# Synthesis and Characterization of a Binuclear Porphyrin Complex as a Model for Heme $a_3$-$Cu_B$ Site of Cytochrome *c* Oxidase

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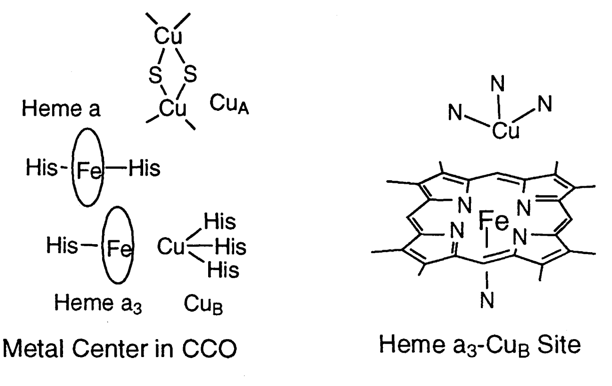
A binuclear complex, **1**, involving copper and heme complexes is synthesized and characterized by paramagnetic $^1$H-NMR, FAB mass, and ESR spectroscopies. These measurements indicate a fairly weak magnetic interaction between iron and copper ions when a bridge ligand is absent.

Mitochondrial cytochrome c oxidase (CCO) is a terminal enzyme in the respiratory chain and catalyzes the reduction of molecular oxygen to water.[1] The enzyme contains active metal centers; heme a and $Cu_A$ which comprise the electron delivery sites, and heme $a_3$-$Cu_B$ designated as the site of oxygen reduction and of ligand binding. Recently, the crystal structures at 2.8 Å resolution of cytochrome c oxidases from bovine heart[2] and *Paracoccus Denitrificants*[3] have been reported. The metal centers of the enzyme are schematically depicted in scheme 1. In $Cu_A$ site, two copper ions are bridged by two cystein thiolates with bis $\mu$-sulfur-bridge structure, and in heme a, two histidyl imidazoles axially coordinate to heme iron to form a six coordinate complex. On the other hand, the heme $a_3$ is coordinated by a histidyl imidazole to take a five-coordinate structure, and the $Cu_B$ center with three histidyl nitrogen ligands is located 4.5-5.2 Å away from the heme $a_3$. A bridging ligand such as oxygen or chlorine between the heme $a_3$ and the $Cu_B$ has been proposed on the basis of strong antiferromagnetic coupling of both atoms, however, the crystal structural study suggested no detectable bridging ligand between them.[2]

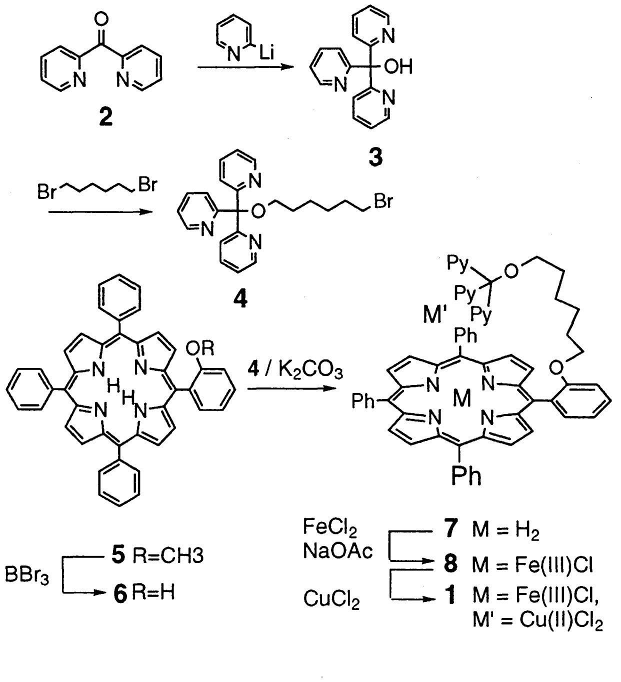
Modeling of the heme $a_3$-$Cu_B$ site can be very helpful in elucidating the reduction mechanism of oxygen by the enzymes. Thus, a number of model complexes having sulfur bridge,[4] imidazolate bridge,[5] and oxo bridge[6] have been prepared because of strong antiferromagnetic coupling in CCO. However, there remains further study to mimic the heme $a_3$-$Cu_B$ site because no bridging ligand has been detected in the crystal structure. In this regard, a heme complex which is covalently connected with a copper complex having three nitrogenous ligands would be useful model to understand the reduction mechanism and the magnetic property of CCO.[7] We report here synthesis and characterization of a heme $a_3$-$Cu_B$ model complex(**1**), in which a copper complex site with three nitrogenous ligands is covalently linked to heme by strong ether bonds.

