Hydrogen-Bonded Dioxygen Adduct of an Iron Porphyrin with an Alkanethiolate Ligand: An Elaborate Model of Cytochrome P450**

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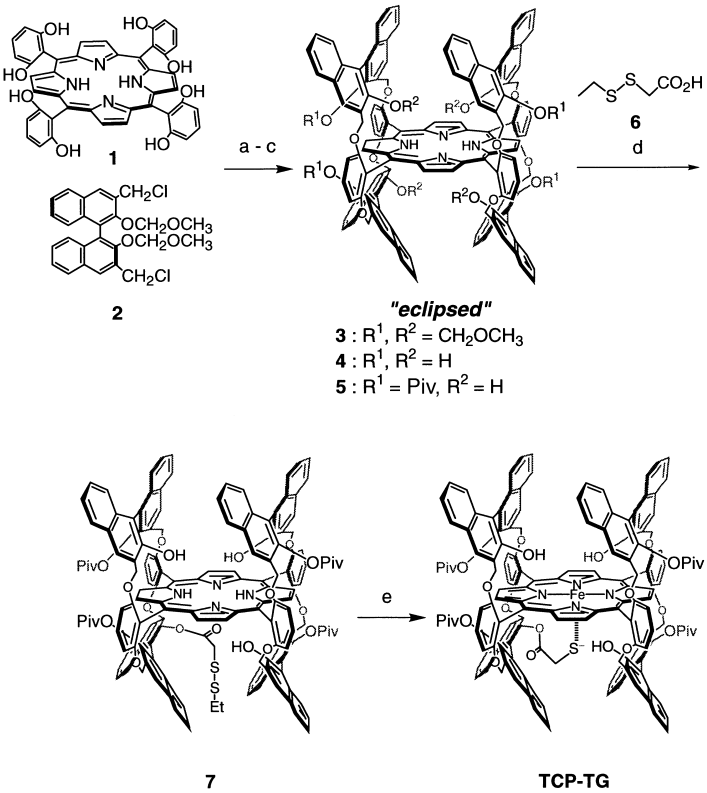
It has been postulated that the reductive activation of dioxygen by cytochrome P450 is assisted mainly by the peripheral amino acids.[1] In the proximal site of cytochrome $P450_{cam}$,[2] for example, the electron-donating thiolate group of Cys357 coordinates axially to iron to increase the electron density on this center. In the distal site, Thr252, Asp251, and water molecules make a hydrogen bonded network with the bound oxygen atom to stabilize the oxy-intermediate and also to relay protons to the terminal oxygen atom.[3] These cooperative functions, the push–pull effect,[4] would enhance the heterolysis, rather than the homolysis, of the putative peroxo moiety to generate a final oxygenating species. However, the molecular mechanism of O–O bond cleavage and the roles of the peripheral amino acids have not been elucidated fully, in spite of intensive studies of both the enzymes and the models. Several hemes with thiolate ligands have been prepared as models of cytochrome P450,[5] but in some previous models, oxidative decomposition of the complexes was unavoidable. There have been only a few examples of dioxygen adducts modeled with thiolate axial ligands[6] and in these models a hydrogen bond to the bound dioxygen has never been observed. In the known adducts,[6b] the non-natural arenethiolate ligands used strongly electron-withdrawing groups for the stabilization, instead of alkanethiolate ligands as in the natural enzymes. A close model applicable for the evaluation of the push–pull effect for oxygen activation requires an advanced structural modification of a porphyrin compound: on the proximal side, an alkanethiolate ligand should be protected sterically from undesirable aerobic oxidation, and on the distal side there should be an oxygen-binding pocket with protic residues in an appropriate position.

In order to mimic the functions of the thiolate coordination and the hydrogen bond, we designed and synthesized a novel cytochrome P450 model heme **TCP-TG** (Scheme 1), a *T*win-*C*oronet *P*orphyrin with a *T*hioglycolate group. This model compound forms a stable adduct with dioxygen.

