Inorganica Chimica Acta, 197 (1992) 121–124

Inorganica
Chimica Acta
LETTER

A new N,O-donor binucleating ligand and its first iron(III) complex as a model for the purple acid phosphatases

Ademir Neves*, Sueli M. D. Erthal
Departamento de Química, Universidade Federal de Santa Catarina, 88049 Florianópolis, SC (Brazil)

Valderes Drago
Departamento de Física, Universidade Federal de Santa Catarina, 88049 Florianópolis, SC (Brazil)

Klaus Griesar and Wolfgang Haase
Institut für Physikalische Chemie, Technische Hochschule Darmstadt, D-6100 Darmstadt (Germany)

(Received January 16, 1992; revised May 12, 1992)

In recent years binuclear iron complexes have received a great deal of attention since binuclear iron units were found in the active sites of iron proteins such as hemerythrin (Hr) [1], ribonucleotide reductase (RR) [2] and purple acid phosphatases (PAPs) [3]. While hemerythrin is the best characterized member of this group [4], the structural, magnetic, spectroscopic and electrochemical properties of purple acid phosphatases (PAPs) are yet less well understood [5]. They constitute a novel class of enzymes that catalyze the hydrolysis of certain phosphate esters, including nucleoside di- and triphosphates and aryl phosphates [6]. The active site of the PAPs isolated from porcine uterus and bovine spleen consists of a homònuclear diiron complex with two accessible oxidation states – a catalytically inactive, purple Fe(III)–Fe(III) form characterized by typical absorption maxima at $\lambda_{max}=550$ nm, and an enzymatically active, pink Fe(III)–Fe(II) form which is blue shifted ($\lambda_{max}=505$ nm) [7]. These bands are assigned as being tyrosinate-to-iron(III) charge-transfer transitions [7]. Resonance Raman spectra of the purple and pink forms using visible excitation, clearly show the presence of tyrosine ring modes [8]. The interconversion of the two forms by using reductants such as ascorbate or dithioerythritol and oxidants such as ferricyanide and hydrogen peroxide and the similar extinction coefficients found in both states, suggest that tyrosinate is coordinated to only one of the iron centers, the non-reducible site [7, 8].

The presence of histidine imidazole is a probable ligand to both iron centers, as has been demonstrated by NMR [9] and ENDOR [10] studies.

Despite the preparation and characterization of several synthetic analogues, most of the works have focused more attention on anion interaction with the active sites [11] and EPR properties [12] of some mixed-valence Fe(II)–Fe(III) complexes. However, with regard to the visible chromophore in PAPs, $[Fe_2O(OBz)(HDP)_2]^+$ [HDP^{-1} = (*N*-(*o*-hydroxybenzyl)-*N*,*N*-bis(2-pyridylmethyl) amine)] [13] is the unique example of a binuclear Fe(III)–Fe(III) complex with one phenolate per iron center, which exhibits a purple colour ($\lambda_{max}=522$ nm), comparable to those found for the Fe(III)–Fe(III) enzymes [13]. No redox properties have been described for this complex.

In this study, we report the synthesis and some physicochemical properties of the first binuclear iron complex, which readily shows similar UV–Vis properties like those observed in the purple and pink forms of PAPs. Moreover, binuclear iron complexes containing the (μ-alkoxo)(μ-carboxylato)$_2$ structural entity are exceedingly rare [14].

Experimental

Synthesis of N,N′,N,N′-bis[(2-hydroxybenzyl)(2-methylpyridyl)]-2-ol-1,3-propanediamine ($H_3BBPPNOL$)

2-OH-SALPN. This compound was prepared by the method of Mazurek *et al.* [15].

N,N′-bis(2-hydroxybenzyl)-2-ol-1,3-propanediamine ($H_3BBPNOL$). To a solution of 2-OH-SALPN (44.6 g; 151 mmol) in methanol (300 ml) was added slowly $NaBH_4$ (2.9 g; 75 mmol) with stirring. The methanol was removed by rotary evaporation and the sirup obtained was dissolved in chloroform (300 ml), washed with a brine solution and dried over $MgSO_4$. The chloroform was removed by rotary evaporation and the oil obtained was dried under vacuum. Yield 42 g; 70% with respect to 2-OH-SALPN. ^1H NMR ($CDCl_3$; δ): 6.7–7.5(m, 8H, phenyl); 3.6–4.3(s, 5H, $N-CH_2-R$ and $R_2-CH-OH$); 2.3–3.0(m, 4H, $(N-CH_2)_2$).

