ESRF	**Experiment title:** Uranium speciation in eucaryotic and procaryotic cells	**Experiment number:** 30-02-726
Beamline: BM 30B	**Date of experiment:** from: 14-09-2005 to: 20-09-2005	**Date of report:** 28-09-2005
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Aims of the experiment and scientific background

Uranium is a naturally occuring heavy metal. Its extensive use in the nuclear cycle and for military applications has focused attention on its health effects and on its potential ecotoxicological impact. It is now established that speciation has a great influence in U dissemination in the biosphere and in its toxicity. Moreover, some bacteria are able to metabolize it and certainly to modifiy its chemical form. Due to their rapid growing and their ubiquity, they represent an important source of U dissemination in the biosphere. U dissemination leads to three types of possible animal and human exposure: either ingestion or inhalation or directly through a wound. After an acute exposure, U rapidly transits from the site of contamination to the blood, which distribute it in the body to target organs, namely kidneys and bones.

The main goal of the experiment was to determine U speciation after accumulation either in **bacteria** and in animal cells. For this purpose, we exposed either *Ralstonia metallidurans* CH34 bacterial strain (procaryotic cells), or NRK-52E (**kidney**, rat) and ROS (**bone**, rat) eucaryotic cells to two U(VI) chemical forms: U(VI)-bicarbonate and U(VI)-citrate. Quantification of metal uptake was monitored by ICP-MS analyses. Our results proved the ability of bacteria and animal cells to ad- or absorb large amounts of U. Moreover, electron microscopy observations and nuclear microprobe analyses of exposed rat renal cells revealed the existence of two U physical/chemical forms: on the one hand some dense precipitates are visible, and on the other hand soluble U is also present in cell cytoplasm. The second aim of our experiment was thus to precisely identify these two chemical forms, and to compare it to U chemical form in bacterial/eucaryotic cell exposure media.

X-ray absorption spectroscopy (EXAFS) of lyophilized cells at the U L_{III}-edge was performed on BM30B.

Experimental method

NRK52E (rat, renal proximal tubule) and ROS (rat, osteoblasts-like) cultured cells were continuously grown at 37 °C, 5% CO_2 in DMEM (Dulbecco's Modified Eagle's Medium) cell culture medium supplemented with 10% (v/v) fetal calf serum. Cells were washed twice with serum free cell culture medium, and exposed either to 300-600 µM U(VI)-bicarbonate or to 150-300 µM U(VI)-citrate diluted in serum free culture medium. After a 24 h incubation period, cells were washed twice with 10 mM $NaHCO_3$ and scrapped from their support. After centrifugation, the pellet was frozen and then lyophilized by highering temperature from –10 °C to 20 °C in 3h under a 0.37 mbar vacuum. The samples were pressed as 5-mm diameter pellets. EXAFS spectra were recorded at U L_{III}-edge in fluorescence mode (samples) or by transmission (references) using a 30 elements solid state Ge detector (Canberra). The monochromator was a Si(220) double crystal.

As no sample evolution was detected during the measurement, acquisitions were conducted at room temperature. At least 6 spectra for each sample were recorded and averaged to improve the statistics. The EXAFS oscillations were isolated from the raw, averaged data by removal of the pre-edge background, approximated by a first-order polynomial, followed by μ_0-removal *via* spline fitting techniques (SEDEM and Athena). Curve-fitting amplitudes and phases will be calculated in a second step.

Results

Bacteria:

U is known to precipitate on phosphate residues of Gram-positive cell wall components. Moreover, bacterial strains known as dissimilatory metal-reducing bacteria (DMRB) reduce U(VI) to the less mobile uraninite (UO_2). *Ralstonia metallidurans* CH34 was shown to resist U concentration up to 8 mM and to accumulate nearly 30% of the initial dose.

XANES and EXAFS spectra of bacteria exposed to U(VI)-bicarbonate and U(VI)-citrate are shown in figure 1.

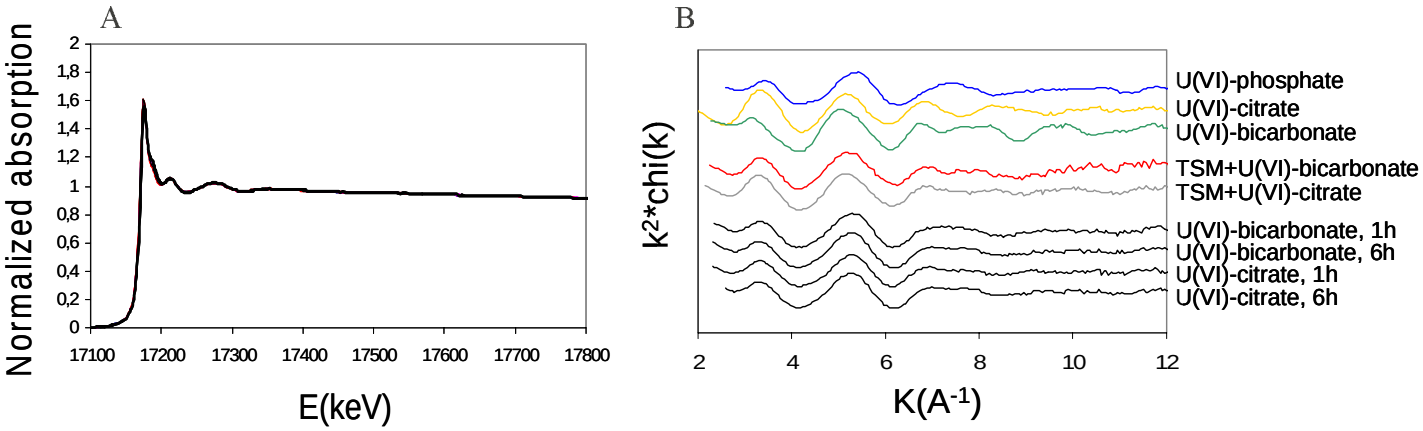

Fig. 1. U L_{III}-edge XANES spectra (A) and the corresponding k^2-weighted EXAFS oscillations (B) of Ralstonia metallidurans CH34 bacterial strain exposed to 1 mM U(VI)-bicarbonate or 1 mM U(VI)-citrate (black) compared to 1 mM U(VI)-bicarbonate (red) and –citrate (grey) diluted in TSM bacteria culture medium and to U(VI)-bicarbonate (green), -citrate(yellow) and –phosphate (blue) standards.

