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
Beamline: BM 20	**Date of experiment**: from: 06.11.2009 to: 09.11.2009	**Date of report**: 07.06.2010
Shifts:	**Local contact(s)**: Dr. A. Rossberg	*Received at ROBL:*

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Report:

INTRODUCTION. Highly ordered and self-assembling proteinaceous surface layers (S-layers) are widespread structures of procaryotic cell envelopes [1, 2]. In many archaea, as in the case of the acidophilic creanarchaeon *Sulfolobus acidocaldarius*, studied in this work, they represent the only cell wall component. Up to date nothing is known about the possible role of archaeal S-layers for the protection of the cells against toxic metals and radionuclides. In this study the interactions of the S-layer of *S. acidocaldarius* with U(VI) was investigated to differentiate its role in the complexation of this radionuclide.

EXPERIMENTAL. *S. acidocaldarius* was cultivated at pH 2.5 and 70 °C in a mineral salt medium [3] supplemented with 0.1% tryptone and 0.005% yeast extract. Cells were harvested at the end of the logarithmic growth phase and cell lysis was performed with 0.15% SDS according to Michel *et al.* (1980) [4]. For the complete removal of the cytoplasmic membrane the suspension was treated with 2% SDS and stirred overnight. After centrifugation, the lower dark part of the resulting pellet was discarded, whereas the upper white part was resuspended in HEPES buffer (pH 7) containing 2 mM EDTA and 2% SDS and incubated once again for 30 min at 60 °C. Subsequently the suspension was centrifuged again and the upper part of the resulting pellet, containing the isolated S-layer polymers, was resuspended in distilled water. SDS was removed by five wash steps with distilled water. Uranium was added to the S-layer polymers in form of uranyl nitrate (0.5 mM and 0.05mM $UO_2(NO_3)_2 \cdot 6\ H_2O$ at pH 4.5 and pH 6, respectively) and shaken at room temperature for 48 h. The sample preparation and the set-up of the X-ray absorption spectroscopic measurements were performed analogously to the XAS studies conducted with whole cells of the strain [5, 6].

RESULTS. As shown in Figure 1 the absorption edge in the XANES spectra of both samples is located at ~17166 eV and therewith corresponds well to the edge position of the U(VI) reference solution. In addition, a peak located at 17188 eV, arising from the multiple scattering contribution of the two axial oxygen atoms of U(VI) [7], was observed in the NEXAFS region of

both spectra. These findings clearly demonstrate that uranium is present in both samples as U(VI).

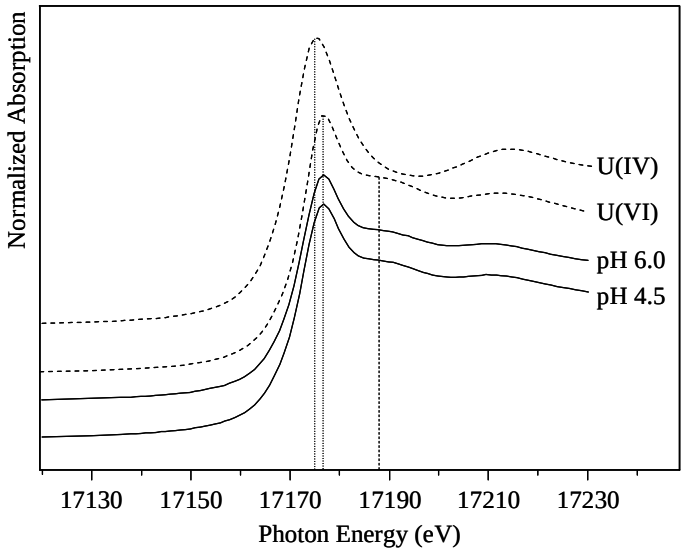

Fig. 1. Uranium L_{III}-edge XANES spectra recorded from the uranium complexes formed at the S-layer protein of *S. acidocaldarius* DSM 639 at pH 4.5 and pH 6 together with those of two reference solutions, one of U(VI) and another one of U(IV). For comparison, the position of the white line of U(IV) and U(VI). Moreover, the position of the XANES peak, which represents the multiple scattering path of the axial oxygen atoms of U(VI) (~17188 eV), is marked by a dashed line.

