The Rossendorf Beamline at ESRF

Forschungszentrum Dresden Rossendorf

Experiment Report Form

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Should you wish to make more general comments on the experiment, enclose these on a separate sheet, and send both the Report and comments to the ROBL team.

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Reports shall be submitted not later than 6 month after the experiment.

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 / experiment 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.
- bear in mind that the double-page report will be reduced to 71% of its original size, A4 format. A type-face such as ”Times” or ”Arial” , 14 points, with a 1.5 line spacing between lines for the text produces a report which can be read easily.

Note that requests for further beam time must always be accompanied by a report on previous measurements.

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ROBL-CRG	**Experiment title:** Interaction of actinide cations with metalloproteins	**Experiment number**: 20-01-644
Beamline: BM 20	**Date of experiment**: from: 30/06/2008 to: 01/07/08	**Date of report**: 27/11/2008
Shifts: 3	**Local contact(s)**: C. Hennig	*Received at ROBL:*

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Report:

Transferrins are monomeric glycoproteins of about 80 kDa with a single polypeptide chain of 670-700 amino acids, used for the solubilization, sequestration, and transport of ferric iron. However, they can accommodate a wide variety of other metal ions, including most of the first row transition elements, several of the second and third rows, group 13 metal ions, lanthanides and actinides [1]. It should be noted that transferrins have a strong preference for cations with a high positive charge. The polypeptide chain is first of all folded into two globular lobes, representing the N-terminal and C-terminal halves of the molecule. The two lobes are joined by a short connecting polypeptide. Each lobe contains a single iron binding site, and each has essentially the same folding. It is composed of two separately folded domains, which confers the protein a great flexibility. When Fe(III) is complexed in a site of transferrin, the lobe is closed, as its two domains draw nearer. Target organs' cells of transferrin possess membrane receptors which selectively bind the diferric form of transferrin (holotransferrin), where both lobes are closed, to the detriment of its free form (apotransferrin), fully open.

A few Extended X-ray Absorption Fine Structure (EXAFS) studies of transferrins have already been carried out, mainly by Garrat *et al*. [2]. These studies, undertaken with ovotransferrin and serum transferrin lead to a coordination number of six low-Z ligands with bond distances of 1.9-2.1 Å, consistent with X ray crystallography studies. Harris *et al.* [3] have studied thorium(IV) complexation with transferrin, with nitrilotriacetic acid (NTA) as a synergistic anion, using a difference ultraviolet spectroscopy method. They showed that at physiological pH, transferrin binds two thorium ions at non-equivalent sites. Complexation of plutonium(IV) with transferrin has also been confirmed [4]. Although UV difference spectra are equivocal, it seems likely that two Pu(IV) are bound. However, *in vitro* studies have shown that Pu(IV) apparently does not induce the closure of the transferrin lobes, and that $Pu(IV)_2$-Tf was not recognised by membrane receptors of transferrin of liver cells [5].

Although the EXAFS spectrum of holotransferrin has been reported in the literature as stated above, no complete description of the environment has been provided in aqueous phase yet. Study at the molecular level of the complexation of actinide(IV) by apotransferrin is also lacking. This is the purpose of this project (see also

experimental report 06-Sep-05) to investigate the complexation of An(IV)NTA_2 molecules by apotransferrin (NTA = nitrilotriacetic acid), where NTA is a protecting ligand against hydrolysis.

Our results on the structure in solution of the iron binding site in holotransferrin are reported in reference [6]. Best EXAFS model for data fitting was deduced from a screening of the Protein Data Bank over all the crystallographic structures of transferrin proteins. They reflect a distorded octahedral polyhedron has suggested by average of the crystal structures reported in the PDB.
We have also investigated the influence of the actinide cation (from Th to Pu) on the actinide(IV)/transferrin complex [7]. Various theoretical models were used and combined to the EXAFS data to obtain a possible schematic view of the actinide binding site.

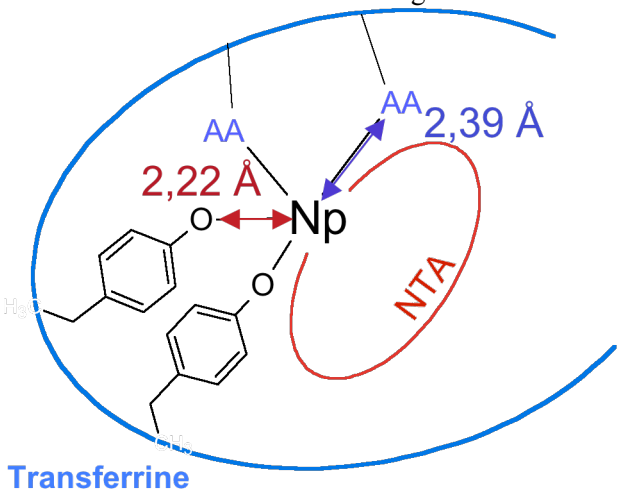

Fig. 1 : schematic possible structure of the neptunium/transferrein complex, one lobe. Distances were obtained from EXAFS fits.

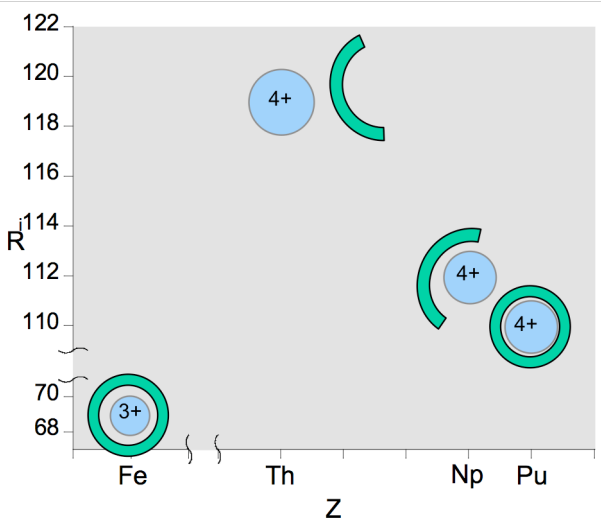

Fig. 2 : conformation of the transferrin lobe from EXAFS data and neutron scattering. Z = cation atomic number; Ri = cation ionic size; circle = close lobe, arc = open lobe.

Figure 1 shows one possible schematic view of the actinide environment in one of the two lobes (both lobes are considered here identical). Similar metric parameters where obtained with EXAFS for Np and Pu. Np(IV) and Pu(IV) form stable complexes with an increase in complexation percentage from pH = 6.5 to 7.5, the maximum being at physiological pH. Surprisingly, in our experimental condition, Th(IV) is never complexed by apotransferrin and stays in the Th(IV)NTA_2 form in solution. Complementary neutron small angle scattering measurements have also been carried out and suggest a change in the protein tertiary structure from Np to Pu (see Figure 2). This very promising result should how be confirmed by further neutron measurements. All these results will be described in detail in an incoming paper.

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