Oxygenation Reactions of Cytochrome Oxidase Models. Evidence for Hematin Formation in Mononuclear and Heterobinuclear Complexes

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Summary Controlled potential electrolysis, chemical reduction, or synthesis is used to prepare the Fe^{III}/Cu^{I} and Fe^{II}/Cu^{I} complexes of *meso*-tetra-αααα-*o*-(nicotinamidophenyl)porphyrin; on exposure to oxygen the Cu-free derivative and the fully reduced heterobinuclear complexes undergo irreversible oxidation to the hematins Fe(P)–OH–(N_4) and Fe(P)–OH–Cu$(N_4)^{2+}$, respectively.

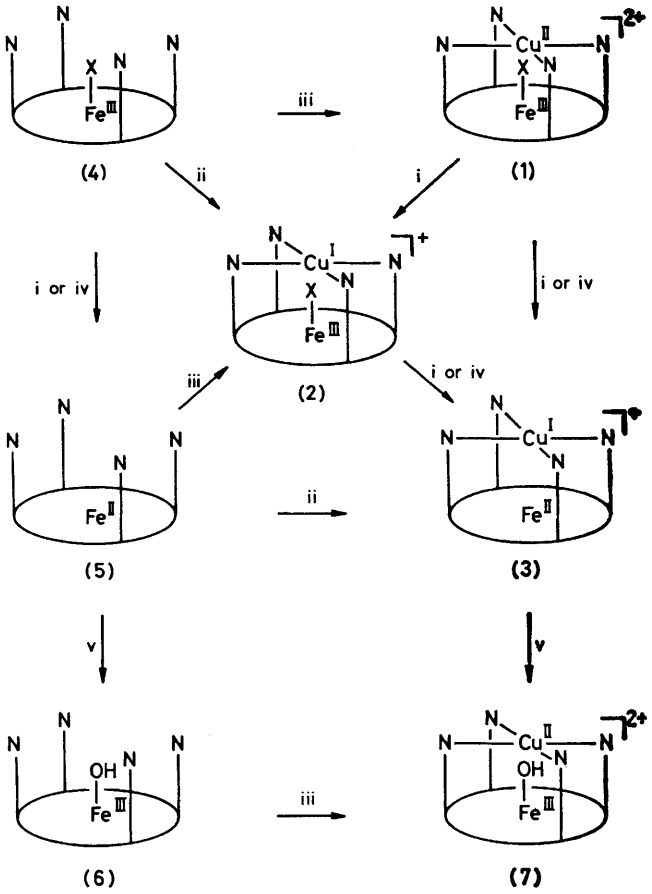
DESPITE considerable efforts by many groups over several decades, the active-site structure and mechanism of action of cytochrome c oxidase have remained undefined; the present state of knowledge has been summarised in a number of recent reviews.[1] We[2] and others[3] have adopted the synthetic model approach in an attempt to elucidate the structure of the active site of the enzyme in its fully oxidised or resting state. In particular, efforts have centred around the synthesis of complexes containing a magnetically coupled Fe^{III}–Cu^{II} moiety, in response to suggestions[4,5] and evidence[6] that such a situation exists at the oxygen binding site of the enzyme. An alternative model[7] postulating an un-

coupled high-spin Fe^{IV} porphyrin and Cu^{I} centre appears not to have received much support, although little is yet known about the chemistry and spin-state of Fe^{IV} porphyrins.[8]

There is an urgent need to characterise at a molecular level the redox states and oxygen-containing intermediates involved in the catalytic cycle of cytochrome oxidase. We have shown[2,9,10] that a model system consisting of an Fe^{III} porphyrin with an appended Cu^{II}-containing tetrapyridine ligand system with various bridging groups has limited applicability as a model to describe the magnetic properties of the fully oxidised enzyme. Nevertheless, in the fully reduced form of this model system, the Fe–Cu distance (expected to be *ca.* 5 Å),[2] might allow reaction with dioxygen in a manner similar to that proposed[5] for cytochrome oxidase. We report here some results of the study of the reduced model system with oxygen.

Cyclic voltammetric and a.c. polarographic measurements on solutions of $[Fe(P)–Cl–Cu(N_4)](ClO_4)_2$† (**1**) (Pt button, 10^{-4} M in CH_2Cl_2 containing 0·1 M tetrabutylammonium perchlorate) show two quasi-reversible 1-electron reduction processes for the Cu^{II}/Cu^{I} couple at $E_{\frac{1}{2}} = 0{\cdot}31$ V and for the Fe^{III}/Fe^{II} couple at $E_{\frac{1}{2}} = 0{\cdot}04$ V (*vs.* saturated calomel electrode). Controlled potential electrolysis could therefore be used to produce solutions of (**2**) (λ_{max} 417, 511, and 575 nm; e.s.r. at 4·2 K, *g ca.* 6 and 2; no Cu^{II} signals), and the fully reduced species (**3**) (λ_{max} 425, 438(sh), 525, and 564 nm; e.s.r.-inactive to 4·2 K), but not $[Fe^{II}(P)–Cu^{II}(N_4)]^{2+}$. Complexes (**2**) and (**3**) were also generated by chemical reductions or by insertion of Cu^{I} into the complexes (**4**) or (**5**) (Scheme). Attempted insertion of Cu^{II} into (**5**) resulted in the Fe^{III}/Cu^{I} product (**2**) by means of an internal redox reaction, consistent with the above assignment of electrode potentials.

