Siderophore-Mediated Chemistry and Microbial Uptake of Plutonium

Mary P. Neu

Metal ions can interact with microorganisms via a range of mechanisms. For example, metals can sorb directly to an organisms' cell wall, or can react with microbal byproducts, such as extracellular polymers. A classic system of microbial-metal interaction involves low molecular weight organic ligands (siderophores) which are excreted and used by plants and microbes to acquire iron.

All microorganisms, except the *Lactobacilli,* have nutritional requirements for Fe(III) that are not met in aqueous aerobic environments. Under those conditions, Fe(III) has a low solubility (at neutral pH, typical concentrations are about 10^{-18} molar) so that its bioavailability it limited. In order to acquire sufficient iron, bacteria synthesize and secrete siderophores that can chelate Fe(III) and carry it into the cell via specific high-affinity uptake receptors. The siderophores are typically multidentate, oxygen-donor ligands that usually have hydroxamate, catecholate, or carboxylate moieties. Although they are designed to have an extremely high affinity for Fe^{+3}, siderophores can bind other "hard" ions, such as Al(III), Zn(II), Ga(III), Cr(III),, and, Pu(III,IV). (Hard metal ions have high charge to ionic radius ratios and form strong inner sphere complexes with ligands containing "hard" donor atoms, such as oxygen.)

We are examining the redox and coordination chemistry of plutonium with siderophores in order to understand how they could affect actinide biogeochemistry. We have investigated the ability of hydroxamate siderophores to coordinate Pu(IV) and solubilize the Pu(IV) solid, $Pu(OH)_4(s)$, at neutral pH, and have studied the potential for common soil aerobes to interact with plutonium by siderophore uptake mechanisms. We have focused on two tri.-hydroxamate desferrioxamine (DFO) siderophores—desferrioxamine E (DFE) and desferrioxamine B (DFB)—because they are the most-well studied and are readily available.

We have prepared and structurally characterized the first plutonium-siderophore complex, $Al(H_2O)_6[Pu(DFE)(H_2O)_3]_2(CF_3SO_3)_5 \cdot 14H_2O$ (Neu et al. 2000). In fact, our work is the first structural characterization of any plutonium biomolecule. (This complex also contains the first verified nine-coordinate Pu^{4+} ion.) X-ray crystallographic data reveal that the crystal structures of the free ligand, the Fe(III) complex, and the Pu(IV)-DFO complex have interesting similarities. As seen in Figure 2, the DFE occupies approximately one-half of the plutonium coordination sphere. Three water molecules bind to the plutonium in the remaining space. The polytopal geometry of the plutonium coordination sphere is a slightly distorted tricapped trigonal prism; the three bound waters and three oximate oxygens form trigonal planes while the three carbonyl oxygens cap the prismatic faces.

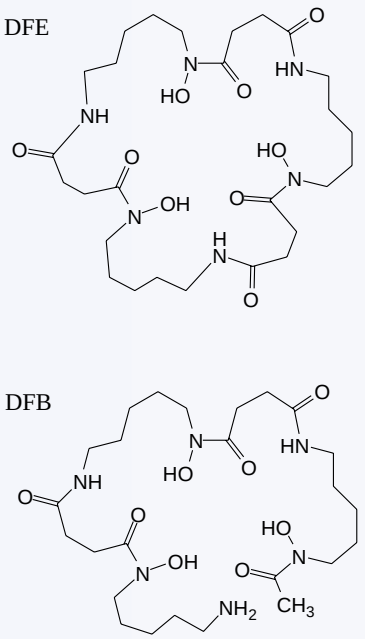

Figure 1. Hydroxamate Siderophores
The chemical structures of two desferrioxamine siderophores are shown above; the linear trihydroxamate produced by *S. pilosus*, Desferrioxamine B (DFB), and the cyclic trihydroxamate produced by *P. stutzeri.*, Desferrioxamine E (DFE).

Ruggerio et al. (2000) found that Pu(IV)-DFO is thermodynamically the most stable complex among all the possible Pu-DFO complexes: regardless of the initial state of the plutonium, eventually the Pu(IV)-DFO complex forms. If Pu(III) is present initially, it is rapidly oxidized by DFO to Pu(IV). Plutonium(V) and Pu(VI) are reduced to Pu(IV). At neutral pH and higher, the reduction to Pu(IV) is instantaneous. Under acidic conditions, Pu(VI) is rapidly reduced to Pu(V), followed by a slow reduction to Pu(IV), with the rate dependent on the pH and the DFO concentration. Stoichiometric titration of Pu(VI) into a DFO solution showed that up to 12 equivalents of plutonium could be reduced per DFO, showing that DFO is a powerful reductant for Pu(VI). In contrast, U(VI)-DFO is inert to reduction.

The formation constant, β, for the Pu(IV)-DFO complex formed at neutral pH has been estimated to be extremely high, $\log \beta = 30.8$, for DFB (Jarvis, et al., 1991). That constant is higher than those known for many organic chelators, such as EDTA, citrate, and tiron. High complex-formation constants are generally equated with effective solubilization of the metal ion by the ligand.

