An X-ray Absorption Fine Structure study of Au adsorbed onto the non-metabolizing cells of two soil bacterial species

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Abstract

Gram-positive and Gram-negative bacterial cells can remove Au from Au(III)–chloride solutions, and the extent of removal is strongly pH dependent. In order to determine the removal mechanisms, X-ray Absorption Fine Structure (XAFS) spectroscopy experiments were conducted on non-metabolizing biomass of Bacillus subtilis and Pseudomonas putida with fixed Au(III) concentrations over a range of bacterial concentrations and pH values. X-ray Absorption Near Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS) data on both bacterial species indicate that more than 90% of the Au atoms on the bacterial cell walls were reduced to Au(I). In contrast to what has been observed for Au(III) interaction with metabolizing bacterial cells, no Au(0) or Au–Au nearest neighbors were observed in our experimental systems. All of the removed Au was present as adsorbed bacterial surface complexes. For both species, the XAFS data suggest that although Au–chloride–hydroxide aqueous complexes dominate the speciation of Au in solution, Au on the bacterial cell wall is characterized predominantly by binding of Au atoms to sulfhydryl functional groups and amine and/or carboxyl functional groups, and the relative importance of the sulfhydryl groups increases with increasing pH and with decreasing Au loading. The XAFS data for both microorganism species suggest that adsorption is the first step in the formation of Au nanoparticles by bacteria, and the results enhance our ability to account for the behavior of Au in bacteria-bearing geologic systems.

1. INTRODUCTION

Microorganisms play an important role in the biogeochemical cycling of Au, and biominalization of Au has been suggested as a mechanism responsible for the formation of secondary Au deposits in soil environments (e.g., Watterson, 1992; Bischoff, 1994, 1997; Falconer et al., 2006; Reith et al., 2007). Studies of Au(I) and Au(III) bioaccumulation by a wide range of types of microorganisms (bacteria, fungi and yeasts) indicate that the extent of Au removal from solution decreases with increasing pH, that bacteria show a greater affinity for Au than do the other types of organisms studied, and that Au uptake can be reversed by exposure of the biomass to a 0.1 M thiourea solution (Nakajima, 2003; Tsuruta, 2004). A number of laboratory studies have observed Au(0) nanoparticle formation from Au(I) and Au(III) solutions in the presence of metabolizing bacteria (Beveridge and Murray, 1976; Kashfi et al., 2001; Karthikeyan and Beveridge, 2002; Lengke and Southam, 2005, 2006; Reith et al., 2005; Lengke et al., 2006a,b; Reith et al., 2006; Lengke et al., 2007). However, the mechanisms of Au reduction and precipitation, and the potential role of Au adsorption onto the cells in this process, are not well understood.

Previous XAFS studies have investigated the valence state and speciation of Au in aqueous solutions (Farges et al., 1993; Pokrovski et al., 2009a,b) and on mineral and microbial surfaces (Berrodier et al., 2004; Lengke et al., 2007). Farges et al. (1993) studied the speciation of Au(III) in aqueous chloride solutions, and demonstrated that...
Au(III) is present as 4-coordinated, square-planar complexes. At low pH, AuCl₄⁻ is the dominant complex, and with increasing pH, chloride ligands are replaced by hydroxide ligands. Pokrovski et al. (2009a, b) studied Au complexation and speciation in chloride- and in sulfur-bearing hydrothermal fluids. Their XANES and EXAFS results suggest that in Au-chloride systems, Au(III)Cl₄ is the dominant Au species at low temperature (~150 °C), while at higher temperatures, Au(III) reduces to Au(I), in the form of a linear Au(I)Cl₄ complex, and to Au(0). Furthermore, in sulfur-bearing hydrothermal fluids under acidic and neutral-to-basic conditions, the atomic environment of dissolved Au involves two sulfur atoms in a linear geometry around Au(I) at similar atomic distances, with an average distance of 2.29 ± 0.01 Å. Berrodier et al., 2004 investigated Au(III) adsorbed from chloride solutions onto ferrihydrite, goethite, and boehmite mineral surfaces, reporting that Au(III) is adsorbed onto these surfaces dominantly as inner-sphere, square-planar complexes. The surface speciation of Au on these surfaces varies with pH, with Au(III)O₄ surface complexes at pH >6 and Au(III)(O,Cl)₄ at pH <6, and with the number of Cl ligands increasing with decreasing pH. In this study, Au(I) and Au(0) were not observed. Lengke et al. (2007) found precipitation of Au(0) nanoparticles from Au(III)-chloride solution by metabolizing cyanobacteria, and indicated that organic sulfur released from cyanobacteria bound with the reduced Au(I) to form an intermediate Au(I)-sulfide species before Au(0) nanoparticles formed. These previous studies indicate that at ambient temperature, Au(III) adsorbed onto the mineral surfaces studied does not change valence state, but that interaction of metabolizing bacteria with aqueous Au(III) can lead to Au reduction to Au(I) and eventually to Au(0) by the biomass. Although it is clear that bacteria can cause Au reduction, the role of adsorption in this process and the molecular binding environments of Au on bacterial cell walls have not been determined.

