[Chem. Pharm. