatic systems after photochemical oxidation to Hg²⁺ [2,3]. In suboxic and anoxic zones Hg(II) is converted to mono-methyl mercury – CH₃Hg⁺ – after Hg(II) biouptake by methylating microorganisms [3]. This chemical form of Hg is prone to bioaccumulation and biomagnifies through aquatic food webs, causing more fish consumption advisories than any other pollutant [1].

The increasing release of anthropogenic Hg into the environment, primarily as a result of the burning of coal [4,5], has prompted research to better understand the processes that control the microbial biouptake of Hg(II) [6]. For this purpose, we are employing a whole cell E. coli biosensor to assess the role of various ligands in controlling Hg(II) transfer through the bacterial cell membrane (i.e. biouptake). Recently, we have shown using XANES spectroscopy, that when aminopolycarboxylate ligands, such as EDTA, bind Hg(II) in solution, a ligand exchange reaction takes place between EDTA and thiol moieties at the cell membrane [7]. These XAS experiments were however performed at a higher Hg concentration than those in biouptake experiments and were limited to the collection of the XANES. The purpose of this report is to show that one can probe the coordination environment of Hg(II) in bacterial cells at relevant Hg(II) concentrations (50 nM Hg in solution) and cell densities used in biouptake experiments.
2. Material and Methods

2.1. Sample preparation

Bacterial cells of *E. coli* – strain ATCC® 25922™ – were first grown in Lysogeny broth (LB) overnight at 37°C. One mL of the cell suspension was subsequently collected from the LB medium, washed once with a minimal salt medium (MSM), and resuspended in an equivalent volume of MSM. A small aliquot (10-20 µL) was transferred to 400 mL MSM, and the cells were grown in MSM for ~24 hours until early exponential growth phase characterized by an OD$_{600}$ of 0.2 (~2×10$^8$ cells per mL). At that stage, the cell suspension was washed twice with and resuspended in an equivalent volume of a third minimally complexing medium (MCM) containing glucose as a carbon source. After adding Hg(NO$_3$)$_2$ to achieve final concentrations of 50 and 500 nM Hg(II) in cell suspensions (~3 and ~30 µg Hg per g cells, wet weight), the microbial biouptake of Hg(II) takes place, as is evidenced from our previous results with an *E. coli* based whole cell biosensor [7]. After 3 hours of Hg(II) exposure, the cells were washed twice in 0.1 M NaClO$_4$ and collected as a cell pellet on a 2.5 cm diameter cellulose nitrate filter that was sealed between 2 strips of Kapton tape. The samples were prepared just prior to beamtime, and the bacterial activity was quenched by flash freezing in LN$_2$. The samples remained frozen at a temperature of ~80°C prior to XAS data collection. Reference spectra for Hg-thiol standards were prepared following the protocol presented in Jalilevhand et al. [8].

2.2. XAFS experiments

XAFS experiments were performed at the Sector 5 of the Advanced Photon Source using DND-CAT bending magnet beamline equipped with a Si(111) double crystal monochromator. Harmonics were rejected by detuning by about 40%. Data were collected at the Hg L$_{III}$-edge, $I_0$ was monitored with Oxford ion chambers of 16 cm path-length and the fluorescence signals were collected using a Vortex ME4 silicon drift detector. The frozen cell samples were rapidly positioned in a THMS600 freezing stage – Linkam Scientific Instruments Ltd., UK – maintained at ~5°C. Up to 40 scans of 40 minute duration were collected for the samples exposed to the lowest concentrations of dissolved Hg and the data were reduced and interpreted with Athena [9]. Reference spectra were acquired in transmission as well as in fluorescence mode, processed with Athena and fit with Artemis [9] to verify their quality.

3. Results

XANES spectra are shown in Figure 1 and the EXAFS spectra in Figure 2. Among the various Hg-thiol reference compounds that were prepared, two explain best the spectra obtained for the cells in exponential growth phase. These standards are the following:

(i) the two-fold thiol coordinated Hg – Hg(SR)$_2$ – a precipitate that forms in a solution of Hg(NO$_3$)$_2$ and excess cysteine at acidic pH with the stoichiometry: Hg(Cys)$_2$(s) and,

(ii) the four-fold thiol coordinated Hg – Hg(SR)$_4$ – obtained from the reaction of 72 mM Hg(II) with 635 mM cysteine at alkaline pH: Hg(Cys)$_4$(aq).

In the XANES spectra of the Hg-thiol references, two minor spectral features distinguish Hg(SR)$_2$ from Hg(SR)$_4$. The two-fold coordinated Hg-thiol presents two shoulders located at about 12,290 eV and 12,300 eV, whereas the four-fold does not. In addition, the derivative of the normalized XANES of Hg(SR)$_2$ contains a peak at around 12,295 eV that is clearly not present in Hg(SR)$_4$. Linear combination fitting of the XANES derivative of the Hg-cell samples suggest that these samples contain mixtures of both reference coordination environment (data not shown). The XANES derivative fit provides 70% Hg(SR)$_2$ and 30% Hg(SR)$_4$ for cells exposed to 50 nM Hg and 54% Hg(SR)$_2$ and 46% Hg(SR)$_4$ for cells exposed to 500 nM Hg. However, the lack of pronounced edge features in the XANES makes the fit results very sensitive to data processing. Thus, the EXAFS were analyzed as well for comparison.
Figure 1. XANES spectra of the reference compounds as well as the spectra from exponential phase cells exposed to 500 nM Hg and 50 nM Hg in solution. The derivative of the normalized XANES is shown in the inset.

