

Cytochrome c Oxidase Models. A Magnetically Coupled Heme–Copper Complex

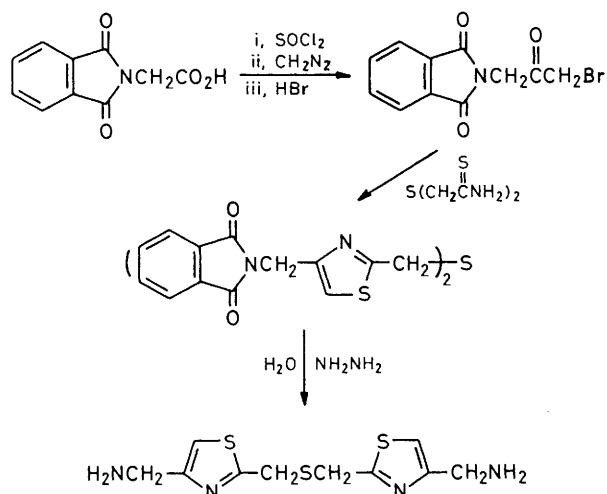
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Heme model compounds have been synthesized incorporating a high spin iron(III) ion and a copper(II) ion to form a Fe–O–Cu complex showing antiferromagnetic coupling with $-3J = 132 \pm 5 \text{ cm}^{-1}$.

Cytochrome c oxidase is the terminal component of the mitochondrial respiratory chain linking electron transport to oxygen reduction. The enzyme is equipped with an unusual complement of metals consisting of two heme a molecules and two copper ions. Although the molecular mechanism by which oxygen is reduced to water is far from clear, a rich body of

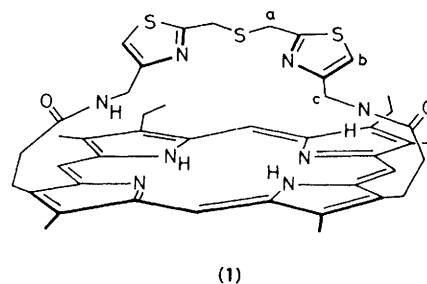
evidence has established that dioxygen interacts with one pair of heme and copper only, referred to as heme a_3 and Cu a_3 .¹ The second pair of heme and copper functions as an electron shuttle, similar to other cytochromes in the electron-transport chain. It is at the heme a_3 –Cu a_3 binding site that dioxygen is bound and reduced. In the resting (fully oxidized) state of the



Scheme 1

enzyme, heme a_3 -Cu a_3 exhibits properties revealing that the two metal centres are strongly antiferromagnetically coupled. The magnitude of the exchange coupling has been estimated to be $>200\text{ cm}^{-1}$.² This large coupling phenomenon has therefore inspired a number of proposals suggesting an imidazolate,³ oxo,⁴ or mercapto⁵ group bridging between the heme iron and copper. Recently, attempts have been made to synthesize model compounds incorporating these structural features.⁶⁻⁹ However almost all heme-copper model systems failed to show any significant magnetic interactions between the two metal ions. The negative results may be attributable to several factors: the Fe-Cu distance is sometimes too large and the ligand framework too rigid to allow flexibility; the copper(II) ion is often held in a square-planar geometry with mismatched magnetic orbitals between the metals. In one case at least (a N_2S_2 thiaza¹⁰ crowned porphyrin¹¹) the copper binding was so weak that dissociation of Cu^{II} presented a serious problem.¹² An apparent solution would be to build a non-square-planar copper ligand possessing a good affinity for Cu^{II} and structurally flexible enough to assume a strain-free interaction with a pendant heme group. These conditions have been met in the heme-tridentate ligand system reported herein.

The requisite diamino thiazole sulphide was synthesized according to Scheme 1 where each step had a yield of at least 90%. All compounds were characterized by ^1H n.m.r. and mass spectrometry. The diamine was treated with an equimolar quantity of mesoporphyrin XII¹³ diacid chloride in CH_2Cl_2 using a high-dilution technique.¹¹ The resultant thiazole-strapped porphyrin (1) was purified by chromatography (silica gel, 6% $\text{MeOH}-\text{CH}_2\text{Cl}_2$) and crystallized from CH_2Cl_2 - MeOH , yield 72%.[†] ^1H N.m.r. spectroscopy indicated that the protons a, b, and c resonate at δ 0.86, 5.80, and 3.97, respectively; these are displaced significantly upfield from their original positions in the uncoupled amine, thereby establishing the indicated strap-on-top structure. A further proof of the configuration was provided by e.s.r. spectroscopy. Insertion of copper using copper(II) acetate in CH_2Cl_2 - MeOH led to a bis- Cu^{II} complex. The dipolar interaction of the two paramagnetic copper ions resulted in the triplet e.s.r. spectrum shown in Figure 1. The apparent zero-field splitting D value suggested a Cu-Cu distance of no greater than 4.3 \AA .¹⁴



(1)

