dried under a nitrogen stream, redissolved in 40 µL 75 mM TRIS/HCl buffer (pH 7.4), which contained 0.05 % Triton X-100 and 2.5 mM MgCl$_2$, and mixed with 10 µL of enzyme preparation. Together with control experiments, the probes were preincubated for 90, 60, 45, 30 and 15 min at 37 °C. After addition of 10 nmol $^{14}$C-sphingomyelin (ca. 40000 cpm (counts per minute)) in 50 µL of the same buffer, the reaction was incubated for another 30 min. The reaction was stopped by the addition of 750 µL chloroform/methanol (1/1). After addition of 200 µL water, the lipids were extracted and the radioactivity of the polar upper phase, which contained $^{14}$C-phosphorylcholine, was determined by scintillation counting.

The determination of A-SMase proceeded analogously, with use of an Mg$^{2+}$-free sodium acetate buffer (pH 4.5).

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[1] T. Kolter, K. Sandhoff, *Angew. Chem.* **1999**, *111*, 1632–1670; *Angew. Chem. Int. Ed.* **1999**, *38*, 1532–1568.

[2] Y. A. Hannun in *Sphingolipid-Mediated Signal Transduction* (Ed.: Y. A. Hannun), Springer, New York, **1997**, pp. 1–18.

[3] G. van Echten-Deckert, A. Giannis, A. Schwarz, A. H. Futerman, K. Sandhoff, *J. Biol. Chem.* **1998**, *273*, 1184–1191.

[4] M. J. Lee, J. R. Van Brocklyn, S. Thangada, C. H. Liu, A. R. Hand, R. Menzeleev, S. Spiegel, T. Hla, *Science* **1998**, *279*, 1552–1555.

[5] R. N. Kolesnick, M. Kronke, *Annu. Rev. Physiol.* **1998**, *60*, 643–665.

[6] Y. A. Hannun, *Science* **1996**, *274*, 1855–1859.

[7] L. R. Ballou in *Sphingolipid-Mediated Signal Transduction* (Ed.: Y. A. Hannun), Springer, New York, **1997**, pp. 35–51.

[8] K. Hofmann, V. M. Dixit, *Trends Biochem. Sci.* **1998**, *23*, 374–377.

[9] K. Hofmann, V. M. Dixit, *Trends Biochem. Sci.* **1999**, *24*, 227.

[10] S. Tomiuk, K. Hofmann, M. Nix, M. Zumbansen, W. Stoffel, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3638–3643. The authors report that the highest activities of N-SMase are found in the central nervous system and in the intestine.

[11] R. Kolesnick, Y. A. Hannun, *Trends Biochem. Sci.* **1999**, *24*, 224–225 (and discussion on p. 227).

[12] a) B. Liu, N. Andrieu-Abadie, T. Levade, P. Zhang, L. M. Obeid, Y. A. Hannun, *J. Biol. Chem.* **1998**, *273*, 11313–11320; b) S. Jayadev, C. M. Linardic, Y. A. Hannun, *J. Biol. Chem.* **1994**, *269*, 5757–5763.

[13] M. Tanaka, F. Nara, K. Suzuki-Konagai, T. Hosoya, T. Ogita, *J. Am. Chem. Soc.* **1997**, *119*, 7871–7872.

[14] F. Nara, M. Tanaka, T. Hosoya, K. Suzuki-Konagai, T. Ogita, *J. Antibiot.* **1999**, *52*, 525–530.

[15] Recently the known natural product alutenusin was found to be a competitive and specific inhibitor of N-SMase ($K_i = 25$ µM): R. Uchida, H. Tomoda, Y. Dong, S. Omura, *J. Antibiot.* **1999**, *52*, 572–574.

[16] W. P. Taylor, T. S. Widlanski, *Chem. Biol.* **1995**, *2*, 713–718.

