A comparative study of actinide complexation in three ligand systems with increasing complexity

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Abstract. The complexation of thorium, neptunium and plutonium at oxidation state +IV with three ligands of increasing complexity has been investigated. These ligands are relevant for bioinorganic systems. The first ligand is the small nitrilotriacetic acid that often play the role of protecting ligands against hydrolysis. EXAFS results for the Th to Pu series have been correlated to quantum chemical calculations and show an homogeneous behavior of the actinide at oxidation state +IV. For larger ligands, steric effects may become significant and one can ask how the ligand may accommodate the large actinide cation coordination sphere. Model pentapeptides have been synthesized and tested as complexing agents. Comparison with NTA shows that the molecular arrangements are radically different. The third ligand system is transferrin, a diferric metalloprotein that is well known to coordinate a large variety of cations from transition metals of f-elements. Metalloproteins bear primary, secondary and tertiary structures that all play a crucial role in bonding. At a given oxidation state (+IV), but for various atomic numbers (Th, Np, Pu) EXAFS data at the cation LIII edge exhibit significant coordination discrepancies that are related to a changes in protein geometry. In that sense, the metalloprotein may be viewed as a complex system.

1. Introduction
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.

When tightly bound to protein ligands, metal ions are critical to the function, structure, and stability of proteins, by only allowing specific interactions to take place and/or selective chemistry to occur. Metallobiomolecules can thus be considered as elaborated inorganic complexes with well-designed metal active site structures. Besides these functional aspects, metallic interactions strongly influence the folding of a peptide or protein into a stable tertiary and/or quaternary structure. Furthermore, cooperative interactions originating from the tertiary structure of the protein are mostly not well understood to date and may be at the origin of the selectivity of the binding site. Although the various interaction processes between the metallic cation and the protein are widely studied in all the fields of biochemistry, focus on the specific actinide family is more seldom.

Actinide elements display a very rich chemistry because of the specific properties of their 5f and 6d valence electrons. In oxidation state +IV, actinides behave mostly as hard acids in the Pearson classification, resulting in strong interactions with hard donor groups such as carboxylates. Although their ability for complexation is relatively high compared to oxidation state +III, their tendency to hydrolysis is also very strong, often resulting in mixed hydroxy species when dealing with physiological conditions [1]. In case of internal contamination, various studies have shown the affinity of actinides with transferrin, calmodulin or albumin metalloproteins. Indeed, it has been suggested that actinide(IV) were chelated in the iron complexation site of the transferrin [2], composed of two tyrosines, one histidine and one aspartic acid. Concerning actinide-calmodulin interaction, it has been brought to light that plutonium(III) binds specifically in the calcium binding site [3], constituted of five oxygen atoms from the side chain of aspartate and glutamate aminoacids and one oxygen atom from the carbonyl group of a peptide bound. Moreover, a screening of structures of uranyl-protein complexes in the Protein Data Bank (PDB) has been carried out by Van Horn et al.[4] The coordination donors include aspartyl and glutamyl carboxylate, tyrosinate and amide oxygen atoms. Acidic amino-acids (Asp and Glu) or free carboxylate termini are indicated as binding uranium in most of the reported structures. Pible et al. have recently developed an in silico method in order to localize possible complexation sites of uranyl in 3D-structures of proteins [5]. This study unsurprisingly brings out that oxygen atoms are the preferential ligands for uranyl, with a mean distance of 2.51 Å. Among the amino-acid considered as the most favourable to uranyl chelation are aspartic and glutamic acids (with the two oxygen atoms of their carboxylate function), and tyrosine, serine and threonine (with their hydroxide function). All these metal binding sites involve carboxylate functional groups from aspartic amino acid residues.

This proceeding presents a summary of systems that we have been under study in our group since four years. This is a condensed summary of already published or submitted data. We intend here to present a discussion between three different systems with increasing order of ligand complexity: nitrilotriacetic acid (NTA), a simple linear penta-peptide, acetyl-aspartyl-aspartyl-prolyl-aspartyl-amide (Ac-Asp-Asp-Asp-Asp-NH₂, named PPI further in the text), and transferrin, a metalloprotein that is well known to carry iron in human serum.

1) NTA is a small aminocarboxylic acid that bears three carboxylate groups and is often used as a protecting ligand against hydrolysis of actinides(IV).
2) Our selected peptide presents several advantages: i) The proline residue defines a symmetric peptide with some flexibility and potential turn [6,7]. This facilitates the interaction of the aspartate side chain with cations albeit the short length might moderate tertiary effects in a rough approximation; ii) comparison between carboxylate functions engaged in a small peptidic chain and carboxylic acids is facilitated by the large number of crystal structures available in the literature of actinide(IV)-carboxylate complexes, iii) the peptide size is small enough to neglect in a first approximation tertiary structural effects. Furthermore, related aspartyl-rich di and tripeptides have already been reported to bind Al(III) cation [8].
3) Transferrin is a good example of real complex metalloprotein that has been widely studied as serum iron carried.
Details about these three systems (together with experimental conditions) will be found in the referenced articles.

