Inorganica Chimica Acta, *179* (1991) 7–10

Inorganica Chimica Acta
LETTER

Reinvestigation of a μ-imidazolato [Mn(III)–Cu(II)] metalloporphyrin complex with novel ESR characteristics

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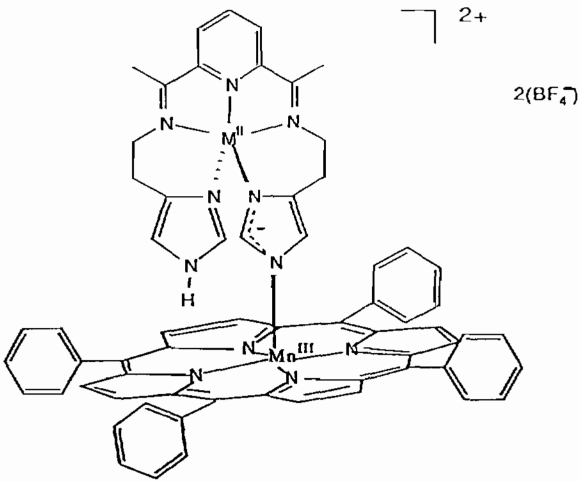
(Received October 8, 1990; revised December 6, 1990)

Cytochrome *c* oxidase contains two iron hemes (*a* and a_3) and at least two copper atoms, Cu_D and Cu_U (D and U = ESR detectable and undetectable). Heme *a* and Cu_D are thought to be about 20 Å apart and some distance from the closely-coupled binuclear active site, a [heme a_3/Cu_U] unit [1]. In the oxidized resting state (2 Fe^{III}, 2 Cu^{II}), the relative ESR inactivity of the active site [2] apparently results from a strong antiferromagnetic coupling interaction ($-J \geqslant 200$ cm^{-1}), through some bridging ligand between the high-spin heme a_3 ($S = 5/2$) and Cu_U ($S = 1/2$) metal sites, to give an integer spin $S = 2$ ground state [2, 3]. In a comprehensive model, first suggested by Palmer *et al.*, an imidazolate anion (imid$^-$) from a histidine residue was proposed as a reasonable bridge for fostering strong antiferromagnetic coupling at the active site [4]. There is no question about the bridging capability of the imidazolate anion [5–7], but there is doubt about the ability of the anion to mediate a 'strong' antiferromagnetic exchange interaction between metal centers. Imidazolate-bridged complexes of copper(II) possess moderate antiferromagnetic coupling of up to $-J = 90$ cm^{-1} [5], but in general, μ-imidazolato complexes involving metalloporphyrin metal centers (e.g., oxidase model compounds) have displayed much weaker interactions of $-J < 10$ cm^{-1} [6, 8]. One possible exception to this generalization has been a binuclear '[Mn^{II}(imid)Cu^{II}]' porphyrin compound which we initially reported to possess strong coupling across an imidazolate bridge between Mn^{II} ($S = 5/2$) and Cu^{II} ($S = 1/2$) [9]. This report stands as nearly the lone exception to a growing consensus that imidazolate is only a weak to moderate mediator of magnetic exchange interactions, and the basic conclusions of the report have been recently questioned [8]. In view of the controversy, and due to the continuing importance of imidazolate-bridged metal centers to metalloprotein chemistry, this communication re-examines and extends our original report. In this endeavor, we have uncovered an [Mn^{III}(imid)Cu^{II}] porphyrin compound with surprising ESR characteristics in view of the electronic ground state as implied by a variable-temperature magnetic susceptibility study.



Compound 1: M = Cu^{II}
Compound 2: M = Zn^{II}

Fig. 1. Schematic representation of the imidazolate-bridged binuclear manganese(III) porphyrin compounds (M^{II} = Cu, Zn).

The μ-imidazolato [Mn–Cu] metalloporphyrin complex of interest (compound **1** of Fig. 1) has been synthesized, under an inert atmosphere, by the reaction of [Mn^{II}(TPP)] (1 mmol; TPP^{2-} = tetraphenylporphyinate), [Cu^{II}(imidH)$_2$DAP](BF_4)$_2$ [10] (1.2 mmol) and Proton Sponge (1 mmol) in CH_2Cl_2/10% CH_3CN under reflux for 12 h. The solvent was then removed under vacuum. Purification of the resulting crystalline solid was accomplished by column chromatography using silica gel and a CH_2Cl_2 eluent to remove 'unreacted' manganese porphyrin as a leading band**. A second minor band was then eluted using a CH_2Cl_2/CH_3OH (25%) solvent system. The band was collected and the solvent removed under vacuum to yield a dark green crystalline solid. The material was washed well with CCl_4 and re-

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**The isolated solid was a manganese porphyrin compound(s), but without BF_4^- anions as demonstrated by the lack of a broad $\nu(BF_4^-)$ IR band around 1050 cm^{-1}.