[17] Regarding the synthesis and reactivity of similar spiroepoxides, see: a) H. D. Becker, T. Bremholt, E. Adler, *Tetrahedron Lett.* **1972**, *41*, 4205–4208; b) S. Danishefsky, M. D. Shair, *J. Org. Chem.* **1996**, *61*, 16–44; c) E. J. Corey, J. P. Dittami, *J. Am. Chem. Soc.* **1985**, *107*, 256–257; d) H. Waldmann, K. Hinterding, P. Herrlich, H. J. Rahmsdorf, A. Knebel, *Angew. Chem.* **1997**, *109*, 1553–1555; *Angew. Chem.Int. Ed. Engl.* **1997**, *36*, 1541–1542; e) K. Hinterding, A. Knebel, P. Herrlich, H. Waldmann, *Bioorg. Med. Chem.* **1998**, *6*, 1153–1162.

[18] Physical Data of **6**: $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.85$ (t, 3H, $J = 6.8$ Hz, CH$_3$), 1.23 (m, 12H, 6 × CH$_2$), 1.57 (m, 2H, CH$_2$) 2.21 (t, 2H, $J = 7{,}5$ Hz, COCH$_2$), 3.65 (m, 1H, CH*H*$_{\text{epox.}}$), 3.86 (m, 2H, COC*H*-H$_{\text{epox.}}$,*C*HHOH), 4.31–4.49 (m, 3H, $\alpha$-CH, OH, CH*H*OH), 6.29 (d, 0.47H, $J = 3.5$ Hz, H$_{\text{ring}}$), 6.32 (d, 0.53H, $J = 3.2$ Hz, H$_{\text{ring}}$), 6.77 (br., 0.47H, H$_{\text{ring}}$), 6.79 (br., 0.53H, H$_{\text{ring}}$), 6.93–7.05 (m, 2H, H$_{\text{ring}}$, NH), 8.00 (s, 0.53H, NH) 8.03 (s, 0.47H, NH); $^{13}$C NMR: (100.6 MHz, CDCl$_3$, major diastereomer): $\delta = 14.1$, 22.6, 25.6, 29.2, 29.3, 29.4, 31.8, 36.3, 54.7, 59.4, 62.5, 80.1, 130.6, 138.7, 140.3, 144.6, 170.1, 174.7, 185.2; charactaristic signals of the minor diastereomer: $\delta = 54.8$, 59.3, 62.6, 80.0, 130.4, 138.4, 144.4, 170.2, 174.8, 185.1; high resolution EI-MS (155 °C): calculated for C$_{20}$H$_{30}$N$_2$O$_5$, 378.2139; found, 378.2194; $R_f = 0.49$ (dichloromethane/methanol, 10/1).

[19] Two Mg$^{2+}$-dependent neutral sphingomyelinases have been cloned so far: a) Ref. [10] and b) S. Chatterjee, US-96-774104-961224, **1998**; S. Chatterjee, CAN-129:91437, **1998**.

[20] F. Nara, M. Tanaka, S. Masuda-Inoue, Y. Yamasato, H. Doi-Yoshioka, K. Suzuki-Konagai, S. Kumakura, T. Ogita, *J. Antibiot.* **1999**, *52*, 531–535.

[21] R. W. Ledeen, G. Chakraborty, *Neurochem. Res.* **1998**, *23*, 277–289.

[22] B. Liu, Y. A. Hannun, *J. Biol. Chem.* **1997**, *272*, 16281–16287.

