XAFS Study of U-Bacterial Cell Wall Interaction

S. D. Kelly,[1] K. M. Kemner,[1] J. B. Fein,[2] D. A. Fowle,[3] M. I. Boyanov,[2] B. A. Bunker,[2] N. Yee[2]

[1] *Environmental Research Division, Argonne National Laboratory, Argonne, IL, U.S.A.*

[2] *University of Notre Dame, Notre Dame, IN, U.S.A.*

[3] *Present address: University of Windsor, Windsor, Ontario, Canada*

Introduction

Bacteria are ubiquitous in near-surface geologic systems, and they can affect the distribution and fate of metals in these systems through adsorption reactions between metals and bacterial cell walls. Many studies have addressed relative binding capacities of metals to cell walls. Recently, Fein et al.[1] developed a chemical equilibrium approach to quantifing metal adsorption onto cell walls, treating the sorption as a surface complexation phenomenon. The authors used acid-base titrations to determine site-specific stability constants. This approach implies that different functional groups are deprotonated in different pH ranges and absorb metals from solution in those ranges. Fowle et al.[2] observed a similar pH dependence for UO_2^{+2} adsorption, but they also observed significant uranyl adsorption at low pH, which they ascribed to interaction between the uranyl cation and a neutrally charged phosphoryl group on the cell wall. However, all of these models are based on macroscopic adsorption data, and the nature and mechanism of metal binding to the cell walls have not been determined. X-ray absorption fine structure (XAFS) measurements at the complex metal absorption edge can distinguish between the different functional groups thought to be important in metal uptake, thereby proving or disproving proposed surface complexation models.

Methods

Samples of U sorbed to cultures of *Bacillus subtilis* were prepared by exposing aqueous U(VI) solutions to a known concentration of biomass, so that the ratio of metal to cell surface area remained constant. To probe pH-dependent changes in the sorption mechanism, samples were prepared as a function of pH, from pH 1 to 5. The biomass solution was centrifuged, rinsed, and recentrifuged, and fluorescence XAFS measurements were made on the resulting wet, homogeneous pastes. All XAFS measurements were made at the MRCAT sector 10-ID beamline at the Advanced Photon Source.[3]

The energy of the incident x-rays was selected by using a double-crystal monochromator, and higher harmonics were rejected by using an Rh mirror. The incident x-ray intensity was sampled by using a Stern-Heald detector[4] filled with Ar gas. Linearity tests[5] indicated less than 0.3% nonlinearity for a 50% decrease in incident x-ray intensity. The incident x-ray intensity varied by less than 15% throughout the energy range of the XAFS measurements. Multiple scans were collected at six different locations on the sample to reduce radiation exposure. The sample was exposed for approximately one minute for each of the two to five measurements at each location. No time-dependent change in the XAFS data was observed for any of the samples. An example of the measured x-ray absorption coefficient for the U L3-edge is shown in Fig. 1a.

The codes contained in the UWXAFS package were used to analyze the data. The smoothly varying background was subtracted from the XAFS data by using the AUTOBK program[6] (see Fig. 1a). The result of averaging six χ(k) data sets is shown in the inset of Fig. 1a.

Results and Discussion

The theoretical models, generated by FEFF7,[7] were based on the crystal structure of hydrogen uranyl phosphate tetrahydrate[8,9] and sodium uranyl(VI) triacetate.[10] First models for hydrated uranyl, uranyl acetate, and uranyl phosphate standards were developed. These models were then combined and used to generate a model for the U-*B. subtilis* data. An example of the data and results of the fit to the data are shown in Fig 1b. The best-fit values for the fit to the U-*B. subtilis* sample and the uranyl standards are similar, giving confidence in this model for the U-*B. subtilis* data. The results for the number of phosphoryl and carboxyl functional groups bound to the urnayl as a function of pH are shown in Fig. 2. These results indicate that EXAFS can be used to distinguish hydroxyl, carboxyl, and phosphoryl bonding of U to the *B. subtilis* biomass and are consistent with the surface complexation model proposed by Fein et al.[1] and Fowle et al.,[2] in which protonated phosphoryl groups complex U at pH 1.67.

