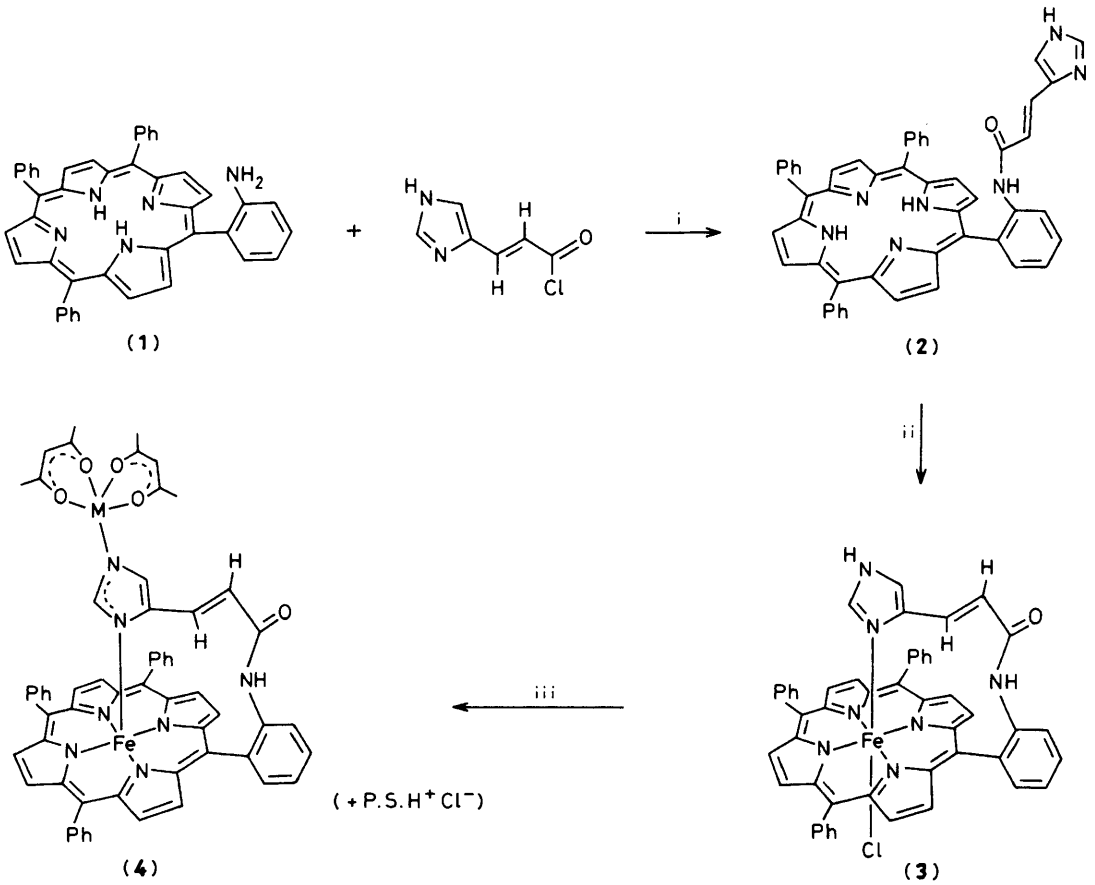
Cytochrome c Oxidase Models. A μ-Imidazolato Complex of Copper(II) and Iron(III) Derived from an Appended-tail Porphyrin

Robert J. Saxton and Lon J. Wilson*

Department of Chemistry, William Marsh Rice University, P.O. Box 1892, Houston, Texas 77251, U.S.A.

An appended-tail Fe^{III} porphyrin complex derived from mono-NH_2TPPH_2 ($TPPH_2$=tetraphenylporphyrin) and urocanic acid chloride has been prepared and used as a precursor for synthesizing a μ-imidazolato binuclear species of Fe^{III} and Cu^{II} as a cytochrome c oxidase model compound.