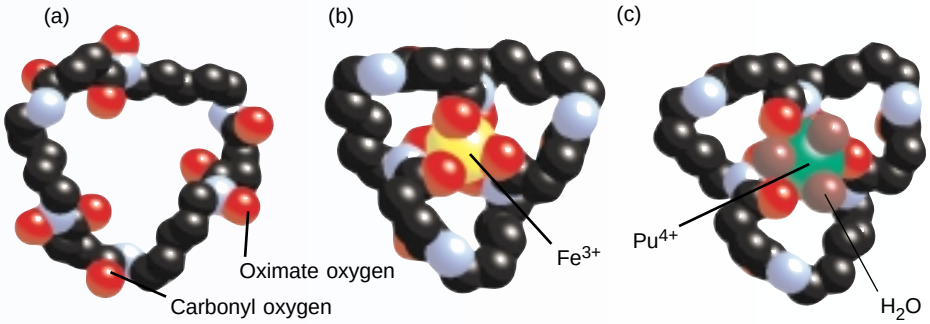

Figure 2. Sidereophore Complexation
(a) A space filling model of DFE.* (b) Fe-DFE complex. A twist of the carbon backbone allows the three oximate oxygens and the three carbonyl oxygens to form a "cavity" that securely holds the Fe^{3+} ion. (c) The Pu-DFE complex is structurally similar to Fe-DFE. The large Pu^{4+} ion protudes slightly from the cavity. Three water molecules remain bound to the ion.**

*Van der Helm, D., and M. Poling. 1976. *J. Am. Chem. Soc.* **98**: 82. **Hossain, M. B., D. van der Helm, and M. Poling. 1983. *Acta Cryst. B* **39**: 258.

However, the DFO siderophores are far less effective at solubilizing Pu(OH)4(s) in buffered neutral solution compared to the organic chelators, even though the latter have lower Pu(IV) complex-formation constants (Cleveland, 1991). Pu(IV) hydroxide can be slowly solubilized by EDTA at a rate of approximately 1.1 μmoles per day (μmol/day), and by citrate and tiron at rates of 0.2 μmol/day, and 0.1 μmol/day, respectively. In contrast, the rates of solubilization by DFE and DFB are 50 to 500 times slower than EDTA. Additionally, EDTA solubilization of Pu(OH)4(s) was 10 times slower after pre-treating the plutonium with DFB. These surprising results suggest that the DFO siderophores are passivating the surface of the Pu(IV) solid and inhibiting solubilization.

We also found that DFB can mediate plutonium association with bacteria (John et al. 2000). As an example, the soil isolate *Aureobacterium flavescens* (JG-9) is a siderophore auxotroph, producing no siderophore of its own but requiring one to obtain iron. We verified that all iron and plutonium uptake by *A. flavescens* is strictly associated with the added siderophore DFB, that is, no uptake occurs without its addition. Uptake of Fe-DFB or Pu-DFB also needed living, metabolically active bacteria; heat-killed or metabolically inhibited cells showed little association with the Fe-DFB or Pu-DFB complex. These results points to similar energy-dependant uptake processes by which the iron and plutonium are transported to the cell interior. Interestingly, DFB-mediated uptake of plutonium is about two orders of magnitude lower than with iron and exhibits a different time dependence. Whereas iron uptake is fastest at the beginning, generally slowing and leveling out to approximately 100 nanomoles per milligram of bacteria after 1 hr, plutonium uptake increases linearly until it reaches a peak rate of 25 nanomoles per milligram of bacteria after 10 hours.

We have examined competition between Fe-DFB and Pu-DFB uptake by adding different quantities of the metal complexes at varying times. We are currently determining the location of iron and plutonium on and within the cell by using various chemical treatments of the cells. Our results indicate differences in recognition, uptake, and final location of the iron and plutonium. The Fe-DFB complex is rapidly recognized by the uptake channel receptors and is translocated into the cell interior. While the Pu-DFB complex appears to be recognized by the same binding site(s) with approximately the same affiinity, it is only slowly transported across the cell membrane into the interior.

Hydroxamate siderophores are naturally present in the environment (Powel et al. 1980). They have a high binding affinity for Pu(IV), a large reducing capacity for Pu(VI) and Pu(V), and they inhibit the solubilization of plutonium solids. Our recent work shows that Pu-DFO complexes are recognized by microbial metal-siderophore binding sites and may be taken into cells. These varied and dynamic interactions suggest that these strong metal chelators will significantly affect plutonium biogeochemistry.

Further Reading

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Mary Neu earned B.S. degrees in math and chemistry and graduated *magna cum laude* from the University of Alaska, where she was named the Outstanding Student in the Physical Sciences in 1986. She received a Ph. D. in inorganic and nuclear chemistry from the University of California at Berkeley in 1993 under the direction of Professors Darleane Hoffman and Ken Raymond. After a year as a University of California post-doctoral research fellow at Los Alamos, she joined the technical staff in 1995. Mary currently directs and performs research in the Actinide, Catalysis and Separations Chemistry group in the Chemistry, Science, and Technology Division. Her areas of interest include inorganic, environmental, bioinorganic and radiochemistry, with an emphasis on plutonium and other actinides.