Cytochrome c Oxidase Models. A μ-Imidazolato Complex from Copper(II) and Tetraphenylporphyrinatomanganese(II) with the Magnetic and E.S.R. Signature of the Cytochrome c Oxidase Active Site

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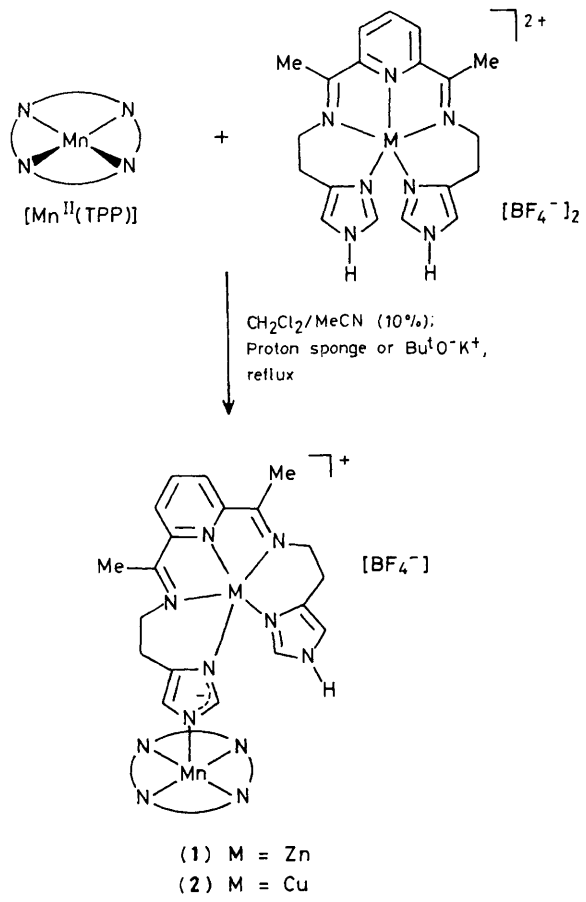
A μ-imidazolato heterobimetallic compound derived from an imidazolate-bearing Cu^{II} complex (S = 1/2) and [Mn^{II}(TPP)] (S = 5/2) possesses the magnetic and e.s.r.-silent signature of the cytochrome c oxidase active site in its resting state; as such, the [Mn(imidazolato)Cu]$^+$ centre appears to mimic electronically the binuclear [Cu_u^{2+} (S = 1/2)/Cyt. a_3^{3+} (S = 5/2)] active site of the enzyme.

Cytochrome c oxidase, as the terminal enzyme in the electron-transfer chain of aerobes, catalyses the 4e$^-$ reduction of dioxygen to water ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ + energy), with the energy being stored in the ADP–ATP cycle.[1] The enzyme contains two haems and two copper sites, with the active site being binuclear in one haem (Cyt. a_3) and one copper (Cu_u). Much recent interest has focused on the electronic and structural properties of this heterobimetallic site, especially with the enzyme in its fully-oxidized or resting state. In this state, the binuclear [Cu_u^{2+}/Cyt. a_3^{3+}] site exhibits magnetic and e.s.r.-silent properties commensurate with an S = 2 ground state, arising presumably from strong *anti*ferromagnetic coupling ($-J \geqslant 200$ cm^{-1}) between S = 1/2 (Cu_u^{2+}) and S = 5/2 (Cyt. a_3^{3+}).[2,3] A Mössbauer spectroscopic study of ^{57}Fe-enriched oxidase from a bacterial source (*Thermus thermophilis*)[4] has challenged the S = 2 assignment of earlier magnetic susceptibility measurements, but we have recently reaffirmed the earlier susceptibility results for three different preparations of bovine resting oxidase.[5]

Biochemically reasonable bridges, *i.e.* [Cu_u^{2+}–(B)–Cyt.a_3^{3+}], such as B = imidazolato (imid$^-$) from histidine,[6] oxo from O_2, H_2O, or tyrosine,[7,8] and mercapto from cysteine[9,10] have all been proposed as potential mediators of the strong *anti*ferromagnetic coupling between Cu_u^{2+} and Cyt.a_3^{3+}, and several such [Cu^{2+}–(B)–Fe^{3+}] model compounds have been reported by others[11—20] and ourselves.[21—24] In general, these model compounds have shown $-J \ll 200$ cm^{-1} for B = imid$^-$, and have thus argued in favour of a single atom bridge instead of a multi-atom case like imidazolate. In this communication we report a B = imid$^-$ model compound derived from S = 5/2 tetraphenylporphyrinatomanganese(II) [Mn^{II}(TPP)] (a spin mimic of Cyt.a_3^{3+}) and an S = 1/2 imidazolate-bearing Cu^{II} complex [(**2**) in Scheme 1] which exactly replicates the e.s.r.-silent and magnetic properties of the resting oxidase active site.

