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Research paper

Synthesis and properties of a heterobimetallic iron-manganese complex and its comparison with homobimetallic analogues

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ABSTRACT

Heterobimetallic cofactors containing one manganese and one iron ion have recently been found within the di-metal carboxylate protein family. Herein we report the synthesis and characterization of three binuclear metal complexes with Fe-Fe, Mn-Mn, and Fe-Mn metal composition. All three complexes use the same ligand framework, the BPMP ligand (HBPMP = 2,6-bis[(bis (-2-pyridylmethyl)amine) methyl]-4-methylphenol)) with two additional acetate ligands bridging the two metals. In terms of stability towards metal exchange, the Fe-Mn is more stable than the Mn-Mn complex but less stable than the Fe-Fe complex. Cyclic voltammetry shows that the Fe-Mn complex behaves markedly different than the homobimetallic complexes. The Fe-Mn complex also shows higher reactivity with O_2 than both the Fe-Fe and the Mn-Mn counterparts.

1. Introduction

Nature has incorporated transition metals in the active sites of many enzymes that are involved in a diverse range of chemical reactions. For some metalloenzymes, two different metals are present in the active site. Examples of these heterobimetallic enzymes are the [NiFe]-hydrogenases that catalyse both the oxidation of hydrogen and the reduction of protons [1], the cytochrome-c oxygenases that reduce dioxygen [2], and the Cu-Zn superoxide dismutases that catalyse the disproportionation of superoxide to dioxygen and hydrogen peroxide [3–5].

One class of enzymes that has recently been shown to have members with heterobimetallic cofactors is the di-metal carboxylate protein family. This family primarily has two Fe ions in the active site and the best studied members are the ribonucleotide reductase (RNR) R2 proteins [6–7], and soluble methane monooxygenases [8–9]. These enzymes have a carboxylate-bridged di-metal centre in their active sites and are involved in dioxygen activation [10].

RNR is one of the most important enzymes for life and is present in all living organisms, from bacteria to mammals (including humans). RNR converts the four standard ribonucleotides to their deoxyribonucleotide counterparts and thereby provides the precursors needed for both synthesis and repair of DNA [6,11].

Three main classes of RNR have been identified according to how they react when exposed to oxygen [6–7]. The first class is oxygen-dependent, the second class works both in aerobic and anaerobic conditions, while the third class is oxygen-sensitive. Class I is the most related to this study and has been further subdivided into Class Ia containing the classical motif with two iron ions in the active site, Class Ib that can also function with two manganese ions, and Class Ic containing one iron and one manganese ion [6,12–15].

Class I RNRs are composed of two non-identical proteins called R1 and R2 [6]. The R1 subunit has the binding site for the substrate and effector molecules that alter the activity. The R2 subunit contains the bimetallic centre that initiates the reaction with oxygen to create a radical that is first stabilized as a tyrosyl radical ($Y^{\cdot}$), and subsequently shuttled to a cysteine residue in the R1 subunit during catalysis [16–18].

The Class Ic R2 proteins, e.g. CtR2c present in the *Chlamydia trachomatis* [19], lacks the radical harbouring tyrosine [13–14]. The one-electron oxidizing equivalent is instead proposed to be stored as a Fe(III)-Mn(IV) species that replaces the Fe(III)-Fe(III)-$Y^{\cdot}$ cofactor in standard R2s [20–24].

A similar Fe-Mn cofactor was found in another human pathogen, *Mycobacterium tuberculosis* [25]. This R2c like protein exhibits an unprecedented tyrosine – valine cross-link in the vicinity of the iron ion that results from a two-electron oxidation, probably catalysed by the metal centre. Interestingly, it was also shown that not only the Fe(III)-

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Mn(IV) form is stable to incubations with H_2O_2, but the reduced forms are efficiently activated by H_2O_2 treatment [25–27].

Synthetic binuclear model complexes containing iron and/or manganese ions could be used to gain further insight into how heterobimetallic cofactors are assembled and how they differ from the more common homobimetallic counterparts. To date, only a few heterobimetallic compounds which contain a Fe-Mn centre have been synthesized and characterized but not as heterometallic active site mimics [28–31].

The main goal of this study is to examine the relative stability and reactivity towards molecular oxygen of three binuclear complexes using the same ligand framework, but with different metal composition – Fe-Fe, Fe-Mn, and Mn-Mn, respectively. For this, the BPMP ligand (HBPMP = 2,6-bis[bis(2-pyridylmethyl)aminomethyl]-4-methylphenol) was chosen. BPMP binds two metal ions with a bridging phenolate and the coordination sphere of the two metal ions can be completed with e.g. two additional bridging acetate groups. The three complexes, $[(BPMP)Fe_2(OAc)_2](PF_6)$ (**1**), $[(BPMP)Mn_2(OAc)_2](PF_6)$ (**2**), and $[(BPMP)FeMn(OAc)_2](PF_6)(HPF_6)$ (**3**) have been synthesized and characterized and their stability and reactivity with molecular oxygen has been investigated (Scheme 1).

