Experiment title: Interaction between bacterial S-layers and uranium using EXAFS

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Report:

Introduction. The natural isolate Bacillus sphaericus JG-A12, recovered from a uranium mining waste pile, and the reference strain B. sphaericus NCTC 9602 possess surface layers (S-layer) as outermost cell wall components /1/. S-layers are highly ordered paracrystalline protein layers on the surface of many bacteria and archaea. It has been demonstrated that S-layers interact with different metal ions /2-4/. In this respect the S-layer of the uranium mining waste pile isolate B. sphaericus JG-A12 is of special interest because it may possess a high affinity to a large variety of toxic metals and may play an important role in uranium detoxification. In addition, the S-layer proteins of B. sphaericus JG-A12 and NCTC 9602 differ at their N-terminal domains significantly from all other S-layers studied up to date /1/. In this study we examined the interaction of uranium with native and recrystallised S-layers of B. sphaericus JG-A12 and NCTC 9602 to identify the chemical groups of the protein, which are responsible for metal binding.

Experimental. The incubation of the S-layers with uranium was carried out at pH 4.5. As S-layer lattices are not stable at pH values <5, the purified lattices were stabilized with 1-Ethyl-3-(N,N’-dimethyl-aminopropyl)-carbodiimid (EDC). 20 mg native or recrystallised S-layers were incubated with 9x10⁻⁴ M uranium nitrate in 0.9 % NaClO₄, pH 4.5. After incubation, the S-layers were washed in 0.9 % NaClO₄, pH 4.5 and in aqua. dest., pH 4.5. The pellets were resuspended in water, dried in a vacuum incubator at 30 °C and powdered.

Results and discussion. In all samples uranium is coordinated by two axial oxygen atoms (Oₐx) at a distance of 1.77-1.78 Å. The second and the third shells are issue from the contribution of two oxygen at the equatorial plan. However the third one could be modeled to nitrogen atom, as well (EXAFS could not distinguish between O and N atoms). All FTs contain a shoulder at about 2.5 Å, which can be well fit by C neighbors at 2.89-2.91 Å. This distance is typical for carbonate groups coordinated to
U(VI) in a bidentate fashion. Moreover, if this FT peak originates from bidentate carboxyl ligands, the U-O\textsubscript{eq} bond distance should be approximately 2.46-2.49 Å which is the case in this work. Fig. 1 and 2, a weak peak appears at about R + ΔR = 3 Å (not corrected from phase shift) in the FT, which was modeled to the interaction of uranium with phosphorous giving a distance of 3.64-3.67 Å. A fifth peak at about 3.6 Å was modeled with a single scattering phosphorous shell giving a contribution at about 3.87-3.89 Å.

In summary, EXAFS analysis of the uranium complexes formed by native and recrystallised S-layers of \textit{B. sphaericus} JG-A12 and \textit{B. sphaericus} NCTC 9602 demonstrated the implication of carboxyl and phosphate groups in the interaction with uranium.

References