Cytochrome Oxidase Models: a Binuclear Iron–Copper Site with a Bipyrimidine Bridge

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Summary An [Fe (high-spin) $^{2+}$–(bipyrimidine)–Cu^{2+}] site has been synthesized and investigated as an electronic model for the proposed [Cyt.a_3–(imidazolate)–Cu] active site in cytochrome oxidase.

Cytochrome oxidase, being the terminal oxidation – reduction enzyme in mitochrondrial respiration,[1,2] is one of the most important, yet enigmatic metalloenzyme. Recent e.s.r.,[2] m.c.d.,[3] and magnetic susceptibility[4] investigations together have strongly indicated a structure for the dioxygen-binding Cyt.a_3^{2+} active site as being an imidazolate-bridged [Cyt.a_3^{2+}–(imid)–Cu^+] binuclear centre capable of a two-electron reduction of ligated dioxygen ($\frac{1}{2}O_2 + 2e^- + 2H^+ \rightarrow H_2O$). In the fully oxidized or resting form of the enzyme, the proposed structure of this unusual mixed-metal site is shown in Figure 1.[5] In this state the Cu^{2+} ($S = 1/2$) ion is undetectable by e.s.r. spectroscopy, ostensibly owing to strong antiferromagnetic coupling ($-J \geqslant 200\ cm^{-1}$) with the formally Fe^{3+} ($S = 5/2$) haem, resulting in a spin-coupled $S = 2$ ground state for the [Fe^{3+}–(imid)–Cu^{2+}] unit.[2,4] The construction of synthetic model compounds represents the most viable approach† to test this proposed model for the active site in oxidase, and, thus, we communicate our initial results which have produced the first such [Fe^{2+}–(B)–Cu^{2+}] site for examination where B is the aromatic heterocyclic bridge, 2,2′-bipyrimidine (bipym). To our knowledge this report characterizes the first well defined [Fe–Cu] binuclear species to be synthesized, and, in general, represents one of the few studies involving a binuclear system containing two different metal ions.[6]

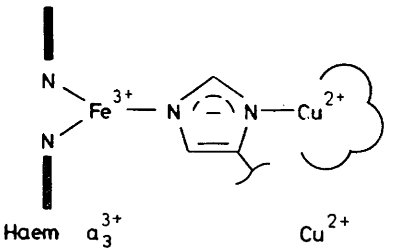

Figure 1. Proposed structure of the active site in cytochrome oxidase. The case shown is for the fully oxidized resting enzyme.

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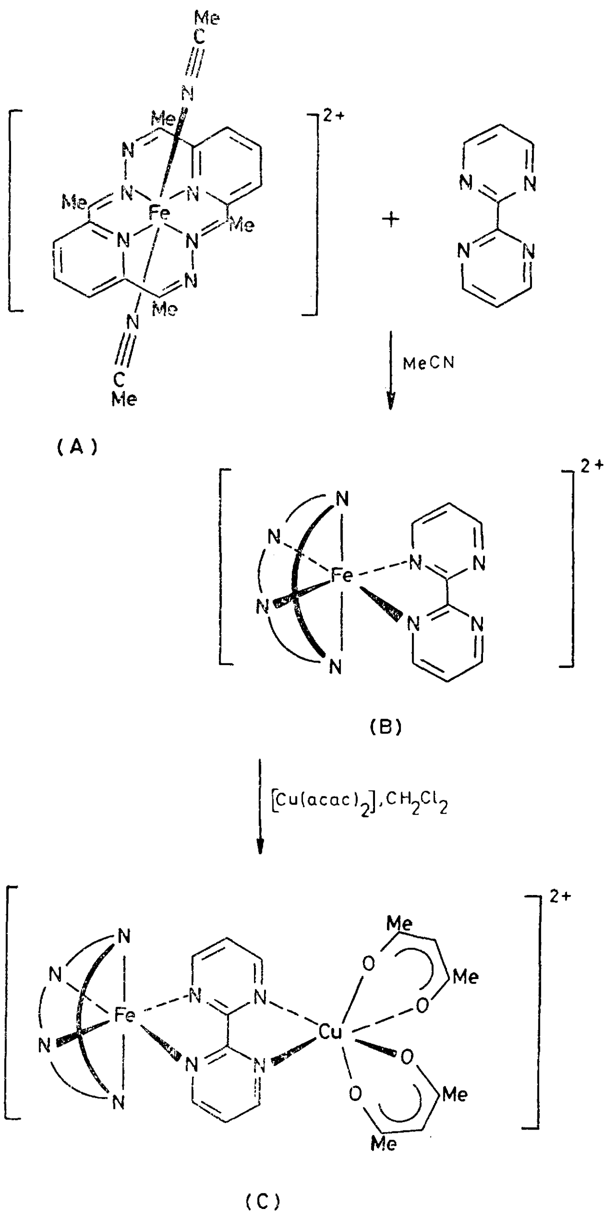
† The size and complexity of the protein (mol. wt. *ca.* 2 × 10^5 and membrane-bound), precludes the possibility of the structure being resolved by *X*-ray crystallography, although the EXAFS technique could yield some information in this regard.