2. Experimental section

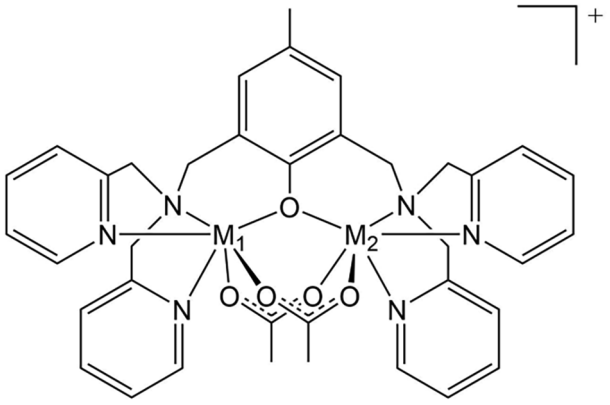
2.1. Synthesis

2.1.1. General synthetic procedures

All reagents and solvents were purchased from commercial sources. The 2,6-bis-(hydroxymethyl)-4-methylphenol was purified by recrystallization in acetone before use. All the solvents used were distilled under N_2. Acetonitrile was distilled over CaH_2, THF was distilled over sodium-benzophenone. The dry solvents were collected and transferred to dry flasks using a cannula before every reaction. Methanol was distilled over CaH_2 and collected under argon in a Schlenk flask with 4 Å molecular sieves. $[Fe(CH_3CN)_6](PF_6)_2$ was synthesized following a procedure from the literature [32].

2.1.2. Synthesis of 2,6-bis(chloromethyl)-4-methylphenol

The synthesis followed a modified literature procedure [33]. To a solution of 2,6-bis-(hydroxymethyl)-4-methylphenol (2.54 g, 15.1 mmol) in 50 ml of CH_2Cl_2, 100 ml of conc. HCl was added. The mixture was left to stir for 24 h. The compound was extracted with CH_2Cl_2; the organic phase was collected and dried with $MgSO_4$ and removed under reduced pressure to give yellow crystals. Yield: 2.54 g (82%) ^{1}H NMR (CD_3OD): δ 7.10 (s, 2H, ArH), 4.67 (s, 4H, CH_2) 2.25 (s, 3H, CH_3).



1 M_1 = Fe, M_2 = Fe
2 M_1 = Mn, M_2 = Mn
3 M_1 = Fe, M_2 = Mn

Scheme 1. Schematic structure of the cation of **1–3**.

2.1.3. Synthesis of di-(2-picolyl)amine (DPA)

The synthesis followed a modified literature procedure [34]. 2-amino-methylpyridine (4.5 ml, 46.2 mmol) was put in a round bottom flask with 20 ml of dry methanol. In another round bottom flask, the 2-pyridinecarboxaldehyde (4.95 g, 46.2 mmol) was dissolved in 20 ml of dry methanol and transferred in a dropping funnel. The pyridine carboxaldehyde was added to the 2-amino-methylpyridine slowly at 0 °C under inert conditions. After allowing the solution to go to room temperature, it was again cooled to 0 °C and $NaBH_4$ (1.78 g, 47.1 mmol) was added. The solution was allowed to go to room temperature again and left stirring overnight. The solution was poured into ice (20 ml), the pH was adjusted to 3–4 with conc. HCl, and extracted with CH_2Cl_2 until the water phase became colourless (5 × 50 ml). The water phase was neutralized with K_2CO_3 and extracted with CH_2Cl_2 3 times. The solvent was dried with $MgSO_4$ and removed under vacuum followed by a further purification through column in alumina deactivated with 4% of water, the eluent was a mixture of CH_2Cl_2/CH_3OH/TEA in the ratio 75/2/1. Yield: 4.9 g (53.3%) ^{1}H NMR (CD_3OD): δ 8.35 (s, 2H, PyH) 7.43 (s, 2H, PyH) 7.16 (s, 2H, PyH) 6.95 (s, 2H PyH) 4.56 (s, NH) 3.78 (4H, CH_2)

2.1.4. Synthesis of (2,6-bis[(bis(-2-pyridylmethyl)amine)methyl]-4-methylphenol) (HBPMP)

The synthesis followed a modified literature procedure [35]. A mixture of DPA (4.21 g, 20.68 mmol) and triethylamine (4.18 g, 41.3 mmol) was dissolved in THF and added dropwise to a solution of 2,6-bis(chloromethyl)-4-methylphenol (2.12 g, 10.3 mmol) in THF at 0 °C. After the complete addition of the DPA mixture the solution was warmed up to room temperature and left stirring for 4 days. The triethylamine salt precipitate was removed by filtration and washed 3 times with THF, and the combined filtrates were concentrated under vacuum. The residue was dissolved in 50 ml of water and extracted with CH_2Cl_2 (3 × 50 ml). The extracts were combined, dried with $MgSO_4$ and the solvent was removed under vacuum. The product was obtained as a yellow oily residue. Yield: 5.24 g (95.6%). ^{1}H NMR ($CDCl_3$): δ 8.42 (4H, m), 7.68 (m, 4H), 7.53 (dd, J = 7.8, 3.2, 4H) 7.22 (m, 4H), 6.92 (d, J = 3.2, 2H) 3.79 (d, J = 3.3, 8H) 3.71 (d, J = 3.2, 4H) 2.19 (d, J = 3.3, 3H)

