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Polyethyleneimine Methylenecarboxylate: a macromolecular DTPA analogue to chelate plutonium(IV)

Florian Lahrouch^{a,d}, Bruno Siberchicot^b, Laurane Leost^a, Jean Aupiais^b, André Rossberg^c, Christoph Hennig^c, Christophe Den Auwer^{*a} and Christophe Di Giorgio

Up until now, molecular chelating agents, such as the diethylenetriamine pentaacetic acid (DTPA) have been the standard method for actinide human decorporation. Mainly active in blood serum, their distribution within the body is thus limited. To treat a wider range of organs affected by plutonium contamination, a potential new class of macromolecular decorporation agents is being studied. Polyethyleneimine methylenecarboxylate (PEI-MC) is one of such example. It is being considered here because of its capacity for targeting the liver and bones.

Since the discovery of plutonium (Pu) by Seaborg and coworkers in 1940, the development of civil and military industries has led to an important production of this element. Pu presents a strong chemical and radiological toxicity, no matter the isotope. In the case of accidental plutonium contamination in humans (excepting inhalation), distribution inside the body *via* the blood system leads to retention inside target organs, mainly the skeleton and liver.¹ Plutonium, mostly at the +IV oxidation state in environmental conditions, has a strong affinity with the highly vascularized hematopoietic marrow located in the bones² and for the ferric proteins such as the ferritin³, largely present in liver. Furthermore, the severe radiotoxicity of plutonium is at the origin of severe tissue damage and can trigger bone and liver cancers or leukemia.^{4,5} Such accumulation of heavy metals is not specific to plutonium but its radioactivity builds up additional radiological effects.

The diethylene triamine penta acetic acid (DTPA) injected as Ca(II) or Zn(II) salt form is, at this time, the most effective decorporation agent able to promote plutonium (IV) excretion.⁶ However, this molecule is limited because of its weak selectivity

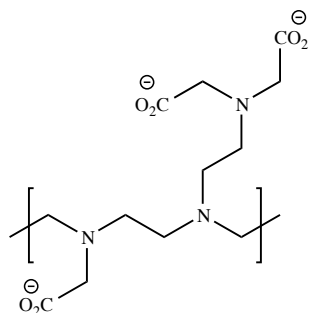


Fig. 1: Representation of the monomeric unit of PEI-MC as if functionalization were equal to 100% and with an arbitrary protonation state.

towards Pu and a narrow distribution within the body,⁷⁻¹⁰ being active mainly in serum.^{11,12} Although the retention time of Pu(IV) in serum is less than few hours (one day after injection, less than 1% of Pu is present in the blood), a remedy is needed for the fraction which goes beyond the blood system to target organs like bone or liver.¹³ We are focusing here on this fraction that may be stored in target organs. Extra-serum complexation may be achieved with indirect targeting correlated to the particular biodistribution of polymers (which are usually retained mechanically in the main organs due to their size).¹⁴ Soluble polymers and other macromolecular decorporation agents could therefore initiate a breakthrough in the field of nuclear toxicology. Additionally, decorporation efficiency (selectivity, body distribution, complexation) could be enhanced by a greater abundance of the chelating sites per area unit and a relative flexibility of the polymeric backbone.

The polyethylene imine (PEI) macromolecule has been extensively studied in medicinal chemistry.¹⁵ In our previous work we demonstrated the interest of using carboxylate macromolecular functionalized polyethylene imine (PEI-MC, Figure 1) as a chelating agent of uranyl (VI) in model conditions.¹⁶ We are now considering PEI-MC for Pu(IV) human decorporation.

