



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.

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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 iron metalloprotein with actinide(IV) : the transferrin and ferritin case.

Experiment number:
CH 1764

Beamline: BM20	Date of experiment: from: 05/11/04 to: 09/11/04	Date of report: 06/12/04
Shifts: 12	Local contact(s): H. Funke	<i>Received at ESRF:</i>

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Report:

Actinide cations and in particular Pu and Np are of first concern in case of human contamination for their ability to bind to proteic domains with great affinity [1]. Although many studies have been focussing on the complexation mechanisms of uranyl by organic or mineral systems as humic acids or clays [2,3], few of them have focussed on the sub-molecular level understanding of uptake by metalloproteins of major interests. Focus on ferritin (F) and serum transferrin (Tf), two metalloproteins that are responsible in advanced eukaryote cell of 15-20% and 10% of iron storage and transport respectively, has been motivated by preliminary screening of the major metalloproteins sensitive to actinide contamination. Serum transferrin is a regulator of Fe(III) carriage in blood [4] with two possible complexation sites. The iron metal binding site is made of a distorted octahedron with two tyrosyl phenolates, one histidyl imidazole, one aspartate carboxyl and one required synergistic carbonate anion. Tf can bind a wide variety of d-block transition metals, as well as actinides and lanthanides [5-7]. For instance Taylor *et al.* have suggested that the protein is able to stabilize the tetravalent states and form stable M^{4+} -Tf complexes [8]. Ferritin is generally speaking responsible for iron storage inside the cell [9]. The metalloprotein is constituted of a protein coat of 24 elliptic subunits containing an iron oxyhydroxide core associated to phosphate groups (up to 4500 iron atoms). Kinetic studies of ^{241}Am incorporation in the hepatopancreas of *Homarus gammarus* [10] have shown that ferritin was the first protein to be contaminated and the last to decorporate the americium ion. Uranium loading of apoferritin has also been reported [11].

This report presents preliminary EXAFS and spectrophotometric results related to the interaction of Np(IV) (results on Pu(IV) are under progress) with apo serum transferrin and equin spleen ferritin. It is presented as a summary of two articles that have been recently submitted and to which the reader will be able to refer to for details [12,13].

To avoid hydrolysis at physiological pH, Np(IV) and Pu(IV) in initial stock solution were complexed by the synergistic nitrilotriacetic acid (NTA) anion. Figure 1 shows the characteristic absorption band of Np(IV) aqua and Np(IV)(NTA)₂ complexes at 960 and 980 nm respectively. The presence of a band at 724 nm in the visible range and the absence of any band at 610 nm confirms that the spectral evolution can not be attributed to the oxidation of Np(IV) into Np(V) but is characteristic of the Np(IV)-NTA complex. The evolution of the absorption band of Np(IV)(NTA)₂ at 980 nm to a band at 995 nm is characteristic of the formation of a new complex between Np(IV) and transferrin.

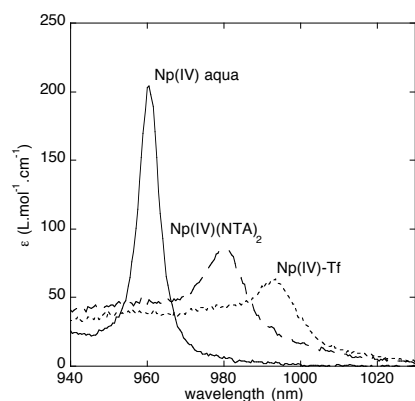


Fig. 1 : Spectrophotometric spectra of Np(IV) in aqueous solution, Np(IV)(NTA)₂ complex and Np(IV)-Tf complex.

