Cytochrome c oxidase models. A novel dinuclear iron–copper complex derived from a covalently modified deuteroporphyrin–L-histidine–bis(benzimidazole) ligand

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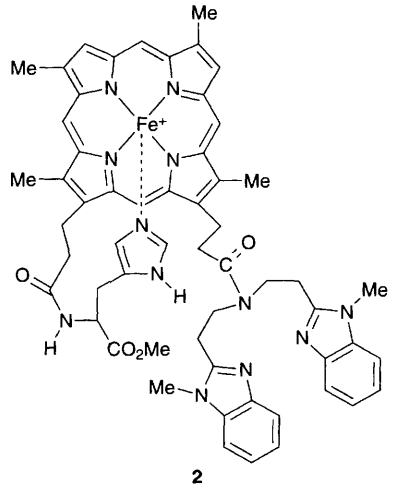
The iron(III)–copper(II) complex derived from a covalently modified deuteroporphyrin at both carboxylate ends of the two propionic acid side chains is described as a model for the dioxygen reduction site of cytochrome c oxidase; it exhibits a weak coupling between the metal centres, and its fully reduced $Fe^{II}Cu^{I}$ form reacts smoothly and nondestructively with dioxygen at room temperature to produce the $Fe^{III}Cu^{II}$ species.

Cytochrome c oxidase is the terminal oxidase of the electron-transfer system coupling the exergonic four-electron reduction of dioxygen with vectorial proton translocation across the membrane.[1] The three-dimensional X-ray structures at 2.8 Å resolution of the enzyme from bovine heart[2] and from *Paracoccus denitrificans*[3] are now available. They contain five catalytically active metal centres which comprise a bis(imidazole)-coordinated haem a, an unprecedented [2Cu–2S–Cys] cluster as the Cu_A site, and a dinuclear haem a_3/Cu_B centre consisting of a five-coordinate haem, with an axial imidazole, and a tris(imidazole)-ligated copper atom. The Cu_A and haem a centres act as simple electron delivery sites, while the haem a_3/Cu_B couple is the site of dioxygen reduction.[4] Some recent advances in the biomimetic chemistry of the haem/Cu site focused on μ-oxo bridged (porphyrin)iron(III)–copper(II) complexes, where the bridge assembles two mononuclear Fe^{III} and Cu^{II} precursors, as mimics of the 'resting' oxidized form of the enzyme.[5] More recent reports described dinuclear haem–copper complexes derived from covalently modified, dinucleating porphyrin ligands.[6,7] The former[6] undergoes irreversible oxygenation to give a stable dioxygen adduct, while the complex reported by us is oxidized by dioxygen nondestructively at −45 °C to produce the $Fe^{III}Cu^{II}$ species.[7] We report here a novel dinuclear haem–copper complex derived from a natural porphyrin which, besides the arm carrying the nitrogen donors for the copper atom, contains a covalently linked L-histidine residue as the iron axial ligand, thereby improving the significance of the current models for the cytochrome c oxidase active centre.

The deuterohaemin derivative DH–HisBB **2** was obtained from deuterohaemin-2(18)-L-histidine methyl ester (DH–His, **1**)[8,9] by condensation with *N,N*-bis-[2-(1′-methyl-2′-benzimidazolyl)ethyl]amine (BB)[10] to the remaining propionic acid substituent of the porphyrin ring.† The FABMS spectrum of DH–HisBB shows the molecular ion cluster centred at *m/z* 1030 in perfect agreement with the simulated spectrum. The presence of the imidazole group intramolecularly bound as the iron axial ligand promotes the preferential folding of the arm carrying the two benzimidazole groups towards the opposite part of the porphyrin plane, as shown for related deuterohaemin-L-histidine-peptide complexes.[8] This is reflected by reduced affinity for exogenous imidazole by DH–HisBB (K = 1600 dm^3 mol^{-1} in MeOH) with respect to DH–His (K = 2300 dm^3 mol^{-1}).

Binding of Cu^{2+} to DH–HisBB produces the dinuclear complex DH–HisBB–Cu. The EPR spectral parameters obtained from a frozen Me_2SO solution at 123 K ($g_{\|}$ = 2.289, $g_{\perp}$ = 2.068, $A_{\|}$ = 167 × 10^{-4} cm^{-1}) are indicative of a tetragonal stereochemistry with N,O ligation for the Cu^{II} ion. The ^1H NMR spectrum of DH–HisBB in $(CD_3)_2SO$ dramatically changes upon Cu^{2+} binding. The haem methyl resonances in the range δ 45–55, for the high-spin Fe^{III} centre, shift to δ 22 (broad signal) in the presence of Cu^{2+}. The FABMS spectrum of the DH–HisBB–Cu complex (*p*-nitrobenzyl alcohol matrix) shows a prominent cluster of peaks centred at *m/z* 1093, which presumably corresponds to the reduced $Fe^{II}Cu^{I}$ form, together with a minor cluster of peaks at *m/z* 1109, corresponding to an oxo or hydroxo adduct of the complex.

