First Synthetic NO−Heme−Thiolate Complex Relevant to Nitric Oxide Synthase and Cytochrome P450nor

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Nitric oxide (NO) is an endogenous compound that has various bioactivities, and heme proteins play important roles in the biosynthesis of NO and in signal transduction mediated by NO. Therefore studies into the binding of NO by synthetic iron porphyrins should aid our understanding of how NO interacts with heme-containing biomolecules. Synthetic NO−heme−imidazole complexes as models of nitrosylhemoglobin,[1] nitrosylmyoglobin,[2] and soluble guanylate cyclase[3] have already been well studied. A synthetic NO−heme−thiolate complex is also expected to be useful as a model of nitric oxide synthase (NOS) and P450nor, but because of the high reactivity of NO with thiolate, such a complex has not been obtained. NOS is a heme−thiolate-containing enzyme responsible for catalyzing the oxidation of a guanidino nitrogen of L-arginine to produce NO, and it is known that NO itself strongly binds to heme. In fact, an Fe^{II}−NO complex is formed in part during the catalytic reaction of NOS and markedly reduces the activity.[4] P450nor is a unique enzyme that catalyzes reductive dimerization of NO to N_2O.[5] A number of mechanistic studies have been conducted since the finding of P450nor. A plausible reaction mechanism was proposed by Shiro et al.,[6] but has not been confirmed. Considering the above circumstances, a synthetic NO−heme−thiolate complex could be a valuable experimental tool.

We have already succeeded in the synthesis of a stable Fe^{III}-porphyrin−alkanethiolate complex (**SR**, Scheme 1), in which the sulfur atom is sterically protected from reactive molecules such as O_2 and NO by bulky groups.[7] We report here some spectroscopic and electrochemical properties of the first synthetic NO−heme−thiolate complex, prepared by the use of **SR**.

The **SR**(Fe^{III})−NO complex was prepared by bubbling a small excess of NO into a benzene solution of **SR**(Fe^{III}) under an argon atmosphere. The electronic absorption spectra of the solution changed through isosbestic points as shown in Figure 1a, suggesting that NO coordinates to **SR**(Fe^{III}) and forms **SR**(Fe^{III})−NO.[6a] Since this **SR**(Fe^{III})−NO reverted to ligand-free **SR**(Fe^{III}) when argon was bubbled through the solution, it was confirmed that NO coordinates reversibly to the Fe atom of **SR**, as illustrated in Scheme 1, without modification of the thiolate (e.g., formation of a nitrosothiol).

The ν(N−O) mode in **SR**−NO was detected at 1826 cm^{-1} by IR spectroscopy, and it shifted to 1792 cm^{-1} upon substitution by ^{15}NO. This ν(N−O) mode of **SR**−NO was not affected to a noticeable extent by changing the solvent, which corresponds to changing the environment of the sixth coordination site of the enzymes. The ν(Fe−N) mode in **SR**−NO was examined by resonance Raman spectroscopy at $\lambda_{ex} = 413.1$ nm using the same conditions as in the case of several P450−NO complexes.[8] A peak was detected at 510 cm^{-1}, and it shifted to 508 cm^{-1} upon substitution by ^{15}NO. These vibrational spectra show that ν(N−O) and ν(Fe−N) of **SR**−NO are fairly close to those of natural

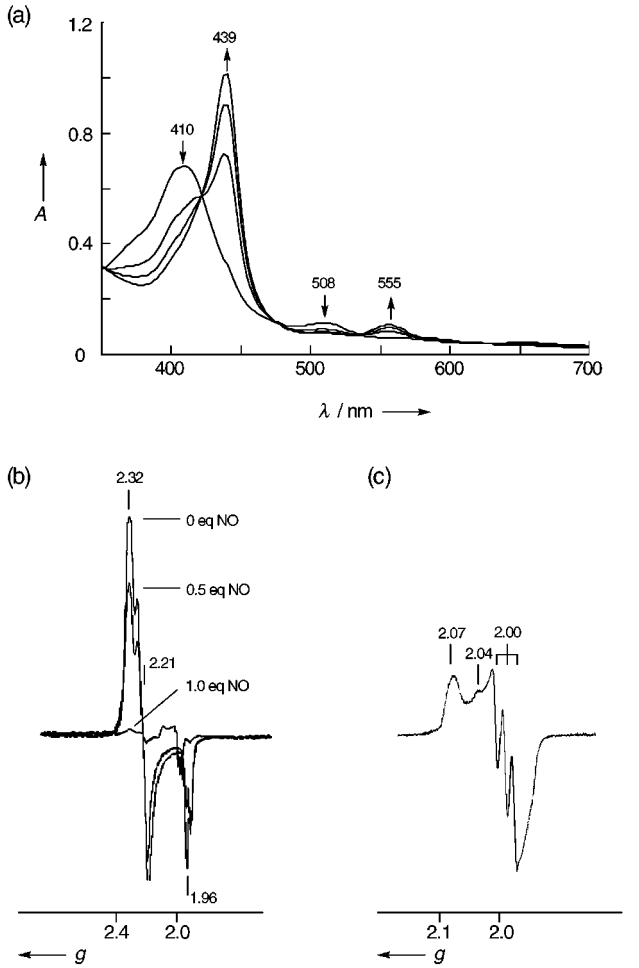

Figure 1. (a) Electronic absorption spectral change of **SR** (10^{-5} M in benzene at 300 K) by bubbling a small excess of NO (measured at 1-min intervals for 3 min) and (b) EPR spectral change of **SR** (10^{-3} M in toluene at 77 K) upon gradual addition of NO and (c) subsequent addition of $NaBH_4$ and 18-crown-6 to (b). In spectrum c, the g_3 signal is not separated.