Iron insertion into the porphyrin was accomplished using the usual FeSO_4 - HOAc method,¹⁵ followed by repeated washing with 10% HCl to ensure that no metal was bound to the thiazoles. The hemin chloride thus prepared can react with zinc chloride or copper(II) chloride to give analytically pure FeMCl_3L , L = strapped porphyrin ligand. If the hemin chloride, dissolved in CH_2Cl_2 - MeOH , was added to ammoniacal copper(II) acetate in aqueous methanol, a solid (2) was obtained which has the composition $\text{FeCu}(\text{O})(\text{H}_2\text{O})(\text{OAc})\text{L}$. I.r. spectroscopy (KBr pellet) of this sample showed a peak at 880 cm^{-1} , which is absent in the spectrum of FeCuCl_3L and which is characteristic of the M-O-M antisymmetric stretching vibration.¹⁶ The visible spectrum of (2) appeared to be very similar to that of μ -oxo Fe^{III} porphyrin dimers (600 nm, sh; 564 nm; 386 nm). Compound (2) was reasonably stable and could be dissolved in CH_2Cl_2 and re-evaporated without undergoing any change. To determine whether compound (2) is a Fe-O-Fe dimer, a CH_2Cl_2 solution of this compound was shaken with 5% aqueous NaOH . The resultant material, compound (3), retained the same composition and its i.r. (882 cm^{-1}) and absorption spectra showed no significant variations; however its magnetic properties differed drastically from solid (2). The magnetic susceptibilities of solid (2) measured by a SQUID susceptometer and the corresponding magnetic moments are shown in Figure 2(a); μ_{eff} values of compound (3) as well as the Fe-Zn complex are shown in Figure 2(b).

The magnetic behaviour of solid (2) is best understood by assuming a high-spin Fe^{III} ($S_1 = 5/2$) coupled with Cu^{II} ($S_2 = 1/2$). Such a spin coupling should result in two states: $S = 3$ and $S = 2$, with an energy gap of $|3J|$.[‡] The molar susceptibility can be calculated using the simplified Van Vleck's equation (1),¹⁷ where N , β , and K have their standard meaning.

$$\chi = \frac{2N\beta^2}{kT} \cdot \frac{5g_1^2 + 14g_2^2 \exp(3J/kT)}{5 + 7 \exp(3J/kT)} \quad (1)$$

The experimental data can then be fitted with a non-linear least-squares KINFIT program¹⁸ to provide the following parameters: $-3J = 132 \pm 5\text{ cm}^{-1}$, $g_1 = 2.14$, and $g_2 = 1.82$. The calculated best fit is also shown in Figure 2(a). The overall features of the μ_{eff} curve are similar to those of $\text{Cu}^{\text{II}}-\text{Mn}^{\text{II}}$ and $\text{Cu}^{\text{II}}-\text{Fe}^{\text{III}}$ complexes which have been found to exhibit energy gaps of 79.2 and 289 cm^{-1} , respectively.^{19,20} The curve shows that below *ca.* 50 K, the $S = 3$ excited state is completely depopulated. At temperatures below 20 K, μ_{eff} drops rapidly owing to the zero-field splitting of the ground state. The susceptibility of the iron-zinc complex obeys the Curie-Weiss law and is representative of an isolated high-spin iron

[†] Compound (1) gave a satisfactory elemental analysis.

[‡] If the spin Hamiltonian is defined as $H = -JS_1 \cdot S_2$. The energy gap would be $|6J|$, if $H = -2JS_1 \cdot S_2$.

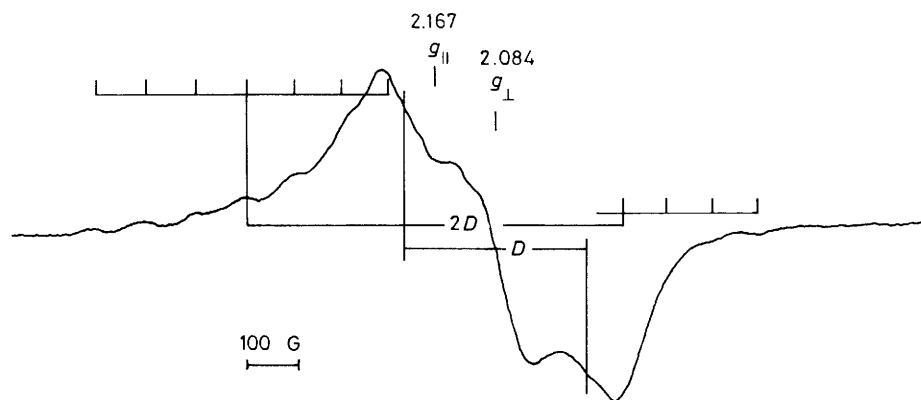


Figure 1. E.s.r. spectrum of the bis-Cu^{II} complex in CH₂Cl₂ at 77 K. The seven-line hyperfine pattern derived from the two copper(II) nuclei ($I = 3/2$) can be discerned on the left; only the two outermost lines are seen on the right.