2.1.5. Synthesis of $[(BPMP)Fe_2(OAc)_2](PF_6)$ (1)

The synthesis followed a modified literature procedure [35]. HBPMP (1.10 g, 2.08 mmol) was put in a 100 ml round bottom flask and dissolved in dry acetonitrile under nitrogen flow. $Fe(CH_3COO)_2$ (0.72 g, 4.16 mmol) and NH_4PF_6 (0.34 g, 2.10 mmol) were added. The solvent was removed under vacuum and the residue was re-dissolved in CH_2Cl_2. The solution was transferred to another flask with a cannula with a filter avoiding transfer of the $NH_4(CH_3COO)$. The solvent was removed by vacuum, leaving a red-brown solid. Yield 1.80 g (95%).

2.1.6. Synthesis of $[(BPMP)Mn_2(OAc)_2](PF_6)$ (2)

The synthesis followed a modified procedure literature procedure [36]. HBPMP (1.53 g, 2.89 mmol) was put in a 100 ml round bottom flask and dissolved in dry acetonitrile under nitrogen flow. Mn $(CH_3COO)_2$ (1.41 g, 5.77 mmol) and NH_4PF_6 (0.52 g, 3.19 mmol) were added. The solvent was removed under vacuum and the residue was re-dissolved in CH_2Cl_2. The solution was transferred to another flask with a cannula avoiding transfer of the $NH_4(CH_3COO)$, and the solvent was removed the under vacuum, giving a yellow precipitate. Yield 1.90 g (73%).

2.1.7. Synthesis of $[(BPMP)FeMn(OAc)_2](PF_6)(HPF_6)$ (3)

Under inert condition HBPMP (2.61 g, 4.92 mmol) was dissolved in 20 ml of dry and oxygen free acetonitrile. $Mn(CH_3COO)_2$ (0.84 g,

4.89 mmol) and $[Fe(CH_3CN)_6](PF_6)_2$ (2.83 g, 4.89 mmol) was added in that order. This procedure avoids the presence of species other than what are needed for **3**. The mixture was left stirring for 1 h and the solvent was removed under vacuum, giving a dark blue precipitate. Yield 4.22 g (4.02 mmol, 82%). The dark blue precipitate could be recrystallized from an acetonitrile/diethyl ether mixture. Elemental analysis theoretical: C, 42.39%; H, 3.75%; N, 8.02%; found : C, 41.20%; H 3.85%; N 8.21%. Metal ratio, Fe:Mn 1:1.2.

All metal complexes were stored in an inert atmosphere (glove box) until use.

2.2. Instrumentation

^{1}H NMR spectra was recorded with JEOL-400 MHz spectrometer at 293 K. Chemical shift are given in ppm and referenced internally to the residual solvent signal. *UV–Vis spectroscopy* was performed on a Varian Cary 50. *EPR spectroscopy* was performed using a Bruker ESR-500 spectrometer equipped with a dual mode DM9807 resonator an ESR900 cryostat and an Oxfords ITC503 temperature controller. EPR conditions: Microwave frequency, 9.59 GHz, modulation frequency 100 kHz, modulation amplitude 20 G. *HR-MS* was performed by Organisch-Chemisches Institut der Westfälischen Willhelms-Universität, Munster Germany. *IR-spectroscopy* was measured on a PerkinElmer Spectrum One, FT-IR spectrometer with a PerkinElmer universal ATR sampling accessory. *Elemental analysis* was performed by Analytische Laboratorien Gmbh, Lindlar, Germany. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was measured on a PerkinElmer Avio 200ICP-OES.

2.2.1. Electrochemistry

The measurements where done using an AUTOLAB potentiostat with a three-electrode setup in a glove box, controlled using the GPES electrochemical interface (eco chemie). The working electrode was a glassy carbon disc (3 mm diameter), the counter electrode was a glassy carbon rod and the reference electrode was an Ag wire in a solution of 10 mmol of $AgNO_3$ in CH_3CN. The working electrode was routinely polished inside the glove box with alumina (porosity 0.05 μm) in CH_3CN slurry on a felt surface before the use. The scan was typically −0.8 V to 1.2 V at 100 mV/s with 1 mM complexes in dry acetonitrile and 0.1 M $TBAClO_4$ as electrolyte unless otherwise indicated.

2.2.2. Mössbauer spectroscopy.

Mössbauer spectra were recorded with a conventional Mössbauer spectrometer operated in the constant acceleration mode in conjunction with a 512-channel analyzer (WissEl GmbH). The isomer shift is given with reference to α-iron at room temperature. A continuous flow cryostat (Oxford Instruments) was used to cool the samples to 77 K. Spectral analysis was performed with the public domain program Vinda running on an Excel 2003® platform using Lorentzian line shapes with the line width Γ [37].