The Mössbauer spectrum of DH–HisBB–Cu in the solid state at 4.2 K consists of a quadrupole doublet with isomer shift of 0.45 mm s^{-1} and quadrupole splitting of 0.80 mm s^{-1} typical of high-spin ferric haem.[11] The absence of magnetic hyperfine interactions at 4.2 K (in zero field) suggests that the high-spin iron(III) ion is coupled to Cu^{II} to yield a complex with an integer spin system.‡ At temperatures > 20 K the quadrupole doublet gradually becomes broad and asymmetric. This temperature behaviour indicates that the iron(III) and copper(II) centres are no longer tightly coupled above 20 K, which means that the coupling constant in the dimer system is very weak. The current data, therefore, bring experimental support to the weak coupling model that can be used, as an alternative to the strong coupling model,[11,12] to interpret the magnetic properties of cytochrome c oxidase. We are currently investigating the temperature dependence behaviour of the $Fe^{III}Cu^{II}$ complex by EPR, Mössbauer



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and magnetic susceptibility to gain a better understanding of the spin coupling problem and its relevance to cytochrome c oxidase.

Preliminary investigation of the redox properties of DH–HisBB–Cu elicits an interesting behaviour. The Cu^{II} ion can be selectively reduced by ascorbate as shown by disappearance of its EPR signal and thus, like for cytochrome c oxidase,[1] its redox potential must be higher than that of the iron centre. Anaerobic treatment of a Me_2SO solution of DH–HisBB–Cu (*ca.* 5×10^{-6} mol dm^{-3}) with a small amount of aqueous dithionite yields the fully reduced $Fe^{II}Cu^{I}$ complex. When this solution is exposed to dioxygen a smooth oxidation reaction to the oxidized $Fe^{III}Cu^{II}$ species is observed at room temperature *without* significant porphyrin destruction (Fig. 1). This behaviour is very unusual for iron porphyrins which lack aromatic substituents at *meso* positions and, in fact, when the iron(II) form of DH–HisBB is subjected to the same reaction, complete porphyrin degradation occurs. In the haem–copper system a rapid two-electron transfer from the $Fe^{II}Cu^{I}$ couple to dioxygen can occur, releasing hydrogen peroxide, which in turn may be reduced by a second $Fe^{II}Cu^{I}$ molecule (Scheme 1)

By contrast, the proton-assisted autoxidation pathway undergone by reduced DH–HisBB leads to degradative porphyrin oxidation,[13] probably through odd-electron reduced oxygen species. It is therefore anticipated that the smooth reactivity of the reduced DH–HisBB–Cu complex towards dioxygen depends on the formation of a peroxide-bound intermediate complex. Our current efforts are attempting to characterize such a relevant intermediate species.

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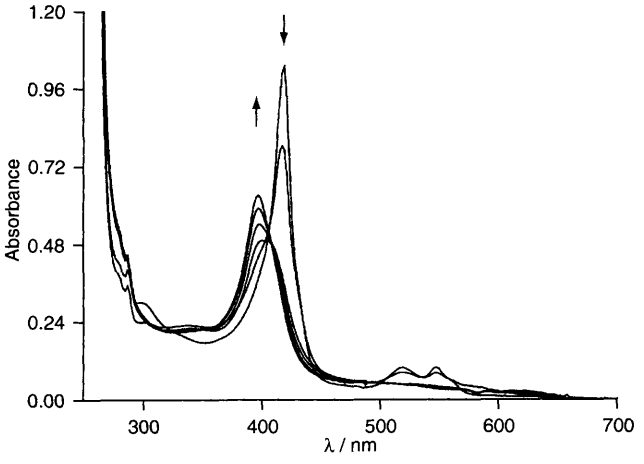

Fig. 1 UV–VIS spectra recorded during the oxidation of the reduced $Fe^{II}Cu^{I}$ form of the DH–HisBB–Cu complex, obtained upon treatment of a Me_2SO solution (*ca.* 5×10^{-6} mol dm^{-3}) of the complex with aqueous dithionite, with air at room temperature

$$Fe^{II}Cu^{I} + O_2 \longrightarrow Fe^{III}Cu^{II} + H_2O_2$$

$$Fe^{II}Cu^{I} + H_2O_2 \longrightarrow Fe^{III}Cu^{II} + 2H_2O$$

Scheme 1

Footnotes

† A mixture of deuterohaemin-L-histidine methyl ester[8] (300 mg, 0.39 mmol) and dried hydroxybenzotriazole (126 mg, 0.93 mmol) was dissolved in dry dmf (5 cm^3) and cooled to 0 °C. Then, *N,N*-dicyclohexylcarbodiimmide (190 mg, 0.93 mmol) and, after 1 h, *N,N*-bis[2-(1′-methyl-2′-benzimidazolyl)ethyl]amine[10] (310 mg, 0.93 mmol) were added under stirring. The reaction was continued for 4 h at 0 °C and 20 h at room temp. The mixture was then poured into diethyl ether (250 cm^3) under vigorous stirring and the precipitate thus formed was collected by filtration. The crude product was purified by chromatography on a silica gel column (4 × 20 cm). Using a mixture of acetic acid–*n*-butanol–water (4 : 2 : 1 *v/v/v*) as eluent the unreacted deuterohaemin-L-histidine methyl ester was eluted first, while the product was retained by the column. The product was then eluted with methanol–dichloromethane (1 : 1 *v/v*) (yield *ca.* 40%). UV–VIS, λ_{max}/nm (Me_2SO) 358 (ε/dm^{-3} mol^{-1} cm^{-1} 25500), 394 (97500), 498 (5800), 618 (2700).