Scheme. Synthetic scheme for the (Fe^{2+}–Cu^{2+}) [and (Fe^{2+}–Zn^{2+})] binuclear compounds.

Goedkin *et al.*[7] first reported the synthesis of the macrocyclic Fe compound (A) in the Scheme. When (A) as the ClO_4^- salt reacts with bipym in acetonitrile the solution gradually changes from green to red, from which analytically pure (B) can be isolated. This compound contains the folded form of the macrocycle and the co-ordinated bipym ligand, and is a low-spin Fe (l.s.)$^{2+}$ species as characterized by its magnetic moment (μ_{eff} = 0·8 B.M. room temp.) and Mössbauer [δ(SNP) *ca.* 0·48 mm s^{-1}; ΔE_Q *ca.* 0·38 mm s^{-1}] and ^{1}H n.m.r. spectra.‡ The subsequent reaction of (B) with [Cu(acac)$_2$] in methylene chloride gives a purple solution from which analytically pure (C) is obtained as the ClO_4^- salt in ⩾ 60% yield. Compound (C) is the first discrete [Fe–(B)–Cu] binuclear species to be prepared and the first instance where bipym serves to bridge unlike metal centres.[8] Use of [Zn(acac)$_2$] in the synthesis produces the analogous [Fe–(bipym)–Zn](ClO$_4$)$_2$ derivative.

Room temperature magnetic moments of 5·46 B.M. (Fe^{2+}–Cu^{2+}) and 5·04 B.M. (Fe^{2+}–Zn^{2+}) for the two derivatives are consistent with an essentially high-spin state for Fe^{2+} in each case. Thus, chelation of (B) with [Cu(acac)$_2$] {or [Zn(acac)$_2$]} to generate (C) also produces a spin state change at the Fe^{2+} centre. It is interesting to note that the Cyt.a_3 porphyrin site in oxidase, whether in the Fe^{2+} or Fe^{3+} state, is also always assumed to be high-spin in its protein function. In solution, this change in spin state for the present synthetic system is reflected by the $E_{1/2}$(Fe^{2+} → Fe^{3+}) redox activity of the species, where the Fe(h.s.)$^{2+}$ centres in (C) and the (Fe^{2+}–Zn^{2+}) derivative [$E_{1/2}$ *ca.* +0·70 V(S.C.E.) for both] are oxidized at 600 mV lower in potential than the Fe(l.s.)$^{2+}$ centre in (B) [$E_{1/2}$ *ca.* +1·3 V(S.C.E.)].§

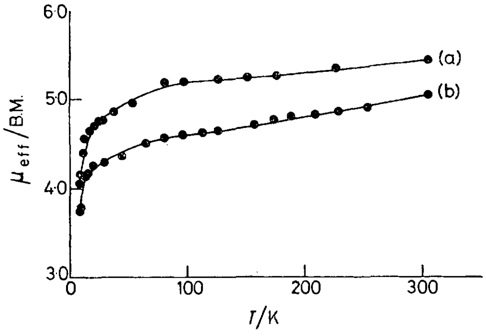


Figure 2. μ_{eff} vs. temperature for the (Fe^{2+}–Cu^{2+}) (curve a) and (Fe^{2+}–Zn^{2+}) (curve b) compounds as ClO_4^- salts.