2.3. Titration of 2 with Fe^{2+}

Complex **2** (1 mM) was dissolved in 5 ml of acetonitrile (with 0.1 M $TBAClO_4$) and placed in an electrochemical cell. 150 μL of a solution of $[Fe(CH_3CN)_6](PF_6)_2$ (15 mM) in acetonitrile was added to the cell and the CV collected. The working electrode was polished, and the procedure was repeated until 2 equiv. of $[Fe(CH_3CN)_6](PF_6)_2$ had been added.

2.4. Oxygen activation

In the glove box, 0.15 mM solutions of **1**–**3** were prepared in dry and oxygen free acetonitrile. 3.5 ml of each solution was put in gas-tight cuvettes and removed from the glove box. Using a gas-tight syringe oxygen gas was injected into the cuvettes (3–250 μL) and a UV–Vis spectrum was collected. The procedure was repeated until the total volume of oxygen injected was 1 ml (5 ml for **3**).

3. Results and discussion

3.1. Synthesis

The ligand HBPMP was synthesized following a literature procedure [34]. Crystalline 2,6-bis-(hydroxymethyl)-4-methylphenol was chlorinated with HCl in the 2,6 positions and subsequently di-(2-picolyl) amine (DPA) was added to complete the ligand framework.

The synthesis of the homobimetallic and heterobimetallic complexes followed a modified version of the procedure published by Borovik et al. [35]. For the synthesis of **1** the ligand was dissolved in acetonitrile and two equivalents of $Fe(CH_3COO)_2$ were added. Ammonium hexafluorophosphate was added and CH_2Cl_2 was used to precipitate **1** with PF_6^- as the counter ion. The same procedure was used to synthesize **2** using $Mn(CH_3COO)_2$ as the metal source. The syntheses were performed under inert conditions and the products stored in a glove-box in an oxygen- and water-free environment.

For the synthesis of the heterobimetallic complex **3**, the ligand was dissolved in dry acetonitrile and one equivalent of $Mn(CH_3COO)_2$ was added as a source of manganese and acetate bridges followed by the addition of $Fe(CH_3CN)_6(PF_6)_2$ as the source of iron and counter ion [32]. The order in which the metal salts are added is crucial since manganese is more labile in the ligand. This is shown in 3.6 by exchange studies with **1** and **2** separately, and is also expected from the Irvin-William series [38]. Therefore, the addition of manganese before iron minimises the formation of undesired homobimetallic side products. Surprisingly elemental analysis shows the presence of two PF_6^- counter ions in the product. Since the complex was prepared under anaerobic conditions, and since the Mössbauer spectroscopy described in 3.4 shows the presence of 80% Fe(II) we do not believe that the complex forms in a Fe(III)-Mn(II) oxidation state, even if the mass spectrometry in 3.2 shows the presence of the oxidized complex. Also the formation of the Fe(II)-Mn(III) oxidation state is very unlikely, and the presence of a Mn(II) signal in the EPR spectrum shown in 3.3 is not supporting this assignment. Instead we propose that the complex forms in the Fe(II)-Mn(II) oxidation state and that is it isolated together with and extra PF_6^- anion and presumably an extra proton as cation.

3.2. Mass spectrometry

The HR-MS of **3** showed a peak at m/z = 379.08 with z = 2 corresponding to the $[(BPMP)FeMn(OAc)_2]^{2+}$ ion (Fig. S1) presumably in a Fe(III)-Mn(II) oxidation state. The isotope pattern indicates the presence of an impurity (< 20%) of the Fe-Fe complex (**1**). No peak at m/z = 758 corresponding to the $[(BPMP)FeMn(OAc)_2]^+$ ion could be observed, likely due to oxidation of the complex when exposed to oxygen. A similar behaviour was observed for **1** where only the Fe(III)-Fe(II) form is observed (Fig. S2), but not for **2** where the Mn(II)-Mn(II) form is the only one detected (Fig. S3).

3.3. IR spectroscopy

The mid-range IR spectra of **1**, **2**, and **3** in solid state are very similar (Fig. S4). Three bands originating from coordinated acetate and pyridine ligands are present in all three spectra, at ~1420, ~1480 and ~1590 cm^{-1} in **1** and **2**, and at ~1435, ~1480 and ~1605 cm^{-1} in **3** [39]. Since these bands are present in all three complexes we can conclude that **3** must have a structure very similar to that of **1** and **2**.

