Structure of a New Tetranuclear Iron(III) Complex with an Oxo-Bridge; Factors to Govern Formation and Stability of Oxo-Bridged Iron(III) Species in the L-Subunit of Ferritin

Yuichi Sutoh, Yuko Okawamukai, Satoshi Nishino, and Yuzo Nishida[*]

Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990-8560, Japan. Fax: +81-23-628-4591. E-mail: yuzo@sci.kj.yamagata-u.ac.jp

[*] Author for correspondence and reprint requests

We have investigated the reaction products of several iron(III) compounds with hydrogen peroxide, and have found that hydrogen peroxide promotes the formation of an oxo-bridged iron(III) species in the presence of methanol (electron donor), and carboxyl groups of the ligand systems play a role to give the tetranuclear iron(III) compound containing a bent Fe-O-Fe unit (O: oxo oxygen atom). Based on the present results and the facts that L-chains of human ferritins lack ferroxidase activity, but are richer in carboxyl groups (glutamates) exposed on the cavity surface, it seems reasonable to conclude that (i) the hydrogen peroxide released in the H-subunit may contribute to the formation of a diferric oxo-hydrate in the L-subunit, (ii) the formation of a bent oxo-bridged iron(III) species is essentially important in the L-subunit, and (iii) rich carboxyl groups in L-subunits contribute to facilitate iron nucleation and mineralization through the capture and activation of the peroxide ion, and formation of a stable bent oxo-bridged iron(III) species.

Key words: Tetranuclear Iron(III) Complex with Bent Oxo-Bridged Unit, Hydrogen Peroxide, Glutamates of L-Subunit Ferritin

Introduction

Ferritins are a class of iron storage and mineralization proteins found throughout the animal, plant, and microbial kingdoms (Harrison and Arosio, 1996; Chasteen and Harrison, 1999). Iron is stored within the protein shell of ferritin as a hydrous ferric oxide nanoparticle with a structure similar to that of the mineral "ferrihydrite". Mammalian ferritins are of two types, H and L, which have complementary functions in iron uptake. The H-chain contains a dinuclear ferroxidase site and catalyzes the oxidation of ferrous ion by O_2-producing H_2O_2, and the formation of a diferric oxo-hydrate biomineral proceeds in the L-subunit. Recent detection of a peroxodiferric intermediate in the ferroxidase reaction in the H-subunit firmly establishes the ferritin ferroxidase site as very similar to sites in the O_2-activating diiron enzyme such as methane monooxygenase (MMOH) (Zhao et al., 1997; Liu et al., 1995). The similarities among the peroxodiferric intermediates of MMOH and site-specific mutants of R2, and ferritin (H-subunit) include a distinct blue color from a peroxo → Fe charge transfer band (Feig and Lippard, 1994; Waller and Lipscomb, 1996). It has been generally recognized that hydrogen peroxide formed in the H-subunit ferritin is released from the site (Hwang et al., 2000) and thus the role of hydrogen peroxide is believed to be negligible in the ferritins at present; this is quite different from the case of MMOH where the decay of the peroxo-intermediate has been proposed to yield a high-valent Fe oxidant in the O_2-activating enzymes that oxidizes organic substrates (Waller and Lipscomb, 1996; Liu et al., 1997).

In our previous paper, we have reported that some binuclear iron(III) complexes with an alk-oxo-bridge [ligands: H(HPTP) and H(HPTB)] react with hydrogen peroxide, to give a blue species (Nishida et al., 1985, 1992; Weiss and Dick, 1994; Nishino et al., 1999), which is confirmed as a peroxodiferric species as shown below (Scheme I); we also reported the detailed chemical properties of the blue intermediate (Nishida and Takeuchi, 1987; Nishino et al., 1999; Kobayashi et al., 2000).