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crystallized from CH_2Cl_2/hexane to yield compound **1***. The product gave satisfactory elemental analyses (C, H, N, Mn, Cu) for compound **1**, as formulated, with one CH_2Cl_2 molecule of crystallization**. The [Mn–Zn] derivative (compound **2** of Fig. 1) was obtained by an analogous procedure, and it too possessed satisfactory elemental analyses, assuming one CH_3OH molecule of crystallization**. As a precaution, all of the above procedures were performed under a N_2 or Ar atmosphere. As shown in Fig. 1, both compounds are formulated as Mn(III) porphyrin compounds with $[Mn^{III}(imid)M^{II}]^{2+}$ cores and BF_4^- counter ions. This formulation now differs from our initial report of Mn^{II}-containing compounds since the chromatographic procedure (new to this work) yields only manganese porphyrin products characterized by Soret bands around 380 and 460 nm (λ_{max} 458, 380 for **1** and 456, 381 for **2**), a clear indicator of Mn(III) and not Mn(II) [13, 14]. Apparently, $[Mn^{II}(TPP)]$ becomes unavoidably oxidized during the lengthy reaction and work-up time required to produce and isolate the binuclear compounds in reasonable yield. The blue shift to $\leqslant$460 nm for compounds **1** and **2** of the 475 nm band of $[Mn^{III}(TPP)Cl]$ is consistent with observations that more basic axial field ligands (in this case μ-imid$^-$ versus Cl^-) produce a blue-shifted Soret band [15]. The samples in our initial work were not subjected to the chromatographic step, and they displayed two absorption bands in the 460–475 nm range, suggesting the presence of more than one Mn(III) porphyrin compound. Efforts to prepare compounds **1** and **2** directly from $[Mn^{III}(TPP)Cl]$ instead of $[Mn^{II}(TPP)]$ gave only trace amounts of product, and, regardless of attempts to maintain rigorously anaerobic conditions, no Mn(II) porphyrin compounds were obtained in detectable amounts as determined by electronic spectroscopy. Since compounds **1** and **2** were both prepared in small but similar yield (5% for **1**; 6% for **2**), it is assumed that the potentially redox-active $[Cu^{II}(imidH)_2DAP]^{2+}$ cation does not determine the distribution of products in any special way relative to its Zn(II) counterpart. In addition, the presence of Cu(II), and not Cu(I), in compound **1** is supported by the variable-temperature magnetic susceptibility study below.

Figure 2 presents μ_{eff} versus temperature (30–290 K) data by the Faraday method [16] for compounds **1** and **2**. For compound **2**, μ_{eff} is relatively temperature invariant, ranging between 5.15 μ_B at 291 K and 4.68 at 32 K. This result is consistent with a high-spin $[Mn^{III}(imid)Zn^{II}]^{2+}$ core in **2**, since the spin-only value for $S=2$ Mn(III) is 4.9 μ_B. The μ_{eff} value at 290 K for compound **1** with its $[Mn^{III}(imid)Cu^{II}]^{2+}$ core is 6.10 μ_B. This value is reasonable for a $[Mn^{III}(imid)Cu^{II}]^{2+}$ core with isolated or weakly-interacting Mn^{III} ($S=2$) and Cu^{II} ($S=1/2$) centers. However, as the temperature is lowered, the magnetic behavior of **1** is dissimilar to that of **2** in that it is distinctly non-Curie with μ_{eff} gradually decreasing from 6.10 (290 K) to 3.06 (26 K) μ_B. This is the classic pattern for an antiferromagnetic interaction, and as such, the data have been fit [17] to a standard isotropic spin Hamiltonian of the form $H=-JS_1S_2$ (solid line in Fig. 2) to yield $-J\simeq 60$ cm^{-1} for the intramolecular interaction. Of course, an intramolecular interaction is possible only in compound **1** ($S=1/2$, Cu^{II}) and not in **2** where the diamagnetic Zn(II) center serves as a 'magnetic blank'; likewise, if the copper center in **1** were Cu(I) and not Cu(II), magnetic behavior like that for compound **2** should result. The value for $-J$ in **1** is the largest yet found for a mixed-metal, μ-imidazolato metalloporphyrin compound [6, 8], but it is substantially less than values obtained for some homobinuclear imidazolate-

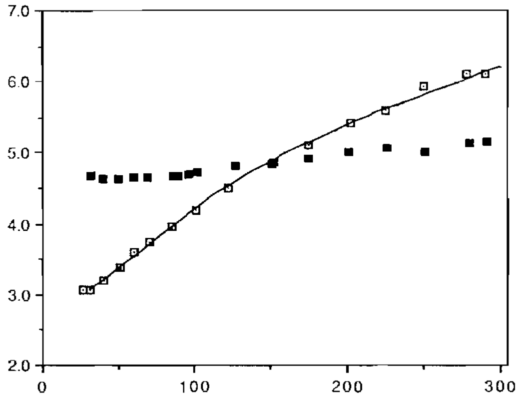

Fig. 2. The μ_{eff} (μ_B) vs. T (K) data for compound **1** (□) and compound **2** (■). The solid line is the theoretical fit with $-J=60$ cm^{-1} and $g=2.0$.

