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Forschungszentrum Dresden Rossendorf

Experiment Report Form

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Instructions for preparing your Report

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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:** Actinide selective recognition by biomimetic molecules	**Experiment number**: 20-01-659
Beamline: BM 20	**Date of experiment**: from: 18/09/08 to: 22/09/08	**Date of report**: 20/11/2008
Shifts: 12	**Local contact(s)**: **C. Hennig**	*Received at ROBL:*

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Report:

Most data available on the interaction of actinides with biological systems are based on macroscopic measurements, with very few structural information at the molecular level. However, in case of accidental release of radionuclides, internal contamination with actinides (Th, U, Np, Pu, Am) under either acute or chronic conditions has the potential to induce both radiological and chemical toxicity. For instance Pu(IV) retention in the human body is 50% in bone and 30% on liver.[1] 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 very seldom due to the combined intricacy of metallo biochemistry and actinide chemistry.[2] However, such "molecular speciation" related to actinide in biomolecules is of considerable interest to understand the potential transport of radionuclide inside living organisms. It also has an important input in providing guidance on the structure, affinity and design of potential specific chelating agents synthesized and used for the elimination of incorporated radionuclides. One of the strategies to understand the interaction of actinide elements with biomolecules is to consider metallobiomolecules as elaborated coordination complexes with well-designed metal active sites. Comparison with biorelevent "analogues", as Fe(III) has revealed a very promising tool in order to rationalize the selective propensity of organic ligands to chelate actinide cations. More generally, a spread belief is that the coordination properties of some actinides at oxidation state +IV (Th, Np, Pu) in biological systems compare with that of iron, particularly Fe(III). For instance, the chemical similarity between Pu(IV) and Fe(III) has been observed in the siderophore-mediated uptake of Pu by *Microbacterium flavescens*[3] or in the transferrin system.[4] But, in fact, very few studies directly addressed that issue with biomimetic compounds.

Our approach was to consider simple organic ligands that bear some of the functional groups of a protein binding site without the intricacy of tertiary structure properties. A simple linear penta-peptide, acetyl-aspartyl-aspartyl-prolyl-aspartyl-aspartyl-amide (Ac-Asp-Asp-Pro-Asp-Asp-NH_2, named PP1 further in the text), was then chosen as a model.[5]

EXAFS spectra of M-PP1 (M = Fe, Th, Np, Pu) have been recorded at various metal to ligand ratios and various pH (see experimental reports 2001659_BM20_0207 and CH2539_BM20_0208). The pseudo radial distribution function corresponding to the EXAFS spectra of all An-PP1 (An = Th, Np, Pu) show similar features: the first shell is split into two main contributions, and a M-M contribution clearly appears at distance > 3.5 Å. Thus, one can assume that the metal-peptide complexes are at least binuclear. The first low-Z contribution of the first shell, being at a very short distance, could be attributed to μ_2-oxo or μ_2-hydroxo bridges between the actinide

cations. The Fourier transform of the EXAFS spectrum of Fe-PP1 also possesses a Fe – Fe contribution around 3 Å. Consequently, all the EXAFS spectra were adjusted in a similar way, assuming that the complexes form linear chains of $[M(\mu_2\text{-}O(H))_2(PP1)_x]$ moieties (M = Fe, Th, Np, Pu). It can be noticed that, whatever the sample, the first oxygen contribution is at a shorter distance than classical M – O(aquo) distances. Indeed, the M – O distance is 0.09 Å shorter for Fe-PP1 than for Fe aquo,[6] and respectively 0.15 Å and 0.17 Å shorter in the case of Np-PP1 and Pu-PP1, compared to the corresponding aquo species.[7] This supports the assumption that μ-oxo or μ-hydroxo bridges occur. Notably, the M – M distance in M-PP1 complexes are significantly shorter than the one in the hydrolysis products (coll) : $\Delta(d^{Coll}_{Th-Th} - d^{ThPP1}_{Th-Th})$ = 0.08 - 0.10 Å $\Delta(d^{Np(OH)_5}_{Np-Np} - d^{NpPP1}_{Np-Np})$= 0.1 Å and $\Delta(d^{Coll}_{Pu-Pu} - d^{PuPP1}_{Pu-Pu})$= 0.06 - 0.08 Å. The nuclearity of the M-PP1 complexes was also estimated. Fe-PP1 nuclearity is around 2, which confirms the fact, pointed out with mass spectroscopy, that the iron complex is binuclear. Similar nuclearities were obtained for Np-PP1, Pu-PP1 and Th-PP1 with values higher than 2, suggesting that the complexes form oligomeric chains. However the exact value of nuclearity is difficult to estimate and no significant differences were observed between Th, Np and Pu. The EXAFS data also show a second shell of oxygen atoms, which can be attributed to the peptide interaction with the cation. According to the NMR study, this interaction occurs through the carboxylato functions and vicinity of the amido functions of the peptide bounds in a way that is not fully understood yet. Note that the carboxylato oxygen atoms of the peptide, as hard bases, usually interact with hard acids like iron(III) or actinide(IV).
The combination of the EXAFS best fit parameters and other spectroscopic data strongly suggests that the interaction of PP1 with Fe(III), Th(IV), Np(IV) and Pu(IV) cations relies on a original type of peptidic complexes. The formation of such molecular species prevents the actinide and iron cations from the hydrolysis that usually occurs under comparable conditions.

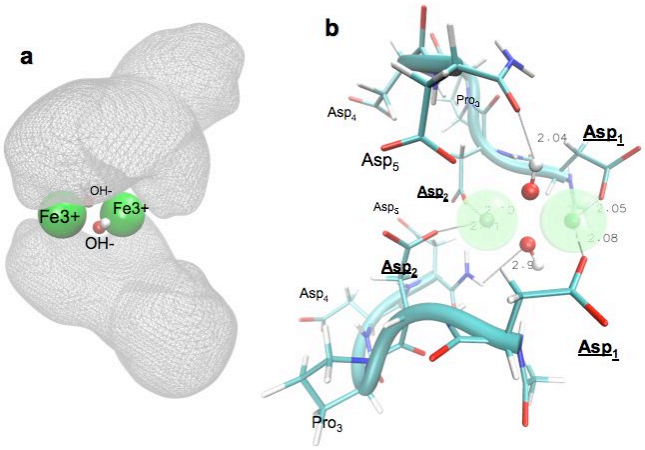

Fig. 1 : a) 3D volumetric densities maps of the peptide around the Fe^{3+}/OH^- ionic system which demonstrated, for a same threshold value, the possibility to place two copies of the β-turn folded peptide without any steric clash. b) Model in which Asp1 and Asp2 are complexing the Fe^{3+} cations.

Finally, for Fe^{3+}, a 3D statistical molecular dynamics calculation was performed which aim was to determine the most favorable orientations of the peptide around the ionic system. For each independent trajectory, the method consisted to fit all the obtained snapshots on the Fe^{3+} ions and then to statistically analyze all the resulting positions of the peptide through atomic densities maps in 3D. Visualization of these maps in 3D using increasing threshold values lead to statistically equivalent positions of the peptide around the ionic system. For each independent trajectory, these analyses revealed the possibility to place two copies of PP1 around both Fe^{3+} ions without any steric clash (Figure 1a), and in agreement with the experimental observations. Such a model is represented in Figure 2b in which each Fe^{3+} interacts with two Asp residues.

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