Based on our results (Ito et al., 1996, 1997), we have proposed that the high oxidant potential of the blue peroxodiferric species of MMOH appears only when it is activated through interaction with the peripheral groups such as carboxyl residue existing nearby or activated by both the peripheral
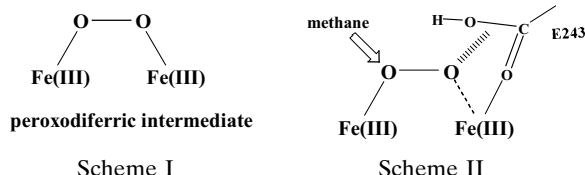
Scheme I

Scheme II

groups and the substrate (see Scheme II) (Nishida, 1998, 2004). This may support the discussion that the facile release of peroxide ions from the peroxodiferric species of H-subunit in human ferritin should be due to the absence of peripheral groups such as glutamates as observed for MMOH.

In the L-subunit of human ferritin diferric oxohydrate biominerals are formed (Harrison and Arosio, 1996; Chasteen and Harrison, 1999), but the mechanism remains unknown. Since it has been recognized that the degradation of the peroxodiferric species of the R2 subunit of ribonucleotide reductase proceeds with the acceptance of electron(s) (Ravi *et al.*, 1994), it seems quite likely that the hydrogen peroxide released in the H-subunit may play an important role in the formation of a diferric oxo-hydrate biomineral in the L-subunit. In order to confirm this we have investigated the reaction products of several iron(III) compounds with hydrogen peroxide (Nishino *et al.*, 2002), and have found that (i) hydrogen peroxide promotes the formation of an oxo-bridged iron(III) species in the presence of methanol (electron donor), and (ii) carboxyl groups in the ligand systems play a valuable role to give a stable oxo-bridged iron(III) species with a bent-structure. Present results seem to be highly important to elucidate the facts that L-chains lack ferroxidase activity, but are richer in carboxyl groups (glutamates) exposed on the cavity surface (Harrison and Arosio, 1996; Chasteen and Harrison, 1999).

Experimental

Materials

Iron(III) compounds with H(dpa) and H(dpal) (see Fig. 1) were obtained according to literature methods.
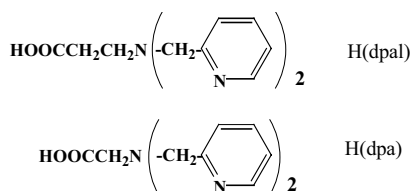

Fig. 1. The chemical structures of H(dpal) and H(dpa).

Measurements

ESI-mass spectra (positive pattern) were obtained with an ESI-MS PE SCIEX API 300 instrument in water/methanol (4:1) solutions containing an iron(III) compound at Institute for Molecular Science (Okazaki, Japan) (Nishino *et al.*, 2002). The $H_2{}^{18}O_2$ (2% solution, ^{18}O content 92%) was purchased from ICON (New York).

Preparation and crystal structure determination of $Fe_4O_2(dpal)_4(PF_6)_4 \cdot H_2O$

To the water/methanol solution (v/v, 1:1) containing $Fe(dpal)Cl_2$ (500 mg) the hydrogen peroxide solution (30%, 2 ml) was added and the resulted solution was kept to stand for 10 d. Green crystals precipitated were once recrystallized from methanol/water (v/v, 3:1). The crystal structure of a green crystal of $Fe_4O_2(dpal)_4(PF_6)_4 \cdot H_2O$ was determined by X-ray diffractometry (CCDC: 193952): space group $C2/c$ (#15), monoclinic, $a = 25.425(2)$ Å, $b = 12.7880(9)$ Å, $c = 25.930(3)$ Å, $\beta = 113.825(3)°$; the complex is centrosymmetric. R ($= \Sigma||F_o|-|F_c||/\Sigma|F_o|$) = 0.072 for 7958 observed reflections [$I > 2\sigma(I)$].