2. Discussion

2.1. Nitrilotriacetate ligand
Nitrilotriacetate acid is a tridentate aminocarboxylic ligand (NTA) bearing three identical carboxylic groups. Interestingly, coordination can also occur via the tertiary nitrogen atom in order to complete the metal polyhedron. As a result, this nitrogen atom often binds in a distorted way with longer bond length when two NTA molecules bind to the cation. Depending on the size of the cation, an additional water molecule may be inserted in the first coordination sphere. EXAFS data at the actinide cation L3 edge together with spectrophotometry and quantum chemical calculations have been undertaken on the An(NTA)2 complex in aqueous solution (An = Th(IV), U(IV), Np(IV), Pu(IV)). Figure 1 shows one of the possible structures obtained from quantum chemical calculations. Details about these data will be found in the following reference [9].

![Fig. 1: Structures of AnIV-NTA2 complex from quantum chemical calculations. Oxygen atoms are in red, nitrogen atoms are in blue, the actinide cation is in pale blue.](image)

Given the usual uncertainty associated with the amplitude values in EXAFS ($S_0^2$ times N) the number of neighbors was not varied and set constant to 6, the number of oxygen atoms in the first coordination sphere (6 or 7 depending on the presence or not of one water molecule in the first coordination sphere). The presence of one water molecule in the cation first coordination sphere (which contributes to roughly 10% of the first shell total amplitude) is insignificant from the EXAFS point of view given the uncertainty usually associated with amplitude adjustment (between 15 and 20% at best). According to the quantum chemical model, the two nitrogen atoms of the NTA ligands are about 0.3 Å further away from the cation than the oxygen atoms of the carboxylate groups are. Given this typical difference in distances, the relative amplitude weight of the two nitrogen atoms to the cation first coordination sphere (about 20%) and the similarity of the backscattering phases and amplitudes between oxygen and nitrogen, the presence of a weak beating node in the EXAFS spectrum should appear around 16 Å⁻¹. Therefore according to the geometry of the model clusters, the two nitrogen atoms coming from the two NTA ligands will not contribute significantly to the EXAFS signal of the first coordination shell. Our results are consistent with a cation polyhedron with only 6 oxygen atoms from the carboxylate functions directly in the first coordination sphere (with or without an additional water molecule). The average cation-oxygen distance of the first shell decreases from 2.39(1) Å ($\sigma^2 = 0.0052(8)$ Å²) to 2.32(1) Å ($\sigma^2 = 0.0087(10)$ Å²) from Th to Pu in agreement with the well known actinidic contraction usually observed in coordination chemistry.
In an attempt to better understand the role of the two nitrogen atoms in the coordination sphere from the beginning to the end of this chemical series, a multi-shell fitting approach using a parameterized fitting procedure was also undertaken. This fitting procedure has been applied to both Th and Pu complexes for which the difference in coordination sphere is expected to be most significant. The Debye Waller factors associated to the nitrogen shell are abnormally large: $\sigma^2(\text{Th}) = 0.0413(400) \text{ Å}^2$ and $\sigma^2(\text{Pu}) = 0.0124(48) \text{ Å}^2$ (the value and uncertainty for Th suggests that the nitrogen shell is indeed insignificant) suggesting again that this shell does not contribute much to the fit. Interestingly, the value for Th is threefold larger than that for Pu, suggesting that both nitrogen atoms are more significant in the plutonium fit than in the thorium one. Removal of the two nitrogen atoms in the fit model, everything else being kept identical lead to a larger degradation of the $r$ factor for Pu (6.3 % instead of 3.9 %) than for Th (7.1 % instead of 6.4 %). In conclusion, we have suggested that the actinide polyhedron is in a (6+2)$_{NTA}$ or $1_{\text{wat}}+(6+2)_{NTA}$ configuration. Improvement of the fit quality by inclusion in the parameterized fit with the nitrogen shell suggests the appearance of a distinct second shell as the atomic number increases. This observation stresses the role of the cationic size in actinide coordination, specially where electrostatic isotropic cation-ligand interactions dominate. In a schematic and probably simplistic way, this contrasts with first row transition metal coordination when preferred orientations in space are set by orbital hybridization.

