Novel Iron Porphyrin−Alkanethiolate Complex with Intramolecular NH···S Hydrogen Bond: Synthesis, Spectroscopy, and Reactivity

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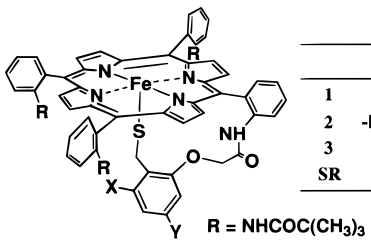
Among heme enzymes, cytochrome P450 and NO synthase (NOS) have strong oxidizing ability and unusual structure, in that their heme irons have thiolate coordination. Consequently, much interest has been focused on their structure−function relationship.[1] We have synthesized the first synthetic heme thiolate (**SR** complex[2]) which retains thiolate coordination during catalytic oxidation and have found several remarkable thiolate axial ligand effects.[2]

Recently, the presence of an NH···S hydrogen bond in the active site of P450 and NOS has been suggested, based on the analysis of their crystal structure.[3] Such a bond should markedly affect the chemistry of the heme thiolate. Ueyama and co-workers have reported synthetic structural models of heme arenethiolate with an NH···S hydrogen bond,[4] but their paper did not include data about the influence of the NH···S hydrogen bond on the catalytic activity of the heme thiolate.

We report here a novel iron porphyrin−alkanethiolate complex with an intramolecular NH···S hydrogen bond that we synthesized in order to examine the influence of the NH···S hydrogen bond on catalytic oxidation. Complex **1** was designed to form an NH···S hydrogen bond by introducing amide NH in the vicinity of the thiolate, while complexes **2** and **3** were designed not to form an NH···S hydrogen bond by replacing amide NH with N-methyl or by introducing acetamide in a position apart from the sulfur atom (Figure 1).[5]

Complexes **1**−**3** were characterized by FAB MS, IR, EPR, electronic absorption spectroscopy, resonance Raman spectroscopy, and X-ray crystal structure analysis. The absorption spectra of the ferrous−CO complexes of **1**−**3** exhibited typical hyperporphyrin spectra for a thiolate-ligated iron(II) porphyrin−CO complex (Table 1). The Soret band of the ferrous−CO complex of **1** (456 nm, which arises from a transition between the lone pair p orbital of the thiolate and the e_g orbital of heme) was considerably blue-shifted compared to that of the other complexes, indicating electron deficiency of thiolate in complex **1** arising from the NH···S hydrogen bond.



	X	Y
1	-NHCOCH$_3$	H
2	-N(CH$_3$)COCH$_3$	H
3	H	-NHCOCH$_3$
SR	H	H

Figure 1. Structures of complexes **1**−**3** and **SR**.

Table 1. λ_{max} of Absorption Spectrum and Fe^{III}/Fe^{II} Redox Couple (V) of **1**−**3** and **SR**

	λ_{max} (log ϵ) of absorption spectrum (nm)		Fe^{III}/Fe^{II} redox couple (V)[c]
	Fe^{III} [a]	Fe^{II}−CO[b]	
1	430 (5.04)	380 (4.77), 456 (5.08)	−0.41
	539 (4.06)	556 (4.01)	
2	429 (4.96)	391 (4.73), 465 (4.82)	−0.56
	537 (3.95)	558 (3.77)	
3	425 (5.10)	388 (4.78), 460 (4.94)	−0.53
	537 (4.04)	557 (3.86)	
SR	428 (5.03)	385 (4.76), 460 (4.94)	−0.52
	538 (3.98)	562 (3.77)	

[a] In dimethyl sulfoxide. [b] 30 min after the addition of $NaBH_4$ under a CO atmosphere in dimethyl sulfoxide. [c] In 0.1 M *n*-tetrabutylammonium perchlorate/CH_2Cl_2, at Pt electrode vs SCE reference.

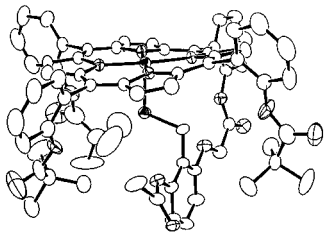


Figure 2. Molecular structure (ORTEP drawing; 25% probability level) of **1**. Fe and S atoms are shown as octant shading ellipsoids, N and O as octant ellipsoids.