In this study, we measured the valence state and speciation of the Au that was removed from Au(III)-chloride solutions by non-metabolizing common soil bacteria: the Gram-positive species Bacillus subtilis, and the Gram-negative species Pseudomonas putida. We conducted the XAFS experiments as a function of pH at two different Au loadings. Our objectives were to determine the functional groups involved in the initial binding of Au to the bacterial cell walls, and to determine whether the binding mechanism changes as a function of Au loading and pH.

2. MATERIALS AND METHODS

2.1. Bacteria growth and sorption experiments

B. subtilis and P. putida bacterial cells were incubated aerobically in 3 mL of tryptase soy broth (TSB) growth medium with the addition of 0.5% yeast extract at 32 °C for 24 h, and then transferred to 2 L of the same growth medium and incubated for another 24 h. The bacteria were harvested by centrifugation at 8100g for 30 min, then transferred to test tubes, and washed five times in 0.1 M NaClO₄ electrolyte. After the final wash, the bacterial pellet was transferred to a pre-weighed test tube and resuspended with small amount of clean 0.1 M NaClO₄ electrolyte. The suspension was centrifuged at 8100g for two 30-min intervals, decanting the bacteria-free supernatant after each interval, and the wet mass of the bacterial pellet was determined.

All Au standards and samples were made from diluting 1000 ppm Au ICP-OES standard solution (1000 ± 3 ppm Au(III) in 10% HCl) used for inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Assurance grade; Spex Certiprep). Suspensions of non-metabolizing B. subtilis and P. putida cells in 0.1 M NaClO₄ or 0.1 M NaNO₃ (see the Supplementary material) were exposed to 5 ppm Au(III)-chloride solutions for 2 h. The pH values of the suspensions were adjusted using small quantities of 0.1–1.0 M HCl or NaOH and measured using a Thermo Orion model 420A bench-top pH meter. The pH of each experimental solution was monitored every 30 min, and adjusted if required. The final pH was measured after 2 h of total reaction time and reported in Table 1. The chloride concentration is approximately 500 ppm in the suspensions. Sixteen biomass samples were prepared as described in Table 1. The pH values were chosen to represent different Au removal behavior regimes, based on the results from Nakajima (2003) and Tsuruta (2004).

After the bacterial suspensions were mixed for 2 h, they were centrifuged at 8100g for 10 min. The supernatant was filtered through a 0.45 μm disposable nylon membrane, and the concentration of Au remaining in solution was analyzed using ICP-OES. The concentration of adsorbed Au was calculated by the difference in concentration of Au in the starting solution and the final measured concentration of Au in the supernatant. Biomass-free controls were conducted and demonstrated that no significant Au adsorption onto the polypropylene reaction vessels occurred.

2.2. XAFS spectroscopy

The wet biomass pellets from the batch sorption experiments were collected and maintained in an ice-cooled vessel during transport to the beamline. All X-ray absorption spectroscopy measurements were made within 48 h of sample preparation. Fluorescence Au L₃-edge (11,919 eV) XAFS measurements were made on the biomass pellets which were loaded into slotted Teflon holders and covered with Kapton film. The XAFS measurements were performed at the MRCAT sector 10-ID beamline (Segre et al., 2000) at the Advanced Photon Source at Argonne National Laboratory.

The energy of the incident X-ray beam was scanned by using a Si (1 1 1) reflection plane of a cryogenically-cooled double-crystal monochromator. The undulator was tuned to its second harmonic, and tapered to an X-ray energy spread of approximately 3.5 keV to reduce the variation in the incident intensity to less than 15% over the whole scanned energy range. Higher harmonics were rejected using a Rh-coated mirror.

The incident and transmitted X-ray fluxes were measured by ion chambers and the Au L₃-edge fluorescence was monitored by either a 5-grid Stern–Heald–Lytle detector or a 4-element Vortex (ME-4) detector. The incident ion
chamber was filled with 33% nitrogen gas and 67% helium gas. The transmitted and reference ion chambers were filled with 100% nitrogen gas. The fluorescence detector in the Stern–Heald geometry (Stern and Heald, 1983) was filled with argon gas. The signal/background ratio was high enough that no X-ray filter was necessary. The incident X-ray beam profile was 0.6 mm². Linearity tests (Kemner et al., 1994) indicated less than 0.05% nonlinearity for a 50% decrease in incident X-ray intensity. The XAFS data were analyzed using codes from the UW-XAFS package (Stern et al., 1995). Photo-reduction was observed for the third or fourth scan on the same spot of each sample. By comparing XANES spectra, those scans without radiation damage were selected, averaged, normalized and background subtracted using ATHENA (Ravel and Newville, 2005), which is a graphical interface to IFEFFIT (Newville, 2001). Background subtraction used the AUTOBK method with Rbkg, the maximum frequency of the background, set to 1.1 Å⁻¹ and symmetrical Hannings window function was used with dk = 1.0 Å⁻¹ (Newville et al., 1993). Theoretical amplitudes were calculated using Feff6 (Rehr et al., 1992) and experimental data were fit using IFEFFIT. Four of the calculated single-scattering paths, (Au–N, Au–O, Au–S, and Au–C) were used to fit the spectra from the biomass samples, and in order to exclude the possibility of some minor Au–phosphoryl binding, or Au nanoparticle formation, additional Au–P and Au–Au scattering paths were allowed into the fitting model in selected fits. Significant reduction (>50%) in “reduced chi square” ($\chi^2$) and R factor values were used as the standard for the better data-model fitting. The $\chi^2$ is defined as the normal statistical $\chi^2$ divided by the number of degrees of freedom $v$ in the fit, where $v$ is given by the number of “independent points” (determined by the data range used in the analysis) in the data minus the number of parameters allowed float in the fit (Bunker, 2010). The $R$ factor is the sum of the differences of the model and data value squared divided by the sum of the data value squared. The fitting range was set to 1.15–2.9 Å for all the Au biomass sample data sets and 1.15–4.5 Å for all the standards. In order to break correlations between fitting parameters and to reduce the possibility of obtaining wrong fitting results from a single $k$-
weighting value, simultaneous fitting of data sets with multiple $k$-weighting ($k^1, k^2, k^3$) was performed (Kelly et al., 2002).