‡ Small amounts of low-spin iron(III) species due to an aggregated porphyrin complex are detected in the Mössbauer (quadrupole doublet with isomer shift 0.24 mm s^{-1} and quadrupole splitting 2.30 mm s^{-1}) and EPR spectra (g = 2.98, 2.27 and 1.96) at 4.2 K.

References

1 G. T. Babcock and M. Wikström, *Nature*, 1992, **356**, 301; B. G. Malmström, *Acc. Chem. Res.*, 1993, **26**, 332; S. I. Chan and P. M. Li, *Biochemistry*, 1990, **29**, 1; G. Palmer, *J. Bioenerg. Biomembr.*, 1993, **25**, 145.

2 T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono and S. Yoshikawa, *Science*, 1995, **269**, 1069.

3 S. Iwata, C. Ostermeier, B. Ludwig and H. Michel, *Nature*, 1995, **376**, 660.

4 K. C. Minghetti, V. C. Goswitz, N. E. Gabriel, J. J. Hill, C. Barassi, C. D. Georgin, S. I. Chan and R. B. Gennis, *Biochemistry*, 1992, **31**, 6917; J. P. Hosler, S. Ferguson-Miller, M. W. Calhoun, J. W. Thomas, J. Hill, L. Lemieux, J. Ma, C. Georgiu, J. Fetter, J. Shapleigh, M. M. J. Tecklenburg, G. T. Babcock and R. B. Gennis, *J. Bioenerg. Biomembr.*, 1993, **25**, 121; M. Lauraeus, J. E. Morgan and M. Wikström, *Biochemistry*, 1993, **32**, 2664; M. I. Verkhovsky, J. E. Morgan and M. Wikström, *Biochemistry*, 1994, **33**, 3079.

5 A. Nanthakumar, M. S. Nasir, K. D. Karlin, N. Ravi and B. H. Huynh, *J. Am. Chem. Soc.*, 1992, **114**, 6564; A. Nanthakumar, S. Fox, N. N. Murthy, K. D. Karlin, N. Ravi, B. H. Huynh, R. D. Orosz, E. P. Day, K. S. Hagen and N. J. Blackburn, *J. Am. Chem. Soc.*, 1993, **115**, 8513; K. D. Karlin, A. Nanthakumar, S. Fox, N. N. Murthy, N. Ravi, B. H. Huynh, R. D. Orosz and E. P. Day, *J. Am. Chem. Soc.*, 1994, **116**, 4753; S. C. Lee and R. H. Holm, *J. Am. Chem. Soc.*, 1993, **115**, 5833, 11789; M. J. Scott, H. H. Zhang, S. C. Lee, B. Hedman, K. O. Hodgson and R. H. Holm, *J. Am. Chem. Soc.*, 1995, **117**, 568.

6 J. P. Collman, P. C. Herrmann, B. Boitrel, X. Zhang, T. A. Eberspacher, L. Fu, J. Wang, D. L. Rouseau and E. R. Williams, *J. Am. Chem. Soc.*, 1994, **116**, 9783.

7 L. Casella, E. Monzani, M. Gullotti, F. Gliubich and L. De Gioia, *J. Chem. Soc., Dalton Trans.*, 1994, 3203.

8 L. Casella, M. Gullotti, L. De Gioia, R. Bartesaghi and F. Chillemi, *J. Chem. Soc., Dalton Trans.*, 1993, 2233.

9 M. Momenteau, M. Rougee and B. Loock, *Eur. J. Biochem.*, 1976, **71**, 63.

10 L. Casella, M. Gullotti, R. Radaelli and P. Di Gennaro, *J. Chem. Soc., Chem. Commun.*, 1991, 1611.

11 F. M. Rusnak, E. Münck, C. I. Nitsche, B. H. Zimmerman and J. A. Fee, *J. Biol. Chem.*, 1987, **262**, 16 328.

12 E. P. Day, J. Peterson, M. S. Sendova, J. Schoonover and G. Palmer, *Biochemistry*, 1993, **32**, 7855.

13 J. P. Collman, T. R. Halpert and K. S. Suslick, *Metal Ion Activation of Dioxygen*, ed. T. Spiro, Wiley, New York, 1980, p. 1; K. Shikama, *Coord. Chem. Rev.*, 1988, **83**, 73; G. B. Jameson and J. A. Ibers, *Bioinorganic Chemistry*, ed. I. Bertini, H. B. Gray, S. J. Lippard and J. S. Valentine, University Science Books, Mill Valley, CA, 1994, p. 167.

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