2.2. Pentapeptide Asp-Asp-Pro-Asp-NH$_2$

A simple linear penta-peptide, PP1, was then studied as a next step. This peptide bears only carboxylic functional groups (4 groups) with enough chain flexibility to ensure partial folding. We have investigated the role of this pentapeptide in actinide complexation in buffered aqueous solution with Th(IV), Np(IV) and Pu(IV). Again, details about spectrophotometric, NMR and EXAFS data are found in the paper by Jeanson et al.[10]. Figure 2 shows the EXAFS spectra of the Th-PP1, Np-PP1 and Pu-PP1 complexes in aqueous solution.

![EXAFS spectra of samples Th-PP1, Np-PP1 and Pu-PP1.](image_url)
It is clear from Figure 2 that an additional long range metal-metal contribution must be included in the fitting procedure (the Fourier transform, not shown, of these EXAFS spectra confirms the presence of an intense An-An contribution around R+Φ = 3.7 Å). Figure 2 also shows that all three samples exhibit a similar spectrum indicating that similar structures are expected. Combination of this data with spectrophotometric and NMR data strongly suggests that the interaction of PP1 with Th(IV), Np(IV) and Pu(IV) cations yields an original type of peptidic complexes. The formation of such molecular species prevents the actinide cations from hydrolysis that usually occurs under comparable conditions. This is further confirmed by the EXAFS structural parameters of the three complexes, although the An-PP1 complexes share some structural similarities with products of actinide hydrolysis like

\[ \text{[Th}_2(\mu_2-
olongthinspace\text{OH})_2(\text{NO}_3)_6(H_2\text{O})_6]_2(\text{OH}_2)\text{O}_6(H_2\text{O})_6]_{\text{H}_2\text{O}}, [\text{Th}(\mu_2-
olongthinspace\text{OH})_2\text{Cl}_2(H_2\text{O})_6]\text{Cl}_2[11], \text{NH}_4\text{Np(OH)}_5[12] \text{ or Pu hydrolysis products described by DeNecke et al. [13] and Conradson et al. [14]}:\]

- A first short An – O distance (Th-O = 2.0(1) Å at 2.34(1) Å, σ\(^2\) = 0.0115 Å\(^2\); Np-O = 3.4(2) O at 2.23(1) Å, σ\(^2\) = 0.0072 Å\(^2\); Pu-O = 3.3(1) O at 2.22(1) Å, σ\(^2\) = 0.0073 Å\(^2\)), and a long M – M interaction (Th-Th = 2.0 Th at 3.92(1) Å, σ\(^2\) = 0.0010 Å\(^2\); Np-Np = 1.7 Np at 3.80(1) Å, σ\(^2\) = 0.0032 Å\(^2\); Pu-Pu = 1.7 Pu at 3.79(1) Å, σ\(^2\) = 0.0013 Å\(^2\)). Consequently, the first oxygen shell can be attributed to \(\mu\)-hydroxo or \(\mu\)-oxo oxygen atoms. However, significant differences appear. Notably, the An – An distance in M-PP1 complexes are significantly shorter than the one in the hydrolysis products:

\[ \Delta(d^\text{Coll}_{\text{Th-Th}} - d^\text{PP1}_{\text{Th-Th}}) = 0.08 - 0.10 \, \text{Å}. \]

\[ \Delta(d^\text{Coll}_{\text{Np-Np}} - d^\text{PP1}_{\text{Np-Np}}) = 0.1 \, \text{Å} \]

\[ \Delta(d^\text{Coll}_{\text{Pu-Pu}} - d^\text{PP1}_{\text{Pu-Pu}}) = 0.06 - 0.08 \, \text{Å}. \]

From this An-An contribution, the nuclearity of the An-PP1 complexes can be estimated to be higher than 2 although with very large uncertainties because of the asymptotic form of An number of neighbors as a function of nuclearity. 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 carboxylate functions and vicinity of the amido functions of the peptide bounds in a way that is not fully understood yet. Note that the carboxylate oxygen atoms of the peptide, as hard bases, usually interact with hard acids like iron(III) or actinide(IV). However, our experimental conditions might be too acidic for a complete deprotonation of the carboxylic functions (pKa\(_{\text{PP1}}\) ~ 4.4), leading to possible exchange with the solvent.

Figure 3 shows the proposed complexation scheme for the Np-PP1 complex. This schematic drawing reveals the general structure of the complex according to EXAFS and NMR data of reference 10.

![Fig. 3 : Schematized drawing of the Np-PP1 complex.](image)

Although the working pH for the An-PP1 system is relatively higher (around pH = 4) than that for the NTA system (around pH = 1), the resulting structures are radically different (note that EXAFS...
measurements up to pH = 6 for the NTA system showed no significant differences). The main difference is of course the occurrence of a mixed hydroxy-ligand complex with the pentapeptide ligand, meaning that the ability of NTA to protect the An(IV) cation against hydrolysis is better than PP1. Steric properties of the ligand might be the origin of this behavior since the chelating functional groups are identical. In that sense, the proline residue in PP1 defines a symmetric ligand and may play an important role in flexibility. We are currently being working on the synthesis of cyclic peptides in order to test cation encapsulation.