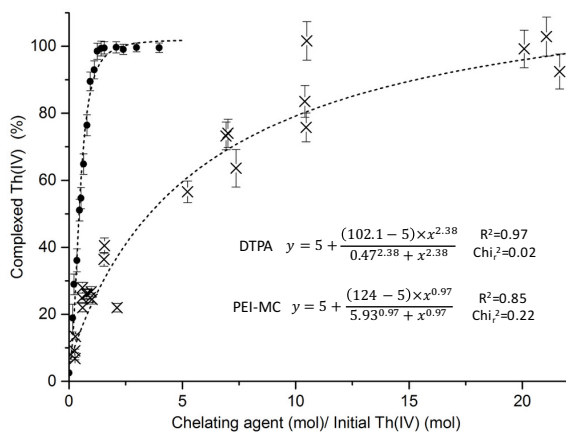


Fig. 2: Th(IV) uptake curves representing the complexation rate of Th(IV) as a function of the normalized molar quantity of PEI-MC (cross) and DTPA (points) at pH 7.0 and their fitting equation (see ESI for details).

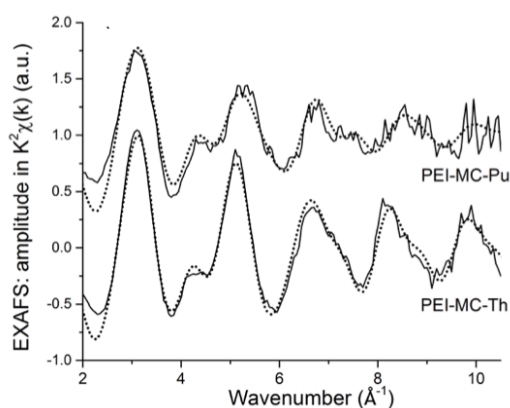


Fig. 3: EXAFS spectra of the PEI-MC-Pu and PEI-MC-Th complexes in pseudo physiological conditions. Solid line = experimental spectrum, dotted line = adjustment. Spectra have been shifted in ordinates for clarity.

With a combination of EXAFS spectroscopy and theoretical calculations, the complexation sites of PEI-MC are described with Pu(IV) and with parent actinide element thorium (IV). Th(IV) is viewed here as a Pu(IV) chemical analogue due to its stability at unique oxidation state +IV. Furthermore, the very low specific activity of ^{232}Th makes it easy to manipulate in the laboratory which is not the case of any of the Pu isotopes.¹⁷ But thorium is of interest in itself because it is the most concentrated natural actinide in the earth's crust and also because it is being looked at as a promising alternative fuel for the next reactor generation.

In this report, we continue to explore the potentiality of PEI-MC as a possible macromolecular analogue of DTPA. The curves representing the uptake rate of Th(IV) by PEI-MC or DTPA in function of the chelating agent quantity normalized by the initial Th(IV) quantity, are shown in Figure 2 (measurements performed in triplicate for each point and in pseudo physiological conditions: pH =7, [NaCl]= 150mM). Note that the uptake curve of Pu(IV) could not be recorded because of radioprotection limitations with such protocol in our laboratory. To avoid Th hydrolysis in these conditions, the uptake curves were recorded in the presence of two molecules of nitrilotriacetic acid (NTA) as a protecting ligand (NTA will also mimic carboxylate metabolites present in the serum as citric acid or even bicarbonates).

The experimental points of the Th complexation with both DTPA and PEI-MC were fitted with a Hill function and compared (experimental details and fitting parameters are reported in Table S1 of ESI). According to the fitting parameters, the complexation molar ratio is equal to 1.24 ± 0.37 for PEI-MC and to 1.02 ± 2 for DTPA. It is worth noting that the DTPA curve reaches its maximum complexation before the PEI-MC curve, as evidenced by the exponent n parameter in the equation which represents the slope at 50% Th(IV) complexation. It is equal to 2.38 ± 0.25 for DTPA and to 0.97 ± 0.17 for PEI-MC. Despite the lower quality of the fit for PEI-MC, Figure 2 shows that DTPA is slightly more effective than PEI-MC at complexing Th(IV) (with respect to one monomeric unit)