3. RESULTS AND DISCUSSION

3.1. Au sorption data

As can be seen in Fig. 1, the extent of Au adsorption varies strongly as a function of pH. In all sample sets studied except for samples with 7.0 g/L P. putida cells, the extent of adsorption was nearly 100% at pH ≈ 3.0, started decreasing at pH ≈ 4.5, and decreased dramatically between pH ≈ 5.5 and 7.5 with increasing pH. These observations are consistent with anion-like adsorption behavior. The bacterial cell wall becomes more negatively charged with increasing pH, and it is likely that increasing electrostatic repulsion between the negatively charged Au(III)–hydroxide–chloride aqueous complexes and the bacteria is responsible for the decrease in adsorption that we observed with increasing pH. As for the samples with 7.0 g/L P. putida cells, the extent of adsorption was approximately 75% at pH ≈ 3.0, decreased at pH ≈ 5.0 and increased to nearly 80% at pH ≈ 6.0. This unusual adsorption behavior may be due to elevated concentrations of bacterial exudates from the higher P. putida cell concentration in these experiments. However, we did not measure dissolved organic carbon concentrations in these samples and therefore additional measurements would be required in order to resolve this issue.

3.2. Analysis of XANES spectra

3.2.1. XANES for Au solution standards, powder compounds and Au foil

As a basis for interpretation of our Au-biomass data, XANES spectra were collected at room temperature for the following standards: Au(III)–chloride (HAuCl₃·3H₂O), Au(III)–acetate (Au(O₂CCH₃)₃), Au(I)–thiosulfate (Au₂S), Au(I)–sulfide (Na₂Au(S₂), 2H₂O), Au(I) thiomalate (NaO₂–CCH₂–CH(SAu)–CO₂Na), Au(I)–chloro(triphenylphosphine) (C₁₅H₁₈AuClP) and 5 μm thick Au foil (Fig. 2a). The three stable oxidation states of Au: Au(0), Au(I), and Au(III) (Patai and Rappoport, 1999) are included in these compounds. A sharp XANES feature characteristic of Au(III) compounds was observed and the absorption edge of Au(III) compounds shifted to about 4 eV lower than Au(I) compounds and Au foil. This sharp pre-edge feature and the shifted absorption edge of Au(III) is known to be caused by a dipole-allowed atomic 2p to 5d transition. This transition is allowed for the atomic d⁰ state of Au(III) but is forbidden for the d¹⁰ state of Au(I) and Au(0) (Pantelouris et al., 1995). Additionally, the post-edge peak at approximately 11,947 eV on Au foil XANES spectra is a clear signature of Au(0). We use these XANES features to determine the oxidation states of Au in the biomass samples. XANES spectra of Au(I)–sulfide, Au(I)–thiosulfate and Au(I)–thiomalate are nearly identical, suggesting a similar Au–S local atomic environment in these compounds. The feature located at ~11,930 eV represents multiple scattering from the S–Au–S linear structure (Elder and Eidsness, 1987; Bau, 1998; Pokrovski et al., 2009b), and this feature can be used as a “fingerprint” of this structure.

A series of 2 mM Au(III)–chloride solutions of pH 3.0, 5.0, 7.0, 9.0 and 11.0 was studied. The Au L₃ edge XANES spectra of the solution standards Fig. 2b) show that Au speciation in solution changes with pH, and the valence state of Au remains as Au(III) in all samples studied. For solutions at pH 3.0 and 5.0, the XANES spectra look nearly identical; the multiple-scattering feature at 11,934 eV is in agreement with Au being present as the square planar AuCl₄⁻ anion (Farges et al., 1993), indicating that the dominant Au species in these two solutions is AuCl₄⁻. For solutions at pH 7.0 and 9.0, the multiple-scattering feature representing AuCl₄⁻ decreases slightly in magnitude and the multiple-scattering feature at 11,945 eV which is related to the Au–OH environment (Berrodier et al., 2004) increases. These changes indicate mixed Au speciation of Au–Cl and Au–OH complexes under these pH conditions. For the solution at pH 11.0, the multiple-scattering feature representing AuCl₄⁻ disappears and the multiple-scattering feature at 11,945 eV indicates that the dominant Au species in the solution is Au(OH)₄⁻. These results are in agreement with the study by Farges et al. (1993) of Au speciation in chloride solutions with higher Au concentrations.

3.2.2. XANES for Au-biomass samples

The Au-L₃ XANES spectra of the Au biomass samples (Fig. 2c and d) confirm the change in Au-speciation and Au valence state as Au is removed from solution. The absence of the characteristic Au(0) peaks at approximately 11,947 eV on all Au-biomass XANES spectra indicates that Au(0) represents less than a few percent of the total Au in these samples, and that virtually all of the Au atoms are present as adsorbed bacterial surface complexes.