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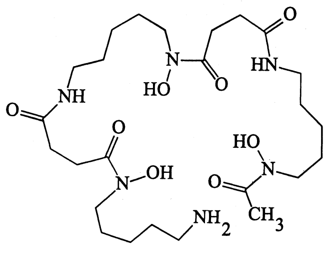
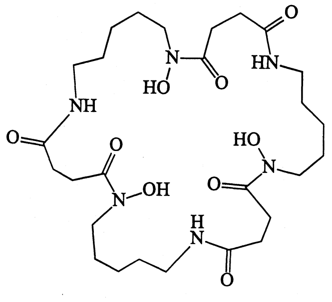
# Structural Characterization of a Plutonium(IV) Siderophore Complex: Single-Crystal Structure of Pu-Desferrioxamine E**

Mary P. Neu,* John H. Matonic, Christy E. Ruggiero, and Brian L. Scott

In order to acquire sufficient essential iron, most microorganisms produce very powerful, low molecular weight chelating agents that can deliver iron into cells via active transport systems. Ferrioxamine siderophores are linear or cyclic hydroxamates that contain 1-amino-5-hydroxyaminopentane as a building block.[1] Desferrioxamine E (DFE) and D$_2$ (DFD$_2$) are the cyclic compounds of this group; the linear compound desferrioxamine B (DFB)[2] has become the drug of choice for iron and aluminum overload by transfusion.[3] Desferrioxamines (DFOs) bind Fe$^{III}$ over a wide pH range (1–12), while showing very little affinity for iron(II) (DFB-Fe$^{III}$: $\log\beta_{110} = 30.6$; DFB-Fe$^{II}$: $\log\beta_{110} = 7.2$).[4] The hexadentate, hard oxygen-donor DFO ligands also strongly bind other hard metal ions.



DFB



DFE

We are studying the complexation and solubilization of plutonium by siderophores. While plutonium does not occur

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naturally, six tons of plutonium have been released into the biosphere since 1941.[5] In addition, numerous U.S. Department of Energy sites have significant plutonium-contamination problems due to accidental releases and past waste-handling practices.[6] Plutonium most likely enters into soils as plutonium(IV) hydroxides and oxides.[7] These forms of plutonium are thought to pose little risk for contaminating ground water and/or becoming bioavailable because they have very low solubility and uncomplexed plutonium(IV) strongly sorbs to mineral surfaces. However, compounds that solubilize the plutonium significantly increase its environmental migration and thereby also increase its bioavailabilty. Ferrioxamines are estimated to be present at 0.01 – 0.1 μM concentrations in soils,[8] and the stability constant for a $Pu^{IV}$ – DFB species is about $\log\beta_{110} = 30.8$.[9]

We have prepared $^{239}Pu^{IV}$ – DFO complexes by reacting $Pu^{III}$, $Pu^{IV}$, $Pu^{V}$, or $Pu^{VI}$ ions with the free ligand. In all cases a $Pu^{IV}$ – DFO species formed. To minimize $Pu^{IV}$ hydrolysis and multiple species formation, the title complex was best prepared by reacting one equivalent of $H_4DFE$ with $Pu^{III}$ in 2 M trifluoromethanesulfonic acid. Following adjustment of the solution to pH 2 with 0.01 M Al in 1 M NaOH, a $Pu^{III}$ – DFE complex formed, which quickly oxidized in air to $Pu^{IV}$ – DFE. The solution was concentrated and crystals formed within a week on storing the solution at 7 °C. An X-ray crystal structure analysis reveals the product to be $[Al(H_2O)_6][Pu(DFE)(H_2O)_3]_2(CF_3SO_3)_5 \cdot 14\,H_2O$, **1**.[10] This is the first plutonium – siderophore complex to be structurally characterized.

The asymmetric unit contains the $[Pu(DFE)(H_2O)_3]^+$ ion (Figure 1), a hexaaquoaluminum(III) ion, four trifluoromethanesulfonate groups and seven water molecules. The nine-coordinate plutonium atom is bound by DFE in approximately one hemisphere and three water molecules in the other. The polytopal geometry of the plutonium coordination sphere (Figure 2) is slightly distorted tricapped-trigonal prism (a twist angle of 10.1° relates the two trigonal planes), in which three water molecules and three oximate oxygen atoms form trigonal planes and three carbonyl oxygen atoms cap the prismatic faces. The four trifluoromethanesulfonate groups form a hydrogen-bonding network with the interstitial water molecules.

