

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

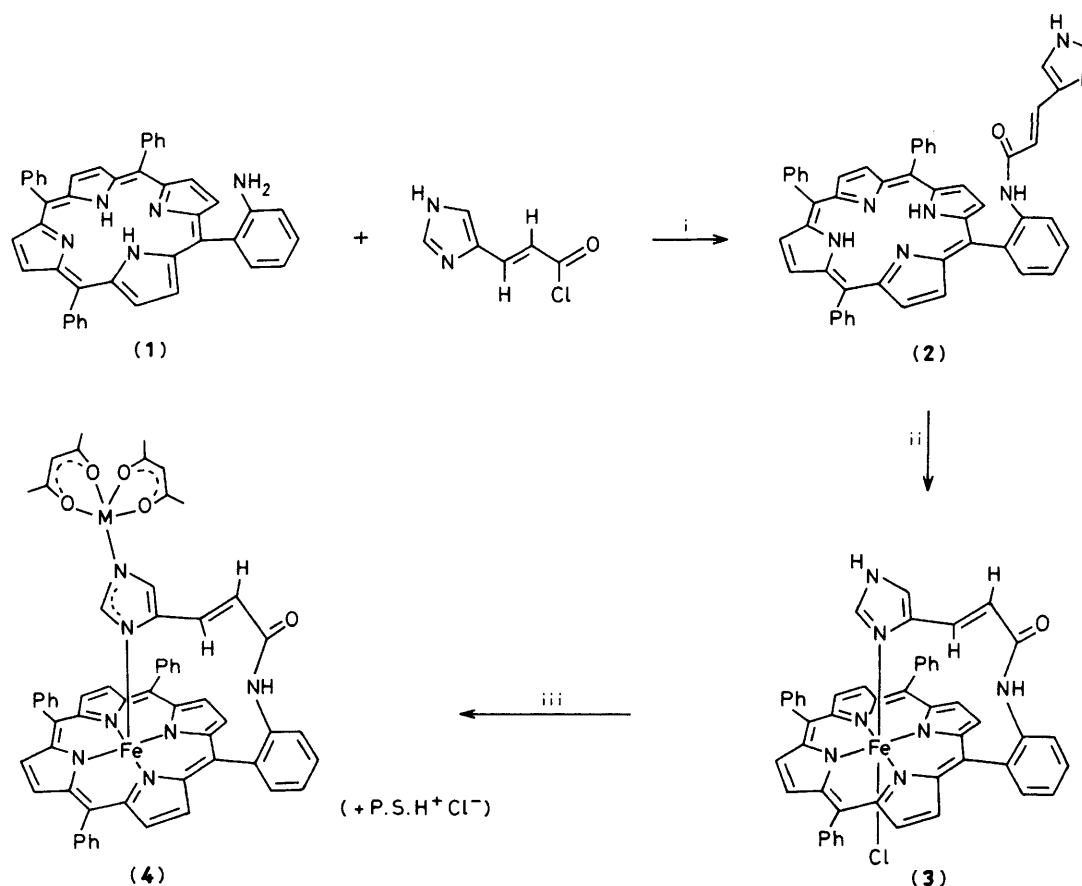
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An appended-tail Fe<sup>III</sup> porphyrin complex derived from mono-NH<sub>2</sub>TPPH<sub>2</sub> (TPPH<sub>2</sub>=tetraphenylporphyrin) and urocanic acid chloride has been prepared and used as a precursor for synthesizing a  $\mu$ -imidazolato binuclear species of Fe<sup>III</sup> and Cu<sup>II</sup> 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<sup>-</sup> reduction of O<sub>2</sub> to H<sub>2</sub>O (O<sub>2</sub> + 4H<sup>+</sup> + 4e<sup>-</sup> → 2H<sub>2</sub>O) with the concomitant release of energy which is stored in the ATP-ADP cycle.<sup>1</sup> Much recent interest in the enzyme has focused on the nature and structure of the active site which appears as binuclear in copper (Cu<sub>II</sub>) and heme (Cyt.a<sub>3</sub>). Furthermore, in the fully oxidized or resting state, this binuclear [Cu<sub>II</sub><sup>2+</sup>/Cyt.a<sub>3</sub><sup>3+</sup>] centre exhibits magnetic and e.s.r. (silent) properties consistent with an *S* = 2 ground state, arising presumably from strong antiferromagnetic coupling ( $-J \geq 200$  cm<sup>-1</sup>) between the *S* = 1/2 (Cu<sub>II</sub><sup>2+</sup>) and *S* = 5/2 (Cyt.a<sub>3</sub><sup>3+</sup>) centres.<sup>2,3</sup> Biochemically feasible bridges, *e.g.*, [Cu<sub>II</sub><sup>2+</sup>-(B)-Cyt.a<sub>3</sub><sup>3+</sup>], such as B = imidazolate (imid<sup>-</sup>) from histidine,<sup>4</sup> oxo from O<sub>2</sub> or tyrosine,<sup>5-7</sup> or mercapto from

