

Biotransformation of uranium complexed with organic ligands

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Abstract. Natural ligands can affect the bacterial metabolism and reductive precipitation of uranium. The metabolism of metal-organic complexes by bacteria depends upon the type of complex formed between the metal and organic ligand. For example, Fe(III) forms a bidentate complex with citric acid, and was readily metabolized by *Pseudomonas fluorescens* under aerobic conditions, whereas the binuclear complex formed between U and citric acid was recalcitrant. When supplied with an electron donor, anaerobic bacteria reduced U(VI)-citrate to U(IV)-citrate with little precipitation of uranium. X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy analysis showed that the reduced uranium was present in solution as mononuclear U(IV)-citrate complex. These results suggest that when reduced uranium is complexed with organic ligands, it can be mobile.

Introduction

Uranium exists in the environment predominantly as U(VI)- and U(IV)-oxidation states. Natural organic complexing agents present at the contaminated sites may not only affect the mobility of uranium, but also its microbial transformation and reductive precipitation. Biotransformation of the complexed radionuclides should precipitate the radionuclides and retard their migration. The mechanisms of microbial reduction and the precipitation of uranyl nitrate and uranyl carbonate are well understood. However, there is a paucity of comparable information on transformation of uranium complexed with natural low-molecular-weight soluble organic ligands.

We investigated the mechanisms of complexation of, and biotransformation of uranium with natural organic ligands, such as ketogluconic, oxalic, malic, citric,

protocatechuic, salicylic, phthalic, and fulvic acids, and catechol. Potentiometric titrations of uranium with the organic ligands confirmed that complexes were formed; EXAF analyses characterized their structures. Ketogluconic acid formed a mononuclear complex with uranium involving the carboxylate group, while malic acid, citric acid, and catechol formed binuclear complexes. Phthalic acid formed a bidentate complex involving the carboxylate group, while catechol bonded to uranium through the two hydroxyl groups. The hydroxycarboxylic acids were bound in a tridentate fashion to uranium through two carboxylate and the hydroxyl groups. In this paper, I examine the biotransformation of uranium complexed with citric acid in aerobic and anaerobic such as denitrifying, iron-reducing, fermentative, and sulfate-reducing environments.

Citric acid and metal complexes

Citric acid, a naturally occurring compound, is a multidentate ligand that forms stable complexes with various metal ions. It forms different types of complexes with transition metals and actinides including bidentate, tridentate, binuclear, or polynuclear complex species (Fig. 1).

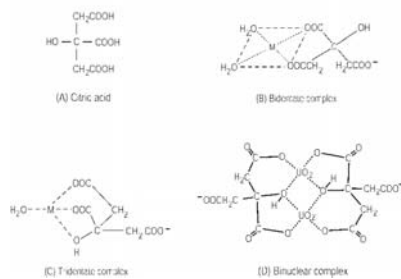


Fig. 1. Metal citrate complexes.

Biotransformation of binary uranyl-citrate complexes under aerobic conditions.

Biodegradation of metal citrate complexes depends upon the type of complex formed between the metal and citric acid; bidentate complexes are readily biodegraded, whereas tridentate complexes are recalcitrant (Francis et al 1992). *Pseudomonas fluorescens* under aerobic conditions readily broke down the bidentate complexes of Fe(III)-, Ni-, and Zn-citrate, but not tridentate Al-, Cd- and Cu-citrate, nor the binuclear U-citrate complexes, which include the hydroxyl group

of citric acid (Fig. 2). The lack of degradation was not due to metal toxicity; rather, limited by the transport and/or metabolism of the complex by the bacteria (Joshi-Tope and Francis, 1993). No relationship was observed between the biodegradability and the stability of the complexes. The tridentate Fe(II)-citrate complex, although recalcitrant, was readily biodegraded after oxidation and hydrolysis to the bidentate Fe(III)-citrate form, denoting a structure-function relationship in the metabolism of the complex (Francis and Dodge 1993). The presence of the free hydroxyl group of citric acid is the key determinant affecting this process. Thus, adding excess citric acid to equimolar (0.52mM) uranyl citrate resulted in the metabolism of the excess citric acid; the uranyl-citrate complex was not toxic to the bacterium. In the presence of 1-, 2-, and 3-fold excess citric acid, the citric acid remaining in each complex after biodegradation was 0.75, 0.80, and 0.83 mM, respectively. The final stoichiometry of U-citric acid in all three treatments was approximately 2:3, indicating the formation of 2:3 U-citric acid complex.

Biotransformation of uranyl-citrate by *Pseudomonas fluorescens* under denitrifying conditions.

