ESRF	**Experiment title:** ***Uranium speciation in eukaryotic and prokaryotic cells***	**Experiment number**: 30 02 824
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Names and affiliations of applicants (* indicates experimentalists):

Barbara GOUGET * CEA/DSM/DRECAM/LPS
Marie CARRIERE * CEA Saclay
Laure AVOSCAN* Bât 637
Guillaume UNTEREINER* F-91191 Gif sur Yvette, France

Introduction

Our research is focused on the resistance of *Cupriavidus metallidurans* CH34, a soil bacterium known to resist to a wide range of metals and *Deinococcus radiodurans*, the most radiation-resistant organism discovered to date, to uranium. Bacteria are cultured in liquid media enriched with U with a controlled speciation: U carbonate, U phosphate or U citrate. U speciation at the time of inoculation with bacteria is estimated using a modelling software based on thermodynamics constants data.

The aim of this experiment was firstly to check U speciation in the various bacterial culture media; before and after contact with bacteria; secondly to follow U speciation after adsorption and/or accumulation on/in the bacteria. In parallel of this work, elemental analysis of uranium in both the culture media and the bacteria pellets are processed to quantify its adorption and/or accumulation ; transmission electron microscopy of ultra-thin cuts of bacteria are observed to check the presence of uranium precipitates inside or outside the bacterial membranes.

Experimental methods

The two bacterial strains were grown in solution in different media. For *C. metallidurans* CH34, Tris Salt Medium (TSM, basic medium used for *Cupriavidus metallidurans* CH34) is used for the uranyl phosphate speciation, TSM with 20% phosphate plus 40mM of bicarbonate for the uranyl carbonate speciation and Citrate Salt Medium (CSM, with 50mM of citrate) for uranyl citrate speciation. For *D. radiodurans*, Tryptone Yeast Glucose medium (TYG) as well as a defined medium (MS) are used with 40mM of bicarbonate for the uranyl carbonate speciation and with 40mM of citrate for uranyl citrate.

The initial uranium concentration in the media was 1mM. At different time of the growth curve (lag phase, exponential phase, stationnary phase and 100h), an aliquot of bacterial suspension was collected. Bacteria and culture media were separated by centrifugation. After centrifugation, the bacterial pellet was frozen and freeze-dried by highering temperature from –10 °C to 20 °C in 3h under a 0.37 mbar vacuum. Samples were grounded and pressed as 5-mm diameter pellets. Liquid culture media were anaylsed in 2 mm quartz capillaries. U L_{III}-edge XAS spectra were recorded in fluorescence mode using a 30 elements solid state Ge detector (Canberra). The monochromator was a Si(220) double crystal.

At least 3 spectra for each sample were recorded. The collected scans for a particular sample were checked for calibration, averaged, and normalized. EXAFS oscillations were isolated by removal of the pre-edge

background, approximated by a first-order polynomial, followed by μ_0-removal *via* spline fitting techniques (Athena software). Spectra were then simulated by linear combinations fitting (LCF) using U reference compounds spectra (uranyl carbonate, uranyl citrate and uranyl phosphate).

Results

EXAFS oscillations of the three references: uranyl carbonate, uranyl citrate and uranyl phosphate, are presented on figure 1. The first peak of k^3*Khi(k)=f(k) uranyl phosphate curve shows a shift in comparison to the uranyl citrate and uranyl carbonate curves. The distinctness between uranyl citrate and uranyl carbonate is located in the second and third peak where uranyl carbonate shows an hollow.