A comparison between the XANES spectra of the standard Au compounds and those of the Au biomass samples (Fig. 2e) suggests significant Au–S binding in all of the biomass samples, and that the Au–S signal is especially strong in samples Group B. The multiple-scattering feature
located at ~11,930 eV in all Au biomass samples likely represents the S–Au–S linear structure that we observed in the Au(I)-sulfide, Au(I)-thiosulfate and Au(I) thiomalate compounds. No significant multiple-scattering feature is seen at 11,934 eV (representing the square planar AuCl$_4$ complex) or at 11,945 eV (representing the Au(OH)$_4$ complex), suggesting that there is negligible Au–Cl or Au–OH binding in the biomass samples.

The sharp pre-edge features of the Au-biomass XANES spectra divide the Au-biomass samples into two groups. For some samples (here denoted Group A, and including samples AuB1, AuB2, AuB3, AuB4, AuP1, AuP2, and...
Au(III)–chloride solution at pH 11.0 (to represent Au(III)), and 5 μm Au foil (to represent Au(0)) in the energy range 11,930–11,934 eV located at 11,930 and 11,934 for linear Au(I)-S binding and square planar Au(III)–Cl binding (Fig. 2e) can be used to distinguish between Au–Cl and Au–S binding in a sample. In addition, the Au(I)–Cl complex is not stable at room temperature and will disproportionate to a mixture of Au(III)–chloride and Au metal (Gammons et al., 1997; Patai and Rappoport, 1999; Lengke et al., 2006a,b). Therefore, Au(I)-Cl can be excluded from models of our experimental system.

### 3.4. Au binding to biomass: EXAFS qualitative analysis

#### 3.4.1. B. subtilis biomass samples (AuB)

The magnitude and real part of the Fourier transformed (FT) data of the Au – B. subtilis biomass samples (AuB) for two different Au/bacteria ratios and four different pH values are shown in Fig. 4c and d. As can be seen by comparing the magnitude and real part of the FT data of the biomass samples and the solution standards at the same pH values (Fig. 5), the amplitude and position of the first peak differ significantly. The amplitude of the first peak in FT data of the solution standards (Fig. 4a) decreases and the peak position shifts to smaller radial distance values as pH increases. In contrast, the amplitude of the first peak in the FT data of the biomass samples increases and the peak position shifts to larger radial distance values as pH increases. These changes with pH can be attributed to a different percentage of Au atoms bound to different functional groups under different pH conditions on bacterial cell walls.

Previous studies have demonstrated that metal sorption onto non-metabolizing bacteria is dominated by carboxyl, phosphoryl and sulfhydryl functional groups on the cells (Hennig et al., 2001; Kelly et al., 2002; Boyanov et al., 2003; Toner et al., 2005; Guiné et al., 2006; Mishra et al., 2009). Considering that 12% (by dry weight) of a typical bacterial cell consists of nitrogen, an important element in proteins (Madigan et al., 2002), nitrogen-containing functional groups such as amine groups may also play a role adsorbing Au atoms in our experiment systems. Just as it is difficult to distinguish between Au–Cl and Au–S bonds using EXAFS, it is also difficult to distinguish Au–O from Au–N bonds in an unknown sample. Therefore, Au–carboxyl, –phosphoryl, and –amine bonds are likely to exhibit similar first shell amplitudes and peak positions in the FT data. Thus, the changes of the amplitude of the first peak in the FT data and the peak position as pH increases seen in the EXAFS data could indicate an increase of sulphydryl...
binding (Au–S), which agrees with the XANES results. Moreover, since the chloride concentration in the solution used in the batch adsorption experiments is high, and EXAFS spectroscopy alone is unable to distinguish between Au–Cl and Au–S bonds, we cannot completely rule out the possibility of a small amount of Au–Cl binding on the bacteria.

FT data for samples with 7.0 g/L bacteria (lower Au loading) show similar changes with respect to pH, but are less pronounced than we observed for the samples with 1.0 g/L bacteria. Comparing samples with different Au loading at the same pH indicates that the first peak amplitude of the FT data of samples with lower Au loading increases and shifts to larger distance compared to samples with higher Au loading. This result suggests that a higher percentage of Au atoms are bound to sulphydryl groups relative to the higher loading conditions.

### 3.4.2. P. putida biomass samples (AuP)

The magnitude and real part of the FT data of P. putida biomass samples (AuP) at two different Au/bacteria ratios and at four different pH values are shown in Fig. 4e and f. Comparisons of the FT data of the two bacterial species show similar peaks and similar trends of the changes in peak amplitude and position, suggesting similar atomic binding environments between these two bacteria. Additionally, at the same pH and Au loading, the magnitude of the first peak of the FT data for P. putida samples AuP1, AuP2, and AuP3 (1.0 g/L bacteria under low pH conditions) increases and the position shifts to higher radial distance values than those of the corresponding B. subtilis (AuB) samples, indicating a higher percentage of Au atoms adsorbed to sulphydryl groups in the AuP samples relative to the AuB samples under the same conditions. The EXAFS spectra of AuP samples 4, 5, (1.0 g/L bacteria under higher pH conditions) 6, 7, and 8 (7.0 g/L bacteria) are nearly identical to each other as well as to the spectrum of the Au(I) thiomalate standard, suggesting significant sulphydryl binding in these samples. There are three resonance signals that can be clearly seen in the magnitude of the FT data of P. putida biomass samples (AuP1–AuP5) (see the Supplementary material) with a larger k range (2.0–14.0 (Å⁻¹)). These three resonance peaks are very likely to represent the mixed N/O and S first coordination shells and second C shell.

The qualitative analysis of the biomass samples indicates that, for all AuB samples and for AuP samples 1, 2 and 3, Au atoms are bound to a mixture of amine/carboxyl/phosphoryl and sulphydryl functional groups on the bacterial cells, and the relative number of Au-bound sulphydryl groups increases with increasing pH. For the rest of the AuP samples, sulphydryl groups appear to be the dominant binding site.

### 3.5. Au binding to biomass: EXAFS quantitative analysis

#### 3.5.1. B. subtilis biomass samples (AuB)

Based on the qualitative analysis discussed above, the EXAFS data from the eight AuB samples were first fit independently using a linear combination of Au–amine and Au–sulphydryl binding. Three single-scattering paths were used: Au–N, Au–S and Au–C. Each data set was fit simultaneously with k weights equal to 1, 2, and 3 in Fourier-transformed R-space to decrease the correlation between the fitting parameters. Modeling the data from the eight AuB samples with these two functional groups yields good fit results. Additionally, to check for the possibility of phosphoryl binding in the samples, a second shell Au–P path (path length allowed to vary between 2.6 and 3.3 Å) was added and the coordination number was allowed to vary. The result of the fit indicates negligible Au–phosphoryl binding, and forcing the Au–P path into the fitting makes the fit results worse, increasing both the $\chi_2^2$ and R-factor significantly. Similar results were obtained when we added an Au–Au path into the fitting. The calculated percentage of Au–Au bonds in the sample was close to zero, indicating that there are no XAFS-observable Au nanoparticles in the sample, a result which is consistent with the XANES data for these samples. Nearly identical fit results can be generated by fitting these data with a linear combination of Au–carboxyl and Au–sulphydryl binding. This is not surprising.
as discussed earlier, N and O have similar backscattering signatures so the contribution of these two first neighbor atoms cannot be distinguished from each other.

By fitting the data from different samples independently, our results indicate that Au atoms in the B. subtilis biomass are bound to a mixture of amine and/or carboxyl and sulfhydryl functional groups. The fitting results also provide constraints on the coordination numbers, bond distances, and Debye–Waller factors of the Au bonds in the samples. However, the correlation between the fitting parameters, such as coordination numbers and Debye–Waller factors or energy shift and bond distances in each individual fit result in large uncertainties for the value of these variables obtained from the fit. For the final fits of the data, all data from these eight samples were fit simultaneously at the $k$-weights of 1, 2, and 3. Based on the fact that the local atomic environments of Au atoms are similar in these eight samples, the energy shift, Debye–Waller factor, and radial distance were constrained to be the same for the same path in all samples in the fitting process. In other words, the structures of Au bound to the various functional groups do not change for these samples, only their relative numbers do. This has the effect of reducing the correlation between fitting parameters, leading to smaller uncertainties in these parameters (Kelly et al., 2002).

The final fitting results for the AuB samples are listed in Table 3, and the magnitude and real part of the FT data and fits are shown in Fig. 6a and b, respectively. The relative numbers of Au bound to either N/O or S as a function of pH for the AuB samples are shown in Fig. 8a. The bond distances suggest an inner-sphere binding mechanism. For samples AuB1, AuB2, AuB3 and AuB4, the number of N and/or O atoms in the first shell decreases with increasing pH and the number of S atoms in the first shell follows a reverse trend. The sum of coordination numbers of N and/or O and S for all the samples is close to two, indicating bidentate binding of the Au atoms. For samples AuB5, AuB6, AuB7, and AuB8, sulfhydryl groups become the dominant binding site and the percentage of Au–amine/carboxyl binding becomes much smaller than in the samples with the higher Au:biomass ratio (AuB1–AuB4). However, the same trend in coordination numbers with respect to pH is observed. As was the case for the samples with the higher Au:biomass ratio, our results are consistent with bidentate binding of Au in these samples, with the sum of the first shell coordination numbers close to two.

In addition to the Au–N, Au–S and Au–C single scattering paths initially included in the fitting, a fourth single scattering path (Au–C$_2$, corresponding to a second-shell carbon in the sulfhydryl group) should in principle be included. Attempts to include this indicated that large disorder in the path distance reduces the amplitude enough to make it unobservable. Guine´ et al. (2006) and Mishra et al. (2009, 2010) studied Zn and Cd adsorption to bacteria and observed Zn and Cd bound to sulfhydryl sites on bacterial surfaces in some samples. No second shell carbon atom in the sulfhydryl group was reported in these studies either, presumably for the same reason.

