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.

ESRF	**Experiment title:** **Structure of the serum transferrin metal binding site upon actinide(IV) uptake.**	**Experiment number**: CH-2083
Beamline: BM29	**Date of experiment**: from: 15/03/2006 to: 19/03/2006	**Date of report**: 30/11/2006
Shifts: 12	**Local contact(s)**: A. Scheinost	*Received at ESRF:*

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Report:

In the field of human toxicology, internal contamination with actinides can induce both radiological and chemical toxicity. Whatever the way of contamination (inhalation, ingestion or wound), the radionuclide is absorbed into, and then transported by blood, before being deposited in its target organs in which it is stored and then slowly eliminated through urines and faeces. Although there is a tremendous volume of data available on the interaction of plutonium with living organisms as plants, nearly all the studies are limited to macroscopic or physiological measurements with no specific information at the molecular level. Molecular approaches have been more seldom due to the combined intricacy of metallo biochemistry and actinide chemistry. Transferrin (Tf), a well known metalloprotein devoted to iron transport in the human serum, has been targeted in the past as one potential carrier of actinide(IV) (An(IV)) in the human body [1]. Since 2005, our project aims to better understand the interaction between An(IV) and Tf at the molecular level [2].

Spectra of the complexes Th(IV)/Tf, U(VI)/Tf, Np(IV)/Tf and Pu(IV)/Tf with synergistic NTA have been recorded since the beginning of this project, at the ROBL beam line (*cf* report 20-01-644) and interpretation is still in progress for both An(IV)/NTA and An(IV)/NTA/Tf series. Those interpretations are complicated by the complexity of the chelation site of transferrin and the large type of coordination polyhedra of the actinide elements.

In the mean time, a reliable model to fit the experimental spectrum of holotransferrin (Fe(III)/Tf) has been developed. A screening of transferrin structures published in the Protein Data Bank (PDB) was carried out. All structures are in agreement with the iron site, *i.e.* two tyrosine residues, one aspartate, one histidine and one synergistic bidentate carbonate anion. However these structures vary from each other. These variations must be due to the experimental methods used to obtain transferrin crystals as for 1GVC, obtained with an excess of NTA. Some structures are also incompletely resolved, like the 1A8E. Moreover our spectra were recorded in solution whereas PDB structures describe transferrin in the solid state. The EXAFS spectrum of each adequate PDB structure was calculated with the feff8 code, and compared to the experimental spectrum of holotransferrin. From this comparison the 1LCT structure was chosen as an acceptable model for the fit. Note that the distinction between the coordination sites of each lobes of transferrin is impossible with EXAFS technique because of their structural similarity. Both iron ions were thus considered equivalent. The

multiple scattering approach was necessary to obtain a satisfactory adjustment of the experimental spectrum (R factor = 0.03). The first contribution was fitted with a shell of 6 oxygen atoms at 2.02 Å (σ^2 = 0.019 Å^2). In order to obtain a good adjustment of the second shell, two types of carbon were taken into account: 5 carbon atoms at 3.09 Å (σ^2 = 0.017 Å^2) and 1 at 2.51 Å (σ^2 = 0.001 Å^2) as well as the corresponding 3-leg scattering paths. It is thus in good agreement with the pseudo-octahedral coordination polyhedron of Fe(III) in holotransferrin.

Parallely, studying the interaction of An(IV) with each amino-acid present in the iron site of transferrin (aspartate, tyrosine and histidine) should help us to better understand the structure of the An-Tf chelation site. Consequently, peptides mimicking parts of the iron site have been synthesized and their interaction with An(IV) investigated by spectrophotometry, and EXAFS. The three AcAsp-Asp-Pro-Asp-AspNH_2, AcHis-Tyr-Pro-His-TyrNH_2 and AcTyr-Tyr-Pro-Tyr-TyrNH_2 pentapeptides, have been investigated. In order to avoid hydrolysis of the actinide cation above the pentapeptide pKas, complexation has been carried out in HEPES buffer. Spectrophotometric measurements have shown the complexation of Np(IV) by the AcAsp-Asp-Pro-Asp-AspNH_2 peptide and not by the AcTyr-Tyr-Pro-Tyr-TyrNH_2 pentapeptide (due to the higher pKa of the phenolate group compared to the carboxylate group). Comparison with the acetate ligand has also been undertaken. To our knowledge, the interaction of actinide(IV) with acetic acid has only been described in the case of Th(IV) [3]. The EXAFS data at the neptunium L_{III} edge have also been recorded at the ROBL beam line. To our surprise, oxidation of Np(IV) into Np(V)O_2^+ has occurred during sample transportation from CEA to ESRF for the Np/acetate complex while the Np/AcAsp-Asp-Pro-Asp-AspNH_2 complex did not show any evolution. Another synthetic route has been employed since then and the Np(IV)/acetate complex has been stabilized. Corresponding Fe(III) complexes have also been prepared and EXAFS data at the Fe K edge have recently been recorded at the FAME beam line of ESRF. The first fitting results on the Np/AcAsp-Asp-Pro-Asp-AspNH_2 complex tend to show the participation of water molecules and bidentate carboxylate groups to the Np(IV) coordination sphere at 2.21 and 2.41 Å respectively (compared to 2.37 and 2.52 Å for the Th(IV)/acetate complex [3]). Surprisingly, the EXAFS spectrum shows an additional contribution at higher distances that has been characterized as a Np-Np contribution at 3.8 Å. To our knowledge this is the first time a polynuclear complex of Np(IV) is observed with such type of ligands. We are presently working on the elaboration of a molecular model to account for these EXAFS data. Parallely, the EXAFS data of the An(IV)/NTA/Tf systems are being investigated.

References

[1] : A. E. Gorden, J. Xu, K. N. Raymond, P. Durbin, *Chem. Rev.* (2003), **103**, 4207.

[2] : A. Jeanson, C. Den Auwer, P. Moisy, C. Vidaud, Proc. Of the NEA/XAS-actinide conferences (2006), Karlsruhe, Germany.

[3] : L. Rao, Z. Zhang, P. L. Zanonato, P. Di Bernardo, A. Bismondo, S. B. Clark, *Dalton Trans.* (2004), 2867.