3.4. EPR spectroscopy

The EPR spectrum of **3** at 5 K in acetonitrile shows a feature at g = 2.12 (Fig. 1) from a Mn(II) species. No signals corresponding to the

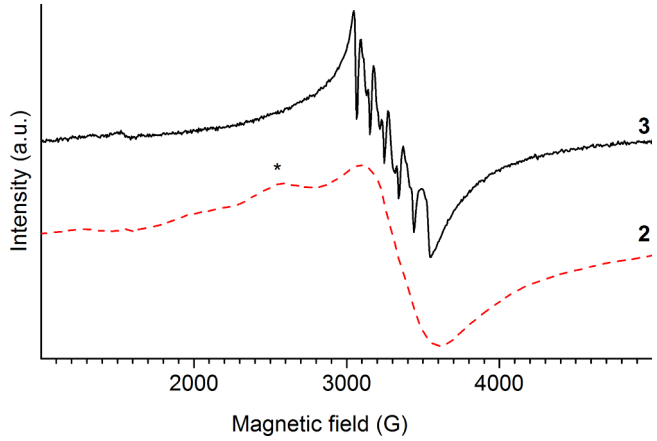


Fig. 1. EPR spectra of **3** (top) and **2** (bottom, dashed) in CH_3CN at 5 K. The characteristic peak from **2** at $g \sim 2.6$ is marked with an asterisk (*). Microwave power, 20 μW.

Mn-Mn species **2**, with an EPR-signal at $g \sim 2.6$ could be observed [36,40].

3.5. Mössbauer spectroscopy

Mössbauer spectroscopy was used to investigate the two iron containing complexes **1** and **3**. The Mössbauer spectrum of **3**, measured at 78 K shows the presence of two different iron components (Fig. 2a). The most abundant species (80%) has an isomer shift δ of 1.13 mms^{-1} and a quadrupole splitting ΔE_Q of 3.16 mms^{-1} (Table S1). These parameters are typical for Fe(II)-high spin (HS) species [41]. The second component with 20% of abundance has $\delta = 0.43$ mms^{-1} and $\Delta E_Q = 0.45$ mms^{-1}, typical of Fe(III)-HS species. The presence of Fe(III) again demonstrates that **3** is easily oxidized. Also in the Mössbauer spectrum of **1** two iron species with different oxidation states could be observed (Fig. 2b), ~62% Fe(II)-HS ($\delta = 1.19$ mms^{-1} and $\Delta E_Q = 2.80$ mms^{-1}) and ~ 38% Fe(III)-HS ($\delta = 0.46$ mms^{-1} and $\Delta E_Q = 0.70$ mms^{-1}) (Table S1). The parameters for Fe(II)-HS and Fe(III)-HS are in good agreement with the previously reported $[(BPMP)Fe(III)Fe(II)(OAc)_2](BF_4)_2{\cdot}H_2O$ (Fe(II): $\delta = 1.16$ mms^{-1}, $\Delta E_Q = 2.42$ mms^{-1}; Fe(III): $\delta = 0.47$ mms^{-1}, $\Delta E_Q = 0.51$ mms^{-1}).[42] The parameters for the Fe(II) and Fe(III) components are not identical for **1** and **3** but similar enough that the Fe(III)-Fe(II) impurity of **1** observed in the HR-MS might be indeed present as Fe(III)-HS in the Mössbauer spectrum of **3**.

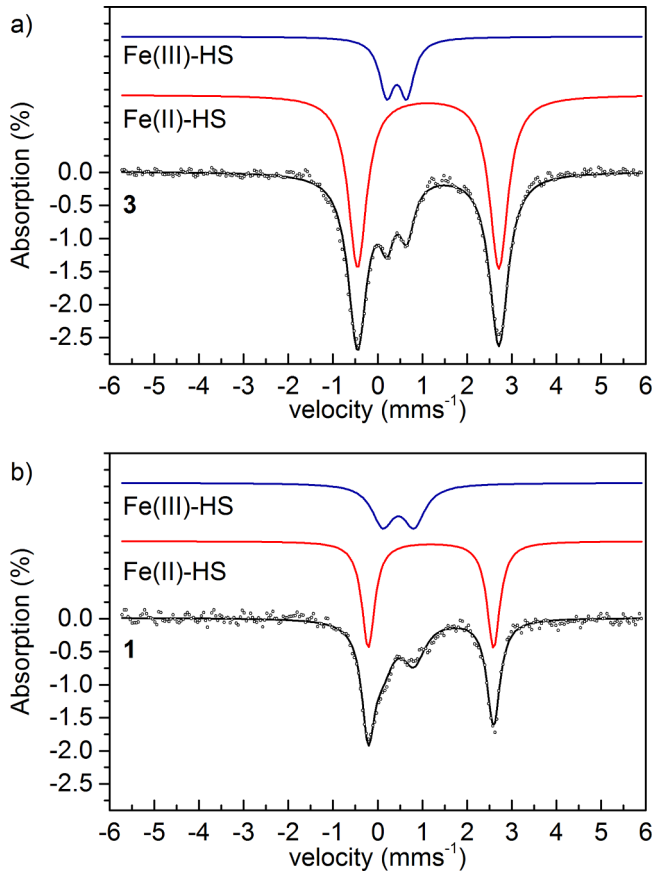


Fig. 2. Mössbauer spectra of a) **3** and b) **1** taken at 78 K. The black solid line is the sum of the subspectra originating from high-spin Fe(II) (middle) and high-spin Fe(III) (top). The parameters used for analysis are displayed in Table S1.