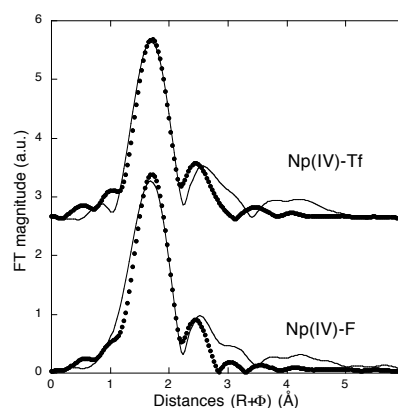


Fig. 2 : Fourier transform of Np(IV)-Tf and Np(IV)-F complexes in aqueous solution at physiological pH.

The L_{III} edge EXAFS spectrum and corresponding Fourier transform of neptunium in Np(IV)-Tf are presented in Figure 2. From the pseudo radial distribution function (R+Φ), backscattering contributions from the first neptunium neighbors and from second shell contributors are clearly observed. In the adjustment of the EXAFS spectrum, the typical coordination number of 8 was set for Np(IV). Two oxygen shells with 5.2 atoms at 2.34(2) Å ($\sigma^2 = 0.007 \text{ \AA}^2$) and 2.8 atoms at 2.56(2) Å ($\sigma^2 = 0.025 \text{ \AA}^2$) and one carbon shell of 7.4 atoms at 3.37(2) Å ($\sigma^2 = 0.009 \text{ \AA}^2$) were necessary to obtain a satisfactory adjustment (R factor = 0.06). The fit quality is very poor above 3 Å because of the high signal to noise ratio and the short data range (only EXAFS data up to 8.5 Å⁻¹ were considered because of the presence of a glitch at 9 Å⁻¹). These structural data shall be compared to the structure of the coordinating lobe in TfFe(III)(NTA) [14] for which iron is coordinated to two tyrosines at a mean distance of 1.83 Å and a tetradentate NTA (3 O at 1.99 Å and 1 N at 2.76 Å) in a highly distorted octahedral symmetry. From a sterical point of view, the global increase of the size of the Np coordination sphere *versus* that of iron(III) can be explained by the increase in ionic radii although the coordination symmetry are radically different between the two cations. From the bond distances point of view, the Np(IV)-Tf interaction may be compared from the one hand to that of Nd(III)-NTA in the crystal structure of Nd(NTA)₂H₂O (2.42 for Nd-O and 2.67 Å for Nd-N) [15] and to the other hand to that of Ce(IV)-Tf in the crystal structure of TfCe(IV)₂ [16]. The shortening of the Np(IV)-Tf distances in the transferrin lobe compared to neodymium is in agreement with i) the shortening of the ionic radii at CN = 8 from Nd³⁺ (R(Nd³⁺) = 1.107 Å) to Np⁴⁺ (R(Np⁴⁺) = 0.980 Å), and ii) the increase of the ionic charge from 3 to 4 if mainly electrostatic interactions are considered.

Complexation of Np(IV)(NTA)₂ by horse ferritin is complicated by the intrinsic intricacy of the ferritin structure made of 12 bundles of four-helical units. Three fixation sites are possible : i) sorption onto the iron oxyhydroxy, ii) phosphate groups or iii) binding to the carboxylic groups from the amino acids of the protein, in particular glutamic and aspartic acids. No *a priori* definite chelation or sorption structural model is available. Figure 2 presents the EXAFS spectrum and corresponding Fourier transform of Np(IV) after uptake by Ferritin in the presence of NTA. Two oxygen shells with 5.0 atoms at 2.35(2) Å ($\sigma^2 = 0.012 \text{ \AA}^2$) and 3.0 atoms at 2.8(1) Å ($\sigma^2 = 0.028 \text{ \AA}^2$) and one carbon shell of 8.0 atoms at 3.3(1) Å ($\sigma^2 = 0.025 \text{ \AA}^2$) were necessary to obtain the best adjustment (R factor = 0.09). Attempts to fit the second coordination sphere with Np-Fe contributions failed, suggesting that Np(IV) does not interact with the oxyhydroxy iron core of the protein but rather with carboxylate functions. However, given the experimental data range (2-8.5 Å⁻¹) interpretation beyond this averaged description of the coordination sphere would be adventurous.

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