Scheme 1 illustrates the synthesis of the two μ-imidazolato manganese porphyrin complexes prepared in the work. In general, the compounds were synthesized by reacting 1 mol equiv. of [Mn^{II}(TPP)], 1.2 mol equiv. of [M^{II}(imidH)$_2$-DAP][BF_4]$_2$ with M = Cu or Zn [the pentadentate ligand is derived from the Schiff base condensation of 2,6-diacetylpyridine (DAP) and two histamine molecules, abbreviated here as (imidH)], and 1 mol equiv. of a base in a CH_2Cl_2/MeCN solvent mixture. Owing to the O_2-sensitive nature of Mn^{II} porphyrin compounds, the syntheses and subsequent handling procedures were performed anaerobically. The reaction in Scheme 1 did not occur in the absence of base, and the related Zn and Cu precursor compounds,



Scheme 1. Synthesis and proposed structure of the μ-imidazolato manganese tetraphenylporphyrin compounds.

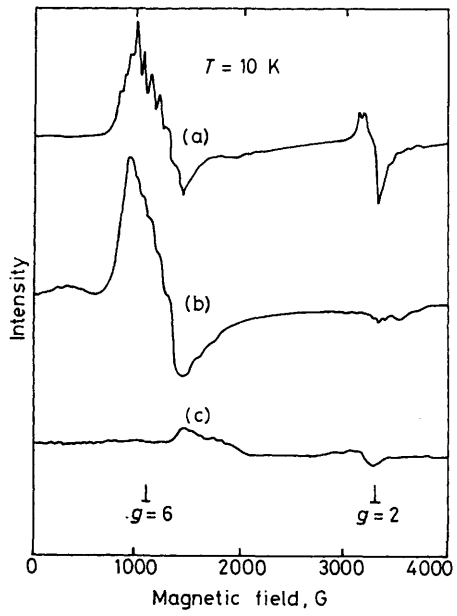


Figure 1. E.s.r. spectra at 10 K of the manganese tetraphenylporphyrin compounds (10^{-4} M) in CH_2Cl_2 glasses: (a) [Mn^{II}(TPP)], (b) compound (**1**), (c) compound (**2**).

[M^{II}(imidH)(py)DAP][BF$_4$]$_2$ (with one pyridine group replacing one imidazole moiety of the pentadentate ligand), also formed μ-imidazolato compounds in the presence, but not the absence, of base. Furthermore, the use of [M^{II}(py)$_2$-DAP][BF$_4$]$_2$ (with two terminal pyridine groups in the ligand) in the reaction produced no binuclear products. Compounds (**1**) and (**2**) were isolated and purified by removing solvent *in vacuo* and recrystallizing the resulting solids from CH_2Cl_2/heptane. Both compounds possessed satisfactory elemental analyses and solution state conductivities in CH_2Cl_2 (40 $\mu\Omega^{-1}$ cm^{-1}) characteristic of uni–univalent electrolyte behaviour as required by their formulation in Scheme 1. Furthermore, cyclic voltammetry,† electronic spectroscopy,‡ and e.s.r. (*vide infra*) studies have shown (**1**) and (**2**) to be discrete, solution-stable species in CH_2Cl_2. This finding contrasts with our previous results for a related [L(TPP)Fe^{III}(imid)Cu^{II}]$^+$ system which exhibited complex behaviour in solution and could therefore only be studied in the solid state.[21] Apparently the greater propensity of [Mn^{II}(TPP)] toward pentaco-ordination aids in the solution stability of (**1**) and (**2**); likewise, a five-co-ordinate Co^{II} compound with a [(TPP)Co^{II}(imid)Ni^{II}] core has also been reported as solution stable in toluene and reactive toward O_2 at the metal centre.[19]