Results

ESI-mass spectra

The ESI-mass spectra showed that the chloride ions of original iron(III) compounds dissociated in the water/methanol solution; in Fig. 2a, the signal at m/z = 357.0 corresponds to $Fe(dpal)(OH)^+$ [in the case of Fe(dpa)-complex, $Fe(dpa)(OH)^+$ is observed at m/z = 343.0]. When hydrogen peroxide was added to this solution, the monomeric species disappeared, and the new signals at m/z = 683.2 and 713.2 have appeared (see Fig. 2b), and these should correspond to an oxo-bridged species (see Scheme III), where the formation of formate (O_2CH) should be due to the oxidation of methanol (see Scheme III). For the detection of two
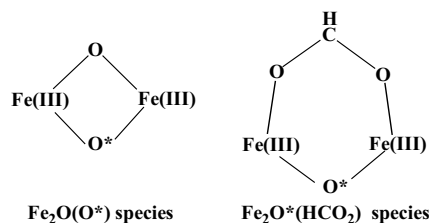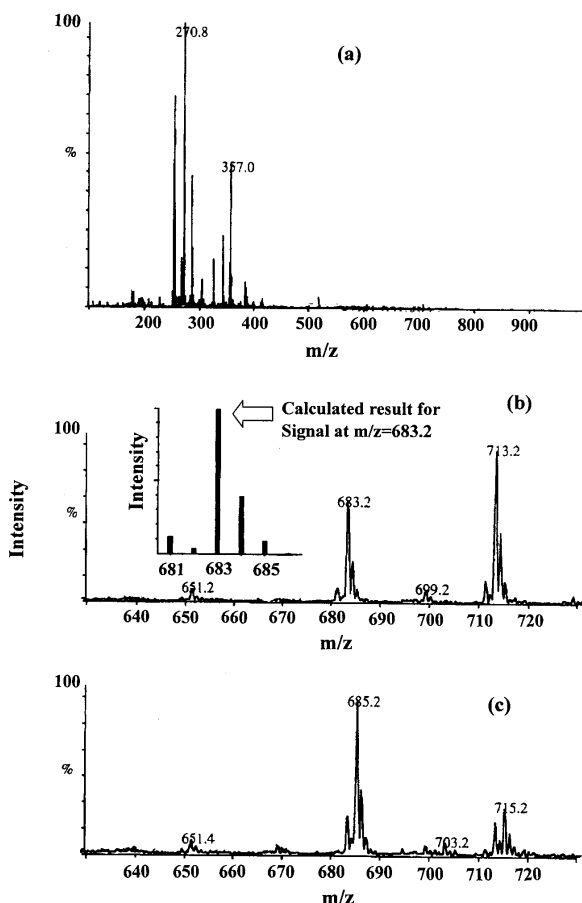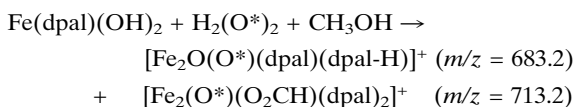

Scheme III

Fig. 2. ESI-mass spectra of the solutions containing the Fe(dpal)Cl$_2$ complex. (a) Fe(dpal)Cl$_2$ complex in water/methanol (4:1). (b) H$_2$^{16}O$_2$ was added to the solution in (a). (c) H$_2$^{18}O$_2$ was added to the solution in (a).

Fig. 3. ESI-mass spectra of the solutions containing the Fe(dpa)Cl$_2$ complex. (a) H$_2$^{16}O$_2$ was added to the solution containing the Fe(dpa)Cl$_2$ complex in water/methanol (4:1). The signals at *m/z* = 655.2 and 685.2 may be attributed to [Fe$_2$O$_2$(dpa)(dpa-H)]$^+$ and [Fe$_2$O(O$_2$CCH)(dpa)$_2$]$^+$, respectively. (b) H$_2$^{18}O$_2$ was added to the solution containing the Fe(dpa)Cl$_2$ complex in water/methanol (4:1).

kinds of an oxo-bridged iron(III) species described in Scheme III, methanol is necessary and thus it seems quite likely that in this reaction methanol is used to reduce the activated hydrogen peroxide coordinated to the iron(III) ions, giving a formate as bridging group.