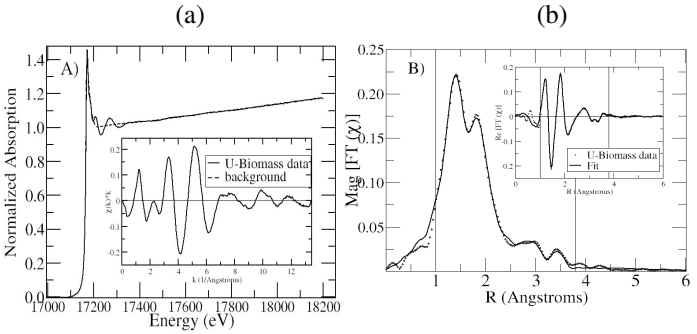

*FIG. 1. (a) Normalized absorption coefficient and inset χ(k) * k data. (b) The magnitude, Mag[FT(χ)], and real part, Re[FT(χ)], of the Fourier transform of the χ(k) * k best-fit model, with data from a U-biomass sample at pH 1.67. The data range Δk = [2.5 : 13.5] Å^{-1} was used in the Fourier transform. The fit range is indicated by the vertical lines.*

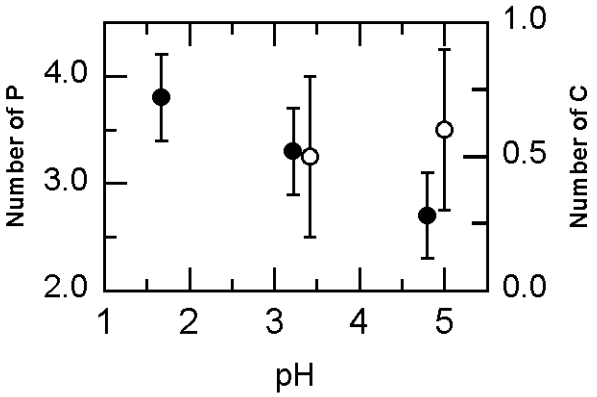

FIG. 2. Numbers of phosphoryl (P; solid cilcles) and carboxyl (C; open circles) functional groups bound to uranyl as a function of pH.

Acknowledgments

Support for S. D. K. and K. M. K. was provided by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Natural and Accelerated Bioremediation Program, under contract W-31-109-Eng-38. This research was also supported by National Science Foundation grant EAR-9905704 and by the Donors of the Petroleum Research Fund, administered by the American Chemical Society. Portions of this work were performed at MRCAT beamline at the Advanced Photon Source. MRCAT is supported by the U.S. Department of Energy under contract DE-FG02-94-ER45525 and by the member institutions. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Energy Research under Contract No. W-31-102-Eng-38.

References

[1] J.B. Fein, C.J. Daughney, N. Yee, and T.A. Davis, Geochim. Cosmochim. Acta **61**, 3319 (1997).

[2] D.A. Fowle, J.B. Fein, and A.M. Martin, Environ. Sci. Technol. **34**, 3737-3741 (2000).

[3] C.U. Segre, N.E. Leyarovska, L.D. Chapman, W.M. Lavender, P.W. Plag, A.S. King, A.J. Kropf, B.A. Bunker, K.M. Kemner, P. Dutta, R.S. Druan, and J. Kaduk, Synchrotron Radiation Instrumentation: Eleventh U.S. Conference **CP521**, 419-422 (2000).

[4] E.A. Stern and S.M. Heald, Rev. Sci. Instrum. **50**, 1579-1583 (1979).

[5] K.M. Kemner, A.J. Kropf, and B.A. Bunker, Rev. Sci. Instrum. **65**, 3667-3669 (1994).

[6] M. Newville, P. Livin, Y. Yacoby, J.J. Rehr, and E.A. Stern, Phys. Rev. B **47**, 14126-14131 (1993).

[7] S.I. Zabinsky, J.J. Rehr, A. Ankudinov, R.C. Albers, and M.J. Eller, Phys. Rev. B **52**, 2995-3009 (1995).

[8] R. Mercier, M. Pham Thi, and P. Colomban, Solid State Ionics **15**, 113-126 (1984).

[9] B. Morosin, Acta Cryst **B34**, 3732-3834 (1978).

[10] D. Templeton, A. Zalkin, H. Ruben, and L. Templeton, Acta. Cryst. **C41**, 1439-1441 (1985).