The isolated U L_{III}-edge k^3-weighted EXAFS spectra and their corresponding Fourier Transforms (FT) are shown in Figure 2. Quantitative fitting of the spectra was performed by using the theoretical phase and amplitude functions calculated with the FEFF8.2 code from a structural model of uranyl triacetate. The best calculated fits for both samples are also shown in Figure 2 and the corresponding structural parameters are summarized in Table 1.

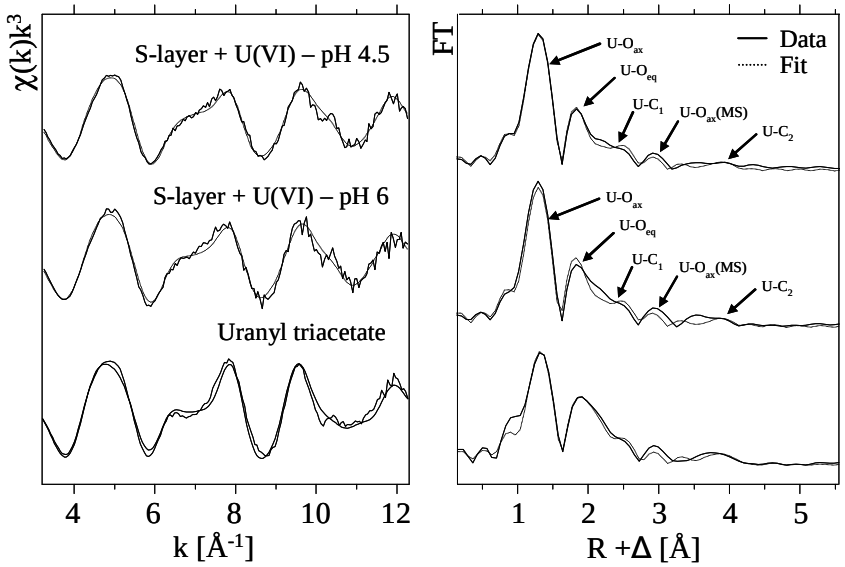

Fig. 2. Uranium L_{III}-edge k^3 weighted EXAFS spectra (left) and the corresponding Fourier transforms (right), along with the best shell fit, of the uranium complexes formed by *S. acidocaldarius* DSM 639 at pH 4.5 and pH 6 and that of the model compound uranyl triacetate.

Table 6. Structural parameters of the uranium complexes formed at the S-layer proteins of *S. acidocaldarius* at pH 4.5 and 6, as well as those of the model compound uranyl triacetate.

Sample	Shell	N[a]	R (Å)[b]	σ^2 (Å^2)[c]	ΔE_0 (eV)
pH 4.5	U-O_{ax}	2.0[d]	1.77(1)	0.0026(1)	2.8(4)
	U-O_{ax} (MS)	2.0[d]	3.54[e]	0.0052[e]	
	U-O_{eq}	4.3(4)	2.42(1)	0.013(1)	
	U-C_1	2.5(2)	2.90(1)	0.0038[d]	
	U-C_2	2.5[f]	4.35(1)	0.0038[d]	
	U-C_1-C_2 (MS)	5.0[f]	4.35[g]	0.009(1)	
	U-C_1-C_2-C_1 (MS)	2.5[f]	4.35[g]	0.009[h]	
pH 6	U-O_{ax}	2.0[d]	1.77(1)	0.0024(1)	2.3(5)
	U-O_{ax} (MS)	2.0[d]	3.54[e]	0.0048[e]	
	U-O_{eq}	5.0(5)	2.43(1)	0.013(1)	
	U-C_1	3.0(3)	2.89(1)	0.0038[d]	
	U-C_2	3.0[f]	4.37(3)	0.0038[d]	
	U-C_1-C_2 (MS)	6.0[f]	4.37[g]	0.012(3)	
	U-C_1-C_2-C_1 (MS)	3.0[f]	4.37[g]	0.012[h]	
Uranyl triacetate	U-O_{ax}	2.0[d]	1.78(1)	0.0023(1)	4.7(4)
	U-O_{ax} (MS)	2.0[d]	3.56[e]	0.0046[e]	
	U-O_{eq}	4.8(3)	2.47(1)	0.0065(6)	
	U-C_1	3.1(3)	2.89(1)	0.0038[d]	
	U-C_2	3.1[f]	4.38(1)	0.0038[d]	
	U-C_1-C_2 (MS)	6.2[f]	4.38[g]	0.008(1)	
	U-C_1-C_2-C_1 (MS)	3.1[f]	4.38[g]	0.008[h]	