Fe(dpal)(OH)$_2$ + H$_2$(O*)$_2$ + CH$_3$OH →

      [Fe$_2$O(O*)(dpal)(dpal-H)]$^+$ (*m/z* = 683.2)

  + [Fe$_2$(O*)(O$_2$CH)(dpal)$_2$]$^+$ (*m/z* = 713.2)

When H$_2$^{18}O$_2$ was added instead of H$_2$^{16}O$_2$, the signals at *m/z* = 683.2 and 713.2 shifted to *m/z* = 685.2 and 715.2, respectively (Fig. 2c). These indicate that the oxygen atom of the oxo-bridged iron(III) species is derived from hydrogen peroxide.
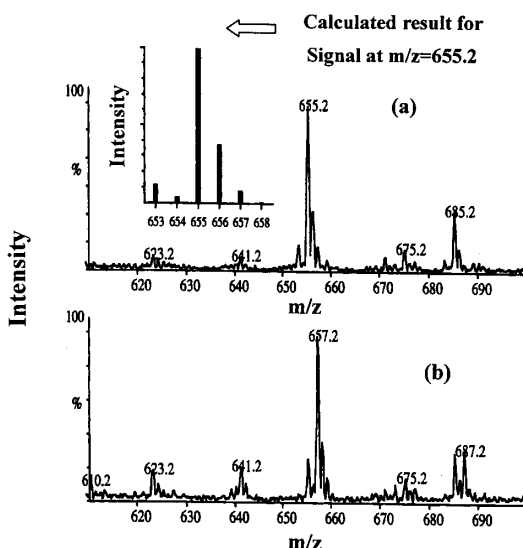
The similar results were also observed for the Fe(dpa)Cl$_2$ complex (see Fig. 3).

Structure of the green crystal

The crystal structure of Fe$_4$O$_2$(dpal)$_4$(PF$_6$)$_4$ is illustrated in Fig. 4, and the selected bond distances (Å) and angles (°) are listed in Table I. It consists of a tetranuclear iron(III) unit (centrosymmetric), where two oxo-bridged iron(III) compounds are bridged by four carboxyl groups derived from the ligand H(dpal). The angles of Fe-O-Fe (O: oxo-atom) are in the range 132 ~ 146.4°.

Discussion

Based on the present results, it seems quite likely that the hydrogen peroxide formed in the H-subunit of human ferritin plays an important role to form the ferric oxide hydrate in the L-sub-unit. As indicated above, the carboxyl groups derived from the glutamates are abundant in the L-subunit, we may assume that the activation of the coordinated hydrogen peroxide should be induced through interaction with the carboxyl groups as pointed out by us (see Scheme IV) (Ito *et al.*, 1996;
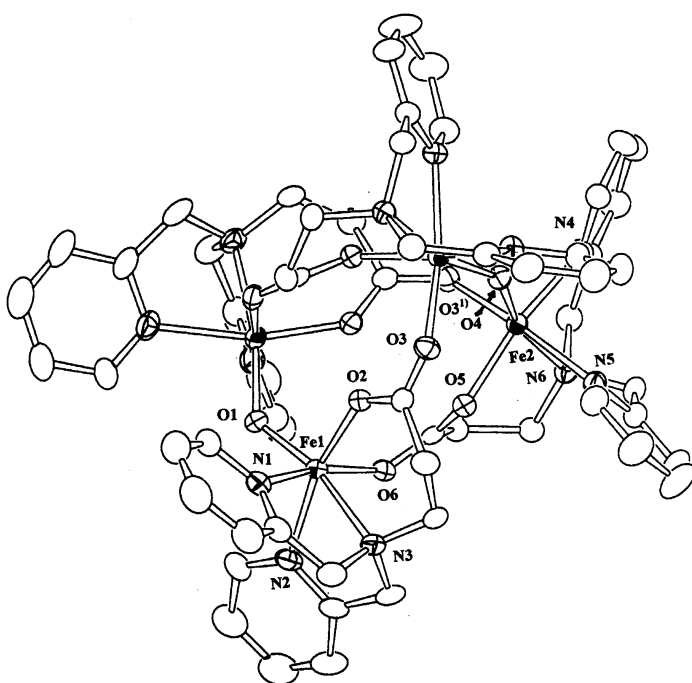
Fig. 4. ORTEP drawing of the $Fe_4O_2(dpal)_4]^{4+}$ ion.