3.5.2. P. putida biomass samples (AuP)

The AuP data are analyzed following a similar approach to that applied to the AuB data. The data from the eight AuP samples were first modeled with a linear combination of Au binding to amine groups and sulfhydryl groups, and were at first fit individually. An Au–P path was then
allowed in the fitting to check the possibility of phosphoryl binding; and an Au–Au path was added to check the possibility of Au nanoparticle formation. For the same reasons as discussed in the previous section, neither XAFS-observable Au–phosphoryl binding nor Au nanoparticle formation were observed in any of these samples. The fit results showed similar Au binding environments in samples AuP1, AuP2, and AuP3 (1.0 g/L bacteria under low pH conditions) to those in AuB samples. Additionally, the data from AuP samples 4, 5, (1.0 g/L bacteria under high pH conditions) 6, 7, and 8 (7.0 g/L bacteria) yielded improved fits with pure sulfhydryl binding relative to models without

Fig. 4. Magnitude of the Fourier transformed (FT) data (FT \( k \) range 2.0–10.2 (Å\(^{-1}\))) for (a) Au solution standards and Au(I) thiomalate powder compound, (c) \( B.\ subtilis \) biomass samples, and (e) \( P.\ putida \) biomass samples; real part of the FT data plotted in \( r \) range 1.2–2.9 Å for (b) Au solution standards and Au(I) thiomalate powder compound, (d) \( B.\ subtilis \) biomass samples, and (f) \( P.\ putida \) biomass samples.
sulfhydryl binding considered. In order to reduce the correlations between fitting parameters, all eight samples were then fit simultaneously at the $k$-weights of 1, 2 and 3. The calculated coordination numbers for N in samples AuP4, AuP5, AuP6, AuP7 and AuP8 are approximately 0, and the calculated coordination numbers for S are close to 2, which is consistent with the individual fit results and indicates pure di-sulfhydryl binding. Finally, samples AuP1, AuP2, and AuP3 were then simultaneously fit with Au–N, Au–S and Au–C paths considered, and samples AuP4, AuP5, AuP6, AuP7, and AuP8 simultaneously fit with a single Au–S path to generate the best fit values.

The final fitting results for the AuP samples are listed in Table 4, and the magnitude and real part of the FT data and fits are shown in Fig. 7a and b, respectively. The relative numbers of Au bound to either N/O or S as a function of pH for the AuP samples are shown in Fig. 8b. The bond distances are consistent with those calculated for the AuB samples. For samples AuP1, AuP2, and AuP3, the number of N atoms in the first shell decreases with increasing pH whereas the number of S atoms in the first shell follows a reverse trend. The sum of coordination numbers of N and S for all AuP samples is close to 2, confirming bidentate binding.

3.6. Discussion

The results from our XAFS experiments indicate that the coordination environment of Au within the biomass samples varies as a function of both solution pH and the extent of Au loading on the bacterial cells. The experiments began with all of the Au in the systems present as aqueous Au(III)-hydroxide-chloride complexes. Exposure to either bacterial species resulted in pH-dependent removal of Au from solution, and virtually all of the Au atoms that became associated with the biomass were reduced to Au(I) by these non-metabolizing cells. In contrast to what has been observed in some studies of Au(III) interaction with metabolizing bacterial cells (e.g., Karthikeyan and Beveridge, 2002; Lengke and Southam, 2005, 2006; Reith and McPhail, 2006; Lengke et al., 2006a,b, 2007; Jian et al., 2009; Reith et al., 2009), complete reduction to Au(0) and nanoparticle formation did not occur in our experiments. No Au(0) (as evidenced by the XANES data) or Au–Au nearest neighbors (from the EXAFS data) was observed in our experimental systems. All the Au in the biomass samples was present as adsorbed species, and the process of adsorption in all samples appears to include the breakdown of aqueous Au(III)-hydroxide-chloride complexes and formation of chloride-free Au complexes with bacterial cell wall functional groups.

The EXAFS data indicate that, to a large extent, similar Au binding mechanisms occur between the two bacterial species studied here, despite their differing cell wall structures. Amine/carboxyl groups and sulfhydryl groups are the main binding sites for the Au in all of the biomass samples studied. For the B. subtilis samples with lower Au loading (5 ppm Au, 7.0 g/L bacteria concentration; AuB5–AuB8), sulfhydryl groups are dominant. For the P. putida samples with lower Au loading (5 ppm Au, 7.0 g/L bacteria concentration; AuP6–AuP8), di-sulfhydryl binding occurs exclusively. For the B. subtilis samples with higher Au loading (5 ppm Au, 1.0 g/L bacteria concentration; AuB1–AuB4) and the P. putida samples with higher Au loading under pH <7 (5 ppm Au, 1.0 g/L bacteria concentration; AuP1–AuP3), amine/carboxyl groups are as important as the sulfhydryl groups in binding the Au. For P. putida samples with higher Au loading at pH >7 (5 ppm Au, 1.0 g/L bacteria concentration; AuP4 and AuP5), di-sulfhydryl binding was observed as well. Additionally, for all the AuB and AuP samples in which Au is bound to more than one type of functional groups, as pH increases (and hence as Au loading decreases), the relative importance of sulfhydryl groups increases.