The synthetic route of **1** was outlined in scheme 2. The copper coordination site of tridentate tris-2-pyridylmethanol(**3**) was obtained in 51% yield from the reaction of bis-2-pyridylketone(**2**) and 2-litiopyridine, prepared from 2-bromopyridine and n-butyllithium, at -80 °C. A hexyl chain was introduced by heating **3** and 1,6-dibromohexane in the presence of sodium hydride in DMF. After evaporation of DMF, **4** was isolated in 45% yield by purification through neutral alumina column (benzene, then dichloromethane-acetone as eluent).

Condensation of *o*-anisaldehyde, benzaldehyde, and pyrrole(1:3:4) in propionic acid gives a mixture of six isomers and polymeric impurity. The porphyrin isomer(**5**) was separated by silica gel column using benzene. After demethylation with $BBr_3$ in dichloromethane at -20 °C, the desired mono hydroxy porphyrin(**6**) was isolated in 4% yield(based on o-anisaldehyde) by silica gel column using benzene, then dichloromethane-acetic acid eluent. Reaction of the porphyrin (**6**) with the ester (**4**) and anhydrous $K_2CO_3$ in DMF gave the tris-pyridyl-porphyrin compound (**7**) in 84% yield.[8]



**Scheme 1.** Illustration of acitve sites of cytochrome c oxidase.



**Scheme 2.** Synthesis of **1**.

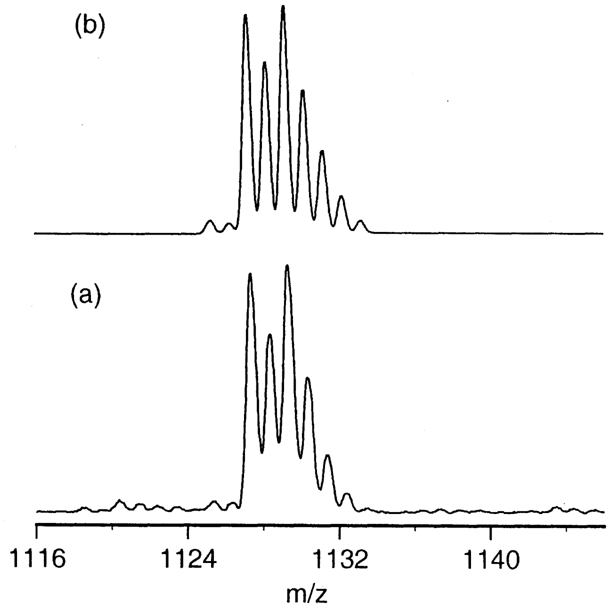


**Figure 1.** FAB-MS spectrum of **1**. (a) the experimental data. (b) computer simulation.

The $^{1}$H-NMR, $^{13}$C-NMR, and 2D-cosy spectra of **7** were fully in agreement with the structure. In the $^{1}$H-NMR spectrum in CDCl$_3$, the methylene protons in hexyl chain of **7** showed downfield shifts compared with those of **4**. This is due to the ring current effect of the porphyrin core of **7**, and suggests that the hexyl chain is brought close to the porphyrin plane. The absorption spectrum of **7** was close to that of **5**, suggesting no interaction of tris-2-pyridyl moiety with the porphyrin.