2.3. Tranferrin metalloprotein
In order to compare with a “real” metalloprotein system, we have undertaken a full study of the complexation of An(IV) with apo-transferrin (apo-Tf), An = Th, Np, Pu. Transferrins are monomeric glycoproteins of about 80 kDa, used for the solubilization, sequestration, and transport of ferric iron. The single polypeptide chain of 670-700 amino acids is folded into two globular lobes joined by a short connecting polypeptide. Both lobes, representing the N-terminal and C-terminal halves of the molecule, contain a single iron binding site and have essentially the same folding. When Fe(III) is complexed inside one of its sites, the transferrin lobe is closed, as its two domains draw nearer. Several holotransferrin structures have been determined by X-Ray diffraction. In both lobes, iron ligands are two tyrosine phenolate oxygen atoms, one histidine imidazole nitrogen atom and one carboxylic oxygen atom from an aspartate residue. A synergistic anion is also required for complexation. This forms a distorted octahedron coordination sphere. In physiological media, carbonate, in its bidentate coordination mode, fulfills the role of synergistic anion. However, some other organic anions, such as oxalate, lactate, or nitrilotriacetate, can play this role in carbonate-free conditions [15]. Pu(IV) and Fe(III) exhibit many similarities in chemical and biological transport as well as distribution properties [16]. Similarities such as ionic potential (z/r) for Fe(III) and Pu(IV) (4.6 and 4.2 e/Å, respectively), formation of highly insoluble hydroxides and first-hydrolysis constants (Pu(OH)₃ and Fe(OH)₂) are just a few examples. The similarity in chemical behavior between Pu(IV) and Fe(III) has also been observed in protein systems like the transferrin one. Indeed, Pu(IV) bound to transferrin has been reported in the iron site location [17,18]. Homology is also expected for Np(IV) and Th(IV), which are chemically relatively similar to Pu(IV).

This work as been reported in full details in a recently submitted article [19]. We provide here a portion of those results. The form of the Np-Tf complex derived from EXAFS data at the neptunium L₃ edge is given for comparison with the NTA and PP1 systems discussed above. This scheme results from a combination of additional spectrophotometric and microfiltration/gamma measurements. In our study, in order to avoid actinide hydrolysis at the protein working pH (from pH = 7 to 8), NTA was first used as a protecting ligand of the An(IV) cation. Therefore the An(NTA)₂ complexes were reacted with apo-Tf at pH close to 7.5 in order to obtain the resulting An-Tf complex. Figure 4 gives the assumed structure of Np-Tf inferred from the neptunium EXAFS data.
From our EXAFS data, two possible structures may be considered: one with the two tyrosine residues of the chelation site, one with two hydroxy anions binding to the neptunium. In addition, two residues form the metal binding site are most probably involved in the neptunium coordination sphere as well as one NTA ligand over 2 before transferrin uptake. Interestingly, comparison with Figure 3 indicates that mixed hydroxy-ligand complexes are obtained in both PP1 and Tf systems. However in the later case, only monomeric complexes occur (absence of Np-Np contribution in the EXAFS spectrum). This can be explained by the chelation ability of transferrin, in contrast to PP1. Most probably, one of the NTA ligand plays the role of synergistic anion, however definite proof of such behavior is still lacking.

3. Perspectives
As defined in the introduction, this proceeding did not intend to expose new data on actinide complex with biological ligands but presents a discussion between three different systems that have been previously described. Ligands exposed here are of increasing complexity, from a nitrilotriacetic, to a penta aspartic peptide to a “real case” metalloprotein transferrin. Only complexation of Th(IV), Np(IV) and Pu(IV) cations is discussed.

With two ligand systems, NTA and Tf, monomeric actinide species have been obtained while with the peptide system oligomeric mixed hydroxy-peptide complexes have been obtained. Interestingly, oligomeric complexes occur with PP1 that is not able to fully encapsulate the cation from a steric point of view, leading to a partial hydrolysis form of the complex. This is an interesting point as both the NTA and the PP1 ligands bear the same carboxylate coordinating functions. Note that dealing with actinide(IV) chemistry, competition between ligand coordination and hydrolysis is always strongly dependent on the complex formation constant and pH versus hydrolysis constant. Transferrin is an interesting case where protein tertiary structure is assumed to play an additional role in cation uptake. We have carried out recent neutron small angle scattering measurements in order to measure the effect of actinide chelation on protein folding. This comparison between the three ligand systems confirms that ligand steric effects are very important. For biological large molecules, this is a general idea that
when going from biological cations (mainly first row transition metals) to exogenic actinide or heavy metals both functional group and tertiary structure effects are to be taken into account.

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