Cytochrome c oxidase contains two heme and two copper sites and is the terminal enzyme in oxidative phosphorylation, catalysing a $4e^-$ reduction of O_2 to H_2O ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$) with the concomitant release of energy which is stored in the ATP–ADP cycle.[1] Much recent interest in the enzyme has focused on the nature and structure of the active site which appears as binuclear in copper (Cu_u) and heme ($Cyt.a_3$). Furthermore, in the fully oxidized or resting state, this binuclear [Cu_u^{2+}/$Cyt.a_3^{3+}$] centre exhibits magnetic and e.s.r. (silent) properties consistent with an $S = 2$ ground state, arising presumably from strong antiferromagnetic coupling ($-J \geqslant 200$ cm^{-1}) between the $S = 1/2$ (Cu_u^{2+}) and $S = 5/2$ ($Cyt.a_3^{3+}$) centres.[2,3] Biochemically feasible bridges, *e.g.*, [Cu_u^{2+}–(B)–$Cyt.a_3^{3+}$], such as B = imidazolate ($imid^-$) from histidine,[4] oxo from O_2 or tyrosine,[5–7] or mercapto from cysteine,[8,9] have all been suggested as possible mediators of the 'strong' magnetic exchange interaction and several such [Cu^{2+}–(B)–Fe^{3+}] model compounds have been reported by others[10–15] and ourselves.[6,16–18] In this communication, we report a B = $imid^-$ model compound where a novel porphyrin ligand, $UroTPPH_2$ ($TPPH_2$ = tetraphenylporphyrin) [(**2**) in Scheme 1], has been synthesized from mono-NH_2TPPH_2 (**1**) and urocanic acid chloride [3-(1*H*-imidazol-4-yl)prop-2-enoyl chloride]. The $UroTPPH_2$ ligand is unusual among appended-tail porphyrins in possessing a site of unsaturation in the tail (lending rigidity) and a terminal imidazole moiety joined at C-4 rather than through N-1. The former property was selected to encourage $S = 5/2$ Fe^{3+} (short, rigid tail) to mimic the $Cyt.a_3^{3+}$ spin state, and the latter to permit $imid^-$ bridging. In general, both properties have been realized,



Scheme 1. Synthesis of (**3**) and (**4**). *Reagents and conditions:* i, DMF, 35 °C, pyridine, 24 h, N_2; ii, $FeCl_2$, DMF, reflux, 30 min; iii, $Cu(acac)_2$, Proton Sponge (P.S.), CH_2Cl_2, reflux, 6 h.

permitting a thoroughly characterized [Fe^{3+}_{hs}(imid)Cu^{2+}] (hs = high spin) species to be isolated as an analytically pure material and studied for the first time in both the solid and solution states as a model compound for the oxidase active site.

Scheme 1 illustrates the synthesis of the metal-free ligand, UroTPPH$_2$ (**2**), the precursor iron(III) complex, [Fe^{III}(UroTPP)Cl] (**3**), and the subsequent reaction with a copper centre [in this case the bis(acetylacetonato) (accac) complex] to form the binuclear complex (**4**). Unlike many reactions of acid chlorides with primary amines, reaction between urocanic acid chloride and (**1**) does not proceed readily at room temperature, but rather requires prolonged stirring under dry N_2 (35 °C for 24 h) to afford complete reaction. In addition, porphyrins are known to photosensitize the production of singlet dioxygen,[19] and reactions involving the attached tail-base were therefore performed in the dark or under dioxygen-free conditions since imidazole moieties are degraded by $^1O_2$.[20] Thus, (**2**) is susceptible to photochemical decomposition while dispersed on a silica gel column, and all column work was also performed excluding light. A typical preparatory reaction for (**2**) involved stirring 1 mol. equiv. of the amine with 5 mol. equiv. of urocanic acid chloride (from the acid and $SOCl_2$ in dimethylformamide, DMF) in the presence of excess of pyridine. UroTPPH$_2$ was then separated from the reaction mixture by chromatography on a silica gel column. Refluxing (**2**) in DMF with anhydrous $FeCl_2$ gave (**3**). Although the metallated complex is less photosensitive, all manipulations were nevertheless also performed in the dark. Purification by silica gel column chromatography and recrystallization from toluene–hexane afforded (**3**) with an $S = 5/2$

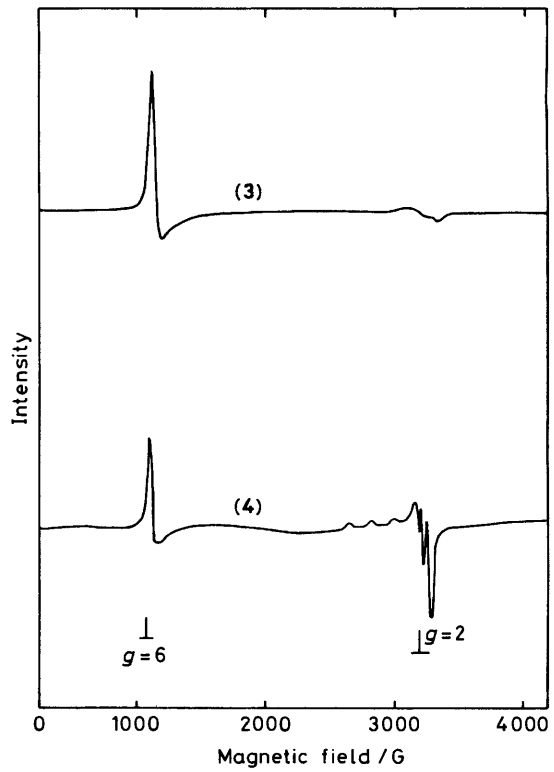


Figure 1. E.s.r. spectra of (**3**) and (**4**) at 10 K in CH_2Cl_2 glasses (1 G = 10^{-4} T).