Figure 2. EXAFS spectra of the reference compounds as well as the spectra from exponential phase cells exposed to 500 nM Hg and 50 nM Hg in solution. Spectral decomposition was performed using the procedure implemented in Athena [9], after verifying that no other Hg reference compound present in our library could be used.

The EXAFS of the Hg(SR)₂ and Hg(SR)₄ references show clear differences in the frequency of oscillation, which is dominated by the first sulfur shell. From EXAFS fitting, the Hg-S bond length is 2.35 ± 0.04 Å for Hg(SR)₂ and 2.50 ± 0.01 Å for Hg(SR)₄, which agrees with...
literature values of 2.32 to 2.36 Å and 2.50 to 2.61 Å for Hg(SR)$_2$ and Hg(SR)$_4$, respectively [10]. Principal component analysis was performed on EXAFS spectra of 6 cell samples with similar Hg concentrations (including the 2 samples in this study) and revealed 2 components. Thus, we conclude that there are 2 coordination environments in our cell samples. These EXAFS spectra are reproduced well as a linear combination of the Hg(SR)$_2$ and Hg(SR)$_4$ references. The speciation of Hg in cells exposed to 500 nM of Hg(II) is explained by considering that 41% of Hg is 2-fold coordinated to thiols and 59% is 4-fold. Whereas for the cells exposed to 50 nM Hg(II), we find that 74% of Hg is 2-fold coordinated to thiols while 26% is 4-fold.

4. Discussion and Conclusions

Previous XAS studies of Hg(II) interactions with bacterial species have focused on exploring the processes that control its adsorption to cell surfaces [11-13]. In these studies, the lowest concentration assessed was 500 µg Hg per g cells (wet weight), and only “non-metabolizing” cells were utilized. The XANES and EXAFS spectra from these investigations showed that Hg(II) is coordinated predominantly to sulphhydril groups at low Hg loadings and predominantly carboxylic groups at higher Hg loadings. Another recent study investigated the oxidation of Hg(0) to Hg(II) by anaerobic bacteria and used XANES spectroscopy to determine the redox state as well as the coordination environment of Hg associated with metabolically active cells [14]. It concluded that the speciation of Hg associated with the cell pellet was a mixture of Hg(SR)$_2$ and Hg(0), but the authors lacked Hg(SR)$_4$ as a reference compound. In the studies where the EXAFS is used to determine Hg(II) coordination to thiols, analysis primarily reveals bond distances and coordination numbers characteristic of the formation of Hg(SR)$_2$ [15].

In our study we have obtained Hg L$_{III}$-edge XANES and EXAFS of cells that were actively metabolizing during Hg(II) exposure, i.e. in exponential growth phase. The XANES results suggest the presence of both Hg(SR)$_2$ and Hg(SR)$_4$ coordination environments in cells, which is confirmed with the EXAFS spectra. As the existence of Hg(SR)$_4$ in bacteria has yet to be reported, we suggest that the formation of this Hg(SR)$_4$ complex is linked to metabolically active cells and potentially the internalization of Hg(II).

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[1] Mergler D, Anderson H A, Chan L H M, Mahaffey K R, Murray M, Sakamoto M et al. 2007 Ambio 36 3
[2] Morel F M M, Kraepiel A M and Amyot M 1998 Annu. Rev. Ecol. Syst. B 29 543
[3] Sunderland E M, Krabbenhoft D P, Moreau J M, Strode S A, Landing W M 2009. Global Biogeochem. Cycles 23 GB2010
[4] Pacyna EG, Pacyna JM, Steenhuisen F, Wilson S 2006 Atmos. Environ. 40 4048
[5] Mason R P, Fitzgerald W F and Morel F M M 1994 Geochim. Cosmochim. Acta 58 3191
[6] Schaefer J K and Morel F M M 2009 Nat. Geosci. 2, 123
[7] Thomas S A, Tong T and Gaillard J-F 2014 Metallomics 6 2213
[8] Jalilvand F, Leung B O, Izadifard M and Damian E 2006 Inorg. Chem. 45 66
[9] Ravel B and Newville M 2005 J. Synchrotron Rad. 12 537
[10] Manceau A and Nagy K L 2008 Dalton T. 11 1421
[11] Dunham-Cheatham S, Farrell B, Mishra B, Myneni S and Fein J B 2014 Chem. Geol. 373 106
[12] Dunham-Cheatham S, Mishra B, Myneni S and Fein J B 2015 Geochim. Cosmochim. Acta 150 1
[13] Mishra B, O’Loughlin E J, Boyanov M B and Kemner K M 2011 Environ. Sci. Technol. 45 9597
[14] Colombo M J, Ha J, Reinfelder J R, Barkay T and Yee N 2014 Chem. Geol. 363 334
[15] Skylberg U, Bloom P R, Qian J, Lin C-M and Bleam W F 2006 Environ. Sci. Technol. 33 1418