Citrate metabolism by *Pseudomonas fluorescens* under denitrifying conditions is facilitated by the enzyme aconitase, as in aerobic metabolism. Breakdown of the various metal-citrate complexes by *P. fluorescens* under denitrifying conditions was similar to that observed aerobically, but the rates were much lower (Figs 3A and B). The bacterium completely degraded bidentate complexes, but did not metabolize the tridentate complexes, and the binuclear U-citrate complex (Joshi-Tope and Francis, unpublished results).

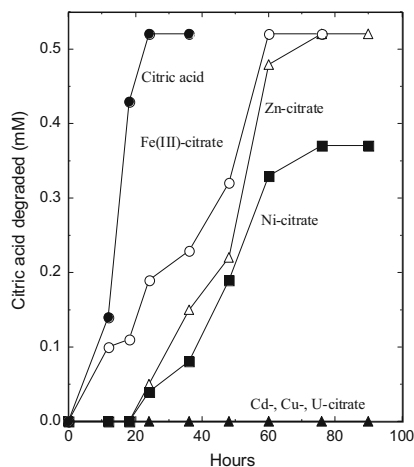


Fig. 2. Biodegradation of metal citrate complexes by *P. fluorescens* under aerobic conditions (Joshi-Tope and Francis, 1993).

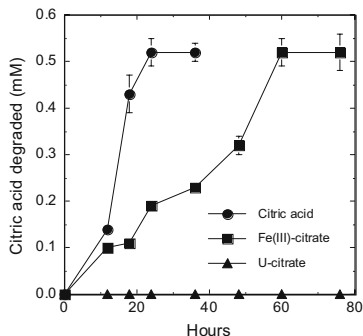


Fig. 3A. Biotransformation of uranyl-citrate by *P. fluorescens* under aerobic conditions.

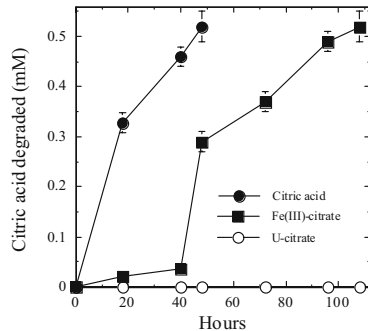


Fig. 3B. Biotransformation of uranyl-citrate by *P. fluorescens* under denitrifying conditions.

Biotransformation of metal-citrates by *Clostridia* under anaerobic conditions

Clostridium sphenoides metabolized the bidentate Fe(III)-, Ni-, and Zn-citrate complexes, converting citric acid to acetic- and butyric-acids, CO₂, and H₂. The metals released from the complexes were present in solution. Fe(III)-citrate was readily metabolized by *C. sphenoides* with the reduction of the ferric to the ferrous form; the latter remained in solution. The tridentate Cd-, Cu-, and U-citrate complexes were not metabolized by the bacterium (Francis et al 2002). In the presence of excess citric acid or glucose, U(VI)-citrate was reduced to U(IV)-citrate that remained in solution (Fig. 4A). These results agree well with observations of the metabolism of metal-citrate complexes by *P. fluorescens* in aerobic and denitrifying environments.

In contrast, *Clostridium* sp. did not metabolize citric acid nor reduce the Fe(III) or U(VI) bound to the acid (Fig. 4B). However, on adding glucose, Fe(III)-citrate was reduced to Fe(II)-citrate, and U(VI)-citrate was reduced to U(IV), which then remained in solution as the U(IV)-citrate complex (Fig. 4B). Glucose was metabolized to acetic- and butyric-acids, CO₂, and H₂.

Sulfate-reducing *Desulfovibrio desulfuricans* and the facultative iron-reducing bacterium *Shewanella halotolerans* anaerobically reduced U(VI) complexed with oxalate or citrate to U(IV), but little uranium was precipitated. The reduced U(IV) remained in solution complexed with oxalate or citrate (Ganesh et al 1997; 1999).

These results show that complexed uranium is readily accessible for the microorganisms as an electron acceptor, despite the bacteria's inability to metabolize the organic ligand complexed to the actinide.