$H_3BBPPNOL$. To a solution of 2-(chloromethyl) pyridine hydrochloride (10.8 g; 33 mmol) in water (100 ml), previously neutralized with $N(Et)_3$ in an ice bath under argon, was added the diamine $H_3BBPNOL$ (10 g; 33 mmol) with stirring. The reaction mixture was heated (60 °C) and further $N(Et)_3$ (23 ml; 165 mmol) was added over a period of 2 h in small portions so

---

*Author to whom correspondence should be addressed.

0020-1693/92/$5.00

that the pH never exceeded 10. The red solution was cooled and extracted with chloroform (3×50 ml), washed with a brine solution (2×50 ml) and dried over $MgSO_4$. The chloroform was removed by rotary evaporation and the oil obtained was dried under vacuum. Yield 14 g; 87% with respect to $H_3BBPNOL$. ^1H NMR ($CDCl_3$; δ): 8.9(m, 2H, two phenolic protons), 6.9–8.3(m, 16H, phenyl and py), 3.9–4.4(m, 9H, N–CH_2–R and R_2–CH–OH), 2.6–3.0 (d, 4H, $(N–CH_2)_2$).

Synthesis of $[Fe_2^{III}(BBPPNOL)(CH_3CO_2)_2][PF_6]\cdot 0.5CH_3OH\cdot 0.5CH_2Cl_2$ ($1\cdot PF_6$)

To a solution of $H_3BBPPNOL$ (2.1 mmol) in methanol was added Et_3N (6.2 mmol), sodium acetate (4.1 mmol) and $Fe(ClO_4)_3\cdot xH_2O$ (4.1 mmol). The clear deep purple solution was heated to 50 °C and stirred for 10 min. After cooling the solution to room temperature, a microcrystalline precipitate was formed, which was filtered off, and washed with cold ethanol and ether*. Metathesis with NH_4PF_6 and recrystallization from CH_3OH/CH_2Cl_2 (1:2) affords a complex that analyses satisfactorily as $1\cdot PF_6$. *Anal.* Calc. for $Fe_2C_{34}H_{38}N_4O_{7.5}PF_6Cl$: C, 44.0; H, 4.1; N, 6.5. Found: C, 44.6; H, 4.2; N, 6.1%.

Results and discussion

The IR spectra of $1\cdot PF_6$ shows one $\nu_a(COO^-)$ at 1550 cm^{-1} and two $\nu_s(COO^-)$ at 1450 and 1420 cm^{-1} indicating the presence of one symmetrical and one unsymmetrical carboxylato groups [16]. The large band at 830 cm^{-1} is attributed to the stretching vibrations of the PF_6^- anion while the broad band around 3400 cm^{-1} may be attributed to the ν(O–H) band of methanol of crystallization. The IR spectra of the corresponding perchlorate salt is quite the same as that of $1\cdot PF_6$ except for the stretching vibrations of the uncoordinated ClO_4^- anion which are in the 1050–1140 cm^{-1} range. The molar conductivity of $1\cdot PF_6$ in acetonitrile determined at 298 K is 153 Ω^{-1} cm^2 mol^{-1}, characteristic of a 1:1 electrolyte [17]. From this information and from the binucleating character of $BBPPNOL^{3-}$, complex $1\cdot PF_6$ can be formulated as $[Fe_2(BBPPNOL)(CH_3COO)_2][PF_6]\cdot 0.5CH_3OH\cdot 0.5CH_2Cl_2$. In order to confirm the binuclear formulation, a series of adequate physicochemical measurements was carried out.

The magnetic susceptibility measurements on a powder sample of $1\cdot ClO_4$ between 4.2 and 284.5 K show that the two iron(III) ions are antiferromagnetically coupled (Fig. 1). The data were fitted based on an isotropic Heisenberg model, $H=-2JS_1\cdot S_2$ ($S_1=S_2=5/2$) by using the following parameters: $g=2.00$ (fixed); $J=-13.7(\pm 0.7)$ cm^{-1}; %imp$=2.1\%\pm 0.2$; $\theta=-7.0$ K. The antiferromagnetic spin coupling constant of -13.7 cm^{-1} lies in the typical range for μ-alkoxo or μ-hydroxo bridged binuclear Fe(III) complexes [14, 18].

