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: Americium speciation in Ferritin protein	**Experiment number**: SC-1349
Beamline:	**Date of experiment**: from: 05/11/04 to: 08/11/04	**Date of report**: 24/02/04
Shifts: 9	**Local contact(s)**: Dr. Harald FUNKE	*Received at ESRF:*

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Preliminary report:

The ferritin protein is an iron storage macromolecule of many species as mammals, marine organisms or plants. It is composed of 24 bundles (Figure 1) linked together by symmetry axis of order 2, 3 and 4, thus forming an inner cavity of 7-8 nm. Although some EXAFS studies have been devoted to the characterization of the inner iron oxyhydroxide core [Wald95], very little is known about its contamination processes by exogeneous metal cations. This project aims to better understand the interaction of an actinide contaminant with this protein. Kinetic studies of ^{241}Am incorporation have shown that ferritin was the first protein to be contaminated and the last to decorporate this radionuclide [Goud94].

Technical difficulties in the laboratory regarding the preparation of suitable starting ^{241}Am reactant obliged us to study another actinide of first interest : ^{237}Np. Note that ^{241}Am is one of the most active radionuclide used in our researches and preparation of adequate starting solutions is always a challenge. Np(IV) starting solution was prepared in nitroso triacetic acid in order to avoid the formation of hydroxydes at working pH = 5.

In order to better understand the interactions between the cation and the protein, two amino acids / cation adducts have been first investigated. The proteic part of the ferritin is mostly composed of carboxylate groups (aspartate, glutamate), although other amino acids may participate to the metal binding (histidine, tyrosine). Therefore the coordination of Np(IV) with aspartate and histidine is a good representative of most of the possible interactions with the proteic part. In case of interaction with the oxyhydroxy iron part, heavy iron backscatter signal is expected. Figure 2 shows that the two EXAFS spectra of aspartate/Np(IV) and histidine/Np(IV) are similar. They have been fitted with comparable structural parameters : 3 O(water) @ 2.31 Å and 5 O(asp) @ 2.35 Å. Note that the total coordination number of neptunium has been fixed to 8 (average coordination number for Np(IV)) and the relative values of water *versus* aspartate has an error

estimated at ± 1. In this model, the aspartate carboxylate group is monodentate (a bidentate model gives a significantly lower fit agreement). Structural data on Np(IV)/carboxylates are scarce. In the structure of tetrakis(Formato-O,O')-neptunium(IV) [Hau76] the coordination number is 8 in a distorted cubic polyhedron. The 4 Np-O first neigbour are located at 2.43 Å while the additional 4 Np-C neigbhours are at 2.50 Å. In another system (uranyl/carboxylic acids), Moll *et al.* have found that water molecules bind at a shorter

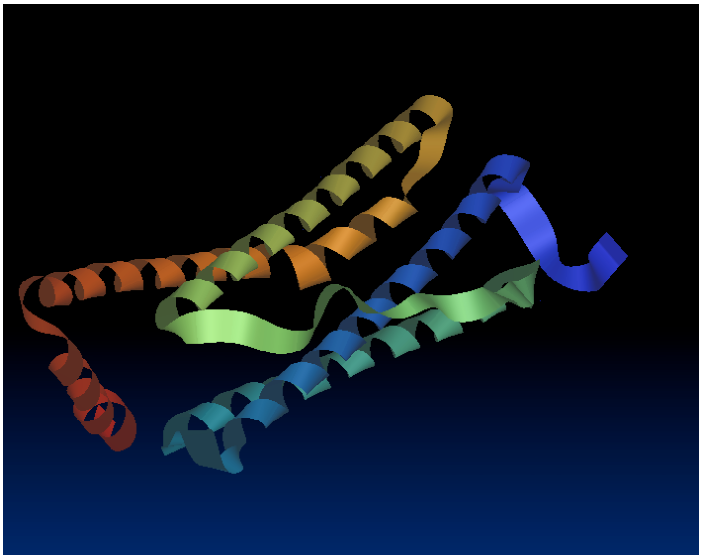

Figure 1 : L-type bundle of spleen horse ferritin (from PDB, ref 1IER).

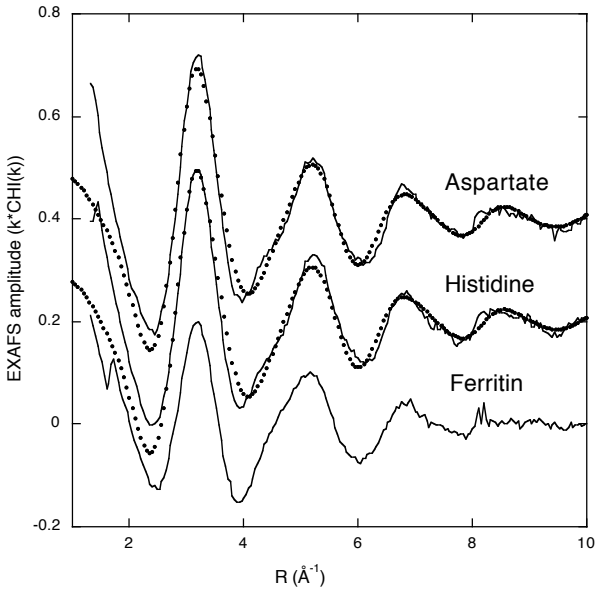

Figure 2 : Raw EXAFS spectra of solutinos of aspartate/Np(IV), histidine/Np(IV) and ferritin/Np(IV).

distance than carboxylates. Complementary IR and RAMAN studies are under investigations to confirm these results. The similarity of the aspartate and histidine spectra shows that the imidazol group does not bind Np(IV) in these conditions and a similar carboxylate coordination is observed.

In the case of ferritin intricate structure, data analysis is complicated by the diversity of the possible coordination sites as well as the lower signal to noise ratio due to the low protein/Np concentration (10^{-4}M). A qualitative analysis of the EXAFS Fourier transform shows that Np(IV) does not bind to the oxyhydroxy iron core but to the proteic part of the protein. Metal coordination sites have been already reported for this protein [Harr96]. At this point of the data analysis we have observed that carboxylate groups participate to the coordination polyhedron. However significant deviation has been found in comparison with the aspartate system. It is believed that other amino acids as tyrosine or histidine also take part to the coordination. In order to perform a quantitative analysis of the neptunium coordination sphere, computer simulations of the possible chelation sites are under investigation.

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[Harr96] : P. M. Harrison, P. Arosio, Biochim. Biophys. Acta (1996), 1275, 161.

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[Wald95] : G. S. Waldo, E. Wright, Z. Whang, J-F. Briat, E. C. Theil, D. E. Sayers, Plant. Physiol. (1995), 109, 797.