 ROBL-CRG	Experiment title: Molecular scale studies on the speciation of U(VI) bound to cells and S-layer proteins of Archaea.	Experiment number: 20-01-688
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Report:

INTRODUCTION. Microorganisms are known to play an important role in the migration behavior of uranium in the environment [1]. In contrast to the well studied interactions of bacteria with uranium, little is known about the processes implicated in the complexation of this radionuclide by representatives of the second microbial domain of life, the “*Archaea*”.

EXPERIMENTAL. *S. acidocaldarius* was cultivated at pH 2.5 and 70 °C in a mineral salt medium [2] supplemented with 0.1% tryptone and 0.005% yeast extract. Cells grown to the late exponential phase were harvested by centrifugation and washed twice with 0.1M NaClO₄. Four parallel cell suspensions were prepared with pH 1.5, 3, 4.5 and 6. They were shaken at room temperature in 10 ml uranium solution (0.5 mM UO₂(NO₃)₂·6 H₂O, pH 1.5, 3, 4.5 or 0.05 mM UO₂(NO₃)₂·6 H₂O at pH 6). The sample preparation and the set-up of the X-ray absorption spectroscopic measurements were performed as described earlier [3].

RESULTS. Quantitative fit results of the EXAFS data indicated that at all acidic conditions studied the adsorbed U(VI) has the common linear trans-dioxo structure: two axial oxygen atoms at about 1.77-1.78 Å, and an equatorial shell of 4 to 6 oxygen atoms at 2.25-2.35 Å. The U-O_{eq1} bond distance is within the range of previously reported values for phosphate bound to uranyl [4,5]. The FT spectra of the U-treated microbial samples contain a FT peak at about R+Δ ~ 2.3 Å, interpreted as a contribution from oxygen neighbors (O_{eq2}). The fourth FT peak, which appears at R+Δ ~ 3 Å (radial distance R = 3.57-3.62 Å) is a result of the back-scattering from

phosphorus atoms. This distance is typical for a monodentate coordination of U(VI) by phosphate [4,5]. The phosphate groups implicated in the coordination of U could have organic origin since the EXAFS spectrum shows high similarity to those resulting from the uranium complexes with organic phosphate groups, e.g. fructose phosphates as shown in Figure 1. At pH 4.5 a high Debye-Waller factor of the U-O_{eq1} shell was calculated. That suggests the existence of more than one oxygen atoms bond in the equatorial plane. The shell may be split into a shorter U-O_{eq1} distance, from the back-scattering contribution of phosphate oxygen(s) in a monodentate binding mode, and a longer bond distance most likely from oxygen(s) of carboxyl groups in a bidentate binding mode.

The U(VI) EXAFS spectrum of the cells treated at pH 6 differs significantly from that obtained after the U treatment at pH 4.5. This spectrum has a high similarity with those arising from inorganic uranyl phosphate mineral phases, like meta-autunite [4]. The structural parameters of these uranium complexes are also in favour of that finding, e.g. the coordination number of the U-P shell is nearly four and the radial distance of the U-O_{eq1} shell is about (2.25 ± 0.02) Å.

