The First Diferric Model Compound for the Uteroferrin–Arsenato Complex

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The X-ray crystal structure and EXAFS measurements are reported for a newly synthesised unsymmetrical dinuclear iron(III) complex representing the first diferric compound with a single bridging arsenato group.

Uteroferrin (Uf) is a diiron enzyme from porcine uteri and is a purple acid phosphatase (PAP). PAPs hydrolyse activated phosphoric esters and anhydrides under acidic conditions.[1] They have a two-metal centre with a tyrosine → Fe^{III} charge transfer transition with a characteristic absorption band in the range of 500–600 nm.[1b] Furthermore, PAPs interact with tetraoxo anions such as arsenate and phosphate, which inhibit their phosphatase activity.[2]

The diiron site in Uf can be found in two oxidation states: an enzymatically active, pink Fe^{III}–Fe^{II} form (Uf_r), and an inactive, purple Fe^{III}–Fe^{III} form (Uf_o). Investigations on the tetraoxo complexes of the PAPs provide new insights into the structure of the diiron site. Recently, the phosphate- and arsenate-bound forms of Uf_o were studied by EXAFS measurements.[3] Due to the higher scattering power of arsenic compared to phosphorus, generally more reliable EXAFS data are obtained for the determination of the Fe···As as compared to the Fe···P distances in the phosphate-bridged analogues.

There are a few dinuclear diferric complexes which serve as models for the phosphate binding,[4–8] but so far no suitable model complex for the arsenate binding has been reported. We now present the first model compound for Uf_o in its arsenate-bound form. This complex contains a single bridging arsenato group which reproduces the coordination mode and the stoichiometry of the PAP–oxoanion interaction proposed by Que *et al.*[3]

The reaction of equimolar amounts of $Fe(ClO_4)_3 \cdot 9H_2O$, $FeCl_3$, Htbpo† and the sodium salt of cacodylic acid in methanol–acetonitrile leads to the formation of red crystals of $[Fe_2(tbpo)(O_2AsMe_2)(Cl)(H_2O)](ClO_4)_3 \cdot 5MeOH \cdot H_2O$ **1** containing a novel (μ-alkoxo)(μ-dimethylarsinato)diiron(III) core. The structure of the cation of **1** is shown in Fig. 1.‡ Each Fe^{III} ion is four-coordinated by the heptadentate ligand $tbpo^-$; the metal ions are bound to two benzimidazole moieties, one tertiary non-aromatic nitrogen atom and the bridging oxygen atom. The metal centre in **1** exhibits a μ-alkoxo-bridged Fe^{III}–Fe^{III} unit with an additional dimethylarsinato bridge. The inequivalent environments of the atoms are completed by the coordination of a water molecule (O4) to Fe(1) and the chloro ligand to Fe(2).

EXAFS spectra of **1** were measured and analysed to obtain a reliable basis for the interpretation of EXAFS data of model compounds in solution and enzyme preparations. In particular the comparison of our results with the results for PAPs like uteroferrin will be possible. Fig. 2 shows the Fourier transform (FT) of the Fe K-edge EXAFS spectrum of **1**.§ The features at $R < 2.5$ Å correspond to the first coordination sphere. The peak at 2.9 Å originates from Fe–C scattering and the peaks from 3.0 to 4.0 Å derive from Fe–C, Fe–As and Fe–Fe absorber–scatterer contributions.

An initial parameter set was chosen with coordination numbers and distances from the crystal structure. The fit was performed with fixed coordination numbers and a variation of E_0, the bond lengths and Debye–Waller factors. The least-squares curve fitting procedure gave results without high correlations between the free parameters. The best fit results gave (CN, atom, r/Å): 1, N/O, 1.89; 3.5, N/O, 2.04; 1, N/O, 2.20; 0.5, Cl, 2.37; 4, C, 2.92; 1, As, 3.30; 1, Fe, 3.58. There is good agreement between the EXAFS and crystallographic results with the exception of the distance of the tertiary nitrogen atom at 2.20 Å. The FT fit can be improved using eight additional C-scatterer contributions in the R-range 3–4 Å but with unreasonably high correlations between the variables within and between the subshells.