There have been few reports of metal binding to amine groups on bacterial cell walls. Amine groups are positively charged under a broad range of pH values; the $\mathrm{pK}_a$ value for amine functional groups on many molecules is near 11.
Table 3
Fit results for eight Au-B. subtilis biomass samples.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>AuB1</th>
<th>AuB2</th>
<th>AuB3</th>
<th>AuB4</th>
<th>AuB5</th>
<th>AuB6</th>
<th>AuB7</th>
<th>AuB8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au–N</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>( R (\text{Å}) ) 1.97 ± 0.01 (^a)</td>
<td>( \sigma^2 (\text{Å}^2) ) 0.002 ± 0.001 (^a)</td>
<td>( E_0 (\text{eV}) ) −1.8 ± 1.1 (^a)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Au–S</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>1.8 ± 0.4</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>( R (\text{Å}) ) 2.29 ± 0.01 (^a)</td>
<td>( \sigma^2 (\text{Å}^2) ) 0.002 ± 0.002 (^a)</td>
<td>( E_0 (\text{eV}) ) 4.3 ± 1.8 (^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au–C</td>
<td>1.8 ± 0.8</td>
<td>1.7 ± 0.9</td>
<td>1.6 ± 0.8</td>
<td>1.4 ± 0.7</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.5</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>( R (\text{Å}) ) 2.98 ± 0.02 (^a)</td>
<td>( \sigma^2 (\text{Å}^2) ) 0.002 ± 0.002 (^a)</td>
<td>( E_0 (\text{eV}) ) −1.5 ± 0.7 (^a)</td>
<td></td>
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</tr>
</tbody>
</table>

\( \chi^2 \)

\( R \) factor 0.005 \(^a\)

\(^a\) Best fit values and goodness of the fit were obtained from a simultaneous fit of eight samples. \( R, \sigma^2, \) and \( E_0 \) of each path were constrained to be the same for the eight samples.

Fig. 6. Data and fits of (a) the magnitude of the FT of B. subtilis biomass data, (b) the real part of the FT of B. subtilis biomass data plotted over the fitting range of 1.15–2.9 Å.

Fig. 7. Data and fits of (a) the magnitude of the FT of P. putida biomass data, (b) the real part of the FT of P. putida biomass data plotted over the fitting range of 1.15–2.9 Å.
In our experimental system, sulfhydryl groups become increasingly important in binding Au in samples with lower Au loading, a result that is consistent with the bacterial adsorption behavior of Zn and Cd (e.g. Guineé et al., 2006; Mishra et al., 2009, 2010). Also, note that at the same Au/bacteria ratio and under similar pH conditions, more Au atoms are bound to sulfhydryl groups in _P. putida_ samples than in _B. subtilis_ samples. This may indicate a higher density of sulfhydryl groups within _B. subtilis_ cells. In most natural geological systems, Au and many other metals are present in low concentrations, and bacterial cell wall sulfhydryl groups may control the bacterial adsorption behavior of these metals.

**Table 4**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>AuP1</th>
<th>AuP2</th>
<th>AuP3</th>
<th>AuP4</th>
<th>AuP5</th>
<th>AuP6</th>
<th>AuP7</th>
<th>AuP8</th>
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<tbody>
<tr>
<td>Au–N</td>
<td>N</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>b</td>
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<td></td>
<td>b</td>
</tr>
<tr>
<td></td>
<td><em>R</em> (Å)</td>
<td>1.97 ± 0.01a</td>
<td>b</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>_σ_2 (Å^2)</td>
<td>0.002 ± 0.001a</td>
<td>b</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>_E_0 (eV)</td>
<td>1.2 ± 0.7b</td>
<td>b</td>
<td></td>
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<tr>
<td>Au–S</td>
<td>N</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>2.0 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td><em>R</em> (Å)</td>
<td>2.29 ± 0.01a</td>
<td>2.30 ± 0.01b</td>
<td>0.002 ± 0.001b</td>
<td>6.5 ± 0.5c</td>
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<tr>
<td></td>
<td>_σ_2 (Å^2)</td>
<td>0.002 ± 0.001a</td>
<td>b</td>
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<tr>
<td></td>
<td>_E_0 (eV)</td>
<td>6.9 ± 2.6b</td>
<td>b</td>
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<tr>
<td>Au–C</td>
<td>N</td>
<td>1.3 ± 0.8</td>
<td>1.3 ± 0.7</td>
<td>1.1 ± 0.5</td>
<td>b</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td><em>R</em> (Å)</td>
<td>2.98 ± 0.02a</td>
<td>b</td>
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<tr>
<td></td>
<td>_σ_2 (Å^2)</td>
<td>0.002 ± 0.002a</td>
<td>b</td>
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<tr>
<td></td>
<td>_E_0 (eV)</td>
<td>0.2 ± 0.8a</td>
<td>b</td>
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<tr>
<td>χ^2</td>
<td>39b</td>
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<tr>
<td>R factor</td>
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<td></td>
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</tr>
</tbody>
</table>

*a* Best fit values and goodness of the fit were obtained from a simultaneous fit of three samples AuP1, AuP2, and AuP3. _R_, _σ_2, and _E_0 of each path were constrained to be the same for the three samples.

*b* Au–N and Au–C paths were not included in fitting samples AuP4–AuP8.

*c* Best fit values and goodness of the fit were obtained from a simultaneous fit of three samples AuP4–AuP8. _R_, _σ_2, and _E_0 of each path were constrained to be the same for the five samples.

(Sorrell, 2005). Most aqueous metal ions also carry positive charges; therefore, aqueous metal cations are not likely to attach to amine groups. However, for metals such as the oxidized forms of aqueous Au, As, Se, and Cr that are present as negatively charged complexes in solution, binding onto amine groups on bacterial cell walls may become important, especially at low pH where electrostatic repulsive forces between the aqueous anions and the negatively charged cell wall weaken.

