Ferritin iron core, Actinide uptake by transferrin and ferritin metalloproteins: two EXAFS studies

C. Barbot, Christophe den Auwer, Guy G. Ouvrard, Isabelle Llorens, Philippe Moisy, Claude Vidaud, Françoise Goudard, P- L. Solari, Valérie Briois, H. Funke

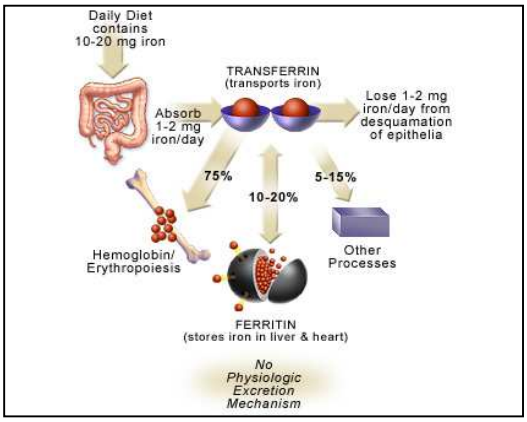
Ferritin iron core, Actinide uptake by transferrin and ferritin metalloproteins: two EXAFS studies

[1]Barbot C., [2]Den Auwer C., [3]Ouvrard G., [2]Llorens I., [2]Moisy P., [4]Vidaud C., [5]Goudard F., [6]Solari P.L., [7]Briois V., [8]Funke H.

[1]Laboratoire de Chimie générale et minérale, Faculté de Médecine-Pharmacie de Rouen,
[2]CEA Marcoule DEN/DRCP/SCPS, Bagnols sur Cèze
[3]Institut des matériaux de Nantes (IMN),Faculté des Sciences, Nantes
[4]CEA Marcoule DSV/DIEP/SBTN, Bagnols sur Cèze
[5]Laboratoire GERMETRAD, Faculté de Pharmacie de Nantes
[6]BM29, ESRF, Grenoble
[7]D44, LURE beamline, Orsay
[8]FZR, Rossedorf beamline (BM20), Grenoble

ABSTRACT

In order to better understand the mechanisms of actinide uptake by specific biomolecules, it is essential to explore the intramolecular interactions between the cation and the protein binding site.
Although this has long been done for widely investigated transition metals, very few studies have been devoted to complexation mechanisms of actinides by active chelation sites of metalloproteins.
In this field, X-ray absorption spectroscopy has been extensively used as a structural and electronic metal cation probe. The two examples that are presented here are related to two metalloproteins in charge of iron transport and storage in eukaryote cells: transferrin and ferritin.
$U(VI)O_2^{2+}$, Np(IV) and Pu(IV) have been selected because of their possible role as contaminant from the geosphere.



EXPERIMENTAL

The stock solution of Np(IV) ([Np]=24 mM, ^{237}Np) was prepared by hydroxylamine (310 mM) reduction (60°C) of Np(V) obtained by dissolution of $Np(V)O_2OH.xH_2O$ in HCl solution. Nitrilotriacetic acid (NTA) complexation was achieved with 2.8 equivalents of ligands at pH 4.
The stock solution of Pu(IV) ([Pu(IV)]=62 mM, ^{239}Pu, was prepared in HCl solution. NTA complexation was achieved with 2 equivalents of ligand with 1M HCl.
The stock solution of $U(VI)O_2^{2+}$ was obtained by dissolving uranium diacetate in pure water (pH=4.0-4.5). A 5 mM solution was obtained by dilution of the previous one in 10 mM sodium acetate (pH=7).
Human serum transferrin and horse spleen ferritin were buffered with N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) sodium salt (HEPES).
Neptunium and plutonium L_{III}-edge EXAFS spectra were recorded at the ROBL beamline (BM20) at ESRF and uranium L_{III}-edge at BM29.
Iron K-edge EXAFS spectra were recorded at the LURE D44 beamline.
Data treatment was carried out using EXAFS98 code [1] concerning actinide uptake and « EXAFS pour le Mac » [2] for evaluating iron structure in ferritin.
For more details on acquisition, data analysis and fitting, see references [3, 4].

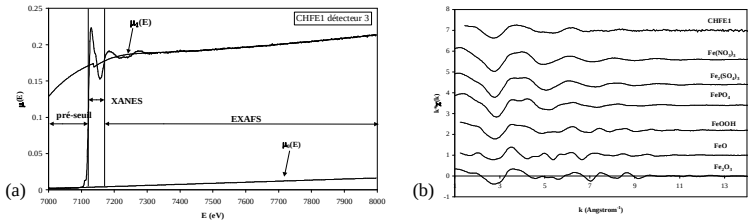
CONCLUSION

Ferritin iron core resembles to that of hematite.