The Fe···Fe distance in **1** of 3.580(2) Å is in accordance with the metal–metal separation found in the (μ-alkoxo)(μ-diphenylphosphinato)-bridged $[Fe_2(tbpo)(O_2PPh_2)(MeOH)(OMe)]^{3+}$ complex [3.545(2) Å][4] and in (μ-alkoxo)bis(μ-diphenylphosphato)diiron(III), $[Fe_2(bhpp)\{O_2P(OPh)_2\}_2]^{2+}$,¶ [3.549(3) Å].[5] This is also valid for the (μ-hydroxo)bis(μ-diphenylphosphato)- and (μ-hydroxo)bis(μ-diphenylphosphi-

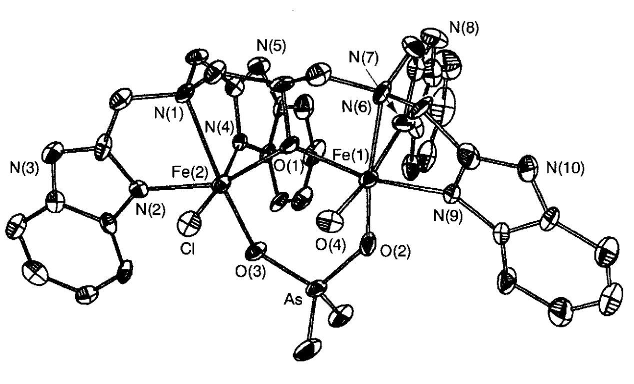

Fig. 1 Structure of the cation of **1** showing 50% probability ellipsoids; hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (°): Fe(1)···Fe(2) 3.580(2), Fe(1)···As 3.276(2), Fe(2)···As 3.336(2), Fe(1)–O(1) 1.971(6), Fe(2)–O(1) 2.025(6), Fe(1)–O(2) 1.911(6), Fe(2)–O(3) 1.919(6), Fe(1)–O(4) 2.088(6), Fe(1)–N(6) 2.269(7), Fe(2)–N(1) 2.288(7), Fe(1)–N(7) 2.077(8), Fe(1)–N(9) 2.055(7), Fe(2)–N(2) 2.063(7), Fe(2)–N(4) 2.086(7), Fe(2)–C(l) 2.368(3), As–O(2) 1.675(6), As–O(3) 1.677(6), Fe(1)–O(1)–Fe(2) 127.3(3), Fe(1)–O(2)–As 131.9(3), Fe(2)–O(3)–As 136.1(4).

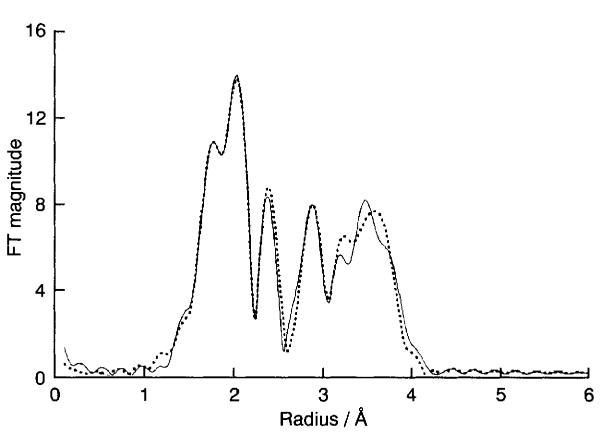

Fig. 2 Experimental (——) and calculated (······) Fourier transforms of the k^3-weighted fine structure (Fe K-edge, k-range 2.8–16 $Å^{-1}$) for **1**. The experimental (Fourier filtered from R = 0.9–4.2 Å) data were fitted using [CN, atom, r/Å (σ^2/$Å^2$)]: 1, N/O, 1.89 (0.0027); 3.5, N/O, 2.04 (0.0025); 1, N/O, 2.20 (0.0031); 0.5, Cl, 2.37 (0.0025); 4, C, 2.92 (0.0039); 1, As, 3.30 (0.0040); 1, Fe, 3.58 (0.0010); (residual, 14%).