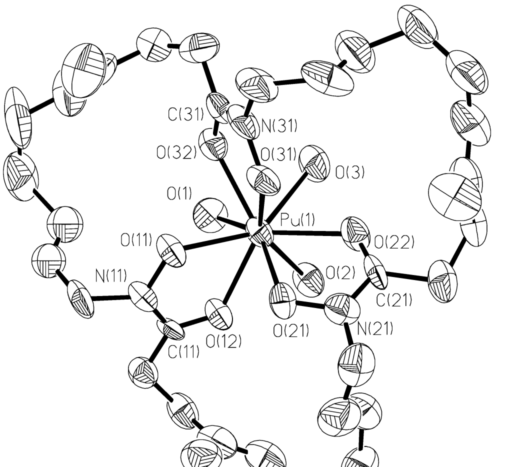

Figure 1. Thermal ellipsoid plot of the Pu cation in **1**. Selected bond lengths [Å] and angles [°]: Pu-O(1) 2.461(12), Pu-O(2) 2.472(11), Pu-O(3) 2.456(12), Pu-O(11) 2.315(10), Pu-O(12) 2.361(10), Pu-O(21) 2.296(11), Pu-O(22) 2.341(11), Pu-O(31) 2.303(11), Pu-O(32) 2.386(12); O(1)-Pu-O(2) 74.5(4), O(1)-Pu-O(11) 85.3(4), O(1)-Pu-O(12) 71.0(4).

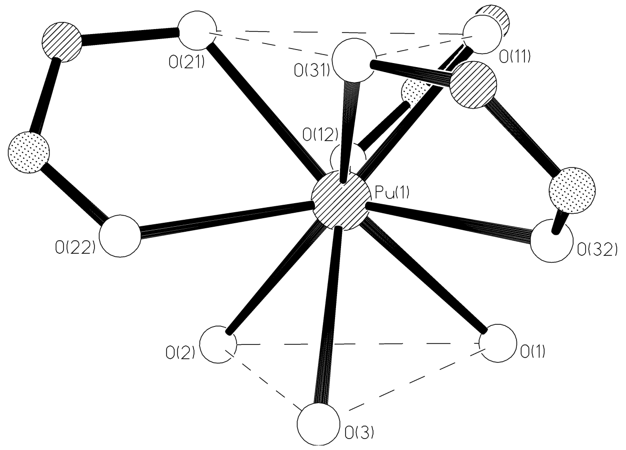

Figure 2. Drawing showing the tricapped trigonal-prismatic geometry.

Characterization of plutonium complexes is scarce; for the most common oxidation state, plutonium(IV), only a few complexes with mono- and bidentate ligands have been characterized by single-crystal X-ray diffraction. Most plutonium(IV) complexes characterized by X-ray diffraction are eight-, ten-, or twelve-coordinate and contain several bidentate ligands arranged in a square-antiprismatic, dodecahedral, or icosohedral geometry, for example, in the oxalato, nitrato, carbonato, and sufonato complexes.[11] Compound **1** is the first discrete molecule containing a nine-coordinate plutonium(IV) ion.

The free DFE ligand and its complex with $Fe^{III}$ have been structurally characterized.[12] An overlay of the $[Fe^{III}(DFE)]$ and $[Pu^{IV}(DFE)]$ complexes is shown in Figure 3. Remarkably, the DFE molecule spans the same diameter in both