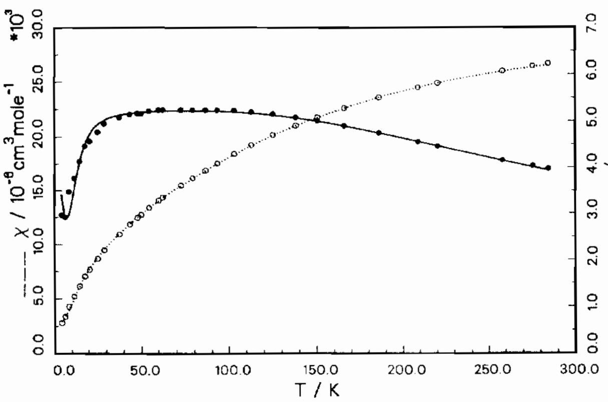

Fig. 1. Magnetic susceptibility of $1\cdot ClO_4$ as a function of temperature (●: experimental data) and temperature dependence of the effective magnetic moment of $1\cdot ClO_4$ (⊙: experimental data).

The electronic spectrum of $1\cdot PF_6$ measured in MeCN solution exhibits features at 327 nm ($\epsilon=3550$ l mol^{-1} cm^{-1}/Fe) and 540 nm ($\epsilon=2200$ l mol^{-1} cm^{-1}/Fe). The lower energy band is assigned by analogy to previously studied iron–phenolate complexes [19], as being a phenolate-to-iron(III) charge transfer (CT) transition. The higher energy band at 327 nm is also assigned to a phenolate-to-iron(III) (CT) transition on the basis of spectroelectrochemical studies, which show that this band disappears upon reduction of $1\cdot PF_6$ to the corresponding Fe(II)Fe(II) complex.

The zero-field Mössbauer spectrum of $1\cdot PF_6$ at 115 and 300 K (Fig. 2) shows two overlapping quadrupole doublets of equal area. The least-squares fits give the following isomer shifts δ (referred to metallic iron at room temperature) and quadrupole splittings ΔE_Q for Fe_A and Fe_B respectively: $\delta^A=0.503$; $\delta^B=0.502$ mm s^{-1} and $\Delta E_Q^A=1.27$; $\Delta E_Q^B=0.89$ mm s^{-1} at 115 K and $\delta^A=0.386$; $\delta^B=0.409$ mm s^{-1} and $\Delta E_Q^A=1.22$; $\Delta E_Q^B=1.00$ mm s^{-1} at 300 K. These values are consistent with a high-spin Fe(III)–Fe(III) formulation [20] in agreement with the magnetic results. The large ΔE_Q values for both sites suggest major distortions from effective octahedral symmetry and are comparable to those observed for uteroferrin and purple acid phosphatase from bovine spleen [8]. From this information, we suggest that the ferric ions Fe_A and Fe_B in $1\cdot PF_6$ are inequivalent. In addition, the observed asymmetry does not change by rotation of the sample with respect to the incident γ-ray, which shows that there are no texture effects.

The cyclic voltammogram of $1\cdot PF_6$ in MeCN is illustrated in Fig. 3(a). The complex shows two quasi-

---

**Anal.* of $1\cdot ClO_4$. Calc. for $Fe_2C_{33}H_{35}N_4O_{11}Cl$: C, 48.9; H, 4.3; N, 6.9. Found: C, 47.0; H, 4.0; N, 7.0%.

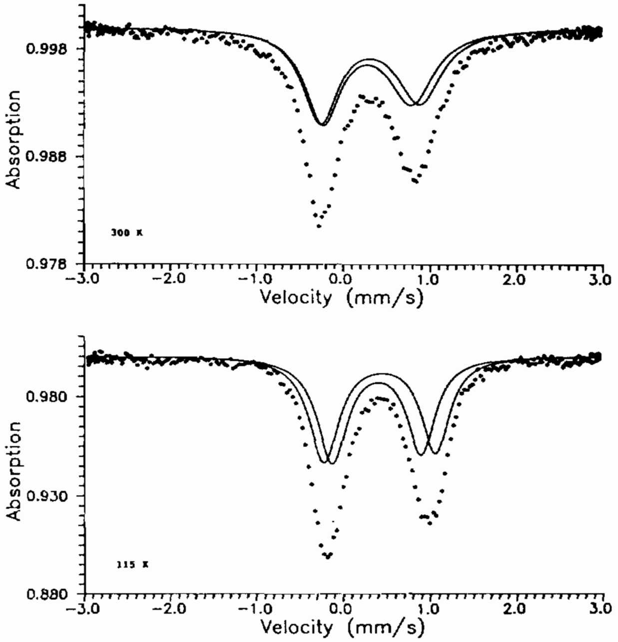

Fig. 2. Mössbauer spectra of a polycrystalline sample of $1 \cdot PF_6$ at 115 and 300 K.