nato)-bridged complexes $[Fe_2(OH)\{O_2P(OPh)_2\}_2(HBpz_3)_2]^+$ and $[Fe_2(OH)(O_2PPh_2)_2(HBpz_3)_2]^+$ with distances of 3.586(1) and 3.560(1) Å, respectively.[6] As a result of the missing bridging ligands the iron···iron distance of 3.700(2) Å in the only (μ-alkoxo)-bridged compound with a terminally bound phosphato group, $[Fe_2(Cl)_2\{O_2P(OPh)_2\}(tbpo)(MeOH)]^{2+}$,[9] is larger than that for **1**. Shorter distances have been observed for (μ-oxo)(μ-oxoanion)- as well as for (μ-oxo)bis(μ-oxoanion)-bridged compounds, which are in the range from 3.198(3) Å for $[Fe_2O(Me_3\text{-tacn})_2\{PO_3(OPh)\}_2]$[7] to 3.421(3) Å for $[Fe_2O(tpa)_2(MoO_4)]^{2+}$,[10] the μ-oxo bridge holding the two metal centres in close proximity.

Complex **1** contains a μ-alkoxo as well as one bidentate bridging arsenato group. Therefore **1** is a good model compound for the proposed structure for the oxidised oxoanion complex of uteroferrin.[3] In contrast the triply arsenato-bridged complex $[Fe_2(Me_3\text{-tacn})_2(HAsO_4)_3]$[7] contains no additional μ-O(R) bridge and consequently this compound can not fulfil the role of a model compound in these points. Complex **1** exhibits an average Fe···As distance of 3.31 Å, a value that is about 0.12 Å shorter than the Fe···As distance found in $[Fe_2(Me_3\text{-tacn})_2(HAsO_4)_3]$ (average 3.43 Å). This difference correlates with the significant difference in the Fe–O–As angles for **1** (average 134°) and for $[Fe_2(Me_3\text{-tacn})_2(HAsO_4)_3]$ (average 150°). In diferric compounds containing phosphato or phosphinato bridges the average Fe···P distance is 3.21 Å.[4–8] In the above described complexes the As–O bond lengths are approximately 0.15 Å larger than the corresponding P–O distances. Assuming that the bond angles for Fe–O–X (X = As, P) (typically for μ-oxo-, μ-hydroxo- and μ-alkoxo-bridged compounds near 130°) are similar, the Fe···X distances should differ by the differences in X–O bond lengths. For example, the Fe···As distance is 3.31 Å for **1** (Fe–O–As = 134°) in comparison to the Fe···P distances in the above mentioned μ-O(R)-bridged synthetic compounds with bidentate bridging phosphato groups (Fe···P = 3.21, average Fe–O–P = 132°). The metal–metal separation and especially the differences in the Fe···X distances (0.1 Å) in the model compounds are in contrast to the values found by Que *et al.* for $Uf_o{\cdot}PO_4$ (Fe···Fe = 3.22 Å, Fe···P = 3.17 Å) and $Uf_o{\cdot}AsO_4$ (Fe···Fe = 3.29 Å, Fe···As = 3.42 Å).[3] Que finds a significantly higher value for the difference in the Fe···X distances (0.25 Å).

The Mössbauer spectra of **1** exhibit two distinct quadrupole doublets. The values for the isomeric shifts (δ) (referred to metallic iron at room temperature) and quadrupole splittings (ΔE_Q) at 4.2 K are: $\delta_1 = 0.35$ mm s^{-1}, $\Delta E_{Q1} = 0.37$ mm s^{-1} and $\delta_2 = 0.35$ mm s^{-1}, $\Delta E_{Q2} = 0.65$ mm s^{-1}. The quadrupole splittings reflect different electric field gradients at the nuclei, suggesting nonequivalent environments at the iron ions, which is in accordance with the crystallographic results for **1**. The magnetic susceptibilities (Faraday method) of a crystalline sample of **1** between 5.1 and 299.5 K show weak antiferromagnetic coupling in the dimer with an effective magnetic moment μ_{eff} of 7.25 μ_B at 299.5 K. The magnetic moment decreases to 0.90 μ_B at 5.1 K. The experimental data were fitted on the basis of a spin-Hamiltonian $\hat{H} = -2J\hat{S}_1\hat{S}_2$ ($S_1 = S_2 = 5/2$) and g = 2.0 (fixed). The coupling constant found for **1** [$J = -10.3(1)$ cm^{-1}] is smaller than the ones found in other μ-alkoxo-bridged diiron(III) compounds with dinucleating ligands.[4,5,9,11]