Compound (**1**) exhibits $\mu_{eff.}$ = 5.84 μ_B at room temperature, a value which gradually decreases to 5.09 μ_B at 20 K. This is slightly lower than that expected (5.9 μ_B) for S = 5/2 Mn^{II}, but it is within the range reported for other S = 5/2 Mn^{II} compounds.[25] Furthermore, the χ_M' *vs.* T^{-1} plot for (**1**) [and (**2**)] is linear between *ca.* 20 and 300 K, indicating Curie–Weiss behaviour. Compound (**2**) contains an additional unpaired electron from the Cu^{II} centre; thus, the expected $\mu_{eff.}$ value for (**2**), in the absence of interaction between S = 5/2 Mn^{II} and S = 1/2 Cu^{II}, is *ca.* 6.3—6.5 μ_B. Somewhat unexpectedly, the experimental magnetic moment for (**2**) at room temperature is 5.11 μ_B, a value which gradually decreases to 4.17 μ_B at 20 K. The depression in the magnetic moment for (**2**) relative to (**1**), through the entire temperature range, could arise from at least four possibilities. The first possibility supposes *auto*reduction of Cu^{II} to Cu^{I} by Mn^{II} to give a [Mn^{III}(imid)Cu^{I}]$^+$ centre in (**2**). The half-wave potential for the Mn^{II}/Mn^{III} couple in [Mn^{II}(TPP)] is −0.27 V (s.c.e./CH_2Cl_2) which is near that of the Cu^{I}/Cu^{II} couple of [Cu^{II}(*imid*H)$_2$DAP]$^{2+}$ of −0.32 V (s.c.e./MeCN) (s.c.e. = saturated calomel electrode).† Thus, *auto*reduction might occur upon formation of a μ-imidazolato compound. If (**2**) possessed a [Mn^{III}(imid)Cu^{I}]$^+$ centre, the expected $\mu_{eff.}$ value should be *ca.* 5.0 μ_B for an S = 2 Mn^{III} species, and this is near the value observed. However, the e.s.r. studies below have definitively established the presence of S = 5/2 Mn^{II} in (**1**), and electronic spectroscopy data‡ indicate comparable electronic structures for the Mn centres in (**1**) and (**2**). A second option is the presence of an S = 3/2 Mn^{II} centre in (**2**) but not in (**1**). This situation leads to a value

† For (**1**), $E_{1/2}$(Mn^{II} ⇌ Mn^{III}) = −0.24 V (s.c.e./CH_2Cl_2) whereas $E_{1/2}$ = −0.27 V for [Mn^{II}(TPP)]. For (**2**), $E_{1/2}$(Mn^{II} ⇌ Mn^{III}) = −0.24 V and $E_{1/2}$(Cu^{I} ⇌ Cu^{II}) = −0.37 V (s.c.e./CH_2Cl_2) whereas $E_{1/2}$ = −0.32 V for [Cu^{II}(imidH)$_2$DAP]$^{2+}$ (s.c.e./MeCN).

‡ For (**1**), $\lambda_{max.}$ ($\varepsilon \times 10^3$) in CH_2Cl_2: 616 nm (10.3 dm^3 mol^{-1} cm^{-1}), 583 (9.0), 532 (7.6), 458 (62.0), 403 (38.1), 347 (33.1). For (**2**): 618 nm (10.0 dm^3 mol^{-1} cm^{-1}), 579 (8.8), 530 (5.7), 460 (85.0), 397 (60.2), 380 (66.4), 348 (62.1). The spectral similarities for (**1**) and (**2**) indicate comparable electronic structures for the Mn centres. For [Mn^{II}-(TPP)]: 602 nm (10.0 dm^3 mol^{-1} cm^{-1}), 562 (11.2), 520 (6.0), 434 (250.1), 359 (55).

of $\mu_{eff.}$ ~5.0 μ_B for (**2**), assuming no magnetic interaction between Mn^{II} ($S = 3/2$) and Cu^{II} ($S = 1/2$) and values of 4.5 μ_B for intermediate-spin Mn^{II} and 2.0 μ_B for Cu^{II}. This possibility cannot be discounted by magnetochemistry alone, but in conjunction with the e.s.r. data below, it appears unlikely since uncoupled $S = 3/2$ Mn^{II} and $S = 1/2$ Cu^{II} in (**2**) should both exhibit e.s.r. spectra. A third option also invokes an intermediate-spin state ($S = 3/2$) for the Mn^{II} centre in (**2**), but assumes a strong *ferro*magnetic interaction ($+J \geq 200$ cm^{-1}) between Mn^{II} ($S = 3/2$) and Cu^{II} ($S = 1/2$) to give a resultant $S = 2$ spin state for the binuclear $[Mn^{II}(imid)Cu^{II}]^+$ centre; this situation would lead also to a value of $\mu_{eff.}$ ~5.0 μ_B. However, no other μ-imidazolato compound to date has displayed *ferro*magnetism, so this possibility is also deemed unlikely. The final option postulates strong *anti*ferromagnetic coupling ($-J \geq 200$ cm^{-1}) between Mn^{II} ($S = 5/2$) and Cu^{II} ($S = 1/2$) to give an $S = 2$ spin-coupled ground state in (**2**) with $\mu_{eff.}$ ~5.0 μ_B. Like the *ferro*magnetic option, an *anti*ferromagnetic option invokes strong magnetic coupling across imidazolate. In this case there is ample evidence, from model compound studies, for *anti*ferromagnetism in μ-imidazolato systems, albeit with $-J \leq 100$ cm^{-1}.[24] An interpretation involving *anti*ferromagnetism is entirely consistent with the present e.s.r. data, especially since (**1**) has been shown by its e.s.r. spectrum to contain $S = 5/2$ Mn^{II} (*vide infra*).