In **TCP-TG**, there exist two hydrophobic cavities surrounded by bulky chiral binaphtyl moieties on both sides of the heme plane. In one of the cavities, the alkanethiolate group coordinates axially to the central iron. In the other side, two



Scheme 1. Synthesis of **TCP-TG**: a) K_2CO_3, THF, *N*-methylpyrrolidone (NMP), 110 °C, 19%; b) trimethylsilyl bromide, CH_2Cl_2, −40 °C, 96%; c) pivaloyl chloride (PivCl = *t*BuCOCl), pyridine, CH_2Cl_2, 97%; d) **6**, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride, 4-dimethylamino-pyridine, CH_2Cl_2, 36%; e) $[Fe(CO)_5]$, I_2, toluene, 50 °C, 41%.

naphtholic hydroxyl groups, which are oriented toward the center of the cavity above the heme, are expected to form a hydrogen bond to bound dioxygen.

The strategy for the synthesis of **TCP-TG** (Scheme 1) is to fix the thiolate moiety into one of the cavities of **5**[7] by a covalent bond. The major framework of the binaphtyl-bridged porphyrin (a "twin-coronet" porphyrin)[8] was prepared by the condensation of *meso*-tetrakis(2,6-dihydroxylphenyl)porphine **1** with the binaphthyl derivative **2** under basic conditions. Only the eclipsed isomer **3**, which is one of the two isomeric products of the condensation reaction,[9] was further converted in the following reactions. The methoxymethyl protecting groups were removed in excellent yield (96%) under acidic conditions. The selective reprotection of the outer four hydroxyl groups of **4** was accomplished with bulky pivaloyl chloride. Ethyldisulfanylacetic acid **6**, a EtS-protected thiolate moiety, was connected to one of the inner hydroxyl groups of **5** through the ester linkage. Finally, the iron ion insertion and the deprotection of the thiolate group were achieved simultaneously to yield the thiolate-coordinated heme **TCP-TG**, which exhibited enough stability for usual manipulation under aerobic conditions. **TCP-TG** was characterized by means of HR-FAB-MS, UV/Vis and ESR (Table 1).

The reduction potential of **TCP-TG** was determined by cyclic voltammetry (Pt, CH_3CN, 0.1 M Bu_4NBF_4). The reversible voltammogram obtained shows the Fe^{II}/Fe^{III} redox couple at −1.03 V (versus Ag/Ag^+). Compared to that of

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Table 1. Selected physical and spectroscopic data for **TCP-TG** and **6**–**9**.

6: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.49$ (s, 2H, SCH_2CO), 2.79 (q, $J = 7.3$ Hz, CH_2), 1.33 (t, $J = 7.3$ Hz, CH_3); HR-MS ($C_4H_8O_2S_2$): calcd: 151.9966; found: 151.9977; elemental analysis: calcd: C 31.56, H 5.30; found: C 31.49, H 5.32.