	1 st coordination shell		2 nd coordination shell		Fit parameters
PEI-MC-Pu	5 O at 2.34 (1) Å $\sigma^2 = 0.0093 \text{ \AA}^2$	3 N at 2.66 (2) Å $\sigma^2 = 0.0171 \text{ \AA}^2$	5 C_{α} at 3.38 (1) Å $\sigma^2 = 0.0116 \text{ \AA}^2$	9 C_{β} at 3.45 (2) Å $\sigma^2 = 0.0116 \text{ \AA}^2$	$S_0^2 = 1.0$ $e_0 = -5.52\text{eV}$ $R_{\text{factor}} = 2.1\%$ $Q = 0.022$
PEI-MC-Th	5 O at 2.40 (1) Å $\sigma^2 = 0.0038 \text{ \AA}^2$	3 N at 2.80 (2) Å $\sigma^2 = 0.0360 \text{ \AA}^2$	5 C_{α} at 3.42 (1) Å $\sigma^2 = 0.0086 \text{ \AA}^2$	9 C_{β} at 3.58 (2) Å $\sigma^2 = 0.0086 \text{ \AA}^2$ 2.5 (5) Cl at 3.88 (2) Å $\sigma^2 = 0.0091 \text{ \AA}^2$	$S_0^2 = 1.0$ $e_0 = 6.26\text{eV}$ $R_{\text{factor}} = 1.0\%$ $Q = 0.05$

Table 1: EXAFS best fit parameters for the PEI-MC-Pu and PEI-MC-Th complexes in pseudo biological conditions. Coordination numbers have been fixed to the 5 + 3 model (numbers in italics). Numbers in brackets are the estimated uncertainties. σ^2 is the Debye Waller factor of the considered scattering path. S_0^2 is the global amplitude factor, e_0 is the energy threshold, and R_{factor} is the agreement factor of the fit in %.

because their complexation sites show some differences. One major difference is that DTPA presents 5 carboxylate functions available on the same amino backbone (5O + 3N) whereas PEI-MC presents, for the same amino backbone, 3 carboxylate functions (3O + 3N). Note that because of the high specific radioactivity of ^{239}Pu , the corresponding uptake curve could not be acquired. To further explore the coordination mode of Th(IV) and Pu(IV) with PEI-MC, the complexation sites in complexes PEI-MC-Pu and PEI-MC-Th were characterized by combining EXAFS spectroscopy and *ab initio* molecular dynamics (*ab Initio* MD). The EXAFS data shown in Figure 3 were recorded at the L_{III} edge of Pu and Th in pseudo physiological conditions (150mM NaCl, pH 6.5) with a An(IV): PEI-MC monomer molar ratio of 0.02 (large excess of chelating monomer). The corresponding moduli of the Fourier

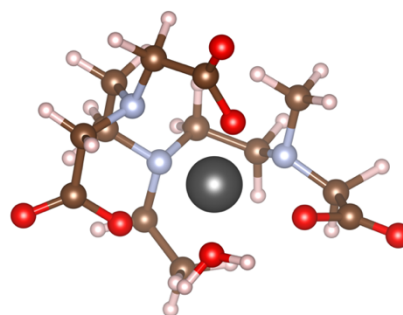


Fig. 4: Snapshot of the PEI-MC-Pu complexation site (3O + 10_{wat} + 3N) in the final stage calculated by *ab Initio* MD. Pu = black, O = red, N = blue, C = brown and H = white

transforms (FT) are shown in Figure S1 of ESI and exhibit for both PEI-MC-Pu and PEI-MC-Th a major contribution between $R+\Phi = 1$ and 2 Å. Other contributions of smaller intensities and of complex pattern are visible around $R+\Phi = 2.4 - 4.0$ Å respectively. The EXAFS data were adjusted in R space following an ideal model of 5O + 3N for the first coordination shell.¹⁸ This number of neighbors, composing the first coordination shell, has been set constant as an assumption CN = 8 (5O + 3N) in order to follow the typical complexation site of DTPA-An(IV).¹⁸ The absence of experimental model compounds and the presence of only light scattering atoms with very similar atomic number (O and N) in the first coordination sphere precludes an accurate description of possible variations within the actinide coordination number greater than 20%. The presence, or not, of additional water molecules within CN = 8 is also impossible to assess either for DTPA or for PEI-MC (in the latter case, 3O coming from 1 monomer of Figure 1). The fitted distances of An(IV)-O and An(IV)-N reported in Table 1 are equal to 2.34 Å and 2.66 Å, respectively, in the Pu(IV) complex and are equal to 2.40 Å and 2.80 Å, respectively, in the Th(IV) complex.