3.6. Electrochemistry

The electrochemistry of **1** and **2** have previously been studied [42–43] and both complexes show two redox waves in the cyclic voltammogram (CV) measured in CH_3CN, corresponding to the M(III)-M(II)/M(II)-M(II) (E_{1a} and E_{2a}) and M(III)-M(III)/M(III)-M(II) (E_{1b} and E_{2b}) couples (Fig. 3). The CV of **3** is more complex than those of **1** and **2** (Fig. 3, blue line). There are two redox events at −0.44 V and +0.28 V (vs Fc^+/Fc) that are at a similar potential to E_{1a} and E_{1b} in **1**, but the the anodic wave corresponding to E_{1a} in **1** is not present in **3**. Instead there is an electrochemically irreversible oxidation wave at 0.08 V that is presumably the anodic counterpart of the reduction at −0.44 V. The substantial irreversibility of the Fe(III)-Mn(II)/Fe(II)-Mn(II) couple

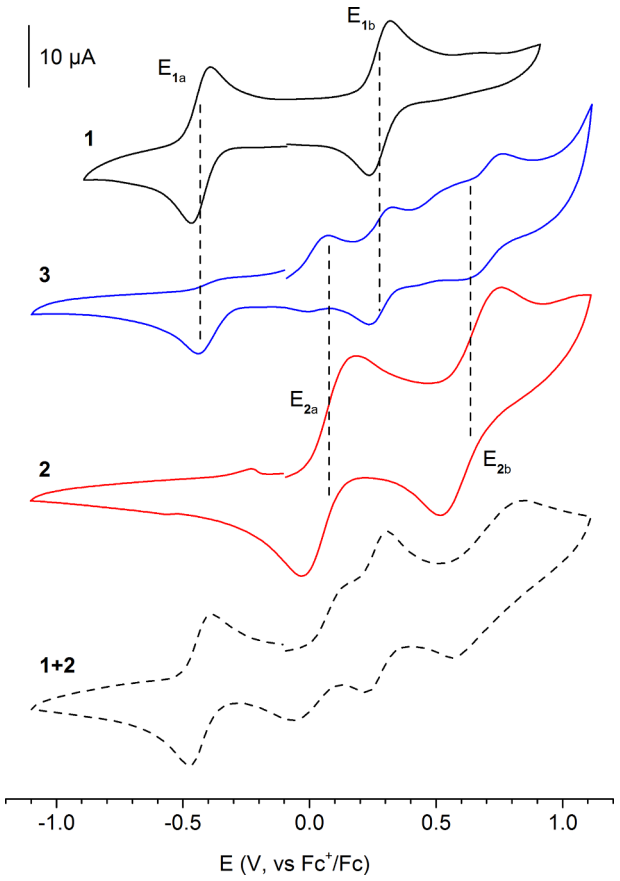


Fig. 3. Cyclic voltammetry of (top to bottom) **1**, **3**, **2**, and a 1:1 mixture of **1** and **2** (dashed) in CH_3CN with 0.1 M $TBA(ClO_4)$ (scan rate 100 mV/s). The half wave potentials for Fe(III)-Fe(II)/Fe(II)-Fe(II) (E_{1a}) and Fe(III)-Fe(III)/Fe(III)-Fe(II) (E_{1b}) from **1** and Mn(III)-Mn(II)/Mn(II)-Mn(II) (E_{2a}) and Mn(III)-Mn(III)/Mn(III)-Mn(II) (E_{2b}) from **2** are indicated by dashed vertical lines.

(ΔE_p = 467 mV) in **3** compared to the reversible Fe(III)-Fe(II)/Fe(II)-Fe(II) couple (ΔE_p = 76 mV) in **1** can also be explained by the hump at −0.007 V on the cathodic part of the CV of **3**, which could be due to a chemical change in the complex before it gets reduced to the Fe(II)-Mn(II) form.

A quasi-reversible redox wave is found at 0.69 V (ΔE_p = 138 mV), this is in turn similar to the E_{2b} (0.64 V) redox wave (ΔE_p = 240 mV) in **2**. A comparison with the CV of a 1:1 mixture of **1** and **2** (Fig. 3, dashed line) that shows (quasi-)reversible redox waves at −0.4 V (ΔE_p = 85 mV), corresponding to E_{1a}, and 0.1 V (ΔE_p = 193 mV), corresponding to E_{2b}, confirms that the CV of **3** is not contaminated to a large extent by the presence of **1** or **2** in the solution.

The CV of **3** indicates that the Fe-Mn complex is somewhat more fluxional than the homobimetallic complexes **1** and **2** and undergoes chemical transformation(s) (e. g. an alteration in the binding mode of the bridging acetates.