Electrochemical investigations on **1** show two quasi-reversible one-electron transfer transitions. The transition at +210 mV *vs.* Ag/AgCl/3 mol dm^{-3} NaCl is assigned to the Fe^{III}–Fe^{III}/Fe^{III}–Fe^{II} couple. The one-electron wave at −40 mV corresponds to the Fe^{II}–Fe^{III}/Fe^{II}–Fe^{II} couple transition. The electronic spectrum of **1** in methanol exhibits a λ_{max} at 375 nm (ε = 6350 dm^3 mol^{-1} cm^{-1}) which arises from a benzimidazole-to-iron charge transfer transition.

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Footnotes

† The polypodal ligand *N,N,N′,N′*-tetrakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane (Htbpo) was synthesised using a novel synthetic pathway. A higher purity of the product was achieved by avoiding the preparation of the hydrochloride intermediate. 1,3-Diamino-2-propanol-*N,N,N′,N′*-tetraacetic acid (8.06 g, 0.025 mol) and 1,2-diaminobenzene (10.81 g, 0.1 mol) were ground to a fine powder and mixed thoroughly. The mixture was heated to 180 °C for 2 h until all water was evaporated. The red glass was dissolved in ethanol and heated at reflux for 30 min. Activated charcoal was added and after 10 min the hot solution was filtered. After cooling the solution, water was added and the remaining crude product was filtered off. The dried precipitate was recrystallised from acetone–water to yield a white powder. Yield: 11.37 g (0.019 mol, 76.8%); mp 169 °C.

‡ *Crystal structure* of **1**: $C_{42}H_{63}AsCl_4Fe_2N_{10}O_{22}$, M = 1388.44, crystal size 0.18 x 0.12 x 0.10 mm, monoclinic, $P2_1/c$, a = 11.452(3), b = 37.359(10), c = 14.123(4) Å, β = 108.62(2)°, V = 5726 Å^3, Z = 4, D_c = 1.61 g cm^{-3}, $2\theta_{max}$ = 44°, Mo-Kα radiation (λ = 0.71073 Å), ω-scan, T = 150 K, 7009 symmetry independent reflections of which 3825 had $I > 2\sigma(I)$; 7008 reflections were used in the refinement, no absorption correction (μ = 1.35 mm^{-1}; max., min. transmission: 0.874, 0.784), the structure was solved using Patterson methods (*SHELXL*-86, G. M. Sheldrick, 1990), refinement on $|F^2|$ (*SHELXL*-93, G. M. Sheldrick, 1993), 4 restraints, 756 independent parameters, calculation of the H atoms in ideal positions, $R1$ = 0.0608 [$I > 2\sigma(I)$], $wR2$ = 0.1601 (all data), max. residual electron density 1.05 e Å^{-3}. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

§ X-Ray absorption data (transmission, 20 K) were recorded with a multi-element solid-state detector on the EXAFS spectrometer of the European Molecular Biology Laboratory at HASYLAB (DESY, Hamburg). Two spectra were averaged after individual energy calibration.[12] A four-segmented cubic spline routine was used for background subtraction. Structural parameters were derived from a curved-wave single-scattering analysis based on theoretical amplitude and phase functions.[13]

¶ Abbreviations used: H_3bhpp, 1,3-bis[(2-hydroxybenzyl)(2-pyridylmethyl)]propan-2-ol; $HBpz_3$, hydridotris(1-pyrazolyl)borate; Me_3-tacn, 1,4,7-trimethyl-1,4,7-triazacyclononane; tpa, tris(2-pyridylmethyl)amine.