Nishida, 1998), which facilitates the electron transfer from the electron donor to the peroxide adduct.

The incorporation of the oxygen atom of the peroxide ion into an oxo-bridged iron(III) species

as confirmed in this study is similar to that observed for ribonucleotide reductase (RNR) (Ling *et al.*, 1994); in the latter case the electron comes from the tyrosine residue of the compound (Ravi *et al.*, 1994). In fact, the presence of tyrosine resi-

Fe1-O1	1.799(1)	Fe1-O2	2.011(2)
Fe1-O6	2.048(2)	Fe1-N1	2.147(3)
Fe1-N2	2.148(3)	Fe1-N3	2.231(3)
Fe2-O3^a	2.055(2)	Fe2-O4	1.788(1)
Fe2-O5	1.989(2)	Fe2-N4	2.153(3)
Fe2-N5	2.158(3)	Fe2-N6	2.260(3)
O1-Fe1-O2	101.0(1)	O1-Fe1-O6	96.18(8)
O1-Fe1-N1	95.53(10)	O1-Fe1-N2	95.1(1)
O1-Fe1-N3	167.0(1)	O2-Fe1-O6	96.25(9)
O2-Fe1-N1	84.6(1)	O2-Fe1-N2	163.9(1)
O2-Fe1-N3	89.6(1)	O6-Fe1-N1	167.9(1)
O6-Fe1-N2	82.4(1)	O6-Fe1-N3	90.3(1)
N1-Fe1-N2	93.4(1)	N1-Fe1-N3	77.6(1)
N2-Fe1-N3	74.4(1)	O3^a-Fe2-O4	96.89(8)
O3^a-Fe2-O5	94.30(10)	O3^a-Fe2-N4	82.2(1)
O3^a-Fe2-N5	167.2(1)	O3^a-Fe2-N6	90.55(10)
O4-Fe2-O5	105.7(1)	O4-Fe2-N4	94.0(1)
O4-Fe2-N5	95.93(9)	O4-Fe2-N6	164.9(1)
O5-Fe2-N4	160.3(1)	O5-Fe2-N5	82.9(1)
O5-Fe2-N6	86.80(10)	N4-Fe2 N5	96.2(1)
N4-Fe2-N6	73.9(1)	N5-Fe2 N6	76.8(1)
Fe1-O1-Fe^a	132.0(2)	Fe2-O4-Fe2^a	146.4(2)

Table I. Bond lengths (Å) and bond angles (°) with standard deviations in parentheses of $Fe_4O_2(dpal)_4(PF_6)_4$.

^{a} Symmetry operation -*X*,*Y*,-*Z*+3/2.
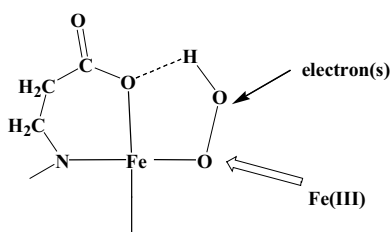
Scheme IV

dues is confirmed for the L-subunit of human ferritin [the glu-27 in the H-subunit is replaced by tyrosine in the HuLF (Harrison and Arosio, 1996; Chasteen and Harrison, 1999)].

Very recently we have observed that the linear oxo-bridged six-coordinated iron(III) complex with the iminodiacetate dianion, [Fe$_2$O(ida)$_4$]$^{4-}$ (Schmitt *et al.*, 2002), is stable below 288 K in solution, but insoluble massive deposits appeared when the temperature of the solution was raised above 308 K (see Scheme V). The structural instability of this complex above 308 K should be due to longer Fe-O (oxo) bond distances (1.796 Å);

Fe-O distances are in the range 1.77–1.78 Å for the stable linear oxo-bridged iron(III) compounds (Ito *et al.*, 1997), and thus a bent Fe-O-Fe structure seems to be necessary to maintain the binuclear unit in solution as observed in this study; in fact Fe-O (O: oxo) distances are long (~ 1.80 Å) in several binuclear iron(III) compounds with a bent structure (Nishida *et al.*, 1994).