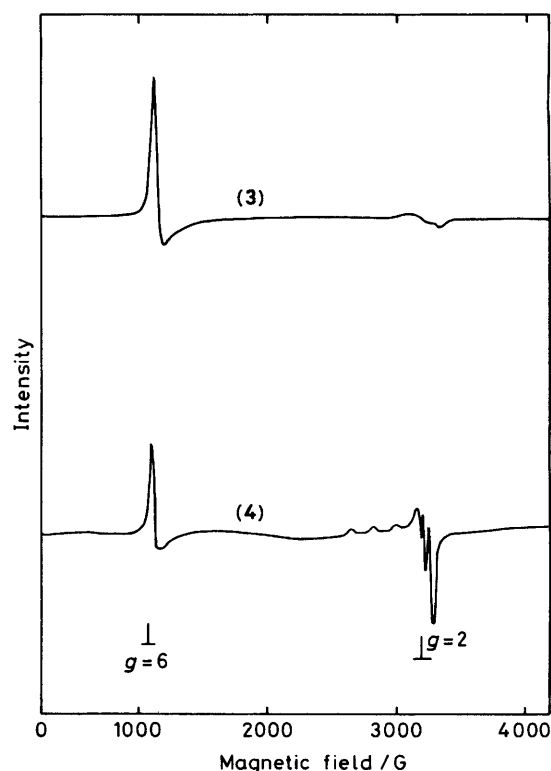
cysteine,<sup>8,9</sup> have all been suggested as possible mediators of the 'strong' magnetic exchange interaction and several such [Cu<sup>2+</sup>-(B)-Fe<sup>3+</sup>] model compounds have been reported by others<sup>10-15</sup> and ourselves.<sup>6,16-18</sup> In this communication, we report a B = imid<sup>-</sup> model compound where a novel porphyrin ligand, UroTPPH<sub>2</sub> (TPPH<sub>2</sub> = tetraphenylporphyrin) [(2) in Scheme 1], has been synthesized from mono-NH<sub>2</sub>TPPH<sub>2</sub> (1) and urocanic acid chloride [3-(1*H*-imidazol-4-yl)prop-2-enoyl chloride]. The UroTPPH<sub>2</sub> 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<sup>3+</sup> (short, rigid tail) to mimic the Cyt.a<sub>3</sub><sup>3+</sup> spin state, and the latter to permit imid<sup>-</sup> 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<sub>2</sub>; ii, FeCl<sub>2</sub>, DMF, reflux, 30 min; iii, Cu(acac)<sub>2</sub>, Proton Sponge (P.S.), CH<sub>2</sub>Cl<sub>2</sub>, reflux, 6 h.

permitting a thoroughly characterized [Fe<sup>3+</sup>(imid)Cu<sup>2+</sup>] (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<sub>2</sub> (2), the precursor iron(III) complex, [Fe<sup>III</sup>-(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<sub>2</sub> (35 °C for 24 h) to afford complete reaction. In addition, porphyrins are known to photosensitize the production of singlet dioxygen,<sup>19</sup> and reactions involving the attached tail-base were therefore performed in the dark or under dioxygen-free conditions since imidazole moieties are degraded by <sup>1</sup>O<sub>2</sub>.<sup>20</sup> 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<sub>2</sub> in dimethylformamide, DMF) in the presence of excess of pyridine. UroTPPH<sub>2</sub> was then separated from the reaction mixture by chromatography on a silica gel column. Refluxing (2) in DMF with anhydrous FeCl<sub>2</sub> 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<sub>2</sub>Cl<sub>2</sub> glasses (1 G = 10<sup>-4</sup> T).