7: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.82$ (d, $J = 4.4$ Hz, 1H, pyrrole β-H), 8.79 (d, $J = 4.9$ Hz, 1H, pyrrole β-H), 8.77 (d, $J = 4.4$ Hz, 1H, pyrrole β-H), 8.76 (d, $J = 4.9$ Hz, 1H, pyrrole β-H), 8.62 (d, $J = 4.9$ Hz, 1H, pyrrole β-H), 8.55 (d, $J = 4.4$ Hz, 1H, pyrrole β-H), 8.30 (d, $J = 4.4$ Hz, 1H, pyrrole β-H), 8.27 (d, $J = 4.9$ Hz, 1H, pyrrole β-H), 7.88–6.50 (m, 46H, arom H), 6.43 (d, $J = 8.8$ Hz, 1H, arom H), 6.34 (d, $J = 8.3$ Hz, 1H, arom H), 6.10 (d, $J = 8.3$ Hz, 1H, arom H), 6.08 (d, $J = 5.9$ Hz, 1H, arom H), 5.87 (m, 2H, arom H), 5.34–4.70 (m, 11H, benzyl CH_2), 4.34 (d, $J = 14.2$ Hz, 1H, benzyl CH_2), 4.22 (d, $J = 13.7$ Hz, 1H, benzyl CH_2), 4.21 (d, $J = 13.2$ Hz, 1H, benzyl CH_2), 2.66 (d, $J = 13.2$ Hz, 1H, benzyl CH_2), 2.52 (d, $J = 14.2$ Hz, 1H, benzyl CH_2), 0.73 (s, 9H, piv CH_3), 0.68 (s, 9H, Piv CH_3), 0.33 (s, 9H, piv CH_3), 0.23 (s, 9H, piv CH_3), −0.44 (m, 1H, SCH_2CH_3), −0.50 (t, $J = 6.8$ Hz, 3H, SCH_2CH_3), −1.20 (m, 1H, SCH_2CH_3), −2.15 (d, $J = 18.6$ Hz, 1H, $COCH_2S$), −2.72 (d, $J = 18.6$ Hz, 1H, $COCH_2S$), −2.94 (s, 2H, NH); UV/Vis (CH_2Cl_2): λ_{max} ($\varepsilon 10^{-3}$) = 325 (19.1), 338 (18.3), 402 (39), 422 (211.6), 518 (11.8), 587 (4), 640 nm (1.4 $M^{-1}cm^{-1}$); IR (neat): $\tilde{\nu} = 3458$, 3333, 3305, 3056, 2958, 2926, 2875, 2853, 1747, 1585, 1457, 1367, 1260, 1231, 1110, 1082, 1025, 796, 748, 719 cm^{-1}; HR-MS ($C_{156}H_{125}O_{21}N_4S_2$): calcd: 2453.8278; found: 2453.8284.

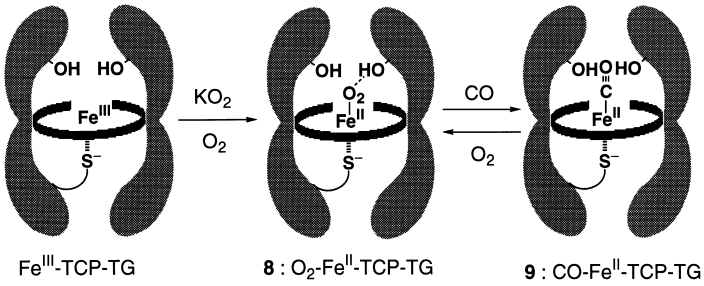
TCP-TG: UV/Vis (CH_2Cl_2): λ_{max} ($\varepsilon 10^{-3}$) = 325 (18.2), 339 (18.7), 363 (17.1), 418 (31.6), 513 (5.8), 575 (2.7), 658 nm (1.7 $M^{-1}cm^{-1}$); IR (neat): $\tilde{\nu} = 3453$, 3062, 2957, 2927, 2873, 1746, 1585, 1456, 1367, 1260, 1229, 1111, 1085, 997, 788, 747, 719 cm^{-1}; ESR (CH_2Cl_2, 7 K): $g = 5.73$, 2.37, 2.26, 2.00, 1.94; HR-MS ($C_{154}H_{118}O_{21}N_4SFe$): calcd: 2446.7359; found: 2446.7417; elemental analysis ($C_{154}H_{117}FeN_4O_{21}S \cdot 8H_2O$): calcd: C 71.37, H 5.17, N 2.16; found: C 71.66, H 5.49, N 1.94.

8: UV/Vis (CH_2Cl_2): λ_{max} (%) = 339 (37), 421 (100), 544 (6), 629 (4).

9: UV/Vis (CH_2Cl_2): λ_{max} (%) = 366 (28), 444 (100), 537 (5).

iron *meso*-tetramesitylporphyrin chloride (−0.64 V), the strongly negatively shifted potential of **TCP-TG** indicates the strong electron donation from the thiolate ligand to the iron atom.