The structure of **1** determined by X-ray crystal structure analysis[8] (Figure 2) shows that the hydrogen atom of the amide group is directed toward the thiolate sulfur atom, and the distances of N- - -S and NH···S and the angle of N−H···S are 3.423(4) Å, 2.800 Å, and 130.73°, respectively. These values indicate that **1** possesses an intramolecular NH···S hydrogen bond. The Fe−S and Fe−N bond distances are listed in Table 2 and are compared with those reported for some synthetic heme thiolates and P450

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(5) To prepare **1**−**3**, [(2-acetylthiomethyl-3-acetamido)phenoxy]acetic acid and other corresponding acids were combined with *meso*-α,α,α,α-tetrakis(*o*-aminophenyl)porphyrin,[6] and then complexes **1**−**3** were synthesized according to the same procedure as used for **SR** in our previous report.[2a]

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(7) The EPR spectra of complexes **1**−**3** showed low-spin signals of a single species, of which the g values indicate that the axial ligand is thiolate anion since their V/Δ values are 1.14−1.30, as obtained by the method of Taylor. Taylor, C. P. S. *Biochim. Biophys. Acta* **1977**, *491*, 137.

(8) Crystal data for **1**: monoclinic, $P2_1/n$, black needles, 0.025 × 0.020 × 0.3 mm, Cu Kα (λ = 1.541 84 Å), Rigaku R-AXIS RAPID Weissenberg IP System, a = 26.387(2) Å b = 17.436(1) Å c = 13.802(1) Å α = 90°, β = 101.650(2)°, γ = 90°, V = 6219.3(8) Å^3, Z = 4, 10 850 unique reflections (2θ max = 136°, completeness = 0.953), $R1$ = 0.0545 for 3058 reflections. In the crystal form, the O atom of the acetamide group ligates to the Fe atom of an adjacent molecule as a sixth ligand.

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Table 2. Selected Bond Distances and Fe−S Modes of Synthetic Heme Thiolates and Native P450s

	Fe−S (Å)	Fe−N (mean, Å)	νFe−S (cm^{-1})
1[a]	2.18	1.98	394
SR	2.20[b]	1.99[b]	366[a]
FeOEP(S−C_6H_5)[c]	2.30	2.06	
$P450_{cam}$ (substrate-bound)	2.20[d]	2.05[d]	350[e]
$P450_{terp}$ (substrate-free)[f]	2.15	1.96	

[a] This work. [b] The bond distances of **SR** were obtained by extended X-ray absorption fine structure spectroscopy.[2a] [c] Reference 9. [d] Reference 3a. [e] Reference 10. [f] Reference 3d.

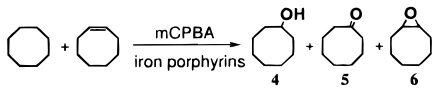
enzymes. Complex **1** is the first example of a synthetic *alkanethiolate*-ligated heme to which the crystal structure analysis has been applied. Bond distances of **1** are much closer to the corresponding ones of P450 enzymes than those of arenethiolate-ligated hemes, as shown in Table 2.

The results from the crystal structure analysis of **1** and EXAFS study of **SR** suggest that the Fe−S bond distance in **1** is shortened by the NH···S hydrogen bond. Therefore, we carried out a resonance Raman investigation to compare the bond distances between **1** and **SR** under the same solution conditions. To identify the Fe−S stretching modes in **SR** and **1**, we incorporated ^{34}S isotope into their thiolate sulfur. The νFe−S signal was examined at $\lambda_{ex} = 363.8$ nm using the same conditions as in the case of P450cam.[10] As shown in Table 2, the Fe−S mode in **1** is shifted to 394 cm^{-1}, compared with 366 cm^{-1} in **SR**. Therefore, it is confirmed that hydrogen bonding shortens the Fe−S bond distance in the case of alkanethiolate-ligated heme, just as previously reported for most of the thiolate non-heme complexes,[11] contrary to the result of the heme-*arenethiolates* reported by Ueyama et al.[4] These results can be explained as follows: NH···S hydrogen bonding shortens the metal−S bond length when the HOMO involves antibonding metal−S interaction.[4b,11] In the case of hemes in which the fifth ligand is a relatively weak electron donor such as Cl^- or ClO_4^-, the HOMO is on the porphyrin ligand.[12] However, when the fifth ligand is a strongly basic one, such as OH^- or CH_3O^-, the HOMO is not on the porphyrin ligand but on the central metal.[12] From these results, it is highly probable that the HOMO of **1** is on the central metal, owing to the highly basic and electron-donating alkanethiolate axial ligand, in contrast to the heme arenethiolates. Therefore, in the case of our complex **1**, NH···S hydrogen bonding shortens the Fe−S bond length, similar to the case of the non-heme complexes.