$\text{Fe}^{\text{III}}$  centre (*vide infra*). Finally, addition of 1 mol. equiv. of [1,2-bis(dimethylamino)naphthalene] (Proton Sponge) or  $\text{Bu}^t\text{O}^-\text{K}^+$  and a 1.1 molar excess of  $[\text{Cu}(\text{acac})_2]$  to (3) in  $\text{CH}_2\text{Cl}_2$  under reflux gave the appropriate imidazolate binuclear complex (4). In a typical preparation of (4), a solution of (3) (0.18 mmol),  $[\text{Cu}(\text{acac})_2]$  (0.20 mmol), and Proton Sponge (0.18 mmol) in  $\text{CH}_2\text{Cl}_2$  (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  $[\text{Cu}(\text{acac})_2]$ . The solid was then recrystallized from  $\text{CH}_2\text{Cl}_2$ -heptane, filtered off, and dried over  $\text{P}_2\text{O}_5$  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  $\mu_{\text{eff.}}$ (298 K, solid) =  $5.4 \mu_{\text{B}}$  and the frozen-solution e.s.r. spectrum shown in Figure 1, (3) displays properties consistent with an  $\text{Fe}^{\text{III}}$   $S = 5/2$  species in both the solid and solution states. Whether the imidazole terminus in (3) is co-ordinated to  $\text{Fe}^{\text{III}}$ , 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  $[\text{Fe}^{\text{III}}(\text{imid})\text{Cu}^{\text{II}}]$  centre in (4) is more certain since base is required for adduct formation of (3) with  $[\text{Cu}(\text{acac})_2]$ , conductivity data in toluene or  $\text{CH}_2\text{Cl}_2$  have established the non-electrolyte behaviour of (4), and, finally, a molecular weight determination of (4) by osmometry in  $\text{CH}_2\text{Cl}_2$  yielded a value of *ca.* 1030 (1066 calc.).

Compound (4) exhibits nearly Curie-Weiss magnetic susceptibility behaviour between 20 and 300 K, with  $\theta = \text{ca.} -2.0$  K. Over this temperature range,  $\mu_{\text{eff.}}$  varies only between  $5.4 \mu_{\text{B}}$  (20 K) and  $5.67 \mu_{\text{B}}$  (298 K). Using a value of  $\mu_{\text{eff.}}$ (298 K) =  $5.4 \mu_{\text{B}}$  for the  $\text{Fe}^{\text{III}}$  centre, as found for (3), and assuming  $\mu_{\text{eff.}} = 2.0 \mu_{\text{B}}$  for  $\text{Cu}^{\text{II}}$ , a room temperature value of *ca.*  $5.8 \mu_{\text{B}}$  can be calculated for an  $[\text{Fe}^{\text{III}}_{\text{hs}}(\text{imid})\text{Cu}^{\text{II}}]$  centre in (4), assuming magnetically non-interacting  $\text{Fe}^{\text{III}}$  and  $\text{Cu}^{\text{II}}$  ion centres. This value compares well with the experimentally determined value of  $5.67 \mu_{\text{B}}$  (298 K). Moreover, the e.s.r. spectrum of (4) in a frozen  $\text{CH}_2\text{Cl}_2$  glass at 10 K displays spectral characteristics consistent with the presence of magnetically dilute  $S = 5/2$   $\text{Fe}^{\text{III}}$  centres [ $g = 6, 2$  (weak)] and  $S = 1/2$   $\text{Cu}^{\text{II}}$  centres ( $g \text{ ca. } 2$ ) in a 1:1 ratio. The well resolved four-line hyperfine pattern on the  $g \text{ ca. } 2$  signal establishes that the signal arises from  $\text{Cu}^{\text{II}}$ , and the narrow line width of the signal is consistent with a magnetically isolated  $\text{Cu}^{\text{II}}$  centre. Thus, the magnetic and e.s.r. properties of this appended-tail  $\mu$ -imidazolato porphyrin species, with essentially non-interacting  $\text{Fe}^{\text{III}}$  and  $\text{Cu}^{\text{II}}$  centres, do not resemble those of the strongly coupled  $[\text{Cu}^{\text{II}}/\text{Cyt.a}_3^{3+}]$  pair in oxidase. This conclusion is similar to other reports for  $[\text{Fe}^{\text{III}}_{\text{hs}}(2\text{-Meimid})\text{Cu}^{\text{II}}]$ ,<sup>10</sup>  $[\text{Fe}^{\text{III}}_{\text{ls}}(\text{imid})\text{Cu}^{\text{II}}]$ <sup>16</sup> ( $l_s = \text{low spin}$ ), and  $[\text{Fe}^{\text{III}}_{\text{hs}}(\text{imid})\text{Co}^{\text{II}}_{\text{ls}}]$ ,<sup>21</sup> species where  $-J$  is  $\ll$

$200 \text{ cm}^{-1}$  across imidazolate, but contrary to conclusions for at least one other  $[\text{Fe}^{\text{III}}(\text{imid})\text{Cu}^{\text{II}}]$  trifluoromethane sulphonate complex<sup>16</sup> 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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