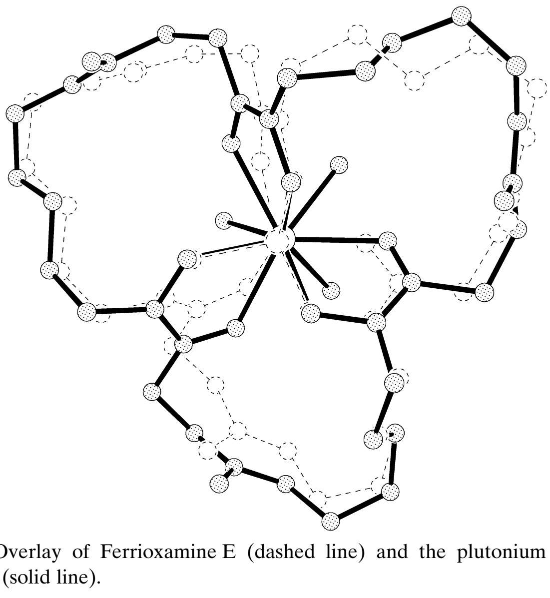

Figure 3. Overlay of Ferrioxamine E (dashed line) and the plutonium cation in **1** (solid line).

complexes, yet has the flexibility to twist the hydroxamate groups roughly 20° relative to the [Fe$^{III}$(DFE)] structure to accommodate the three coordinated water molecules in **1**. This has the effect of making the oxygen atoms that were *trans* (about 180°) in the iron complex to lie in the plutonium complex 140.8(4)° (O(11)-Pu-O(22)), 136.5(4)° (O(21)-Pu-O(32)), and 135.7(4)° (O(12)-Pu-O(31)) apart. This rotation and the larger size of the plutonium atom allows the DFE ligand to span only one half of the atom as compared to fully encapsulating the iron center. The larger plutonium(IV) ion also increases the metal–hydroxamate bonds from 1.938(4)–2.089(3) Å in the iron complex to 2.303–2.375 Å in the plutonium complex, which in turn decreases the average bite angles from 79.1° to 65.6°. The structure has three distinct Pu–O bonds: a Pu–O (water) mean length of 2.463(12) Å and two different Pu–O (hydroxamate) lengths, reflecting a negative charge localized on the oximate oxygen atom. On average, the Pu–O$_N$ distances are 0.06 Å shorter than the Pu–O$_C$ lengths. This is a smaller difference than those reported for other M$^{IV}$–hydroxamato complexes (range from 0.105–0.142 Å.[13]

The *cis-cis* configuration of the oximate oxygen atoms of the DFE molecule relative to one another is the same as that in its iron complex. A correct designation of *trans* or *cis* configuration of the nine-coordinate plutonium is impossible; however, a "pseudo designation" can be inferred from the angles. This configuration may be a result of the hydrogen bonding interactions of the oximate oxygen atoms and the hydrated aluminum cation. The aluminum cation is on an inversion center between two [Pu(DFE)] units. The water molecules from the hydrated Al$^{III}$ ion form strong hydrogen bonds to all three oximate oxygens of the DFE hydroxamate groups (O(21)-O(70) 2.63(5), O(11)-O(72) 2.62(5), O(31)-O(71) 2.61(4) Å).

The DFO ligand has shown extraordinary ability to coordinate a much larger and structurally diverse metal center than that of iron. The unique properties of this ligand, specifically the lipophilic pentane groups and hydrophilic polar carbonyl and hydroxamate groups, appear to dictate a specific molecular structure upon metal complexation. All three polar peptide carbonyl groups lie on the same side of the molecule in both [Pu$^{IV}$(DFE)] and [Fe$^{III}$(DFE)], not the case in the free ligand. This change in molecular conformation may provide a microorganism with a mechanism by which metal complexes are recognized and transported into the cell. Initial studies of the aqueous speciation of plutonium(IV) in the presence of DFB and DFE show the formation of similar products as a function of pH: one at lower pH (<4) and additional species at higher pH (4–10). Studies of the solution properties of **1** and other [Pu$^{IV}$(DFO)] complexes and the bacterial uptake of plutonium in the presence of DFB are in progress.

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[1] *Handbook of Microbial Iron Chelates* (Ed.: G. Winkelmann), CRC, Boca Raton, **1991**.

[2] H. Bickel, G. E. Hall, W. Keller-Schierlein, V. Prelog, E. Vischer, A. Wettstein, *Helv. Chim. Acta* **1960**, *43*, 2129.