Also in support of the presence of the hydrogen bond is that the redox potential is positively shifted only in the case of **1** (Table 1). The cyclic voltammograms of **1** showed a positively shifted Fe^{III}/Fe^{II} redox couple (by ∼0.1 V), in comparison with those of **2**, **3**, and **SR**, which lack the hydrogen bond, and this value is extremely close to those of native P450 forms (−0.42 V, P450cam in high spin state[13]). Each complex showed a clear, reversible redox couple. Since it has been reported that the second electron transfer is the rate-determining step in P450 catalysis,[14] this positive shift indicates that the hydrogen bond present in P450 contributes to the catalytic efficiency.

Although **SR** itself is an exceptionally stable alkanethiolate-ligated heme with respect to O_2, H_2O, and other reactive chemical species, it was found that **1** is even more stable than **2, 3**, and **SR** under air at room temperature in solution by investigation of the change of the EPR spectra. Therefore, we concluded that the NH···S hydrogen bond stabilizes the Fe(III) porphyrin−alkanethiolate structure both by increasing the Fe−S bond order and by inducing a positive shift of the redox potential.

Table 3. Competitive Oxidation of Cyclooctane/Cyclooctene Catalyzed by Iron Porphyrins[a]



	products yield (%)[b]			total yield	alkane/alkene,
iron porphyrin	**4**	**5**	**6**	(%)	(**4** + **5**)/**6**
none	nd[c]	nd	6.64	6.64	
1	5.66	0.34	18.96	24.96	0.32
2	5.25	0.07	6.65	11.97	0.80
3	4.90	1.39	9.91	16.20	0.63
SR	5.01	0.04	8.52	13.58	0.60
SR-Im[d]	0.93	1.42	50.54	52.89	0.05

[a] Reaction conditions: [iron porphyrins] = [mCPBA] = 1.0 mM, [cyclooctane] = 200 mM, [cyclooctene] = 20 mM. All reactions were carried out in CH_2Cl_2 under Ar at −15 °C for 5 min. [b] Yields were based on mCPBA. [c] Not detected. [d] Reference 16.

The most characteristic feature of the reaction of P450 is its high activity as a monooxygenase. It is generally accepted that alkane hydroxylation and alkene epoxidation proceed via different mechanisms,[15] so it is expected that alkane−alkene competitive oxidation can be used as a probe for discrimination of differences in chemical properties among active species derived from iron porphyrins. Thus, competitive oxidation of cyclooctane−cyclooctene was studied in novel heme thiolate−mCPBA systems to examine the effect of the NH···S hydrogen bond. Reaction conditions followed those in our previous report on the reactivity of an active intermediate derived from **SR**.[16] The thiolate ligation of **1**−**3** and **SR** during oxidation reaction was confirmed by measuring the EPR spectrum before and after the reaction. The intermediate of each of **1**−**3** and **SR** was confirmed to be the two-electron-oxidized form by using peroxyphenylacetic acid, which has frequently been used as a probe for this purpose.[17] As shown in Table 3, every heme thiolate effectively oxidized alkane and, like P450,[15] showed a higher ratio of cyclooctanol to cyclooctene oxide compared with **SR-Im**,[2b] which is an axial imidazole-ligated analogue of **SR**. However, significant differences in the ratio of oxidation products were observed between **1** and the other heme thiolates. In the case of **2, 3**, and **SR**, the ratios of cyclooctanol to cyclooctene oxide were ∼0.6. On the other hand, **1** showed a lower ratio than the other heme thiolates. These results indicate that the electronic structure of the active intermediate of **1** is somewhat more advantageous for electrophilic reaction than are those of the other complexes.[18]

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Supporting Information Available: X-ray crystallographic data, in CIF format, are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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