# Synthesis and Characterization of an Iron Porphyrin with a Thiolate Axial Ligand and Its Dioxygen Adduct as a Model for the Active Center of Cytochrome P-450

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(Received April 19, 1999; CL-990305)

Synthesis and characterization of an iron porphyrin axially-coordinated by thiolate were carried out in order to mimic the active center of cytochrome P-450. $O_2$ binds reversibly to the ferrous form at -80 °C. The oxygenated complex showed a $\nu$(Fe-$O_2$) band at 578 cm$^{-1}$ in the resonance Raman spectrum.

Despite intensive studies of cytochrome P-450,[1] the roles of the thiolate ligand on the oxygen activation as well as the molecular mechanism of O-O bond cleavage have not been fully elucidated. Several thiolate-ligated iron pophyrins[2] have been synthesized as models with a view to clarifying the effect of the thiolate ligand. However, in some previous models,[2a-c] unavoidable autoxidation of Fe$^{3+}$-S$^-$ and/or Fe$^{2+}$-S$^-$ complexes under aerobic conditions has made their detailed study difficult. There have been only a few oxygen adducts of thiolate-ligated model hemes.[3] A model applicable for $O_2$ binding/activation study has to fulfill the following structural strict criteria; both $O_2$-binding and thiolate ligand sites should be sterically protected against irreversible autoxidation of both iron and thiolate.

In order to mimic and evaluate the roles of the coordination of the thiolate, we designed and synthesized a novel thiolate-ligated P-450 model complex **11**. We also observed the reversible binding of dioxygen to the ferrous form.

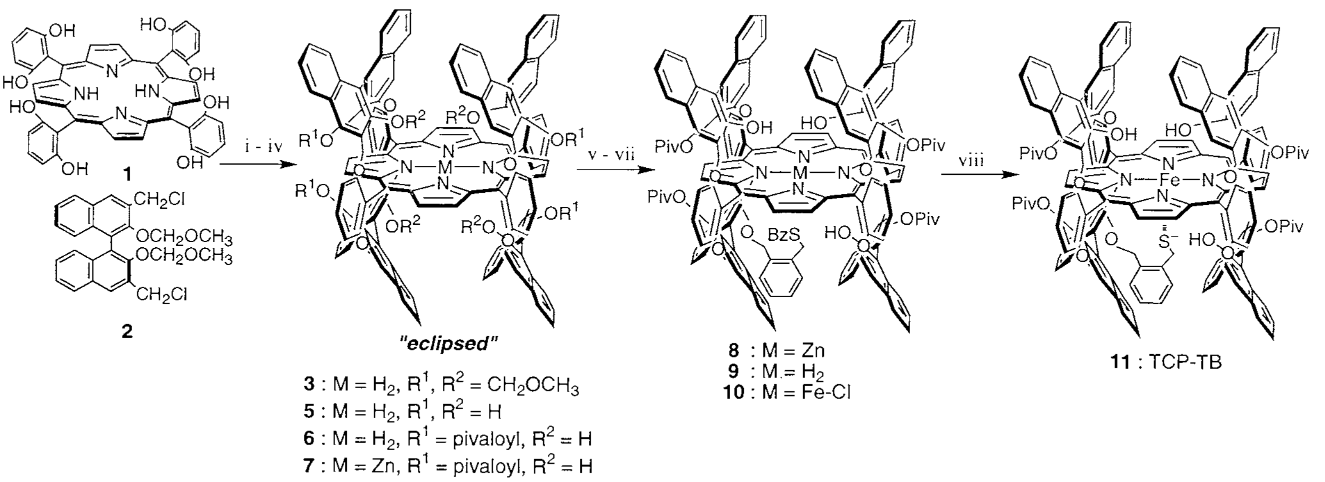
In the present model complex, there exist hydrophobic cavities surrounded by bulky chiral binaphthalene moieties on the both sides of the heme plane. In one cavity, the thiolate group, which is sterically protected from undesirable aerobic oxidation, axially ligates to the central iron.

The strategy for the preparation of the objective model complex is to introduce a thiolate moiety into one of the cavities above tetraarylporphyrin modified with binaphthalene auxiliaries ("twin-coronet" porphyrin).[4] Its major framework is formed by bridging between the ortho positions of the adjacent *meso*-phenyl rings with the binaphthyl derivatives through ethereal linkages.