A number of studies of the reactions of the fully reduced species (**3**) and the copper-free derivative (**5**) with oxygen in solvents such as $CHCl_3$, toluene, or Me_2SO, with or without a donor (axial) ligand, 1-methylimidazole (1-MeIm) have been carried out. The copper-free derivative (**5**) was prepared by crown-ether-complexed sodium dithionite[11] reduction of (**4**) in $CHCl_3$–MeOH solution under argon, precipitation with methanol, and filtration under argon. At ambient temperature in toluene, $CHCl_3$, or Me_2SO, with or without added 1-MeIm (2—5 mol), (**5**) does not reversibly bind dioxygen, but is smoothly oxidised (*e.g.* isosbestic points in Me_2SO at 423, 444, 518, and 565 nm) to the hematin (**6**).‡ This compound is also formed, and is more conveniently prepared, by treatment of the complexes $Fe(P)–X–(N_4)$, (X = Cl^-, Br^-, N_3^-, or CN^-)[2,10] with aqueous base. Analytical, spectroscopic, magnetic, and Mössbauer evidence favours the Fe^{III} hydroxy-formulation rather than the more usual μ-oxo-oligomer. Except for certain protein derivatives (*e.g.* metmyoglobin and methemoglobin) in which dimerisation is sterically prevented, monomeric Fe^{III} porphyrin hydroxo-complexes have not been previously



SCHEME. i, controlled potential electrolysis, ii, solution of $Cu(MeCN)_4{\cdot}BF_4$ in Me_2SO, iii, solution of $Cu(ClO_4)_2$ or $Cu(BF_4)_2$ in Me_2SO or $CHCl_3$–MeOH, iv, crown-ether-complexed $Na_2S_2O_4$ in MeOH or Me_2SO, v, O_2. The ellipse represents the porphyrin ring and the four N's the tetrapyridine 'picket' of *meso*-tetra-αααα-*o*-(nicotinamidophenyl)porphyrin.

isolated.[12] Although the u.v.-visible spectrum of (**6**) [*e.g.* λ_{max} (Me_2SO) 428, 572, 610(sh) nm] is more typical of a μ-oxo-oligomer than a high spin 5-co-ordinate Fe^{III} porphyrin, the i.r. spectrum (Nujol mull) shows bands at 3420 and 3200 cm^{-1} and lacks any bands between 820 and 1000 cm^{-1}; the Fe–O–Fe antisymmetric stretching vibration of all previously reported μ-oxo-oligomers appears in the region 850—900 cm^{-1}.[12] The magnetic moment measured in solution ($CHCl_3$) or the solid state at 300 K is 5·9 μ_B, consistent with high-spin Fe^{III} ($S = 5/2$). The e.s.r. spectrum of (**6**) (solid state or frozen solution) at 77 K and 4·2 K shows strong *g ca.* 6 and weak *g ca.* 2 signals typical of high-spin Fe^{III} porphyrins. Conversely, all Fe^{III} porphyrin μ-oxo oligomers studied to date show a magnetic moment between

† Abbreviations used: $[Fe(P)–Cl–Cu(N_4)]^{2+}$ refers to the cationic species derived from chloro-*meso*-tetra-αααα-*o*-(nicotinamidophenyl)-porphinatoiron(III) (ref. 2) with Cu^{II} ligated to the 4 pyridine N's, and a bridging Cl^-. Likewise, $Fe^{II}(P)–(N_4)$ refers to the Fe^{II} complex of the porphyrin, with a metal-free tetrapyridine 'cap'. Note that in donor solvents such as Me_2SO, the Fe^{II} and/or Fe^{III} porphyrin will most likely be solvated and either 5- or 6-co-ordinate. This is not explicitly shown in the Scheme.

‡ The rate of oxidation is strongly dependent on traces of water in the solvent, *e.g.* in dry toluene, oxidation is incomplete after 2 h; in wet toluene, the reaction is complete within 10 min. In dry solvents the proton source necessary for formation of (**6**) is not obvious. It may be due to adventitious water, despite our efforts to prevent this; alternatively, an initially formed dioxygen adduct may effect hydroxylation of solvent and/or porphyrin.

1·6 and 1·9 μ_B, and no e.s.r. signals, owing to strong antiferromagnetic coupling between two high-spin Fe^{III} ions *via* the oxygen bridge.[12] The Mössbauer spectrum at 4·2 K shows δ 0·41 mm sec^{-1} and ΔE_Q = 0·16 mm sec^{-1}, consistent with an $S = 5/2$ spin state.

Reactions of the fully reduced binuclear complex (**3**) in solution with oxygen are generally complex and depend strongly on such factors as the nature of the solvent, the presence of water, pH, concentration, and time. At ambient temperature in $CHCl_3$ containing 1-MeIm (2 mol), or in Me_2SO containing traces of H_2O, (**3**) is rapidly oxidised (<3 min) to the hydroxo-complex $Fe^{III}(P)$–OH–$Cu^{II}(N_4)^{2+}$ whose spectral properties are identical to those (in the same solvents) of the diperchlorate salt (**7**), independently synthesised from (**6**) and copper(II) perchlorate [(**7**) λ_{max} (Me_2SO) 417, 522(sh), 566, 600(sh), and 660(sh) nm; e.s.r. signals (Me_2SO or 10% MeOH–$CHCl_3$ frozen solution, 4·2 K) for high-spin Fe^{III} and Cu^{II} at *g ca.* 6 and 2, respectively, similar to those of the well-characterised complex (**1**)].[2] Likewise the magnetic moment at 300 K is 5·2 μ_B and the μ_{eff} *vs.* *T* curve closely resembles that of (**1**), where it has been demonstrated that an $S = 3/2, 5/2$ spin equilibrium/spin mixed situation exists on Fe, and that there is no effective exchange interaction between Fe and Cu.

Thus, although the oxidation of (**3**) is somewhat faster than that of (**5**) under similar conditions, the role of Cu in the oxidation is not apparent from the final reaction product. The Cu^{I} may be oxidised by an outer sphere mechanism and need not necessarily proceed by a Cu–O_2 complex.

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