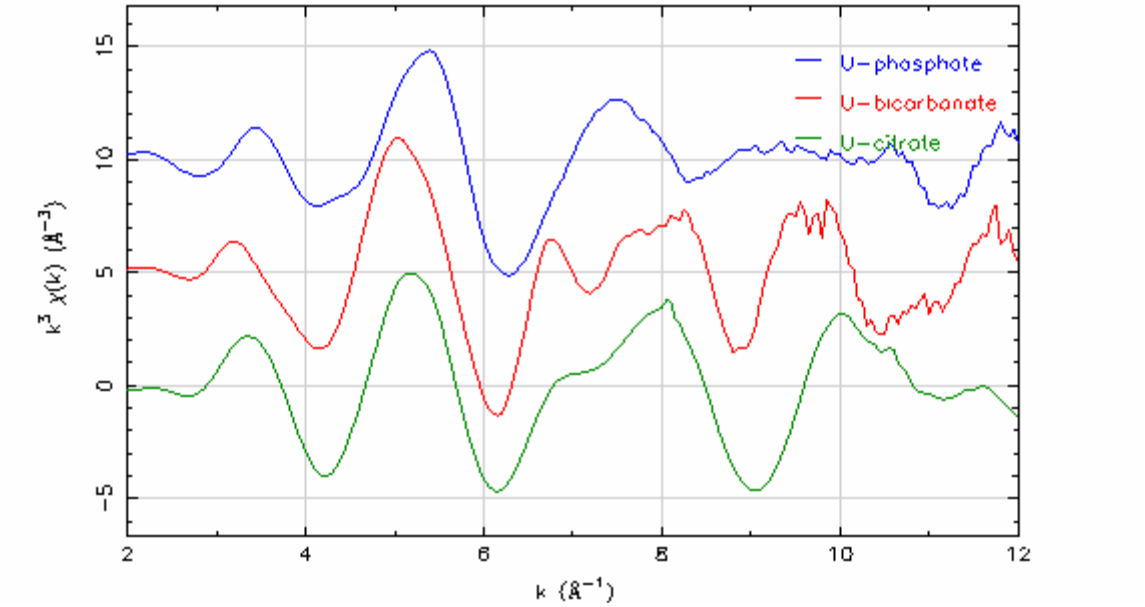

Figure 1: EXAFS oscillations of U reference compounds

EXAFS oscillations of U in the culture media used for bacterial growth of both strains and for each theoritical speciation have been compared to oscillations obtained for the reference compounds. Table 1 shows the results of the linear combination fitting after normalization with the Athena software. R factors are shown in the brackets.

Tableau 1 : Uranium species in culture media

Bacterial strain	***Deinococcus radiodurans***	
Culture medium	**TYG Citrate**	**MS Citrate**
Theoritical speciation	*U citrate*	*U citrate*
Experimental speciation	47% U phosphate 53% U citrate **(0,041)**	17 % U phosphate 13% U carbonate 70% U citrate **(0,033)** 19% U phosphate 81% U citrate **(0,038)**

One or two results fitting with good quality R factors can be obtained for citrate speciation in culture media used for growth of *D. radiodurans*. As theorically expected, experimental speciation gives uranyl present in the minimum culture medium “MS Citrate” as U citrate for 70 to 80%. However, 10 to 15% of U could be complexed to phosphate and/or carbonate coming from the dissolution of atmospheric CO_2.
On the contrary, in the complex medium “TYG Citrate”, linear combinations predict that uranium is complexed to citrate for half part, and complexed to phosphate for the other part.

In the last part of the study, XAS spectra of U in bacterial pellets were collected. As before, linear combination fitting was used to predict uranyl speciation after accumulation or adsorption in or to bacteria (Table 2). R factors are given in the brackets.

Tableau 2 : Uranium species in bacteria

Bacterial strain	***C. metallidurans* CH34**		***Deinococcus radiodurans***		
Culture medium	**TSM 20% phosphate plus bic.**	**CSM**	**TYG Citrate**	**TYG bicarbonate**	**MS Citrate**
Theoritical speciation	*U carbonate*	*U citrate*	*U citrate*	*U carbonate*	*U citrate*
Experimental speciation					
Mid-Exp. phase				100% U phosphate **(0,077)**	100% U phosphate **(0,088)**
End-Exp. phase	78% U phosphate 22% U carbonate **(0,014)**		100% U phosphate **(0,050)**		100% U phosphate **(0,019)**
Stat. phase	69% U phosphate 31% U carbonate **(0,028)**	64% U phosphate 36% U carbonate **(0,023)**	100% U phosphate **(0,068)**	100% U phosphate **(0,036)**	

For *D. radiodurans*, it has been evidenced that whatever the culture medium (complex TYG medium or defined minimum MS medium) and whatever the initial speciation of uranium (citrate or carbonate), bacteria transform uranium in only one species: uranyl phosphate. The biokinetics of this transformation is fast since 100% U phosphate are detected as soon as the mid-expeonential phase of the growth curve. For *C. metallidurans* CH34, uranyl phosphate remains predominant (between 60% and 80%), the other U species being uranyl carbonate.

Conclusions and perspectives

During this experiment, uranium speciation in the culture media could be checked. While uranium carbonate speciation was well controlled, we noted the presence of 20 to 50% of soluble uranyl phosphate in media used for citrate exposure of both cell types. Analysis of uranium speciation directly on bacterial pellets showed that the predominant uranium species in bacteria is a complex with phosphates. Uranyl carbonate is also found in non negligible amounts. 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 examinations of EXAFS oscillations are promising.

References

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