Formation and Characterization of $Pz_3Cu(II)$ - Fe(III) Porphyrin Complex as a Structural Model of Cytochrome *c* Oxidase

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(Received May 8, 1995)

Porphyrins with tripyrazolyl ligand (Pz_3) can form a heteronuclear complex to take advantage of the difference of the coordination strength between two ligands. We synthesized an iron(III) porphyrin-copper(II) complex, which was characterized by means of UV-vis, ESR and MS spectroscopies in comparison with the parent iron(III) porphyrin.

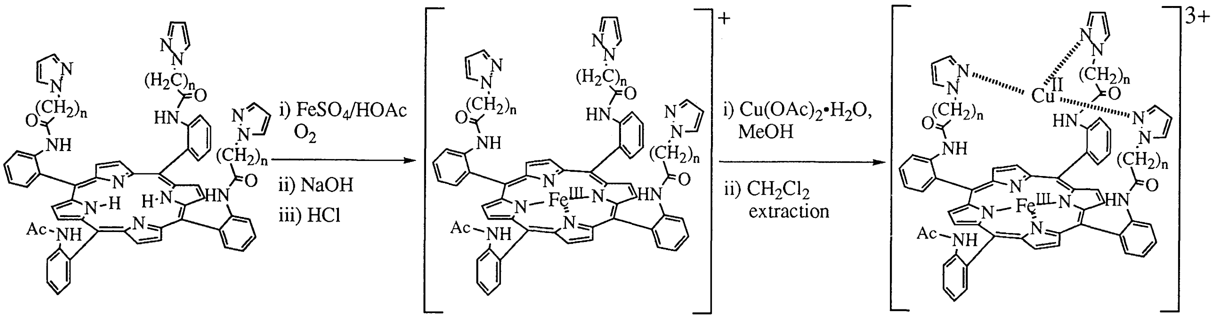
Cytochrome *c* oxidase is the terminal oxidase at the aerobic respiratory chain and catalyzes the four-electron reduction of dioxygen to water. The active site of this enzyme contains a dinuclear Fe/Cu site (heme a_3 - Cu_B),[1] and these transition metal ions couple with the reduction process of O_2. The redox process of the dioxygen was proposed by means of time-resolved Raman spectroscopies as well as the absorption spectra and other kinetic studies.[2] To demonstrate the reaction, the construction of an artificial modeling system is essential. At the present stage, there have been reported a few examples of Cu-heme complexes.[3]

We synthesized the porphyrins bearing tripyrazolyl ligand, which can form heteronuclear complexes to take advantage of the difference of the coordination strength between two ligands. In the natural system, Cu_B is suggested to be held three histidyl nitrogen donors.[1] To realize optimum coordination of Cu^{2+} at the appropriate position on a heme, we chose two different methylene linkages connecting between a porphyrin and pyrazolyl ligands. The designed ligands $\mathbf{C_1}(H_2)$-non and $\mathbf{C_2}(H_2)$-non[4] were prepared by coupling between *meso*-tetra(α,α,α,α-*o*-aminophenyl)porphyrin and the corresponding pyrazolyl carboxylic acids[5,6] in 42 and 33% yields, respectively, with use of EDC-HOBT.

Formation constants of the copper(II) complexes of $\mathbf{C_n}$(Fe)-non in CH_2Cl_2 at 25 °C were measured by means of spectrophotometric titration. Figure 1 shows the UV-vis spectral change of $\mathbf{C_n}$(Fe)-non during the addition of Cu^{2+} (as $CuCl_2$) in methanol. Monotonous spectral change was observed during titration of $\mathbf{C_1}$(Fe)-non indicating isosbestic points at 408.0, 504.5, 541.5, and 653.0 nm. The resultant Soret band showed blue shift from 416.0 to 411.0 nm. The formation constant of $\mathbf{C_1}$(Fe)-Cu was determined to be $K_1=1.04 \times 10^6$ M based on absorbance at 416.0 nm. The same titration of $\mathbf{C_2}$(Fe)-non indicated isosbestic points at 409.0, 509.5, 545.5 and 671.5 nm and the formation constant was obtained as $K_1=1.37 \times 10^5$ M. Thus, $\mathbf{C_1}$(Fe)-Cu is more stable than the $\mathbf{C_2}$ analog.