3.7. Metal exchange

To investigate the relative coordinative stability of Fe and Mn to the HBPMP ligand, titrations of the homobimetallic complexes **1** and **2** with Mn and Fe salts respectively were performed under inert atmosphere in a glove box and followed by cyclic voltammetry. When **1** was titrated with $Mn(ClO_4)_2$ no changes were observed in the CV for the first equivalent of Mn^{2+} (Fig. S5), indicating that the two iron centers stay coordinated to the ligand, and that the complex is stable towards metal exchange. With higher amounts of Mn^{2+} the intensity of the peak currents of the redox waves in the CV decreased drastically but no new redox waves appeared. In contrast, when **2** was titrated with up to 2 equiv. of $Fe(CH_3CN)_6(PF_6)_2$ drastic changes were observed in the CV (Fig. 4). The peaks associated with the Mn(III)-Mn(II)/Mn(II)-Mn(II) and Mn(III)-Mn(III)/Mn(III)-Mn(II) redox events gradually diminished with the addition of Fe^{2+}. The appearance of a cathodic feature at −0.41 V that increases with increasing iron concentration indicates that a new species is formed. In fact, the CVs of **2**, after addition of 1 equiv. of Fe^{2+}, and **3** show large similarities, noticeably the irreversible reduction at ~ −0.4 V suggesting that a Fe-Mn complex has formed during the titration of **2** with Fe^{2+}.

3.8. Oxygen reactivity

Iron and manganese complexes are known to activate molecular oxygen in a large number of natural and synthetic systems [44]. In mononuclear non-heme synthetic systems the reaction of Fe(II) with O_2 have been proposed to form a Fe(III)-super oxide species as a first intermediate. The reaction can continue through the formation of mononuclear Fe(III)-peroxo or Fe(IV)-oxo species, or dimeric Fe(III)-μ(peroxo)-Fe(III) species. Similar species have also been proposed and observed for the activation of O_2 by manganese complexes. The reaction of O_2 with diiron complexes in the Fe(II)-Fe(II) oxidation state can lead to the formation of Fe(III)-Fe(II) complexes [45–46], but also Fe(III)-μ(peroxo)-Fe(III) species have been observed at low temperatures [47].

The high-valent iron or manganese species that can oxidise substrates through hydroxylation or oxygen atom transfer to the product. One such reaction is the oxidation of benzylic protons on a substrate or on the ligand of the metal complex itself. Recently a manganese complex was reported to oxidise its own ligand in this fashion to give an alkoxide and a ketone [48].

The reactivity of **1–3** with molecular oxygen was studied by UV–Vis spectroscopy. Solutions of the complexes (0.15 mM) were prepared in gas tight cuvettes in the glove box and moved to the spectrometer where oxygen gas was injected with a gas-tight syringe. Interestingly, the three complexes react differently with O_2. The Mn(II)-Mn(II) complex **2** does not react with O_2 under the conditions used (Fig. S6). The Fe-Fe complex **1** reacts with the injected O_2 and the peak at 435 nm decreases in intensity over 1 h (Fig. 5). At the same time a band around 300 nm and a broad band around 600 nm increases. The resulting spectrum is very similar to closely related Fe(III)-Fe(II) complexes using the HBPMP ligand and different bridging carboxylates prepared in presence of O_2 [35,42]. The band around 600 nm has been assigned to charge transfer from filled phenolato pπ orbitals to vacant half-filled d (π*) orbitals of Fe(III) [42]. We therefore assign the species formed in the reaction of **1** and O_2 to be $[(BPMP)Fe(III)Fe(II)(OAc)_2]^{2+}$ The two isosbestic points in the UV–vis spectra, show that **1** cleanly converts to the Fe(III)-Fe(II) species.

Complex **3** reacts easily with the injected O_2, the absorption over the whole visible range increases and a band at 570 nm becomes much more pronounced (Fig. 6). The reaction of O_2 with **3** is faster than with **1** and is essentially completed already at the first injection of O_2. Based on the similarity with the UV–vis spectrum of **1** oxidized with O_2 in Fig. 5 we assign the product to be the one-electron oxidized **3**, $[(BPMP)Fe(III)Mn(II)(OAc)_2]^{2+}$

We also used mass spectrometry to investigate the reaction with **1** or **3** with O_2 but no new species other than $[(BPMP)FeFe(OAc)_2]^{2+}$ and $[(BPMP)FeMn(OAc)_2]^{2+}$ could be observed, indicating that ligand oxidation did not occur.

