EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

	Experiment title: **Interaction of protein with uranyl(VI) : the transferrin and calmodulin case.**	**Experiment number**: 30-02-759
Beamline: BM30b	**Date of experiment**: from: 04/10/2006 to: 09/10/2006	**Date of report**: 01/02/2007
Shifts: 12	**Local contact(s)**: H. Palancher	*Received at ESRF:*

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Report:

"Metallomics" stands at the interdisciplinary research field for the promotion of bio-metal science, with emphasis on recent development of chemical speciation of elements in biological samples. It is now well known that the speciation of an element drives its bioavailability and toxicity. Generally, the speciation analysis of a metallic cation in living organisms involves the determination of its chemical forms. However, the structure of these latter remains very often elusive as well as their structure/function relationship. In this field, XAS is an ideally suited probe for the understanding of metallobiomolecules structure-functions relationship at the molecular level and for the speciation of a metallic cation in a biological medium. One of the strategy to understand selective complexation and transport of actinide elements is to observe metallobiomolecules acting as elaborated inorganic complexes with well-designed metal active sites. Among the large variety of metalloproteins involved in carriage processes, transferrin has been shown to bind a wide variety of d-block transition metals as well as actinides like plutonium [1] and lanthanides [2]. Transferrin is a regulator of Fe(III) carriage in blood. It is a glycoprotein of 670 amino acids (81 000 Da) with a tertiary structure made of two equivalent lobes (C and N) with one possible complexation site each. The chemical similarity of behavior between Pu(IV) and Fe(III) has already been observed for instance in the siderophore-mediated uptake of Pu by *Microbacterium flavescens* [3]. However the specific mechanisms that lead to actinide complexation (Th(IV), Np(IV) and Pu(IV); U(IV) is excluded because it is unstable under atmospheric conditions) are still unknown, as well as the structural modifications of the protein metal binding site.

In our search to better understand the uptake mechanisms of actinide(IV) by metalloproteins as transferrin, several approaches have been considered. Originally this proposal addressed the comparison between the interaction of uranyl with transferrin and calmodulin while a parallel study on BM20 (ROBL) focuses on Th(IV), Np(IV) and Pu(IV). Consequently a "global approach" related to the characterization of the actinide coordination sphere upon complexation by the apo- metalloprotein itself and a "biomimetic approach" involving simplified biomimetic peptides has been developed. In the mean time, very interesting results have been obtained at the neptunium and plutonium L_{III} edge on ROBL with pentapeptides that mimic part of the transferrin metal binding site : Aspartate, Tyrosine, Histidine (*cf* experimental reports CH-2083 and 20-01-

659). We have therefore decided to devote this current beam time to understand the complexation of Fe(III) in comparable conditions, *i.e.* with $AcAspAspProAspAspNH_2$, $AcGluGluProGluGluNH_2$, and $AcTyrTyrProTyrTyrNH_2$ (where Asp = aspartate, Glu = glutamate, Tyr = tyrosine). All the complexes were prepared in aqueous solution. Comparison between Asp and Glu allows comparing the role of the side chain flexibility on the cation coordination sphere. We keep in mind that all these data will further have to be compared to the recently obtained model of Fe(III) in holo transferrin [4]. Figure 1 compares the two systems Fe(III)/$AcAspAspProAspAspNH_2$ and Fe(III)/$AcGluGluProGluGluNH_2$ in aqueous solution with two different ligand to cation ratios : 1/1.5 and a large excess of ligand 1/8.

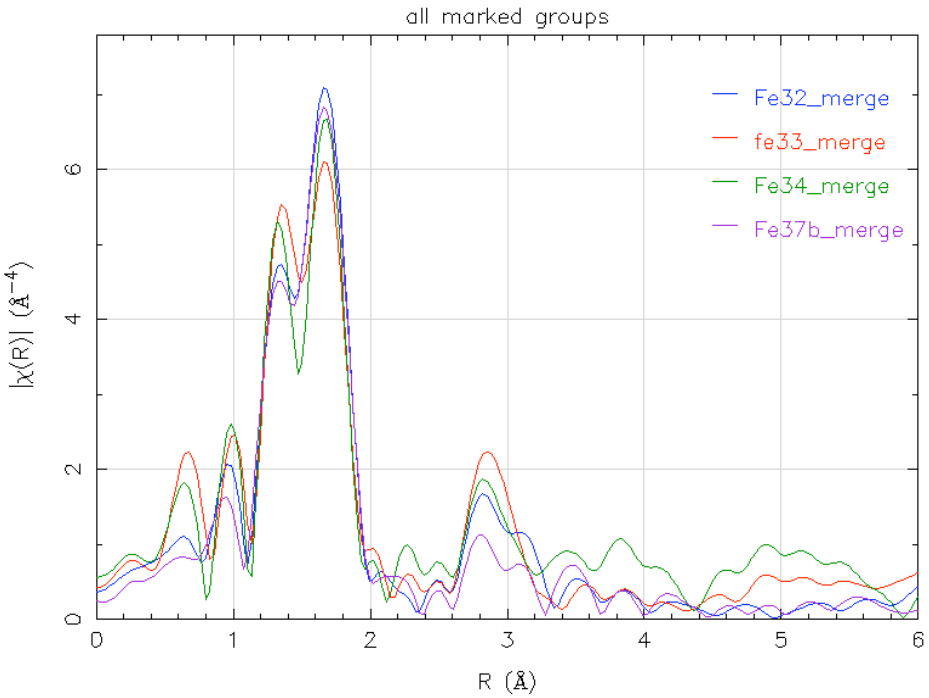

Figure 1 : FT of the EXAFS spectra at the Fe K edge. Fe32 and Fe33 = Fe(III)/$AcAspAspProAspAspNH_2$ with Fe/L = 8 and 1.5 respectively. Fe34 and Fe37 = Fe(III)/$AcAspAspProAspAspNH_2$ with Fe/L = 1.5 and 8 respectively.

Althoug the quantitiative data analysis is still ongoing it is interesting to note that in all cases the iron coordination sphere is splitted with two major distances and an additional intense contribution arrises around R+ϕ=3.9 Å. The two spectra corresponding to the 1/8 ratios are very similar, suggesting that the aspartate and glutamate peptides yield similar complexes. XANES spectra have also been recorded in order to grab additional inormation about the coordination sphere symmetry. For the Fe(III)/$AcTyrTyrProTyrTyrNH_2$ system, the EXAFS spectrum is similar to that of aqueous Fe(III), suggesting that this peptide does not form any complex. This behavior is similar to what has been observed with Np(IV). Finally the Fe(III) acetate complex prepared in glacial acetic acid has also been recorded and will be used as a reference together with solid state iron acetate.

Parallely, all the samples have been characterized by mass spectrometry, chromatography and spectrophotometry in the UV region. All the data are currently being compiled.

[1] : H. Lee, P. J. Sadler, H. Sun, *Eur. J. Biochem.* (1996), **242**, 387.

[2] : Raymond K. N., Pecoraro, V. L., Harris, W. R., Carrano, *C. J.: Proc. Int. Symp.* (Environ. Migr. Long-Lived Radionuclides, 1981, IAEA-SM) 571 (1982).

[3] : M. P. Neu, in *Advances in Plutonium Chemistry* 1967-2000, Ed. D. C. Hohhman, Am. Nucl. Soc. (2002), 169.

[4] : A. Jeanson, C. Den Auwer, P. Moisy, C. Vidaud, OECD-NEA Proceedings on Speciation, Techniques and Facilities for Radioactive Materials at Synchrotron Light Sources, Karlsruhe, September 2006