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*The compound (and its Zn(II) analogue) showed a broad $\nu(BF_4^-)$ IR band centered at 1050 cm^{-1} and a $\nu(C{=}N)$ band between 1580–1600 cm^{-1}. No IR band was found around 300 cm^{-1} for the compound (or its Zn(II) analogue) which would indicate an intact Mn^{III}–Cl bond [11]; $[Mn^{III}(TPP)Cl]$ displays the band at 310 cm^{-1}. The CCl_4 wash was necessary to remove trace amounts of Cr(III) products carried over in the synthesis of $[Mn^{II}(TPP)]$ from $[Mn^{III}(TPP)Cl]$ and $[Cr^{II}(acac)_2]$; one such product was crystallographically determined to be $[Cr^{III}(acac)_3]$: $a=16.58(4)$, $b=15.46(2)$, $c=13.44(2)$ Å, $V=3444$ Å^3, orthorhombic, $Z=8$, $R=0.041$, $R_w=0.060$ [12].

**Elemental analyses (C, H, N, Mn, Cu or Zn) were supplied to the referees and found to be satisfactory. Unfortunately, repeated attempts at crystal growth, over the course of several years, have failed to yield single crystals suitable for an X-ray structural study of either compound.

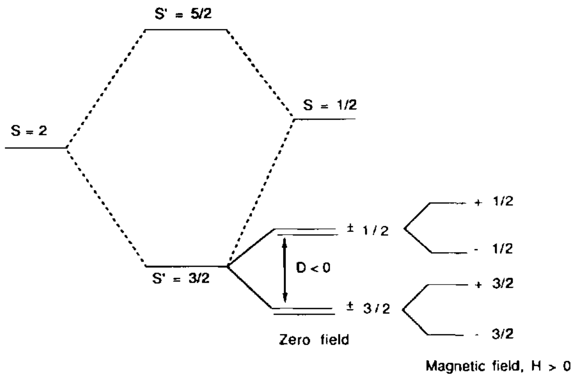

Fig. 3. The proposed energy level diagram for compound 1.

bridged species [5]. This $-J$ value classifies compound 1 as a moderately-coupled system.

As anticipated, compound 2 with its integer spin ground state gave no ESR spectrum (10^{-3} M, CH_2Cl_2 glass, 10 K) in the $g = 2$ to 6 region. For compound 1, the antiferromagnetic coupling between the $S = 2$ and $S = 1/2$ centers results in an $S' = 3/2$ electronic ground state. This half-integer state should be ESR active, but attempts to obtain of spectrum (conditions as above) were singularly unsuccessful*. This rather surprising result seems incongruous with the observed magnetism unless one postulates that the $S' = 3/2$ ground state is characterized by a large and negative zero-field splitting ($D < 0$) as shown in Fig. 3. In this scheme, $D \sim -10$ cm^{-1}, the two unpaired electrons in the $\pm 3/2$ levels would be ESR silent, since energies for the ESR-allowed transitions ($\Delta M_s = 1$) become too large to be observed in the microwave region. Finally, the unpaired electron in the $\pm 1/2$ level would also need to possess a short electronic relaxation time to produce total ESR-silent behavior for the compound. The possible occurrence of negative zero-field splittings in other metalloporphyrin compounds and proteins has just begun to receive consideration [18].

In conclusion, this re-examination of a binuclear [Mn–Cu] metalloporphyrin complex firmly establishes the presence of an $[Mn^{III}(imid)Cu^{II}]^{2+}$ core for the Mn(III) porphyrin compound. The compound possesses antiferromagnetic coupling between $S = 2$ Mn(III) and $S = 1/2$ Cu(II) and across an imidazolate bridge of magnitude $-J = 60$ cm^{-1}. With $-J = 60$ cm^{-1} and an $S' = 3/2$ ground state, the ESR silence of the compound is unexpected and may reflect a rare experimental observation of a negative zero-field splitting for a metalloporphyrin complex. Finally, there now seems to be universal agreement that imidazolate bridges can mediate only weak to moderate antiferromagnetic coupling interactions, a fact that continues to diminish the possibility of a bridging imidazolate ligand in oxidase. This conclusion is also now supported by a range of EXAFS data [19–23] which indicates an [Fe...Cu] separation as short as 3.0 or 3.8 Å, whereas an imidazolate bridge requires > 5.0 Å.

Acknowledgements

We thank the U.S. National Institutes of Health (GM-28451) and the Robert A. Welch Foundation (C-627) for support of this work and acknowledge Professor Graham Palmer for insightful discussions concerning the ESR results.

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*The very weak ESR signal at $g \cong 4$ for compound 1 in ref. 9 is believed to originate from some Cr(III) species which is removed by the CCl_4 wash during work-up in the present work. The $g \cong 6$, 2 signal for compound 2 in ref. 9 is attributed to a trace amount of $[Mn^{II}(TPP)]$.

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