The temperature dependence of μ_{eff} for (C) is shown in Figure 2a where the magnetic moment decreases gradually from 5·46 B.M. (298 K) to 4·56 B.M. at 15 K and then drops more rapidly to 4·04 B.M. at 8·3 K. This pattern could arise from antiferromagnetic exchange between the Fe(h.s.)$^{2+}$ ($S = 2$) and Cu^{2+} ($S = 1/2$) centres like that proposed between (Fe^{3+}–Cu^{2+}) in cytochrome oxidase,[4] an Fe(h.s.) ($S = 2$) $\rightleftarrows$ Fe(l.s.)$^{2+}$($S = 0$) spin-equilibrium process, and/or zero-field splitting of the Fe(h.s.)$^{2+}$ ($S = 2$) state. This last alternative seems to be the major contribution, since the (Fe^{2+}–Zn^{2+}) derivative in Figure 2b exhibits the same general μ_{eff} v.s. T pattern where no exchange interaction is possible and the Mössbauer spectra at 90 K of (Fe^{2+}–Cu^{2+}) and (Fe^{2+}–Zn^{2+}) compounds are essentially unchanged from the spectra at room temperature, a result inconsistent with

‡ The ^{1}H n.m.r. spectrum of (B) as the ClO_4^- salt in (CD$_3$)$_2$CO is consistent with that of an essentially diamagnetic compound of the structure shown in the Scheme: δ(Me) 1·2; δ(py and bipym protons) centred at 3·6 from Me$_4$Si.

§ Electrochemical voltammograms were obtained at 23 ± 2 °C in CH$_2$Cl$_2$ using a PAR model 174 Polarographic Analyzer and a rotating Pt electrode. The solutions were *ca.* 10^{-4} M in the compound and 0·1 M in [Bu$^n_4$N]ClO$_4$ as the supporting electrolyte. $E_{1/2}$ potentials are reported *vs.* the saturated potassium calomel electrode (S.C.E.) and are uncorrected for junction potentials. A standard three-electrode circuit was employed with the reference electrode isolated from the sample solution by a fritted disc.

a spin-equilibrium phenomenon for six-co-ordinate Fe^{2+}.¶ Finally, the electrochemical data (*vide supra*) also argue for identical electronic structures for the Fe(h.s.)$^{2+}$ centres in the two derivatives.

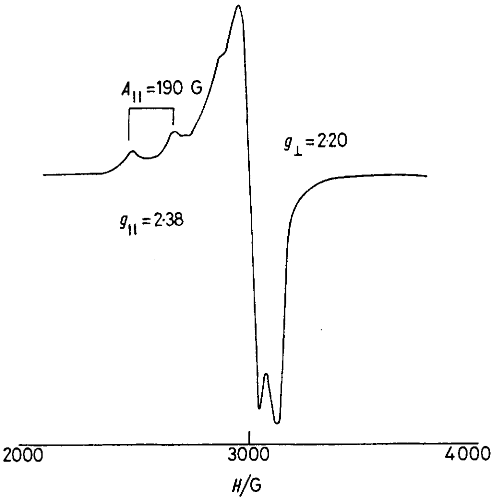


FIGURE 3. *X*-Band electron spin resonance spectrum of compound (C) in a CH_2Cl_2 glass at 8 K.

The e.s.r. spectrum of (C) in a CH_2Cl_2 glass at 8 K appears surprisingly normal for the $S=1/2$ Cu^{2+} centre with $g_{\|}$ = 2·38 and $g_{\perp}$ = 2·20 and $A_{\|}$ = 190 G (Figure 3). Even in the total absence of any direct exchange across the bipym bridge, a highly paramagnetic Fe(h.s.)$^{2+}$ ion at 6—7 Å** might be expected to produce some decrease in intensity[9] or relaxation broadening[10] of the Cu^{2+} signal, but none is evident. In the resting form of cytochrome oxidase, the [Fe^{3+}–Cu^{2+}] distance is not likely to be much shorter (assuming no direct Fe–Cu bond) so that the Cu^{2+} centre in the enzyme is probably e.s.r. inactive owing to ligand-mediated super-exchange with Cyt.a_3^{3+} rather than *via* a through-space dipolar relaxation mechanism. This conclusion seems to be consistent with a [Cyt.a_3^{3+}–(B)–Cu^{2+}] structure for the active site of the enzyme, but does not comment upon the identity of the ligand bridge.[11] The B = 2,2′-bi-imidazolate analogue of (C) may be a more revealing species as to the electronic properties expected for [Fe–(imid)–Cu] centres in general.