Standard deviations as estimated by EXAFSPAK are given in parenthesis
[a] Errors in coordination numbers are ± 25%
[b] Errors in distance are ± 0.02 Å
[c] Debye-Waller factor
[d] Parameter fixed for calculation
[e] Radial distance (R) and Debye-Waller factor (σ^2) linked twice to R and σ^2 of the of the U-O_{ax} path.
[f] Coordination number (N) linked to the N of U-C_1 path.
[g] R linked to R of U-C_2 path.
[h] Debye-Waller factor linked to that of the U-C_1-C_2 (MS) path.

The EXAFS spectra recorded from both samples strongly resemble to each other, which confirms the formation of highly similar uranium complexes at both investigated pH values. In addition, the EXAFS spectra is well in line with that of uranyl triacetate indicating that similar U(VI) complexes were formed at the archaeal S-layer. The most prominent peak of both FT's located at R + Δ ~1.3 Å is assigned to the single backscattering mode (U-O_{ax}) of the two axial oxygen atoms of U(VI). The multiple scattering path (U-O_{ax}-U-O_{ax}) of this axial oxygen shell is also quite important for the fitting of uranyl EXAFS spectra and have an intensity maximum in the FT at R + Δ ~2.9 Å. The second peak of both FT's is attributed to the scattering contribution of the oxygen atoms in the equatorial plane. Fitting results of both investigated samples revealed four to five equatorial oxygen atoms at a radial distance of 2.42 to 2.43 Å. However, the high Debye-Waller factor of 0.013 Å^2 calculated for the U-O_{eq} shell of both samples, indicates that this shell includes equatorial oxygen atoms at different radial distances. In addition, a shell containing two to three carbon atoms (U-C_1) was fitted at a radial distance of 2.89 to 2.90 Å. This distance is typical for the U-C backscattering of U(VI) complexed by the two oxygen atoms of a carboxylate group in a bidentate binding mode. A corresponding complexation modus was already suggested by the EXAFS studies of the uranium complexes formed by whole cells of *S. acidocaldarius* at identical conditions [6]. However, these EXAFS spectra, obtained from whole cells, were rather complex and dominated by a monodentate complexation of U(VI) by organic phosphate groups. Hence, a proper determination of the structural parameters of the U(VI) complexes formed at carboxylate groups was difficult. Comparable complexes, possessing a bidentate coordination of uranium by carbon atoms had also been found at bacterial cells and

their S-layer proteins [8]. With respect to the EXAFS parameters obtained for the U-O_{eq} shell, we assume that the uranyl ion is predominantly complexed by two carboxylate groups. In addition, the uranyl unit exhibit one or two fully hydrated equatorial oxygen atoms. By using this complexation model the high Debye-Waller can be explained by the different radial distances of the equatorial oxygen atoms which are bound to the uranium in a bidentate binding mode - characteristic distance: 2.45-2.51 Å [9] - and that of the equatorial oxygen atoms which exists in their hydrated form - characteristic distance: 2.41-2.42 Å [10, 11]. A second shell of carbon atoms (U-C_2) resulting from the C-atom bearing the carboxylic group was detected at radial distances of (4.35 ± 0.02) Å and (4.37 ± 0.03) Å in the sample incubated at pH 4.5 and pH 6, respectively.

The XAS studies demonstrate that U(VI) forms inner sphere complexes at carboxylate groups of the S-layer proteins in a bidentate binding mode. According to the structural parameters, 1:2 and/or 1:3 uranyl carbonate complexes were formed.

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