Environmental Actinide Mobility: Plutonium and Uranium Interactions with Exopolysaccharides and Siderophores of Aerobic Soil Microbes

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Microorganisms are likely to effect the overall environmental behavior of radionuclides through solubility and speciation changes, biosorption, bioaccumulation or other biotransformations. Our goal is to understand how key interactions with aerobic soil microbes affect the speciation and migration of actinides and how they may be exploited to develop remediation technologies. Initially, we have focused on fundamental interactions of environmentally important plutonium and uranium species with common aerobes and the siderophores and extracellular polymers they produce. Specifically, we are studying: (1) siderophore complexation and uptake using *Streptomyces pilosus*, which produces desferrioxamine B (DFB), *Pseudomonas stutzeri*, which produces desferrioxamine E (DFE), and *Rhodococcus rhodochrous* strain OFS, which produces an uncharacterized catecholate siderophore; and (2) the exopolymer binding using the glutamic acid polymer of *Bacillus lichenformis* and the previously uncharacterized polymer of *Rhodococcus erythropolis.*

By studying siderophores in the presence of actinide solids, we have shown that stability constants are not sufficient for predicting solubilization by complex biomolecules. Surprisingly, the siderophores DFE and DFB do not readily solubilize Pu(IV) hydroxide or oxide. They are 100 times slower than EDTA, despite having significantly higher solution formation constants with Pu(IV). Despite being unable to rapidly solubilize Pu(IV), desferrioxamine-Pu(IV) complexes are thermodynamically favored. No matter what oxidation state of Pu (III, IV, V, VI) is present initially, desferrioxamines rapidly and irreversibly form the Pu(IV)DFO complex at environmentally relevant solution pH. In fact, up to 12 equivalents of Pu(VI) can be reduced per DFE/DFB. We have structurally characterized the first Pu(IV) complex of a biomolecule. We have crystallized a Pu(IV)-DFE complex and found the Pu(IV) is nine coordinate and that DFE spans only one hemisphere of the Pu. The structure has very interesting similarities and differences with the structure of the corresponding Fe(III) complex.

Production of the siderophore of *Rhodococcus rhodochrous* is regulated by iron content of the media. We have optimized production of this siderophore, allowing us to characterize it. Amino acid analysis indicates the presence of arginine in the siderophore. ^{1}H-NMR spectroscopy shows that there are two distinct catechols in the siderophore in a 1:1 ratio. Matrix assisted laser desorption ionization mass spectral (MALDI-MS) results confirm the catecholate functionality and are consistent with a siderophore composed of 2,3 dihydroxybenzoyl arginine. Metal and radionuclide uptake studies of this siderophore and bacteria are underway.

We have purified large quantities of the polyglutamic acid capsule from *Bacillus licheniformis* and determined its stability as a function of temperature and HCl concentration. It is approximately 800 KDa, with approximately 6200 subunits. The surface charge varies with ionic strength and ion type with behavior very different from the surface charge variations typically measured for mineral surfaces. The polymer forms a water soluble U(VI) complex at 1: 10 U:glutamate ratios, but forms insoluble complexes at lower ratios. The conformation of the polymer changes (helical to beta) with varying metal binding, pH and ionic strength.

We have optimized the production and purification of the exopolymer produced by *Rhodococcus erythropolis* that has been identified to be a polysaccharide. The most narrow molecular weight distribution was achieved after 14 hours of growth and is centered at 50 KDa.