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¶ Mössbauer spectra for all well characterized Fe(h.s.)$^{2+}$ ($S=2$) $\rightleftharpoons$ Fe(l.s.)$^{2+}$ ($S=0$) spin-equilibrium compounds in the cross-over region possess both (h.s.) and (l.s.) signals whose intensities vary as a function of the spin isomer concentrations. Since ΔE_Q(h.s.) is normally significantly larger than ΔE_Q(l.s.), this fact usually permits unambiguous identification of the two spin states, if both are present (see for example: M. A. Hoselton, L. J. Wilson, and R. S. Drago, *J. Amer. Chem. Soc.*, 1975, **97**, 1722). The Mössbauer spectrum of (C) is inconsistent with this behaviour, appearing to be approximately the same at room temperature and at *ca.* 90 K with δ(SNP) *ca.* 0·43 mm s^{-1} and ΔE_Q *ca.* 0·48 mm s^{-1}, although the expected similarities in δ and ΔE_Q for the two spin states in the present case [*i.e.*, for low-spin (B), δ = 0·48 mm s^{-1} and ΔE_Q = 0·38 mm s^{-1}] would make unambiguous spin isomer identification tenuous at best.

** From space-filling molecular models assuming Fe–N and Cu–N bond distances of 2·1 and 2·0 Å, respectively.

[1] See for example: K. F. Purcell and J. C. Kotz, 'Inorganic Chemistry,' Saunders, Philadelphia, 1977, p. 1078.

[2] G. Palmer, G. T. Babcock, and L. E. Vickery, *Proc. Nat. Acad. Sci., U.S.A.*, 1976, **73**, 2206.

[3] G. T. Babcock, L. E. Vickery, and G. Palmer, *J. Biol. Chem.*, 1976, **24**, 7907.

[4] G. P. Palmer, G. T. Babcock, L. Garcia-Iniguez, M. F. Tweedle, L. J. Wilson, and L. Vickery, Abstracts of the International Symposium on Mechanisms of Oxidizing Enzymes, La Paz, Mexico, December 1977; M. F. Tweedle, L. J. Wilson, L. Garcia-Iniguez, G. T. Babcock, and G. Palmer, Abstracts of the 174th National Meeting of the American Chemical Society, Anaheim, California, U.S.A., March 1978.

[5] The presence of a similar [Zn^{2+}–(imid)–Cu^{2+}] centre has been documented for superoxide dismutase (J. S. Richardson, K. A. Thomas, B. H. Rubin, and D. C. Richardson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1349; J. S. Richardson, K. A. Thomas, and D. C. Richardson, *Biochem. Phys. Res. Comm.*, 1975, **63**, 986).

[6] See for example: D. A. Krost and G. L. McPherson, *J. Amer. Chem. Soc.*, 1978, **100**, 987.

[7] V. L. Goedken, Y. Park, S. Peng, and J. M. Norris, *J. Amer. Chem. Soc.*, 1974, **96**, 7693.

[8] However, bipyrimidine is known to bridge like metal centres: U. T. Mueller-Westerhoff, *Adv. Chem. Ser.*, 1976, **150**, 41 for Pt–(bipym)–Pt]; M. Hunziker and A. Ludi, *J. Amer. Chem. Soc.*, 1977, **99**, 7370 and E. V. Dose and L. J. Wilson, *Inorg. Chem.*, in the press, for [Ru–(bipym)–Ru].

[9] J. S. Leigh, Jr., *J. Chem. Phys.*, 1970, **52**, 2608; the spectrum in Figure 3 quantitatively accounts for $\geqslant$ 90% of available copper in a sample of known concentration.

[10] See for example: R. S. Drago, 'Physical Methods in Chemistry,' Saunders, Philadelphia, 1977, p. 468.

[11] Since e.s.r. evidence implicates a nitrogen donor atom as part of the bridging group (M. F. J. Blokzijl-Homan and B. F. Van Gelder, *Biochim. Biophys. Acta*, 1971, **234**, 493), a polypeptide nitrogen atom is the only reasonable alternative to imidazolate for the bridge. The study of carefully constructed model compounds {especially [(porphyinato)Fe^{3+}–(B)-Cu^{2+}] systems} is probably the only way definitively to decide this question. Such work is now in progress.