The reaction of methoxymethyl (MOM)-protected (*S*)-binaphthalene derivative **2** and *meso*-tetrakis(2,6-dihydroxyphenyl)porphine **1** gave the two isomeric "twin-coronet" porphyrins, eclipsed **3** and staggered **4**.[5] The MOM protecting groups of the eclipsed isomer **3** were removed under acidic conditions. The selective reprotection of the outside four hydroxyl groups was accomplished with bulky pivaloyl chloride/pyridine. 2-(Iodomethyl)benzyl thiobenzoate as a designed thiolate moiety was fixed onto one of the inside hydroxyl groups of the zinc complex **7**. Finally, the selective deprotection of the thioester group in **10** was achieved with $BuNH_2$ by way of thioester-amide exchange to afford the thiolate-ligated complex **11** (TCP-TB; Twin Coronet Porphyrin with a ThioBenzyloxy group),[6] which showed enough stability to usual manipulation under air.

ESR spectrum of **11** (15 K, toluene) showed a typical signal of six-coordinate low spin ferric heme ($g$ = 2.28, 2.20, 1.98). By Bohan's method,[7] crystal field parameters, tetragonality $|\mu/\lambda|$ and rhombicity $|R/\mu|$, were determined to be 9.29 and 0.326, respectively, from the three $g$ values. According to the classification of donor ligands based upon the crystal field parameters,[8] these values are in the range of six-coordinate low-spin ferric hemes with thiolate anions as axial ligands.

Tetragonality indicates the electron density of Fe ion. The present tetragonality value is much larger than the reported ones.[8] It means very strong electron-donation to the iron mainly from the thiolate ligand and also from the porphyrin macrocycle. The latter ligand is considered to be also fairly electron-donating on account of its eight alkoxy substituents on the *meso*-phenyl rings.



**Scheme 1.** Reagents and conditions : i, $K_2CO_3$, THF, NMP, 110 °C, 19%; ii, Pyridinium *p*-toluenesufonate, 2-butanone, 75 °C, 45%; iii, Pivaloyl chloride, pyridine, $CH_2Cl_2$, 97%; iv, Zn(OAc)$_2$, 72%; v, 2-(Iodomethyl)benzyl thiobenzoate, $K_2CO_3$, NMP, 100 °C, 68%; vi, 4*N*-HCl, 62%; vii, Fe(CO)$_5$, $I_2$, toluene, 50 °C, 78%; viii, $BuNH_2$, $CH_3CN$, 48%.

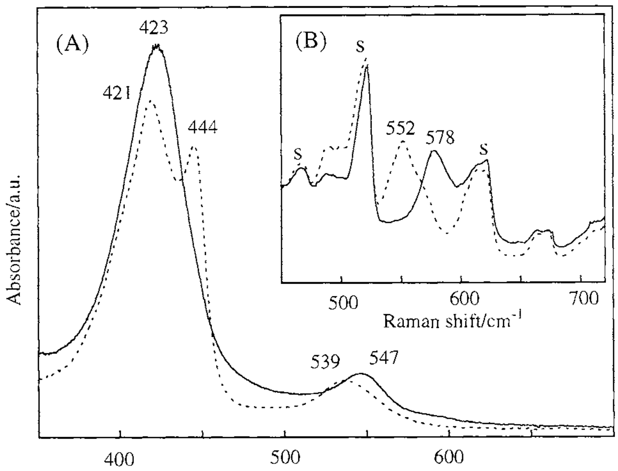


**Figure 1.** (A) UV-vis spectra of ferrous **11** and its $O_2$ adduct (−80 °C, toluene). Solid line : $O_2$ complex; broken line : ferrous **11**. (B) Low frequncy region of resonance Raman spectra of the dioxygen adducts (−80 °C, toluene). Solid line : $+^{16}O_2$; broken line : $+^{18}O_2$. s: Solvent peaks. Ex. 413.1 nm; power 40 mW.

The reduction potentials of **11** was determined by means of cyclic voltammetry (Pt, $CH_3CN$, 0.1 M $n$-$Bu_4NBF_4$). Quasi-reversible voltammogram was obtained to show Fe(III)/Fe(II) and Fe(II)/Fe(I) couples at -1.31 and -1.80 V (vs. $Ag/Ag^+$), respectively. Compared to those of FeTMPCl[9] (-0.64 and -1.45 V), the largely negative-shifted potentials of **11** also indicated the strong electron donation from the thiolate and the porphyrin ligands to the iron atom, as shown above by ESR.