This difference is a consequence of the actinidic contraction, usually observed in f-element coordination chemistry.¹⁹ As mentioned, due to the imprecise fit of the coordination numbers, the presence of possible water molecules in the first coordination sphere is not discussed here. Likewise, the backscattering similarities between O and N atoms combined with their small splitting distances (less than 0.4 Å, inferior to the spectral resolution $2\pi/\Delta k=0.8$ Å) make a more detailed assessment of the contribution of the nitrogen atoms difficult. The high values of the Debye Waller parameters associated with the An(IV)-N paths (Table 1) are a manifestation of the difficulties to localize these nitrogen atoms.

The second coordination shell is mainly composed of carbon atoms from the carboxylate functional groups (C α) and polyethylene imine backbone (C β) (those contributions were linked to the An-N contributions). However, in the PEI-MC-Th complex, the presence of additional chloride ions at a fitted distance of 3.88 Å improved significantly the fit. This contribution originates from the NaCl medium and has been already identified in a similar system.²⁰ In the case of the PEI-MC-Pu complex, the addition of chloride ions is not followed by a significant increase of the fit quality, and the presence of chloride ions in the Pu coordination shell is difficult to assess. A possible reason is the higher noise level of the spectrum of PEI-MC-Pu (1.4×10^{-3} in the k^2 space) compared to PEI-MC-Th (5.9×10^{-4} in the k^2 space) which is due to the low concentration of Pu in the EXAFS cell (see ESI). Nevertheless, in both cases, the use of a second An...C coordination shell was necessary to improve the fit quality of the EXAFS data. Consequently, the An-N distances determined in the fit are largely driven by the second C β , C α coordination sphere.

To further illuminate the An(IV) complexation site with PEI-MC, *ab Initio* MD calculations, using ABINIT code²¹ were performed with a fragment composed of Pu(IV) / Th(IV) complexed to PEI-MC model monomers. In fact, 2 carboxylate functions were removed from the DTPA monomer and terminated with 2 ethyl groups. The monomer, presented in Figure 1, was selected to be representative of the entire PEI-MC which is obtained by the functionalization of the commercial PEI.¹⁶ Following the natural distribution of the amines of type I, II and III (approximately equal to 0.33/0.33/0.33 respectively in this commercial 25 kDa branched PEI²²), this average model monomer is composed of three carboxylate functions and three nitrogen atoms with coordination pattern 3O + 3N.

	An(IV)-O _{PEI-MC}	An(IV)-O _{H2O}	An(IV)-N
PEI-MC-Pu	2.22 Å	2.28 Å	2.68 Å
PEI-MC-Th	2.33 Å	2.32 Å	2.74 Å

Table 2: Interatomic distances calculate by *ab Initio* MD for the hydrated model

The initial calculation, performed at 298 K, in the absence of water molecules (3O + 3N), corroborates the presence of both oxygen and nitrogen atoms in the first coordination shell of Pu(IV) and Th(IV).

All the distances are reported in Table S2 of ESI. The Pu-O and Pu-N distances are calculated at 2.14 Å and 2.59 Å respectively (EXAFS: 2.34 Å and 2.66 Å respectively) and the Th-O and Th-N distances are calculated at 2.20 Å and 2.72 Å respectively (EXAFS: 2.40 Å and 2.80 Å respectively). The difference between the EXAFS and *ab Initio* MD distances can easily be explained by the lighter congestion of the An(IV) coordination shells in the case of the simulation that used the coordination pattern 3O + 3N (instead of 5O + 3N for DTPA). Therefore, a second calculation of *ab Initio* MD had to be performed at 298K with the addition of 22 water molecules in order to let the water fill out the cation coordination sphere. After 2,4 ps of equilibration, one water molecule

completes the first cation coordination shell and distances increase globally (Figure 4 for Pu, S3 for Th; Figure S4 and Table S3 for a summary and schematic representation of both cation coordination sites). For both Pu(IV) and Th(IV) the interatomic distances for this hydrated model are reported in the Table 2.