To mimic the heme a$_3$-Cu$_B$ site in CCO, we inserted iron into the porphyrin core and copper into the tris-2-pyridyl moiety of **7**. Refluxing of **7**, ferrous chloride, and sodium acetate in acetic acid formed an iron porphyrin complex. Further treatment of the iron complex with conc.HCl solution could remove iron from tris-2-pyridyl moiety and resulted in chloro iron(III) porphyrin complex of **8**.[8] The absorption and paramagnetic $^{1}$H-NMR spectra of **8** were close to those of chloro iron(III) TPP complex, showing that pyridine in tris-2-pyridyl moiety does not coordinate to iron in the porphyrin. The binuclear complex, **1**, was prepared by addition of 1 equiv. of copper(II) chloride in methanol into **8** in acetone. The solid compound of **1** was obtained by slow addition of hexane to the solution.

The formation of **1** was confirmed by paramagnetic $^{1}$H-NMR, and FAB mass spectra.[8] The FAB mass spectrum of **1** gave intense peaks due to a cluster of positive ions centered at m/z = 1129.4, corresponding to [**1**]$^{2+}$ formed from dissociation of two chloride anions (Figure 1(a)).[9] The isotope intensity pattern of the peaks closely matched the theoretical pattern as shown in Figure 1(b). Furthermore, the paramagnetic $^{1}$H-NMR of **1** showed broadening of porphyrin peripheral proton signals, suggesting that copper(II) ion is located near iron porphyrin ring. Previous NMR studies of oxo bridging complexes showed downfield shift of pyrrole proton with formation of $\mu$-oxo bridging core between iron and copper.[6] However, the pyrrole shift of **1** hardly changed the position even when copper(II) ion is introduced. This suggests that there is no bridging ligand between iron and copper of **1**, which is similar to the case of CCO. To investigate the magnetic property, we mesuared the ESR spectrum of **1**, where exhibited strong signals at g = 6.0 and 2.0 arising from iron(III) high spin porphyrin complex and copper(II) complex, respectively. These spectral data imply very weak magnetic interaction between iron and copper. Our further efforts will focus on detailed characterization of the present complex and modifying of a non-heme copper site to study structure/function relationship in CCO.

## Reference and Note

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8 **4**: $^{1}$H-NMR in CDCl$_3$; 8.57(m, 3H, 6-H), 7.73(m, 3H, 3-H), 7.67(m, 3H, 4-H), 7.13(m, 3H, 5-H), 3.38(t, 2H, CH$_2$(6) ), 3.36(t, 2H, CH$_2$(1) ), 1.82(m, 2H, CH$_2$(5) ), 1.63(m, 2H, CH$_2$(2)), 1.37(m, 4H, CH$_2$(3,4) ). FAB-MS; m/z 773.4. **7**: $^{1}$H-NMR in CDCl$_3$; 8.81(d, 8H, py-H), 8.43(m, 3H, 6-H), 8.22(m, 3H, 3-H), 8.05(m, 3H, 4-H), 7.75(m, 11H, Ph-H), 7.35(m, 8H, Ph-H), 6.96(m, 3H, 5-H), 3.87(t, 2H, CH$_2$), 1.73(t, 2H, CH$_2$), 0.98(m, 2H, CH$_2$), 0.92(m, 2H, CH$_2$), 0.47(m, 4H, CH$_2$), -2.69(s, 2H, NH). UV-vis. in CH$_2$Cl$_2$ (nm); 645, 590, 549, 515, 418. **8**: $^{1}$H-NMR in CDCl$_3$; 79(8H, py-H), 11.5-13.5(8H, m-H), 6.0(4H, p-H). FAB-MS; m/z 1165.6, cal. 1065.32. UV-vis. in CH$_2$Cl$_2$ (nm); 692, 580, 510, 417, 380. **1**: $^{1}$H-NMR in CDCl$_3$; 79(8H, py-H), 11.8-14.5(8H, m-H), 6.2(p-H). FAB-MS; 1129.4, cal. 1129.24. UV-vis. in CH$_2$Cl$_2$ (nm); 634, 578, 511, 417, 383.

9 The small peaks corresponding to the positive cation that one chloride anion dissociates was also observed at m/z = 1164.