Mass spectra of the resultant $[\mathbf{C_n}(Fe)\text{-}Cu]^{3+}$ complexes[7] exhibited peaks at the mass numbers corresponding to the Cu derivative; $m/z = 1157$ for $[\mathbf{C_1}(Fe)\text{-}Cu]^{3+}$ and 1199 for $[\mathbf{C_2}(Fe)\text{-}Cu]^{3+}$ (Scheme 1). The former complex gave an additional MS peak at $m/z = 1194$, which was assigned to be $[\mathbf{C_1}(Fe)\text{-}CuCl]^{2+}$. The simulated mass peaks in consideration of the naturally abundant isotopes were well accordance with the observed ones. Thus, we concluded the incorporation of the Cl^- ion as a bridging ligand between two metal ions in $[\mathbf{C_1}(Fe)\text{-}CuCl]^{2+}$. The similar μ-chloro bond between Fe^{3+} and Cu^{2+} ions has been reported in nicotinamide linked FeTPP system.[3a] In our instance, the MM+ calculation[8] of the complex indicated that the Fe-Cu separation was estimated to be around 4.88 Å, which is shorter than that of the relating one (4.96 Å)[3a] and is likely to be more stable. On the contrary, the Fe-Cu separation in $[\mathbf{C_2}(Fe)\text{-}Cu]^{3+}$ was also calculated to be 5.02 Å, which could be too long to form μ-chloro bonding and this complex would not give the corresponding molecular ion ($m/z = 1236$) in MS.

The formation of the Cu(II) complexes was further evidenced by ESR (CH_2Cl_2, 10K). The spectrum of $\mathbf{C_1}$(Fe)-non shows the signal $g_\perp = 6.2$ and $g_{//} = 2.0$ corresponding to high spin porphyrin Fe(III) complex (Figure 2). $\mathbf{C_1}$(Fe)-Cu exhibits the typical S = 1/2 copper(II) spectrum ($g = 2.2$) in the tetragonal field with three nitrogen and one Cl^- ligands.[3b,9] However, any distinctive spectral change was not seen in $\mathbf{C_2}$(Fe) species. The copper(II) ion in this complex would dissociate during the preparation of the ESR sample. We



$[\ C_n(Fe)\text{-non}\ ]^+Cl^-$

n=1 : m/z = 1094
n=2 : m/z = 1136

$[\ C_n(Fe)\text{-}Cu\ ]^{3+}Cl^- \cdot 2AcO^-$

n=1 : m/z = 1157, 1194
n=2 : m/z = 1199

Scheme 1.

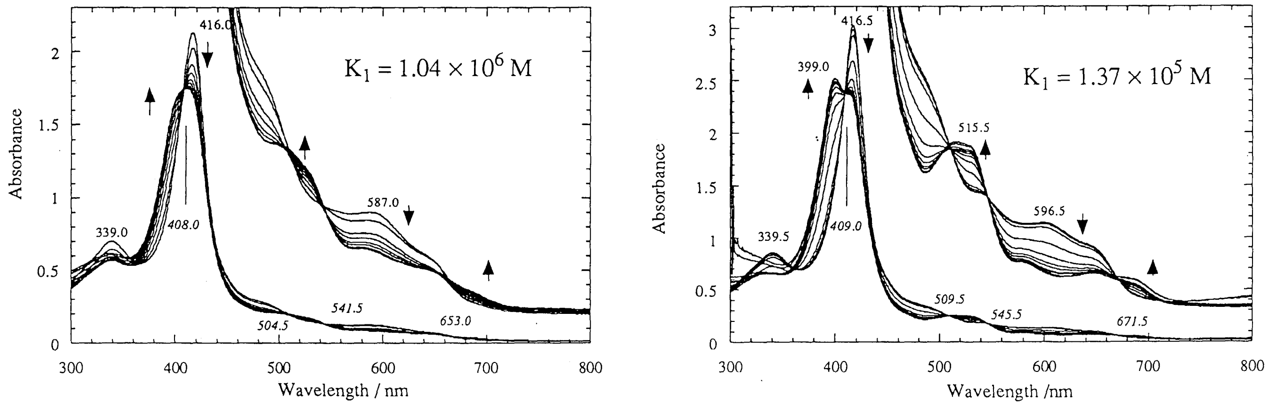


Figure 1. Spectrophotometric titration of (a) C_1(Fe)-non and (b) C_2(Fe)-non with copper(II) chloride in methanol (T= 25 °C). Wavelengths of isosbestic points are shown in italic.