As shown in figure 1, whatever the exposure U(VI) chemical speciation, EXAFS spectra are closely resembling, and present strong similarities with exposure media (TSM+U(VI)-bicarbonate and TSM+U(VI)-citrate). U(VI) speciation in these two exposure media is similar, certainly a combination of U(VI)-phosphate and another form of U. Working with a defined culture medium, it is probable the a second speciation of uranium is U(VI)-sulfate complex.

Animal cells:

It was previously reported that, after exposure to U(VI)-bicarbonate and intracellular accumulation, U(VI) precipitated in a cell compartment called lysosome. X-analysis revealed the presence of U and P, suggesting that the precipitate was U(VI)-phosphate. We also showed that U(VI)-citrate is more toxic than U(VI)-bicarbonate, either to NRK-52E (kidney) and ROS (bone) cells. Assimilation pathways of these two U(VI) chemical forms could thus be different.

EXAFS spectra of NRK-52E and ROS cells exposed to U(VI)-bicarbonate and U(VI)-citrate are shown in figure 2.

In the case of ROS cells, whatever the composition of exposure medium, U(VI) concentration and length of exposure period, the spectra are very near and the local structure of U resemble that of U(VI)-bicarbonate.

Analysis of NRK-52E cells exposed to 300 µM U(VI)-bicarbonate during 24 h leads to a spectrum which might be a combination of U(VI)-bicarbonate and U(VI)-phosphate spectra. On the contrary, the local order of U in NRK-52E cells exposed to 600 µM U(VI)-bicarbonate during 24 h is similar to the one of U(VI)-phosphate. This data confirms the evolution of U local structure in NRK-52E cells as a function of U concentration and exposure duration. As expected, U(VI) has been metabolized by cells and precipitated as a U(VI)-phosphate mineral.

The results should be confirmed after modelling of the Fourier transforms obtained from these data.

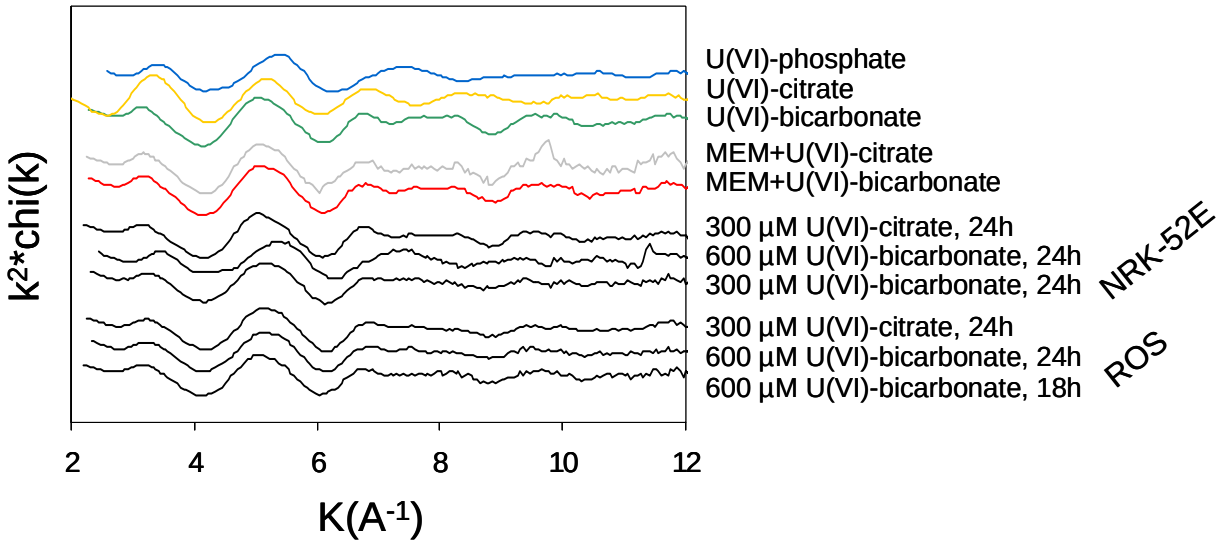

Fig. 2. U L_{III}-edge k^2-weighted EXAFS oscillations of NRK-52E and ROS cells exposed to 300 or 600 µM U(VI)-bicarbonate or -citrate (black) compared to 600 µM U(VI)-bicarbonate (red) and 300 µM U(VI)-citrate (grey) diluted in MEM cell culture medium and to U(VI)-bicarbonate (green), -citrate(yellow) and –phosphate (blue) standards.

Conclusions and perspectives

Fitting of the measured data using a structural model of shells has not been processed yet. However, the first results obtained from XANES spectra and examination of EXAFS oscillations are promising. They should be confirmed by analysis of more samples ranging the whole course of U intoxication for both cell lines. To conclude on bacterial analysis, the spectra of certain standards have to be added to our database (U-sulfate at least). As U local structure in bacteria seem to be the same after 1h and 6h exposure, it would be valuable to analyse kinetics of U accumulation/transformation for shorter exposure periods as bacterial metabolization of U might be much more rapid than expected.

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