References

1 (*a*) M. A. S. Aquino, J.-S. Lim and A. G. Sykes, *J. Chem. Soc., Dalton Trans.*, 1994, 429; (*b*) B. C. Antanaitis and P. Aisen, *Adv. Inorg. Biochem.*, 1983, **5**, 111; (*c*) R. B. Lauffer, B. C. Antanaitis, P. Aisen and L. Que, Jr., *J. Biol. Chem.*, 1983, **258**, 14212; (*d*) J. T. Sage, Y.-M. Xia, P. G. Debrunner, D. T. Keough, J. de Jersey and B. Zerner, *J. Am. Chem. Soc.*, 1989, **111**, 7239 and references cited therein; (*e*) B. A. Averill, J. C. Davis, S. Burman, T. Zirino, J. Sanders-Loehr, T. M. Loehr, J. T. Sage and P. G. Debrunner, *J. Am. Chem. Soc.*, 1987, **109**, 3760; (*f*) M. Dietrich, D. Münstermann, H. Suerbaum and H. Witzel, *Eur. J. Biochem.*, 1991, **199**, 105; (*g*) K. Cichutek, H. Witzel and F. Parak, *Hyperfine Interact.*, 1988, **42**, 885.

2 J. B. Vincent and B. A. Averill, *FASEB J.*, 1990, **4**, 3009.

3 A. E. True, R. C. Scarrow, C. R. Randall, R. C. Holz and L. Que, Jr., *J. Am. Chem. Soc.*, 1993, **115**, 4246.

4 B. Eulering and B. Krebs, 208th ACS National Meeting, Washington DC, August 1994, Abstract INOR 275; manuscript in preparation.

5 B. Krebs, K. Schepers, B. Bremer, G. Henkel, E. Althaus, W. Müller-Warmuth, K. Griesar and W. Haase, *Inorg. Chem.*, 1994, **33**, 1907.

6 P. N. Turowski, W. H. Armstrong, S. Liu, S. N. Brown and S. J. Lippard, *Inorg. Chem.*, 1994, **33**, 636.

7 S. Drüeke, K. Wieghardt, B. Nuber, J. Weiss, H.-P. Fleischhauer, S. Gehring and W. Haase, *J. Am. Chem. Soc.*, 1989, **111**, 8622.

8 W. H. Armstrong and S. J. Lippard, *J. Am. Chem. Soc.*, 1985, **107**, 3730; P. N. Turowski, W. H. Armstrong, M. E. Roth and S. J. Lippard, *J. Am. Chem. Soc.*, 1990, **112**, 681; R. E. Norman, S. Yan, L. Que, Jr., G. Backes, J. Ling, J. Sanders-Loehr, J. H. Zhang and C. J. O'Connor, *J. Am. Chem. Soc.*, 1990, **112**, 1554.

9 B. Bremer, K. Schepers, P. Fleischhauer, W. Haase, G. Henkel and B. Krebs, *J. Chem. Soc., Chem. Commun.*, 1991, 510.

10 R. C. Holz, T. E. Egren, L. L. Pearce, J. H. Zhang, C. J. O'Connor and L. Que, Jr., *Inorg. Chem.*, 1993, **32**, 5844.

11 D. L. Jameson, C.-L. Xie, D. N. Hendrickson, J. A. Potenza and H. J. Schugar, *J. Am. Chem. Soc.*, 1987, **109**, 740; Q. Chen, J. B. Lynch, P. Gomez-Romero, A. Ben-Hussein, G. B. Jameson, C. J. O'Connor and L. Que, Jr., *Inorg. Chem.*, 1988, **27**, 2673; G. D. Fallon, A. Markiewicz, K. S. Murray and T. Quach, *J. Chem. Soc., Chem. Commun.*, 1991, 198; Y. Nishida, M. Nasu and T. Akamatsu, *Z. Naturforsch., B Chem. Sci.*, 1992, **47**, 115; S. Kawata, M. Nakamura, Y. Yamashita, K. Asai, K. Kikuchi, I. Ikemoto, M. Katada and H. Sano, *Chem. Lett.*, 1992, 135; A. Neves, S. M. D. Erthal, V. Drago, K. Griesar and W. Haase, *Inorg. Chim. Acta*, 1992, **197**, 121.

12 S. Priggemeyer, P. Eggers-Borkenstein, F. Ahlers, G. Henkel, M. Körner, H. Witzel, H.-F. Nolting, C. Hermes and B. Krebs, *Inorg. Chem.*, 1995, **34**, 1445; R. F. Pettifer and C. Hermes, *J. Appl. Crystallogr.*, 1985, **18**, 404.

13 N. Binsted, J. W. Campbell, S. J. Gurman and P. C. Stephenson, *EXCURV92 program*, SERC Daresbury Laboratory, 1992.