Fe^{III} centre (*vide infra*). Finally, addition of 1 mol. equiv. of [1,2-bis(dimethylamino)naphthalene] (Proton Sponge) or $Bu^tO^-K^+$ and a 1.1 molar excess of $[Cu(acac)_2]$ to (**3**) in CH_2Cl_2 under reflux gave the appropriate imidazolate binuclear complex (**4**). In a typical preparation of (**4**), a solution of (**3**) (0.18 mmol), $[Cu(acac)_2]$ (0.20 mmol), and Proton Sponge (0.18 mmol) in CH_2Cl_2 (40 ml) was refluxed for 6 h. The solvent was then removed under reduced pressure and the resulting solid washed thoroughly with benzene to remove any unreacted $[Cu(acac)_2]$. The solid was then recrystallized from CH_2Cl_2–heptane, filtered off, and dried over P_2O_5 at room temperature for 12 h. Elemental analyses (C, H, N, Cl, metal) were consistent with the molecular formulations for (**3**) and (**4**) shown in Scheme 1.

With $\mu_{eff.}$(298 K, solid) = 5.4 μ_B and the frozen-solution e.s.r. spectrum shown in Figure 1, (**3**) displays properties consistent with an Fe^{III} $S = 5/2$ species in both the solid and solution states. Whether the imidazole terminus in (**3**) is co-ordinated to Fe^{III}, as shown in Scheme 1, is not indicated by these results, but space-filling molecular models indicate no special problem in accommodating the porphyrin tail in an axial position when the ethylenic hydrogens are in their *trans* configuration. The presence of a binuclear $[Fe^{III}(imid)Cu^{II}]$ centre in (**4**) is more certain since base is required for adduct formation of (**3**) with $[Cu(acac)_2]$, conductivity data in toluene or CH_2Cl_2 have established the non-electrolyte behaviour of (**4**), and, finally, a molecular weight determination of (**4**) by osmometry in CH_2Cl_2 yielded a value of *ca.* 1030 (1066 calc.).

Compound (**4**) exhibits nearly Curie–Weiss magnetic susceptibility behaviour between 20 and 300 K, with $\theta = ca. -2.0$ K. Over this temperature range, $\mu_{eff.}$ varies only between 5.4 μ_B (20 K) and 5.67 μ_B (298 K). Using a value of $\mu_{eff.}$(298 K) = 5.4 μ_B for the Fe^{III} centre, as found for (**3**), and assuming $\mu_{eff.}$ = 2.0 μ_B for Cu^{II}, a room temperature value of *ca.* 5.8 μ_B can be calculated for an $[Fe^{III}_{hs}(imid)Cu^{II}]$ centre in (**4**), assuming magnetically non-interacting Fe^{III} and Cu^{II} ion centres. This value compares well with the experimentally determined value of 5.67 μ_B (298 K). Moreover, the e.s.r. spectrum of (**4**) in a frozen CH_2Cl_2 glass at 10 K displays spectral characteristics consistent with the presence of magnetically dilute $S = 5/2$ Fe^{III} centres [$g = 6$, 2 (weak)] and $S = 1/2$ Cu^{II} centres (g *ca.* 2) in a 1:1 ratio. The well resolved four-line hyperfine pattern on the g *ca.* 2 signal establishes that the signal arises from Cu^{II}, and the narrow line width of the signal is consistent with a magnetically isolated Cu^{II} centre. Thus, the magnetic and e.s.r. properties of this appended-tail μ-imidazolato porphyrin species, with essentially non-interacting Fe^{III} and Cu^{II} centres, do *not* resemble those of the strongly coupled $[Cu_u^{2+}/Cyt.a_3^{3+}]$ pair in oxidase. This conclusion is similar to other reports for $[Fe^{III}_{hs}(2\text{-Meimid})Cu^{II}]$,[10] $[F^{III}_{ls}(imid)Cu^{II}]$[16] (ls = low spin), and $[Fe^{III}_{hs}(imid)Co^{II}_{ls}]$,[21] species where $-J$ is $\ll$ 200 cm^{-1} across imidazolate, but contrary to conclusions for at least one other $[Fe^{III}(imid)Cu^{II}]$ trifluoromethane sulphonate complex[16] that could only be studied in the solid state. Nevertheless, accumulating evidence suggests that imidazolate cannot, in general, foster as strong a magnetic interaction as apparently observed for the active site of resting cytochrome c oxidase.

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