As described in the Introduction, in mammalian ferritins complementary functions of H- and L-subunits make the heteropolymers of two subunits more efficient in the incorporation and mineralization of iron. This is consistent with the fact that when a mutated H-subunit was overexpressed in COS-1 cells, suppression of H-subunit synthesis occurred and homopolymers of the L-subunit became rich, leading to massive iron deposition associated with gastric cancer (Kato *et al.*, 2001; Ferreira *et al.*, 2000). The massive iron deposition observed in the L-subunit homopolymers may be due to an inadequate structural environment around the iron ions as proposed above.
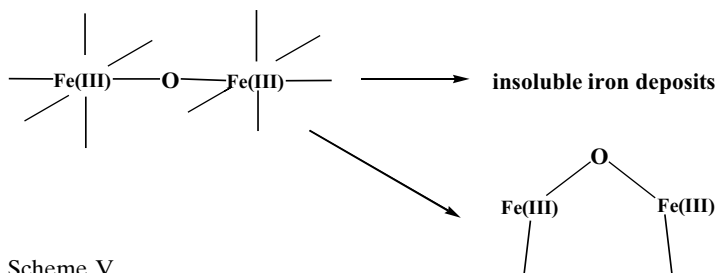
Scheme V

Chasteen N. D. and Harrison P. M. (1999), Mineralization in ferritin: An efficient means of iron storage. J. Struct. Biol. **126**, 182–194.

Feig A. L. and Lippard S. J. (1994), Reactions of nonheme iron(II) centers with dioxygen in biology and chemistry. Chem. Rev. **94**, 759–805.

Ferreira C., Bucchini D., Martin M.-E., Levi S., Arosio P., Grandchamp B., and Beaumont C. (2000), Early embryonic lethality of H ferritin gene deletion in mice. J. Biol. Chem. **275**, 3021–3024.

Harrison P. M. and Arosio P. (1996), The ferritins: molecular properties, iron storage function and cellular regulation. Biochim. Biophys. Acta **1275**, 161–203.

Hwang J., Krebs C., Huynh B. H., Edmondson D. E., Theil E. C., and Penner-Hahn J. E. (2000), A short Fe-Fe distance in peroxodiferric ferritin: Control of Fe substrate versus cofactor decay? Science **287**, 122–125.

Ito S., Okuno T., Matsushima H., Tokii T., and Nishida Y. (1996), Chemical origin of high activity in oxygenation of cylohexane by H$_2$O$_2$ catalyzed by dinuclear iron(III) complexes with amide-containing ligands. J. Chem. Soc., Dalton Trans. **1996**, 4479–4484.

Ito S., Okuno T., Itoh H., Ohba S., Matsuhsima H., Tokii T., and Nishida Y. (1997), Chemical basis for high activity in oxygenation of cyclohexane catalyzed by dinuclear iron(III) complexes with ethereal oxygen containing ligands and hydrogen peroxide system. Z. Naturforsch. **52b**, 719–727.

Kato J., Fujikawa J., Kanda M., Fukuda N., Sasaki K., Takayama K., Kobune T., Takada K., Takimoto R., Hamada H., Ikeda T., and Niitsu Y. (2001), A mutation, in the iron-responsive element of H ferritin mRNA, causing autosomal dominant iron overload. Am. J. Hum. Genet. **69**, 191–197.

Kobayashi T., Nishino S., Masuda H., Einaga H., and Nishida Y. (2000), Detection of complex formation between binuclear iron(III)-peroxide adduct and oligonucleotide by electrospray mass spectroscopy. Inorg. Chem. Commun. **3**, 608–611.