The dioxygen adduct **8** was obtained in the reaction of Fe^{III}–**TCP-TG** with an equimolar amount of KO_2[10] under an oxygen atmosphere (Scheme 2). Addition of KO_2 to the THF



Scheme 2. The formation of the hydrogen bonded dioxygen adduct **8** and CO adduct **9**.

solution of Fe^{III}–**TCP-TG** at −80 °C induced immediately a drastic change in the UV/Vis spectrum. The Soret band ($\lambda_{max} = 424$ nm) decreased in intensity and blue shifted slightly to 421 nm. The Q band absorption ($\lambda_{max} = 530$ nm) greatly diminished. The oxy form transformed into the corresponding CO adduct **9** on exposure to CO, which exhibited a typical hyperporphyrin spectrum with split Soret bands at 366 and 444 nm. This process was reversible. Thus, the Fe^{II} state and the axial coordination of the thiolate are maintained under these conditions without any autoxidation or other oxidative decomposition. The resonance Raman spectrum of the dioxygen adduct exhibited a strong band at 1137 cm^{-1}. By replacing $K^{16}O_2$ and $^{16}O_2$ with $K^{18}O_2$ and $^{18}O_2$, this band shifted to 1073 cm^{-1} (Figure 1). The observed isotopic effect

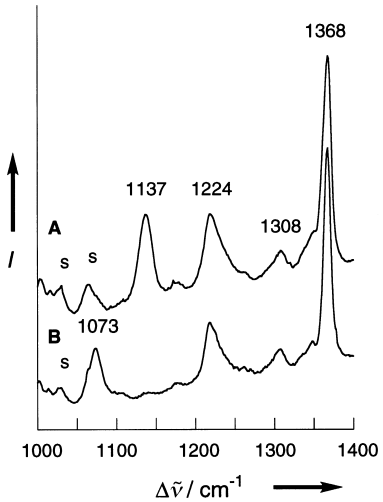


Figure 1. High-frequency region of the resonance Raman spectra of the dioxygen adduct (THF/CH_3CN, −80 °C, $\lambda_{ex} = 413.1$ nm, 20 mW). A: $K^{16}O_2 + {}^{16}O_2$; B: $K^{18}O_2 + {}^{18}O_2$.

(64 cm^{-1}) upon $^{18}O_2$ substitution is in good agreement with the value (65 cm^{-1}) calculated from the harmonic oscillator approximation of the O–O stretching vibration. The 1137 cm^{-1} band is therefore assigned to this ν(O-O) mode. The observed frequency of $\tilde{\nu}$(O-O) is very close to those of oxy-cytochrome $P450_{cam}$ (1140 cm^{-1})[11] and model oxygen adducts (1140, 1147 cm^{-1}).[12] No specific band involving the oxygen ligand was detectable in the low-frequency region with Soret excitation ($\lambda_{ex} = 413.1$, 427.0, 441.6 nm). The Raman active ν(O-O) stretch mode has been known to be characteristic of the dioxygen adduct of thiolate-coordinated heme. In contrast, the ν(O-O) stretch mode of oxy-heme with the nitrogen base as an axial ligand is Raman inactive.[13] A thiolate ligand increases the electron density of the porphyrin π* orbital through the p_π and d_π orbitals to induce the charge-transfer transition from the π* to the empty Fe–O σ* orbital in the oxy form. This leads to the enhancement of ν(O-O) band.[14] Therefore, the present results in the resonance Raman spectra reveal the successful formation of the desired dioxygen adduct of the thiolate-coordinated heme.

This dioxygen adduct was stable even at 0 °C. We assumed that an intramolecular hydrogen bond between the bound oxygen and the hydroxyl groups of the binaphtyl moieties was mainly responsible for its higher stability. To verify this prediction, we investigated the frequency of the O–O stretching mode of the dioxygen complex upon deuterium substitution of the exchangeable protons. The Fe^{III}–**TCP-TG** remained in CH_3OH/CH_3CN or CD_3OD/CH_3CN for several hours and then dried in vacuo overnight, then the resonance Raman spectra were measured. Only the $\tilde{\nu}$(O-O) mode shifted by 2 cm^{-1} to a higher frequency on exchange of CH_3OH by CD_3OD (Figure 2). No other vibrations exhibited