[3] a) R. R. Crichton, *Inorganic Biochemistry of Iron Metabolism*, Ellis Horwood, New York, **1991**, pp. 173–190; b) J. P. Day in *Aluminum and other Trace Elements in Renal Disease* (Ed.: A. Taylor), Baliere Tindall, Eastbourne, **1986**, pp. 184–191.

[4] a) G. Schwarzenbach, A. Schwarzenbach, *Helv. Chim. Acta*. **1963**, *46*, 1390; b) G. Anderegg, F. L'Eplattenier, G. Schwarzenbach, *Helv. Chim. Acta*. **1963**, *46*, 1400.

[5] G. T. Seaborg, W. D. Loveland, *The Elements Beyond Uranium*, Wiley, New York, **1990**, S. 359.

[6] R. G. Riley, J. M. Zachara, DOE/ER-0547-T U.S. DOE, Office of Energy Research, Subsurface Science Program, Washington, **1992**.

[7] "Scientific Basis for Waste Management": J. I. Kim, *Mater. Res. Soc. Symp. Proc.* **1993**, 3–21.

[8] P. E. Powell, G. R. Cline, C. P. P. Reid, P. J. Szanislo, *Nature* **1980**, *287*, 833–834.

[9] N. V. Jarvis, R. D. Hancock, *Inorg. Chim. Acta* **1991**, *182*, 229–232.

[10] X-ray structural analysis of **1**: Bruker P4/PC diffractometer, CCD area detector, Mo$_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), graphite monochrometer, $\phi$- and $\omega$-scan method, SADABS[14] absorption correction. $0.50 \times 0.25 \times 0.10$ mm, space group *Pbcm*, $a = 13.1585(8)$, $b = 23.9480(12)$, $c = 37.521(2)$ Å, $V = 11823.6(12)$ Å$^3$, $Z = 4$, $T = 295$ K. Structure solved by Patterson and difference Fourier methods with the program SHELXS-97,[15] least-squares refinement by SHELXL-97,[16] all atoms except for the F and O atoms of the triflate ions were refined anisotropically; the triflate C–S distances were kept constant at 1.75 Å. Refinement on $F^2$ with $wR_2 = 0.211$ and $R1 = 0.108$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-136339. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[11] a) N. C. Jayadevan, K. D. Singh-Mudher, D. M. Chackraburtty, *Z. Kristallogr.* **1982**, *161*, 7–13; b) M. R. Spirlet, J. Rebizant, C. Apostolidis, B. Kanellakopoulos, E. Dornberger, *Acta Crystallogr. Sect. C.* **1992**, *48*, 1161–1164; c) D. L. Clark, S. D. Conradson, D. W. Keogh, P. D. Palmer, B. L. Scott, C. D. Tait, *Inorg. Chem.* **1998**, *37*, 2893–2899.

[12] a) D. van der Helm, M. Poling, *J. Am. Chem. Soc.* **1976**, *98*, 82–86; b) M. B. Hossain, J. Maf, D. van der Helm, K. Shimizu, *J. Chem. Crystallogr.* **1998**, *28*, 53–56; c) M. B. Hossain, D. van der Helm, M. Poling, *Acta Crystallogr. Sect. B.* **1983**, *39*, 258–263; d) M. B. Hossain, M. A. F. Jalal, D. van der Helm, *Acta Crystallogr. Sect. C.* **1986**, *42*, 1305–1310.

[13] W. L. Smith, K. N. Raymond, *J. Am. Chem. Soc.* **1981**, *103*, 3341–3349, and references therein.

[14] G. M. Sheldrick, Empirical Absorption Correction Program, Universität Göttingen, **1996**.

[15] G. M. Sheldrick, Program for Crystal Structure Solution, Universität Göttingen, **1997**.

[16] G. M. Sheldrick, Program for Crystal Structure Refinement, Universität Göttingen, **1997**.