Ling J., Sahlin M., Sjoberg B.-M., Loehr T. M., and Sanders-Loehr J. (1994), Dioxygen is the source of the μ-oxo bridge in iron ribonucleonide reductase. J. Biol. Chem. **269**, 5595–5601.

Liu K. E., Valentine A. M., Wang D., Huynh B. H., Edmondson D. E., Salifoglou A., and Lippard S. J. (1995), Kinetic and spectroscopic characterization of intermediates and component interactions in reactions of methane monooxygenase from *Methylococcus capsulatus* (Bath). J. Am. Chem. Soc. **117**, 10174–10185.

Liu Y., Nesheim J. C., Paulsen K. E., Stankovich M. T., and Lipscomb J. D. (1997), Role of the methane monooxygenase reductase component in the regulation of catalysis. Biochemistry **36**, 5223–5233.

Nishida Y. (1998), Important role of substrate in activation of dioxygen in biological oxygenases. Trends Inorg. Chem. **5**, 89–103.

Nishida Y. (2004), Oxidative stress and neurodegeneration. Med. Hypothesis Res. **1**, 227–245. http://www.journal-mhr.com/PDF_Files/vol_1_4/1_4_PDFs/1_4_2.pdf

Nishida Y. and Takeuchi M. (1987), Unique reactivity of peroxide ion trapped by binuclear iron(III) complex. Z. Naturforsch. **42b**, 52–54.

Nishida Y., Takeuchi M., Shimo H., and Kida S. (1985), Synthesis and reactivities of binuclear iron(III) complexes with ligands composed of two tridentate chelating groups. Inorg. Chim. Acta **96**, 115–119.

Nishida Y., Nasu M., and Akamatsu T. (1992), Preparation and catalase-like function of a binuclear iron(III) compound with *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane. Z. Naturforsch. **47b**, 115–119.

Nishida Y., Goto A., Akamatsu T., Ohba S., Fujita T., Tokii T., and Okada S. (1994), Iron chelates in biological systems: its relevance to induction of pathogenesis of tissue damage and carcinogenesis. Chem. Lett. **1994**, 641–644.

Nishino S., Hosomi H., Ohba S., Matsushima H., Tokii T., and Nishida Y. (1999), Selective dioxygenation of cyclohexane catalyzed by hydrogen peroxide and dinuclear iron(III) complexes with μ-alkoxo bridges. J. Chem. Soc., Dalton Trans. **1999**, 1509–1513.

Nishino S., Takahashi Y., and Nishida Y. (2002), Important role of hydrogen peroxide for storing of iron ions in human L-subunit ferritin. Inorg. Chem. Commun. **5**, 609–611.

Ravi N., Bollinger J. M. Jr., Huynh B. H., Edmondson D. E., and Stubbe J. (1994), Mechanism of assembly of the tyrosyl radical-diiron(III) cofactor of *E. coli* ribonucleotide reductase. 1. Mössbauer characterization of the diferric radical precursor. J. Am. Chem. Soc. **116**, 8007–8014.

Schmitt W., Jordan P. A., Henderson R. K., Moore G. R., Anson C. E., and Powell A. K. (2002), Synthesis, structures and properties of hydrolytic Al(III) aggregates and Fe(III) analogues formed with iminodiacetate-based chelating ligands. Coord. Chem. Rev. **228**, 115–126.

Waller B. J. and Lipscomb J. D. (1996), Dioxygen activation by enzymes containing binuclear non-heme iron clusters. Chem. Rev. **96**, 2625–2657.

Weiss A. and Dick S. (1994), Der zweikeringe Fe(III)-Komplex von *N,N,N′,N′*-Tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropan – Molekül- und Kristallstruktur und seine Wechselwirkung mit Montmorillonit. Z. Naturforsch. **49b**, 1051–1058.

Zhao Z. W., Treffry A., Quail M. A., Guest J. R., and Harrison P. M. (1997), Catalytic iron(II) oxidation in the non-haem ferritin of *E. coli*; the early intermediate is not an iron tyrosinate. J. Chem. Soc., Dalton Trans. **1997**, 3977–3978.