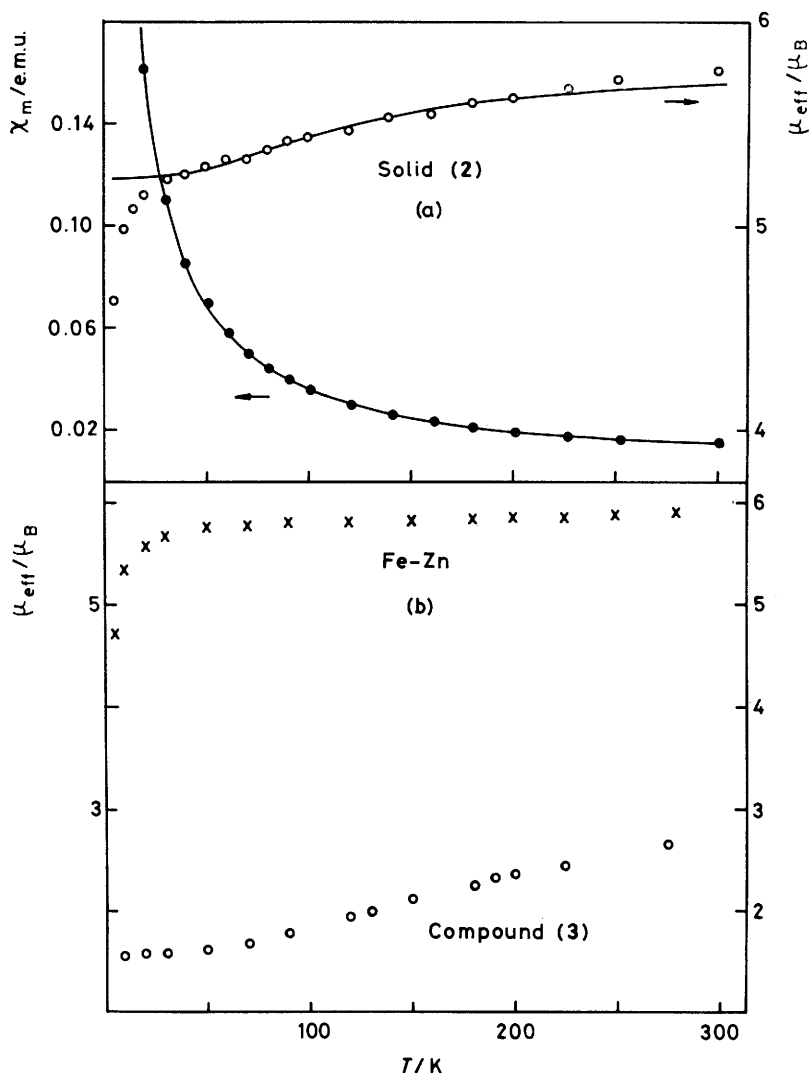
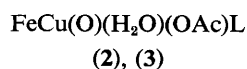


Figure 2. Molar magnetic susceptibility and effective magnetic moment as a function of temperature for (a) solid (2) and (b) the Fe-ZnCl₃L complex and compound (3). The lines represent the fit of the data using $-3J = 132 \text{ cm}^{-1}$. A diamagnetic correction of $-5 \times 10^{-4} \text{ e.m.u. mol}^{-1}$ was applied to the measured data.

($S = 5/2$). The behaviour of compound (3), however, is less straightforward. The χ^{-1} vs. T plot does not show Curie-Weiss



behaviour and the μ_{eff} curve cannot be fitted to any scheme involving spin pairing between iron and copper. This curve may be rationalized by considering a combination of isolated Cu^{II} ($\mu_{\text{eff}} = 1.7 \mu_{\text{B}}$) and half an intermolecular Fe-O-Fe μ -oxo dimer. Combining the i.r. spectroscopic evidence we

therefore assign solid (2) to an intramolecularly oxo-bridged Fe–O–Cu complex and compound (3) to a dimeric Fe–O–Fe complex. E.s.r. spectra also corroborated this assignment. At 77 K in frozen CH₂Cl₂ there was a strong $g = 2$ signal but hardly any $g = 6$ signal for compound (3) whereas for (2), both $g = 6$ and $g = 2$ signals were present but their integrated spin concentration was less than the total Fe and Cu present (<40%). In the absence of definitive X-ray structures, the coordination geometry of copper is unknown although Corey–Pauling–Koltun models suggest a somewhat distorted tetrahedral geometry using the two thiazole N's, the sulphide S, and the oxo group.

Our oxidase model represents the first successful attempt to achieve significant spin-coupling between a copper(II) complex and a high-spin iron(III) heme. The choice of a β -substituted porphyrin instead of a meso-substituted porphyrin may be important since the iron(III) complex of the latter type, especially in the presence of perchlorate or other weak-field ions, often develops spin mixing with the $S = 3/2$ state.^{8,9} The magnitude of coupling present in our model is still small in comparison with the oxidase; perhaps among other things, the presence of an imidazole axial ligand is crucial. The lack of a *trans*-ligand also contributes to the facile formation of the external Fe–O–Fe dimer which severely limits the chemistry that one can achieve with these compounds. The blocking of the sixth site with a ligand should eliminate this problem.†

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† Addition of imidazole base resulted in formation of the hexacoordinate hemichrome and competitive binding for copper.

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