The UV-vis spectrum of ferrous **11** showed a split Soret band ($\lambda_{max}$ = 421, 444 nm) and a single visible band ($\lambda_{max}$ = 539 nm) in a non-polar solvent such as toluene (Figure 1A). This spectrum is quite similar to that of the four-coordinate Fe(II)TPP.[10] It means that the sulfur atom, which is electron-rich itself, has low affinity toward the reduced Fe ion. The CO adduct of ferrous **11** exhibited a single sharp Soret band at 425 nm, and a Q band at 531 nm. This spectrum is almost identical with those of six-coordinate Fe(II)-CO complexes with a thiol axial ligand,[11] and different from the unique hyperporphyrin spectrum for the CO adducts of thiolate-hemes such as P-450. This result suggested that in the CO adduct of **11**, the sulfur atom could coordinate to iron as a thiol form rather than a thiolate one.[12]

Reversible binding of dioxygen to ferrous **11** was confirmed by UV-vis spectroscopy at -80 °C in toluene (Figure 1A). After oxygenation, the substitution with $N_2$ led to the complete recovery of the spectrum of the four-coordinate ferrous form. The oxygen adduct had absorption maxima at 423 and 547 nm. At temperatures above -80 °C, both the oxygen adduct and the four-coordinate ferrous complex were observed. Autoxidation of the ferrous form to the ferric one was significantly slow. The half-life time of the ferrous species was over 1 h even at room temperature.

In the resonance Raman spectrum of the oxygen adduct (Figure 1B), an oxygen-sensitive band was observed at 578 cm$^{-1}$ ($\Delta_{obs}(^{16}O_2/^{18}O_2)$ = 26 cm$^{-1}$; $\Delta_{calc}$ = 26 cm$^{-1}$), which was assignable to $\nu$(Fe-$O_2$). The $\nu_4$ band of the heme core was detected at 1370 cm$^{-1}$ in the high frequency region, but no band of $\nu$(O-O) was found. The present frequency of $\nu$(Fe-$O_2$) was close to those of oxygen adducts with neutral axial ligands (560-580 cm$^{-1}$),[13] and was much higher than those of the thiolate-ligated oxy-hemes (530-540 cm$^{-1}$)[13] and of the relating five-coordinate ones (509 or 525 cm$^{-1}$).[14] Thus, the neutral thiol group could be the sixth axial ligand as observed in the CO adduct.

In conclusion, the validity of the present molecular design for the sufficient protection of the thiolate ligand was demonstrated by the very high stability of **11** toward dioxygen. The ferrous complex showed reversible binding of dioxygen without any oxidative deterioration of the thiol group. Further characterization of the oxygen adduct and preparation of an improved similar model complex are now under way.

This research was financially supported by Grant-in-Aids for COE Research (#08CE2005, to Y. N.), for Scientific Research on Priority Areas (#09235225, to Y. N.) and for Encouragement of Young Scientists (#08740500, to F.T) from the Ministry of Education, Science and Culture, Japan, and by Research Grants to Y. N. from Sumitomo Foundation and to F.T. from Takeda Science Foundation.

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5 The staggered isomer **4** is omitted in Scheme 1, in which compound the positions of the binaphthalene-bridges are different between the upper and lower sides of the heme plane. See Reference 4a.

6 Details of the procedure for the preparation of **11** will be reported elsewhere. **11**: High MS (FAB, NBA) *m/z* Found 2508.7866, Calcd. for $C_{160}H_{124}N_4O_{20}SFe$ 2508.7879; UV-vis ($CH_2Cl_2$, r.t.) $\lambda_{max}$ ($10^{-3}$ $\varepsilon$, dm$^3$mol$^{-1}$cm$^{-1}$) = 326 (32.8), 338 (31.3), 416 (Soret, 61.8), 515 (9.83) and 657 nm (2.69).

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11 The five-coordinate CO adducts of hemes exhibited Soret bands at shorter region, ca. 412 nm, than the six-coordinate ones, ca. 420 nm. Thus, the sixth ligand in the present CO complex is concluded to be the thiol. See Reference 2b.

12 Under CO atmosphere, the addition of various bases to ferrous **11** for deprotonation did not yield a hyperporphyrin spectrum, presumably because of the steric and electronic factors of the thiol group.

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