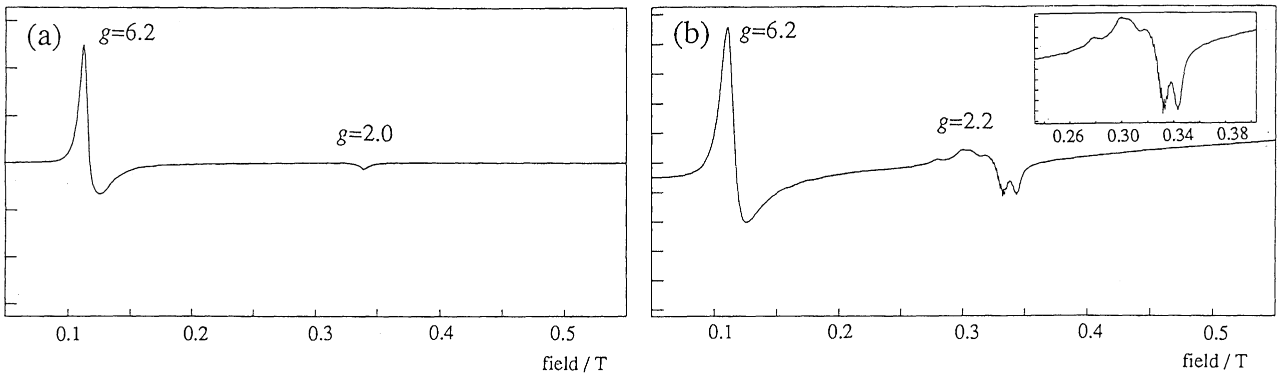


Figure 2. X-Band ESR spectra of (a) C_1(Fe)-non and (b) C_1(Fe)-Cu in CH_2Cl_2 at 10 K. Microwave frequency : 9.47 GHz.

optimized the formation of a heme-Cu complex by changing the chain length of the linker molecule in the tris-pyrazolylphenyl porphyrin.

References and Notes

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4 $C_1(H_2)$-non: ^1H-NMR ($CDCl_3$) 8.83-8.64 (8H, pyrrole β), 8.72-7.14 (16H, phenyl), 7.92-6.38 (9H pyrazole ring), 4.69-4.41 (amide NH), 4.29-4.13 (6H, -CH_2-), 1.02 (3H, Ac), -2.56 (2H, pyrrole NH); MS *m/z* 1041 $(M + H^+)$. $C_2(H_2)$-non: ^1H-NMR ($CDCl_3$) 9.03-8.80 (8H, pyrrole β), 8.68-7.04 (16H, phenyl), 7.96-7.14 (pyrazole ring), 6.62-5.72 (amide NH), 3.91-3.73 (6H, -CH_2-), 1.93-1.84 (6H, -CH_2-), 1.17 (3H, Ac), -2.72 (2H, pyrrole NH); MS *m/z* 1083 $(M + H^+)$.

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7 The insertion of copper(II) was achieved as follows. Each of C_1(Fe)-non and C_2(Fe)-non was dissolved in CH_2Cl_2. To this solution, excess amount of copper(II) acetate in methanol was added and the solvent was stripped to dryness. The residue was redissolved in CH_2Cl_2 and the residual solid was removed by filtration. Hexane was added to the solution and evaporated to give iron(III) porphyrin-copper(II) complex C_n(Fe)-Cu quantitatively (Scheme 1).

8 Molecular mechanics calculation of these complexes was performed using MM+ package inside *HyperChem*™.

9 This spectrum showed remarkable temperature dependency and dipolar relaxation broadening caused the disappearance of the signals above 120 K.