For Pu, two populations of oxygen atoms can be distinguished on the pair distribution functions $G(r)$ (Figure S2ab of ESI), the first at 2.22 Å, corresponding to the carboxylate functions and the second at 2.28 Å, for the water molecule (average 2.23 Å). The nitrogen atoms are positioned around 2.68 Å. In the case of Th, two populations of oxygen atoms are also visible at 2.33 Å and at 2.32 Å corresponding respectively to the carboxylate functions and to the water molecules (average 2.33 Å). The nitrogen atoms are located at 2.74 Å. Note that the inversion of intensities for the O populations from Pu to Th on the $G(r)$ functions (Figure S2a) is due to the distance switch for Th. The resulting An(IV)-O and An(IV)-N distances obtained with this hydrated model are similar to the distances reported for An(IV)-DTPA using EXAFS¹⁸ (Pu(IV)-O = 2.30 Å, Pu(IV)-N = 2.61 Å; Th(IV)-O = 2.40 Å and Th(IV)-N = 2.71 Å) in agreement with the distances reported in this study in Table 1.

Both EXAFS spectroscopy and *ab Initio* MD simulation exhibit An(IV)-O and An(IV)-N distances comparable to those of the DTPA-An(IV) complexes, confirming the implication of both oxygen and nitrogen atoms in the complexation of Th(IV) and Pu(IV). Nonetheless, differences appear with respect to the coordination numbers. On the one hand, the *ab Initio* MD calculations yield a $3O + 1O_{\text{wat}} + 3N$ complexation site (CN = 7) composed of 3O + 3N from the PEI-MC monomer and $1O_{\text{wat}}$ from a water molecule. Note that a coordination number of 7 for both Pu(IV) or Th(IV) is at the lower limit of typical coordination numbers for An(IV) (from 8 to 12 depending on the element and its environment).²³ It is likely that the steric constraints due to the presence of two free ethyl groups in the monomer are sufficient to trigger the hydration of the complexation site. Also, the high flexibility of the monomers is probably able to limit the water molecule insertion. On the other hand, the $5O + 3N$ pattern used for the EXAFS adjustment is consistent for comparison with the DTPA pattern. As mentioned above, however, the presence of additional water molecules in the actinide first coordination shell is not possible to assess with EXAFS.

In conclusion, present investigation reports the complexation of Th(IV) and Pu(IV) with PEI-MC polymer using *Ab Initio* MD calculations and EXAFS spectroscopy in pseudophysiological medium. A similar complexation site for both Pu(IV) and Th(IV) was observed within the expected global contraction from Th to Pu. The presence of O atoms in the first coordination shell of An(IV) was determined by EXAFS spectroscopy. *Ab initio* calculations were also performed and the presence of nitrogen atoms in the first coordination shell of An(IV) were confirmed. Distances in both PEI-MC-Pu and PEI-MC-Th complexes are very similar to the intermolecular distances found in the equivalent DTPA complexes.¹⁸ Considering the specific biodistribution of macromolecular objects, the use of polymeric analogs of molecular chelating agents, as PEI-MC, could be an interesting strategy to modify the body distribution and target specific organs which besides appear as sequestering compartments in case of An contamination. This polymer has also the distinctive ability of being able to not only complex An(IV) - such as Pu(IV) and Th(IV) - but the actinyls such as $\{UO_2^{2+}\}$ as well.¹⁶ Such approaches could then open the field for a new class of decorporating agents in complement with already existing therapy.

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