Scheme 1. Reversible NO Coordination to SR

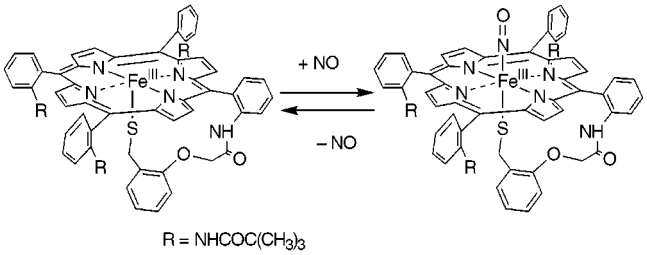

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Table 1. Resonance Raman and Infrared Spectral Data of **SR**− and Various Heme Protein−NO Complexes

	ν(Fe−NO), cm^{-1}	ν(N−O), cm^{-1}	ref
SR(III)−NO	510	1828	this work
SR-HB(III)−NO	515	1837	this work
P450nor(III)−NO	530	1853	8a
P450cam(III)−NO	528	1806	8a,b
NOS(III)−NO	540		8c
CPO[a](III)−NO	538	1868	8a
HRP[b](III)−NO	604		9
Mb(III)−NO	595	1927	9, 10a
Hb(III)−NO	594	1925	9, 10b

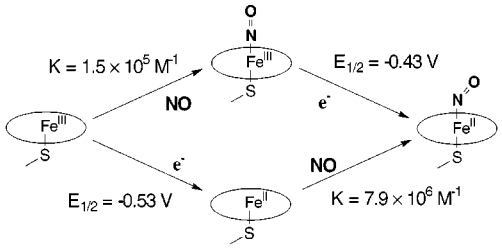
[a] Chloroperoxidase. [b] Horseradish peroxidase.

heme−thiolate-containing enzymes such as P450cam, NOS, and P450nor, and distinct from those of heme−imidazole-containing enzymes, as shown in Table 1. The NO complex of hydrogen-bonding SR (**SR-HB**),[11] which has an intramolecular NH···S hydrogen bond, can also be prepared by the same procedure as used for **SR**−NO, and its ν(Fe−N) mode appeared at 515 cm^{-1}, slightly higher than that of **SR**−NO. This result is consistent with the fact that the wavenumber of the ν(Fe−N) mode of ferric NOS−NO is higher than that of the other P450s, and that NOS has obvious hydrogen bonds toward the thiolate ligand compared with the other P450s.[12]

An EPR spectral change was observed on addition of NO (0 to 1 equiv) to **SR** (10^{-3} M, toluene solution) as shown in Figure 1b. The EPR spectra of **SR** showed a typical low-spin signal of an Fe^{III}porphyrin−thiolate complex, but **SR**−NO is EPR-silent owing to coupling of the two unpaired electrons of Fe and NO. Upon reduction of **SR**−NO with $NaBH_4$/18-crown-6, a characteristic EPR spectrum of six-coordinate thiolate−Fe^{II}porphyrin−NO complex[13] appeared at 77 K (Figure 1c).

Cyclic voltammetry of **SR**−NO at a Pt electrode vs SCE reference in CH_2Cl_2, 0.1 M tetra-*n*-butylammonium perchlorate revealed that **SR**−NO undergoes a reversible one-electron oxidation and a reversible one-electron reduction during the time scale of the experiment. The one-electron reduction potential (Fe^{II}/Fe^{III}) and one-electron oxidation potential (Fe^{III}/Fe^{IV}) of **SR**−NO are −0.43 V and +0.81 V, respectively. These potentials indicate that **SR**−NO is more redox-active than the original **SR** (−0.53 V and +0.94 V, respectively), and support the reaction mechanism for P450nor proposed by Shiro et al.:[6] i.e., the initial step of the reaction is formation of the ferric NO complex with subsequent two-electron reduction, not direct reduction of ferric heme. The thiolate heme and its nitrosyl complex appear to have potentials appropriate for this stepwise reduction.

Scheme 2. Formation of NO Complexes of Ferric and Ferrous SR with Redox Potentials and Equilibrium Constants of the Complexes



The complex formation constant of **SR**(Fe^{III})−NO was determined from the absorption spectral changes. In this experiment, NO was added to **SR** (10^{-5} M CH_2Cl_2 solution) as a CH_2Cl_2 solution, and the concentration of NO was determined by the PTIO method[14] using EPR. The complex formation constant of **SR**(Fe^{II})−NO was calculated by application of the Nernst equation [eq 1].

$$K_{\mathrm{F,red}} = 10^{-[(Em(\mathrm{bound})-Em(\mathrm{free}))nF/(2.303RT)]}K_{\mathrm{F,ox}} \qquad (1)$$

These values are summarized in Scheme 2. The formation constant of the ferrous nitrosyl complex of **SR** (7.9×10^6 M^{-1}) is much lower than those of imidazole ligated hemes, e.g. the formation constant of ferrous protoporphyrin 1-methylimidazole nitrosyl is 5.8×10^{11} M^{-1}.[15] These values indicate that thiolate ligation labilizes the Fe^{II}−NO complex compared with the case of imidazole ligation. Since NOS partially forms an Fe^{II}−NO complex during its catalytic reaction,[4] this result indicates that the thiolate ligation in NOS is advantageous not only for dioxygen activation but also for avoidance of product inhibition by NO.

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Supporting Information Available: Experimental details and results of solvent effect for νN−O and cyclic voltammogram of **SR**-NO (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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