Compounds (**1**) and (**2**) have been examined by e.s.r. spectroscopy in solid solution (CH_2Cl_2 glasses) at 10 K. E.s.r. spectra for (**1**), (**2**), and $[Mn^{II}(TPP)]$ are shown in Figure 1. The spectrum of $[Mn^{II}(TPP)]$ is anisotropic ($g_1 = 6.0$ and $g_2 = 2.04$) with a six-line hyperfine pattern due to ^{55}Mn ($I = 5/2$). Compound (**1**) exhibits a similar e.s.r. spectrum to that of $[Mn^{II}(TPP)]$ with $g_1 = 5.9$ and $g_2 = 2.04$. These g values are typical of $S = 5/2$ systems.[26,27] This result confirms that the Mn porphyrin centre in (**1**) contains $S = 5/2$ Mn^{II}.

Compound (**2**) appears to possess unusual magnetochemical properties when compared to (**1**). Specifically, its $\mu_{eff.}$ value at all temperatures is considerably below that expected for a binuclear compound containing isolated $S = 5/2$ Mn^{II} and $S = 1/2$ Cu^{II} centres. The e.s.r. spectrum of (**2**) in Figure 1 consists of very weak resonances at $g \sim 4$ (Mn) and $g \sim 2$ (Mn/Cu). The $g \sim 4$ value for Mn is considerably different from those of a typical axial $S = 5/2$ Mn^{II} centre. Integration of the $g = 4$ and $g = 2$ signals against e.s.r. signals for known concentrations of $[Mn^{II}(TPP)]$ and $[Cu^{II}(imidH)_2DAP]^{2+}$ in frozen CH_2Cl_2 or MeCN glasses at 10 K indicate that the observed signals in Figure 1c account for <1% of Mn and Cu present. Thus, it can be concluded that (**2**) is effectively e.s.r. silent, with the observed weak signals probably being due to small amounts of Mn and Cu impurities. Attempts to obtain a solid-state e.s.r. spectrum of (**2**) [1% in $(NH_4)_2SO_4$] under the same experimental conditions were also unsuccessful.

Assuming that compounds (**1**) and (**2**) both contain $S = 5/2$ Mn^{II} [as conclusively shown in Figure 1 for (**1**)], the e.s.r.-silent behaviour of (**2**) seems best rationalized in terms of the last possibility, with a fully-coupled $S = 2$ ground state arising *via anti*ferromagnetism. In this context, (**2**) contains a $[Mn^{II}(imid)Cu^{II}]^+$ core with the same magnetic and e.s.r. signature as the $[Cyt.a_3^{3+}$ ($S = 5/2$)/Cu_u^{2+} ($S = 1/2$)] active site of resting oxidase. This interpretation echos the conclusions of Dessens *et al.*,[21] and Desideri *et al.*,[28] in asserting that imidazolate bridges can, in some circumstances, foster strong *anti*ferromagnetic exchange interactions as large as that possibly operating in oxidase. This conclusion is contrary to most other μ-imidazolato model compound results (including some of our own)[24,§] which have indicated an upper limit of *ca.* $-J \sim 100$ cm^{-1} for imidazolate bridges. Clearly, questions still remain to be answered about magnetic interactions across imidazolate bridges. While answers to these questions may bear on the cytochrome c oxidase problem, they may also be moot in view of recent EXAFS results[9,10,29] suggesting an [Fe···Cu] separation of only 3.0—3.8 Å, whereas $imid^-$ requires at least 5 Å.

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§ For the Co^{II} porphyrin analogue of (**2**), $-J \sim 0$ cm^{-1} (ref. 5).

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