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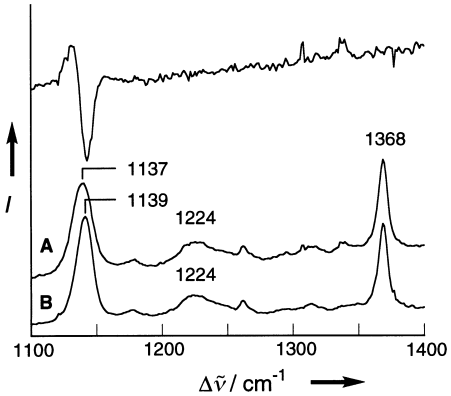

Figure 2. A selected region of the resonance Raman spectra of the dioxygen adduct in CH_3OH (Spectrum A) and in CH_3OD (Spectrum B) (THF/CH_3CN, $-80\,^{\circ}C$, $\lambda_{ex} = 413.1$ nm, 20 mW); the trace above represents the difference spectrum (A – B).

any frequency shifts, which means that there is no perturbation in the porphyrin skeleton on the H/D exchange. Using resonance Raman spectroscopy, Kitagawa and co-workers have already reported evidence for hydrogen bonded dioxygen to the distal histidine in oxycobalt myoglobin and hemoglobin.[15] Our present results agree closely with their observations: the frequencies of ν(O-O), measured in D_2O, were also $2-5\ cm^{-1}$ higher than those measured in H_2O. We therefore conclude that the bound oxygen in **TCP-TG** interacts with the adjacent exchangeable protons and that the dioxygen adduct of **TCP-TG** is stabilized by hydrogen bonds between the dioxygen and the inner hydroxyl groups, as predicted in the molecular design of **TCP-TG** (Scheme 2).

It has been firmly established that hydrogen bonds to bound dioxygen stabilize the oxy-complexes of globins and their model complexes with a nitrogen base as an axial ligand.[16] An analogous hydrogen bond has never been confirmed in cytochrome P450. To the best of our knowledge, the present complex **TCP-TG** shows the first direct evidence for a hydrogen bond to dioxygen in a thiolate-coordinated oxyheme, which has significance for the dioxygen binding and activation in cytochrome P450.[17]

Experimental Section

TCP-TG: **7** (5.3 mg, 2.16×10^{-3} mmol) was dissolved in dry toluene (10 mL) and heated at $50\,^{\circ}C$ under N_2. $[Fe(CO)_5]$ (105 μL, 0.78 mmol) and a solution of I_2 in toluene (6.8 mg, 26.8×10^{-3} mmol) were added. The mixture was stirred overnight in the dark, quenched with water, and then extracted into CH_2Cl_2. After removal of the solvent and drying, the residue was purified by flash chromatography (Merck silica gel 60H, CH_2Cl_2). **TCP-TG** was obtained as a brown solid (3.6 mg, 1.47×10^{-3} mmol, 41 %).

Treatment of **TCP-TG** with KO_2: **TCP-TG** (0.2 mg, 8.2×10^{-5} mmol) was dissolved in THF (0.6 mL, 1.37×10^{-4} M). The solution was cooled to $-80\,^{\circ}C$ and a solution of KO_2 (50 μL, CH_3CN, 1.65×10^{-3} M), which was dissolved with [2.2.2]cryptand, was added into the porphyrin solution. The atmosphere was replaced with O_2. The resulting solution was characterized with UV/Vis absorption and resonance Raman spectroscopy.

Resonance Raman spectra were obtained on a SpectraPro-300i spectrometer (Acton Research Co.) with a 2400-groove grating, a Beamlok 2060 Kr ion laser (Spectra Physics), a holographic supernotch filter (Kaiser Optical), and a LN-1100PB CCD detector (Princeton Instruments) cooled with liquid N_2. Spectra were collected on solvated samples in spinning cells (2 cm diameter, 1500 rpm) at $-80\,^{\circ}C$ at an excitation wavelength $\lambda_{ex} =$ 413.1 nm (20 mW), 90° scattering geometry, and 5 min data accumulation. Peak frequencies were calibrated relative to indene and CCl_4 standards (accurate to $\pm 1\ cm^{-1}$). During each Raman experiment, UV/Vis spectra were simultaneously collected on a PMA-11 CCD spectrophotometer (Hamamatsu) with a Photal MC-2530 (D_2/W_2) light source.

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