reversible redox couples at −0.61 and −1.0 V versus Fc^+/Fc, which can be ascribed to the successive redox reactions of Fe(III)–Fe(III)/Fe(III)–Fe(II) and Fe(III)–Fe(II)/Fe(II)–Fe(II). A large asymmetry of two iron sites in the redox behavior may partly come from a structural asymmetry, although both iron sites have N_2O_4 donor set. The comproportionation constant of 4×10^6 for the equilibrium [Fe(III)–Fe(III)] + [Fe(II)–Fe(II)] $\rightleftharpoons$ 2[Fe(III)–Fe(II)], calculated from the separation of the redox potentials indicates that the mixed-valence complex is considerably stabilized. Thus, we have used a spectroelectrochemical study, at the same conditions as employed in the CV experiments, to explore the accessibility of the Fe(III)–Fe(II) analogue of complex $1 \cdot PF_6$. The spectral change for the reaction of Fe(III)–Fe(III) to Fe(III)–Fe(II) is shown in Fig. 3(b). The $E_{1/2} = -0.61$ V versus Fc^+/Fc and $n = 1.0 \pm 0.1$ electrons values obtained from the Nernst plot (inset of Fig. 3(b)) are in very good agreement with the CV results. The maintenance of a strict isobestic point in successive spectra strongly corroborates for the presence of a single product throughout the course of the electrolysis. However, it is interesting to note that the reduction of $1 \cdot PF_6$ ($\lambda_{max} = 540$ nm) to the corresponding Fe(III)–Fe(II) species ($\lambda_{max} = 484$ nm) is accompanied by essentially no change in the intensity of the absorption band. To interpret this fact, we tentatively suggest that the new band at 484 nm is originated from the overlapping of two (CT) transitions, specifically the phenolate-to-Fe(III) and the Fe(II)-to-pyridine transitions. The lack of appearance of any new band in the 300–400 nm range supports this assignment. A somewhat similar behavior has recently been reported [21] for the mixed-valence $[Fe^{III}Fe^{II}BPMP(OPr)_2]^{2+}$ species ($BPMP^-$ = 2,6-bis-[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenolate), in which the Fe(II)-to-pyridine (CT) transition is shifted to higher energy with respect to the phenolate-to-Fe(III) band.