The observed reactivity with oxygen shows that the heterobimetallic complex reacts differently than a linear combination of the

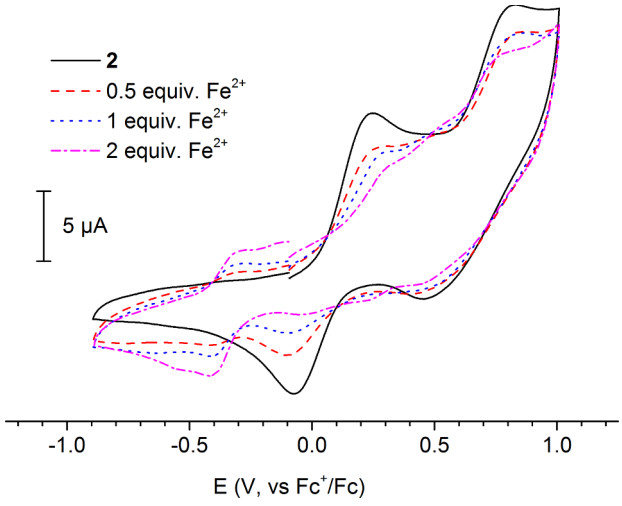


Fig. 4. Cyclic voltammetry in CH_3CN (0.1 M $TBA(ClO_4)$ as electrolyte) following the titration of **2** (1 mM) with $Fe(CH_3CN)_6(PF_6)_2$.

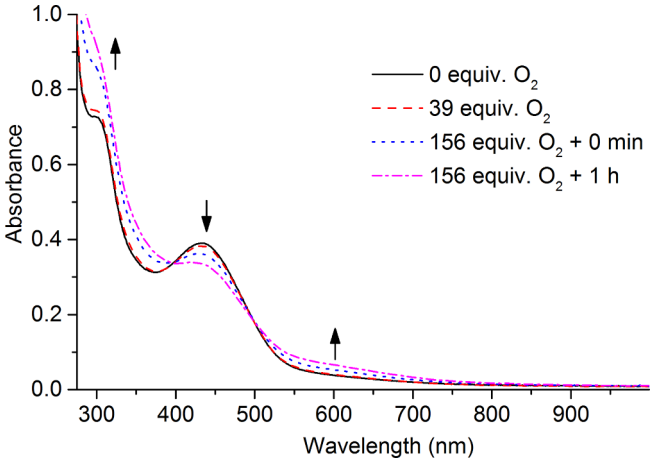


Fig. 5. Complex **1** (0.15 mM) in CH_3CN (solid line) and with added equivalents of $O_{2(g)}$ (coloured traces). The solubility of O_2 in pure acetonitrile at 20 °C is 2.4–2.6 mM [49–50].

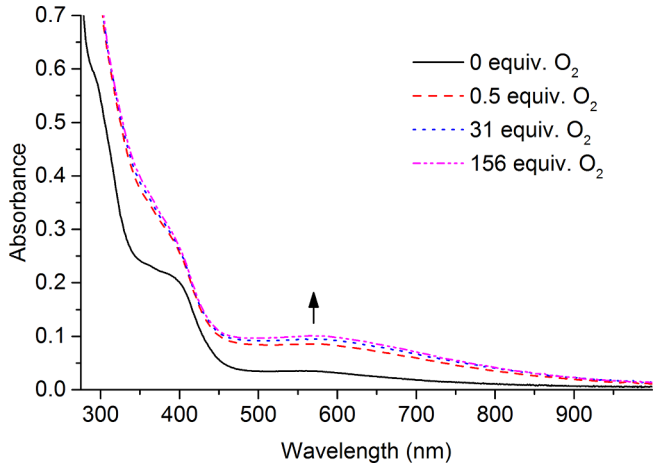


Fig. 6. Complex **3** (0.15 mM) in CH_3CN (solid line) and with added equivalents of $O_{2(g)}$ (coloured traces). The solubility of O_2 in pure acetonitrile at 20 °C is 2.4–2.6 mM [49–50].

two homobimetallic complexes. The presence of two different metals gives rise to higher reactivity than those of the corresponding homobimetallic complexes.

4. Conclusions

We have presented a study of three different dinuclear complexes containing iron and/or manganese. In terms of stability of the complexes towards metal scrambling in the M(II)-M(II) oxidation state, the Fe-Fe complex **1** is the most stable and is not altered by the presence of manganese ions, while the Mn-Mn complex **2** is converted to the Fe-Mn complex (**3**) by the addition of one equivalent of Fe(II) salts. This places the heterometallic complex as the intermediate in terms of stability. In cyclic voltammetry the Fe-Mn complex **3** shows some similarities to both the Fe-Fe and Mn-Mn complexes but has a more complex electrochemical behavior than the homobimetallic reference complexes. The first oxidation wave of **3** is not electrochemically reversible and is probably accompanied with a chemical change in the coordination of the bridging acetate ligands, something that is not observed for the homobimetallic complexes. Also, the reactivity towards oxygen, shows that the heterometallic complex gains new reactivity compared to the homometallic complexes. The Mn-Mn complex is virtually unreactive towards oxygen, while both the Fe-Fe complex and the Fe-Mn complex react with oxygen, with the heterobimetallic Fe-Me complex doing so much more rapidly. Taken together, this study shows that the exchange of one metal ion in homobimetallic complexes in otherwise identical ligand environments can alter the chemical reactivity of the complexes. Further studies of symmetric and asymmetric homo- and heterobimetallic complexes are underway in our lab and will hopefully contribute further to the understanding of the chemistry of heterobimetallic iron-manganese complexes and the necessity why nature chooses such iron-manganese containing co-factors in certain enzymes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2019.03.029.

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