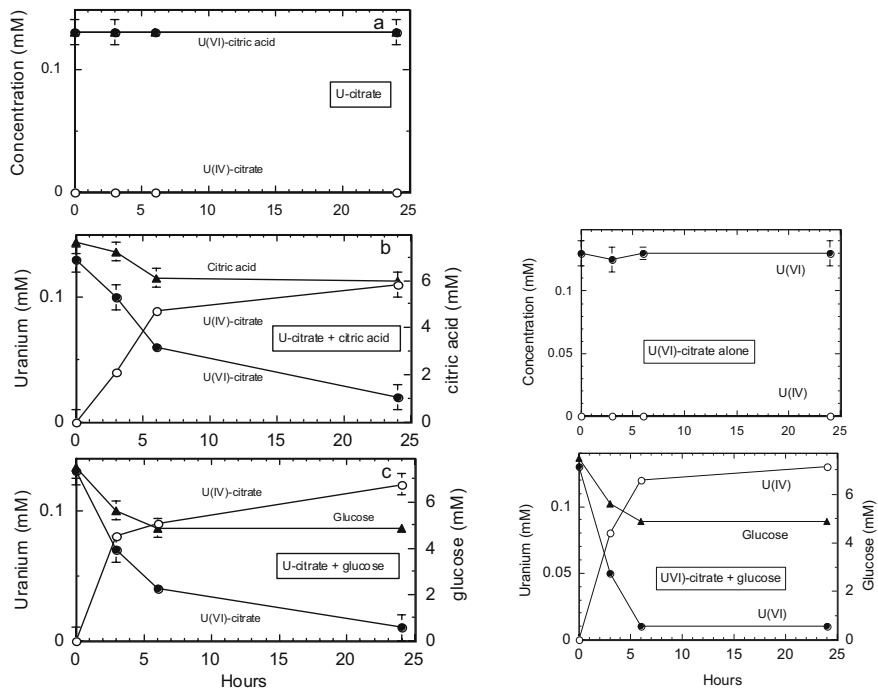


Fig. 4A. Biotransformation of uranyl citrate by *C. sphenoides*.

Fig. 4B. Biotransformation of uranyl citrate by *Clostridium* sp.

Biotransformation of Ternary Fe(III)-U(VI)-citrate complexes.

Citric acid forms ternary mixed-metal complexes with various metal ions involving the hydroxyl and carboxyl groups of citric acid. The presence of 1:1:2 Fe:U: citric acid in solution was confirmed by potentiometric titration, UV-vis spectrophotometry, gel-filtration chromatography, and extended x-ray absorption fine structure (EXAFS) (Fig. 5) analysis (Dodge and Francis, 1997; 2003). Biotransformation studies of Fe-U-citrate complex by *P. fluorescens* showed that the ternary 1:1:2 Fe:U: citric acid complex was resistant. When a one-fold excess of citric acid was added to this complex, it was completely degraded with no change in its stoichiometry. However, with a two-fold excess, a 1:1:1 Fe:U: citric acid complex remained in solution after all the citric acid was biodegraded. Thus, similar to the U-citrate complex, the Fe-U-citrate complex appears to resist biodegradation (Dodge and Francis, 1997). With the persistence of mixed-metal-citrate complexes in wastes and contaminated environments, uranium may thus be mobi-

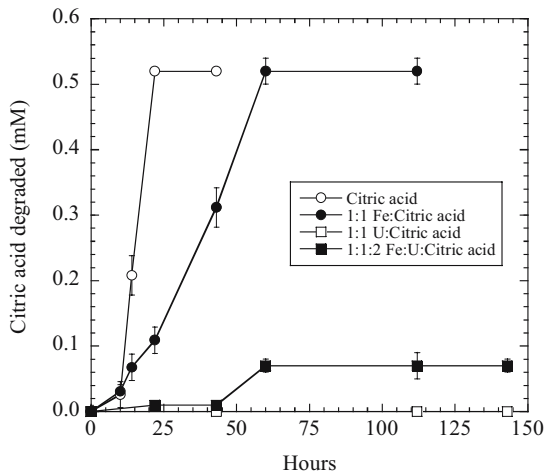


Fig. 5. Biotransformation of 0.52 mM citric acid, 1:1 Fe-citrate, 1:1 U-citric acid, and 1:1:2 Fe-U-citric acid complexes (Dodge and Francis 1997).

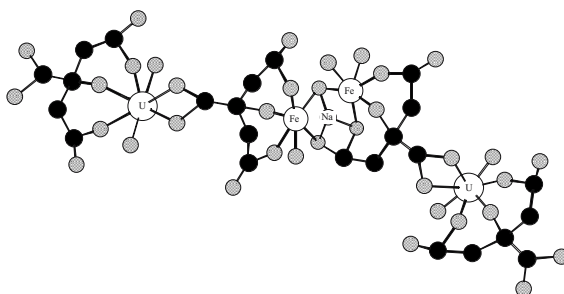


Fig. 6. Proposed structure of the ternary 2:2:4 Fe:U: citric acid complex (Dodge and Francis 2003).

lized. Although a wide variety of microorganisms are present in uranium mining wastes and in natural radioactive mineral deposits, there has been no full evaluation of the extent to which they regulate the mobility of uranium complexed with organic and inorganic ligands. Undoubtedly, a fundamental understanding of the molecular-level mechanisms of interactions of microorganisms with radionuclides associated with natural organic and inorganic ligands and colloids under various microbial- process conditions will aid in developing appropriate strategies for the remediation and long-term stewardship of contaminated sites.

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