In our experimental system, sulfhydryl groups become increasingly important in binding Au in samples with lower Au loading, a result that is consistent with the bacterial adsorption behavior of Zn and Cd (e.g. Guineé et al., 2006; Mishra et al., 2009, 2010). Also, note that at the same Au/bacteria ratio and under similar pH conditions, more Au atoms are bound to sulfhydryl groups in _P. putida_ samples than in _B. subtilis_ samples. This may indicate a higher density of sulfhydryl groups within _B. subtilis_ cells. In most natural geological systems, Au and many other metals are present in low concentrations, and bacterial cell wall sulfhydryl groups may control the bacterial adsorption behavior of these metals.

XAFS results alone cannot differentiate between Au that is adsorbed onto bacterial cell walls and Au that may be present inside the cells. However, our experiments involved only non-metabolizing cells that were thoroughly washed with 0.1 M NaClO_4_ prior to use; no external electron donors or sources of carbon were included in the experiments; and the exposure time was limited to only 2 h. In addition, experiments conducted under similar conditions (Nakajima, 2003; Tsuruta, 2004) demonstrated that Au removal from solution can be readily reversed by exposure of the biomass to a 0.1 M thiourea solution. Therefore, the Au removal from solution that we observed in our experiments was most likely due to adsorption reactions, and the speciation of Au that we determined from the XAFS data most likely reflect the cell wall binding environments for each bacterial species studied.

In our experiments, Au was present initially as aqueous Au(III)–hydroxide–chloride complexes, which were converted during the experiments to Au(I) that was bound to the bacterial cells. The XAS data provide unequivocal evidence for the Au reduction and binding mechanisms, but the data do not provide constraints on the relative timing of the reduction and adsorption reactions that occurred. It is possible that reduction occurs on the cell wall after adsorption of Au(III). We observed no Au–Cl signal in the Au on the biomass, so dechlorination of the aqueous Au–hydroxide–chloride complexes must occur prior to adsorption. Another possibility is that the reduction occurs in solution prior to adsorption of the Au, with electrons supplied to the aqueous Au(III)–hydroxide–chloride complexes by bacterial exudates, forming an intermediate (and unidentified) aqueous Au(I) species which eventually adsorbs onto the bacteria. Speciation studies of the aqueous phase during reduction and measurements of Au reduction potential in the presence of isolated bacterial exudates are needed to distinguish between these two mechanisms.

This study provides constraints on what are likely the initial stages of Au biomineralization and the formation of Au(0) nanoparticles by bacteria. Previous studies have demonstrated that metal and sulfate reducing bacteria can reduce and precipitate aqueous Au(III) to form Au(0) nanoparticles at the solution–cell wall interface (e.g., Kartikayen and Beveridge, 2002; Lengke and Southam, 2005, 2006; Reith and McPhail, 2006; Lengke et al., 2006a,b, 2007). In addition, bacteria have been reported to metabolically reduce Au(III) complexes to Au(0) nanoparticles likely with the formation of intermediate Au(I)–S complexes via fast passive adsorption mechanism (Jian et al.,...
Our results not only provide direct molecular-level evidence of this mechanism, but also suggest that the first step in this process is the adsorption of Au to sulfhydryl and amine and/or carboxyl binding sites on bacterial cell walls, and that these sites may be the locations where Au reduction occurs. Moreover, this research implies that sulfhydryl binding and subsequent reduction of Au on bacterial cell walls may play an important role in affecting the speciation and distribution of Au in near-surface geologic systems.

### 4. CONCLUSIONS

The Au L\textsubscript{III} XAFS measurements of aqueous Au standards, standard Au compounds and biomass samples exposed to Au(III) solutions provide information about the valence state of Au in the biomass samples as well as the average local atomic environment of Au atoms adsorbed to the cell walls. Both XANES and EXAFS data of Au biomass samples indicate that more than 90% of the adsorbed Au, which started as Au(III) in solution, was reduced to Au(I) atoms by non-metabolizing \textit{B. subtilis} and \textit{P. putida} bacterial cells, possibly due to oxidation of electron-transport chain enzymes located within the bacterial cell walls.

In contrast to what has been observed for Au(III) interaction with actively metabolizing metal-reducing bacterial cells, no Au(0) or Au-Au nearest neighbors were observed in our experimental systems. The EXAFS data suggest that dechlorination accompanies bacterial adsorption of Au from Au(III)-hydroxide-chloride solutions under all conditions studied here. The EXAFS data also suggest that Au on the \textit{B. subtilis} and \textit{P. putida} cell walls is present almost exclusively as Au(I) bound to a mixture of amine/carboxyl sites as well as to sulfhydryl functional groups. The relative importance of the sulfhydryl groups increases with increasing pH and with decreasing Au loading on the cell walls. The coordination numbers for all of the biomass samples studied here are consistent with bidentate binding of Au on the bacterial cell walls, and the bond distances indicate inner-sphere binding of the Au. Collectively, the XAFS data presented here provide a framework for understanding the initial stages of formation of Au(0) nanoparticles by bacteria from Au(III) solutions, and the results enhance our ability to account for the behavior of Au in bacteria-bearing geologic systems.

### ACKNOWLEDGEMENTS

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### APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2012.02.030.

### REFERENCES


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