In all cases the actinides are mostly bound to the protein via oxygen donor functions (although nitrogen donor functions as histidine might also participate) resulting in a splitted (CN=8) first coordination sphere. Additional water molecules may also enter the coordination polyhedron.

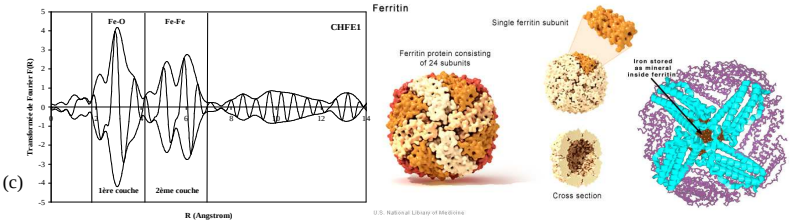
RESULTS : ferritin iron core

Fe K edge (a) and corresponding Fourier transform (b) of horse spleen ferritin and references



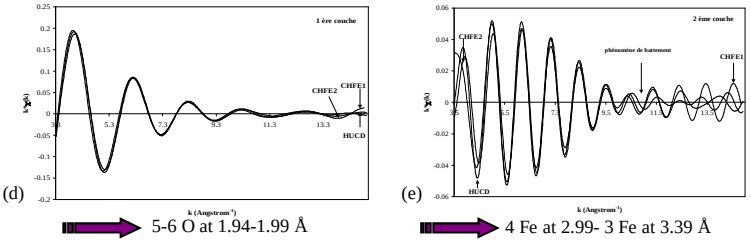
(a) (b)

Radial distribution function of horse spleen ferritin (c), non corrected for phase changes, by Fourier transform, of the data $k^3\chi(k)$.



(c)

Inverse Fourier transform of horse spleen ferritin (d) for first shell (e) for second shell



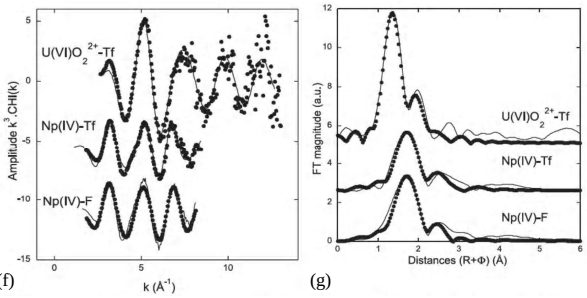
(d) (e)

5-6 O at 1.94-1.99 Å

4 Fe at 2.99- 3 Fe at 3.39 Å

RESULTS : actinide uptake by ferritin and transferrin

Actinide L_{III} edge (f) and corresponding Fourier transform (g) of $U(VI)O_2^{+}$-Tf, Np(IV)-Tf and Np (IV)-F



(f) (g)

Sample	O/N first shell	O/N second shell	C shell	R factor
Np(IV)-Tf	5.7 O/N at 2.34(2) Å σ^2=0.007 Å²	2.3 O/N at 2.5(1) Å σ^2=0.035 Å²	7C at 3.4(1) Å σ^2=0.007 Å²	0.06
$U(VI)O_2^{2+}$-Tf	2 O at 1.78(2) Å σ^2=0.004 Å²	5 O/N at 2.36(2) Å σ^2=0.016 Å²	3 C at 2.9(1) Å σ^2=0.012 Å²	0.04
Np(IV)-Tf	5.0 O/N at 2.35(2) Å σ^2=0.012 Å²	3.0 O/N at 2.8(1) Å σ^2=0.028 Å²	8 C at 3.3(1) Å σ^2=0.025 Å²	0.09

REFERENCES

[1]Michalowicz A., *EXAFS pour le MAC*, in "Logiciels pour la Chimie", edt : Société Francaise de Chimie, Paris, pp 102-103 (1991).
[2]Michalowicz A., EXAFS pour le Mac: a new version of an EXAFS data analysis code for the Macintosh. J. Phys. IV C2 7 (1997) 235.
[3] Den Auwer C., Llorens I., Moisy P., Vidaud C., Goudard F., Barbot C., Solari P.L., Funke H.
Actinide uptake by transferrin and ferritin metalloproteins
Radiochimica acta 93 (2005) 699.
[4] Barbot C. thèse de doctorat. Etude des systèmes organo-minéraux dans leur rapport avec les métaux lourds et les radioéléments. Université de Nantes (2001).