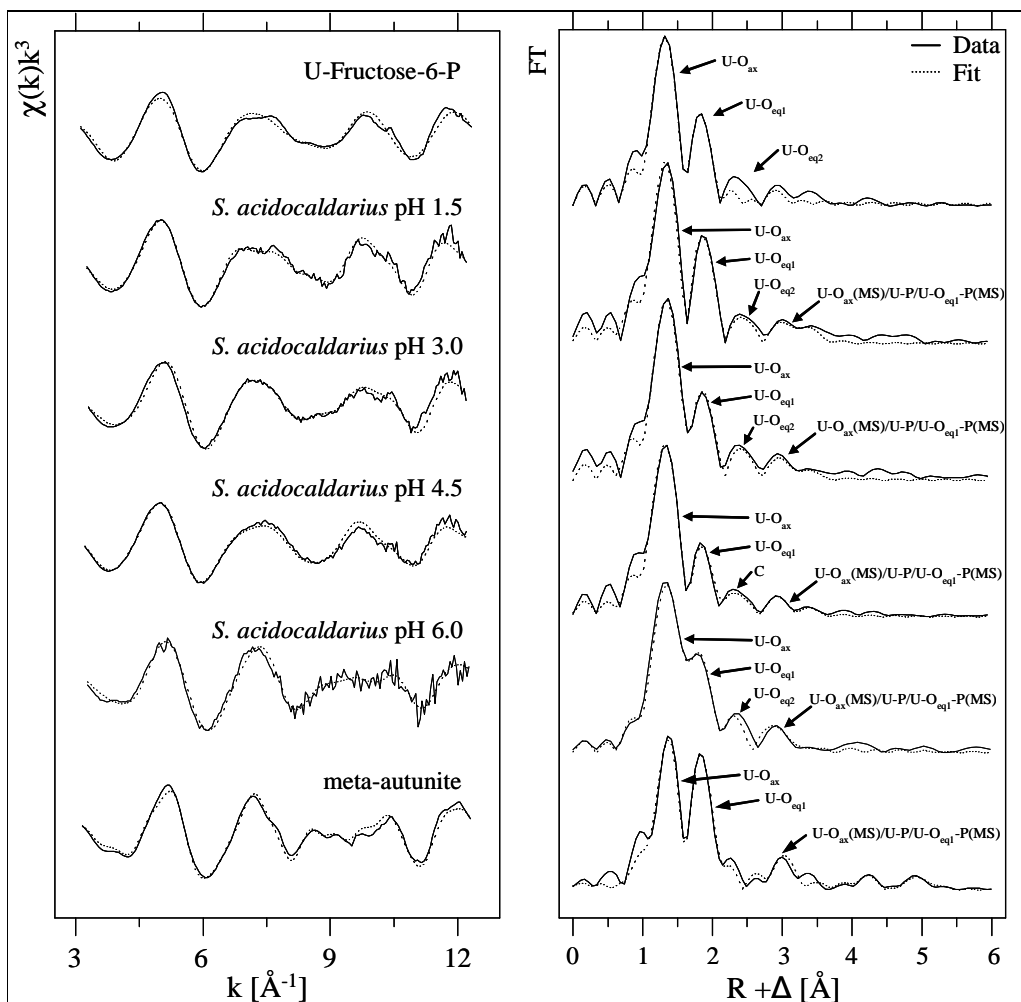


Fig. 1. U L_{III}-edge k^3 weighted EXAFS spectra (left) and the corresponding Fourier Transforms (right) of the uranium complexes formed by *S. acidocaldarius* DSM 639 at different pH values and of the model compounds UO₂-fructose(6)phosphate [6] and meta-autunite [4].

Table 1. Structural parameters of the uranium complexes formed by the cells of *S. acidocaldarius* DSM 639.

Sample	Shell	N ^a	R [Å] ^b	σ^2 [Å ²] ^c	ΔE (eV)
UO ₂ -fructose(6)P ^[6]	U-O _{ax}	2.0 ^d	1.78	0.0015	0.1
	U-O _{eq1}	4.8(5)	2.30	0.0200	
	U-O _{eq2}	1.3(2)	2.88	0.0040 ^d	
pH 1.5	U-O _{ax}	2.0 ^d	1.77(1)	0.0022(2)	-12.0
	U-O _{eq1}	3.9(3)	2.35(1)	0.0066(7)	
	U-O _{eq2}	1.2(2)	2.87(1)	0.0038 ^d	
	U-P	4.6(5)	3.62(1)	0.0080 ^d	
	U-O _{eq1} -P (MS)	9.2 ^e	3.74 ^f	0.0080 ^d	
pH 3.0	U-O _{ax}	2.0 ^d	1.78(1)	0.0023(2)	-13.4
	U-O _{eq1}	4.1(4)	2.30(1)	0.0047(5)	
	U-O _{eq2}	1.2(2)	2.86(1)	0.0038 ^d	
	U-P	3.3(6)	3.59(2)	0.0080 ^d	
	U-O _{eq1} -P (MS)	6.6 ^e	3.70 ^f	0.0080 ^d	
pH 4.5	U-O _{ax}	2.0 ^d	1.78(1)	0.0025(2)	-11.3(4)
	U-O _{eq1}	6.2(8)	2.33(1)	0.017(2)	
	U-C	1.4(3)	2.90(1)	0.0038 ^d	
	U-P	2.2(5)	3.61(2)	0.0080 ^d	
	U-O _{eq1} -P (MS)	4.4 ^e	3.72 ^f	0.0080 ^d	
pH 6	U-O _{ax}	2.0 ^d	1.77(1)	0.0031(2)	-16.4(9)
	U-O _{eq1}	4.7(5)	2.25(1)	0.0058(7)	
	U-O _{eq2}	1.2(3)	2.85(1)	0.0038 ^d	
	U-P	4.9(7)	3.57(2)	0.0080 ^d	
	U-O _{eq1} -P (MS)	9.8 ^e	3.67 ^f	0.0080 ^d	
meta-autunite ^[4]	U-O _{ax}	2.2(1)	1.76	0.0045	-11.0
	U-O _{eq1}	3.9(2)	2.29	0.0026	
	U-P	2.3(3)	3.60	0.008 ^d	

Standard deviations as estimated by EXAFSPAK are given in brackets

^a Errors in coordination numbers are $\pm 25\%$ ^b Errors in distance are ± 0.02 Å^c Debye-Waller factor^d Parameter fixed for calculation^e Coordination number (N) linked twice to the N of the U-P path.^f Radial distance (R) linked to R of the U-P path, according to the model of m-autunite.

In summary, EXAFS analysis of the uranium complexes formed by the cells of *Sulfolobus acidocaldarius* demonstrated that organic and inorganic phosphate groups as well as carboxylic groups are involved in the uranium complexation at acidic conditions.

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