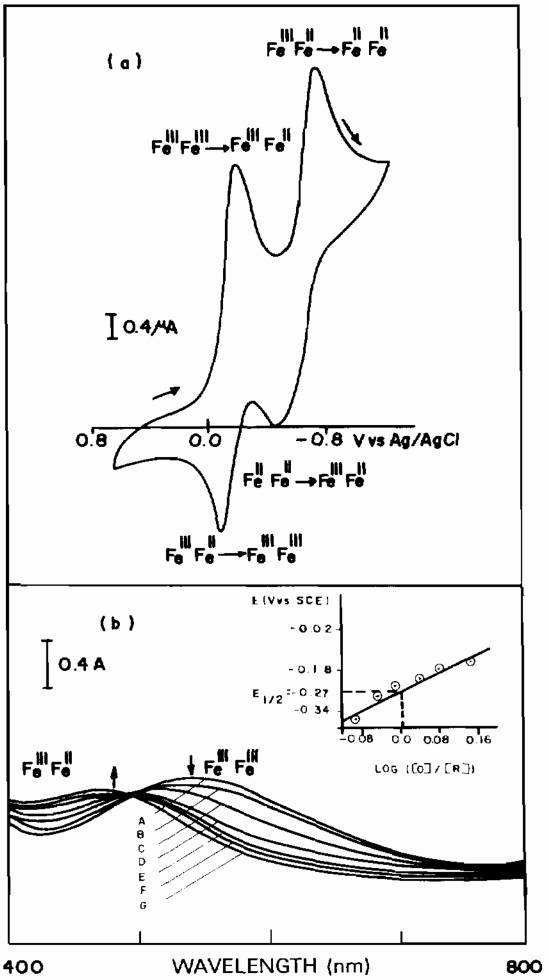

Fig. 3. (a) Cyclic voltammogram of $1 \cdot PF_6$ in MeCN (0.1 M $[Bu_4N][PF_6]$ supporting electrolyte, platinum working electrode, ferrocene internal standard, scan rate 100 mV s^{-1}). (b) Spectra recorded during spectropotentiostatic experiment on $1 \cdot PF_6$ (0.1 M $[Bu_4N][PF_6]$ in MeCN). Applied potentials in V vs. SCE are: A: −0.14; B: −0.18; C: −0.22; D: −0.26; E: −0.30; F: −0.38; G: −0.42.

Based on the IR and Mössbauer spectra, and the electrochemical data, we can assume that the cation **1** present in $1 \cdot PF_6/1 \cdot ClO_4$ complexes has a binuclear structure in which the two iron(III) Fe_A and Fe_B sites are inequivalent as shown in Fig. 4. On Fe_A, the pyridine nitrogen is *trans* to the alkoxo bridge, while a phenolate is *trans* on Fe_B. One of the carboxylato bridges consequently must be *trans* to a phenolate on Fe_A and to

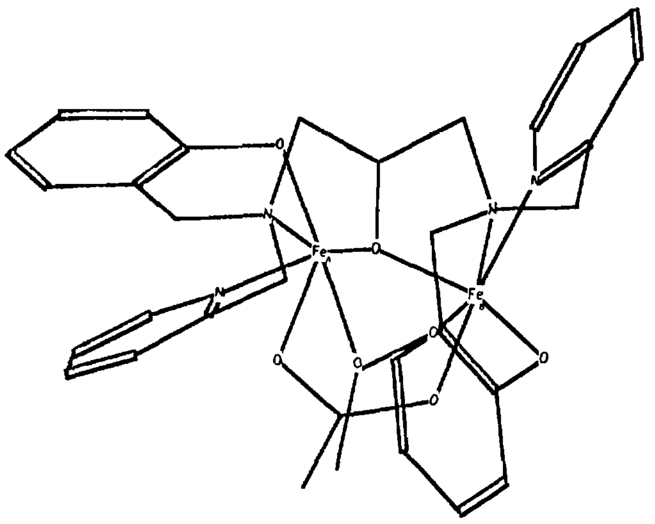

Fig. 4. Proposed structure of the cation $[Fe_2(BBPPNOL)(\mu\text{-}Ac)_2]^+$. The program used was Chem 3D - Mackintosh.

the pyridine on Fe_B, giving rise to an asymmetrically bridged entity.

Finally, the Mössbauer and UV–Vis spectral properties of **1** recall those of the oxidized purple acid phosphatases. Thus **1** serves as a potential model for the visible chromophore of these enzymes. Further studies of such type of dinuclear iron complexes are under current investigation in our group and will be the subject of future reports.

Acknowledgements

This work was supported by grants from CNPq and PADCT (Ministério da Ciência e Tecnologia of Brazil) and KFA (Kern Forschungs Anlage, Germany). One of us (S.M.D.E.) thanks CAPES and Universidade Federal do Paraná for a stipend.

References

1 R. E. Stenkamp, L. C. Siekler and L. H. Jensen, *J. Am. Chem. Soc., 106* (1984) 618; S. Sheriff, W. A. Hendrickson and J. L. Smith, *J. Mol. Biol., 197* (1987) 273.

2 B.-M. Sjöberg and A. Gräsland, *Adv. Inorg. Biochem., 5* (1983) 87; M. Lammers and H. Follman, *Struct. Bonding (Berlin), 54* (1983) 27; T. Joelson, U. Uhlin, H. Eklund, B.-M. Sjöberg, S. Hahne and M. Karlson, *J. Biol. Chem., 259* (1984) 9076.

3 B. C. Antanaitis, T. Strekos and P. Aisen, *J. Biol. Chem., 257* (1982) 3766; K. Doi, B. C. Antanaitis and P. Aisen, *Struct. Bonding (Berlin), 70* (1988) 1.

4 L. Que, Jr. and R. C. Scarrow, in L. Que, Jr. (ed.), *Metal Clusters in Proteins,* ACS Symposium Series 372, American Chemical Society, Washington, DC, 1988, pp. 159–178; S. J. Lippard, *Angew. Chem., Int. Ed. Engl., 27* (1988) 344.

5 M. P. Hendrich, J. DeJersey, D. T. Keough, J. T. Sage and B. Zerner, *Biochim. Biophys. Acta, 745* (1983) 103; S. Burman, J. C. Davis, M. J. Weber and B. A. Averill, *Biochem. Biophys. Res. Commun., 136* (1986) 490; S. Soltysik, S. S. David, M. T. Stankovich and L. Que, Jr., *Recl. Trav. Chim. Pays-Bas, 106* (1987) 252; R. C. Scarrow, J. W. Pyrz and L. Que, Jr., *J. Am. Chem. Soc., 112* (1990) 657; J. B. Vincent, M. C. Crowder and B. A. Averill, *Biochemistry, 30* (1991) 3025; M. Dietrich, D. Münstermann, H. Suerbaum and H. Witzel, *Eur. J. Biochem., 199* (1991) 105.

6 J. B. Vincent and B. A. Averill, *FASEB J., 4* (1990) 3009; J. B. Vincent, G. L. Olivier-Lilley and B. A. Averill, *Chem. Rev., 90* (1990) 1447.

7 B. C. Antanaitis and P. Aisen, *Adv. Inorg. Biochem., 5* (1983) 111.

8 B. A. Averill, J. C. Davis, S. Bueman, T. Zirino, J. Sanders-Loehr, T. M. Loehr, J. T. Sage and P. G. Debruner, *J. Am. Chem. Soc., 109* (1987) 3760.

9 R. B. Laufer, B. C. Antanaitis, P. Aisen and L. Que, Jr., *J. Biol. Chem., 258* (1983) 14212; R. C. Scarrow, J. W. Pyrz and L. Que, Jr., *J. Am. Chem. Soc., 112* (1990) 657.

10 B. C. Antanaitis, J. Peisach, W. B. Mims and P. Aisen, *J. Biol. Chem., 260* (1985) 4572; K. Doi, J. McCracken, J. Peisach and P. Aisen, *J. Biol. Chem., 263* (1988) 5757.

11 S. Drüecke, K. Wieghardt, B. Nuber and J. Weiss, *Inorg. Chem., 28* (1989) 1414.

12 M. Susuki, H. Oshio, A. Uehara, K. Endo, M. Yanaga, S. Kida and K. Saito, *Bull. Chem. Soc. Jpn., 61* (1988) 3907; M. S. Mashuta, R. J. Webb, K. J. Oberhausen, J. F. Richardson, R. M. Buchanan and D. N. Hendrickson, *J. Am. Chem. Soc., 11* (1989) 2745.

13 S. Yan, L. Que, Jr., L. F. Taylor and O. P. Anderson, *J. Am. Chem. Soc., 110* (1988) 5222.

14 Q. Chen, J. B. Lynch, P. Gomes-Romero, A. Ben-Hussein, G. B. Jameson, C. J. O'Connor and L. Que, Jr., *Inorg. Chem., 27* (1988) 2673.

15 W. Mazurek, K. J. Berry, K. S. Murray, M. J. O'Connor, M. R. Snow and A. G. Wedd, *Inorg. Chem., 21* (1982) 3071.

16 G. B. Deacon and R. J. Phillips, *Coord. Chem. Rev., 23* (1980) 227.

17 W. J. Geary, *Coord. Chem. Rev., 7* (1971) 81.

18 S. Ménage and L. Que, Jr., *Inorg. Chem., 29* (1990) 4293; G. D. Fallon, A. Markiewicz, K. S. Murray and T. Quach, *J. Chem. Soc., Chem. Commun.,* (1991) 198; B. Bremer, K. Schepers, P. Fleischhauer, W. Haase, G. Henkel and B. Krebs, *J. Chem. Soc., Chem. Commun.,* (1991) 510.

19 B. P. Gaber, V. Miskoswski and T. G. Spiro, *J. Am. Chem. Soc., 96* (1974) 6868; E. W. Ainscough, A. M. Brodie, J. E. Plowman, K. L. Brown, A. W. Addison and A. R. Gainsford, *Inorg. Chem., 19* (1980) 3655.

20 N. N. Greenwood and T. C. Gibb, *Mössbauer Spectroscopy,* Chapman and Hall, London, pp. 113–168.

21 A. S. Borovik, V. Papaefthymiou, L. F. Taylor, O. P. Anderson and L. Que, Jr., *J. Am. Chem. Soc., 111* (1989) 6183.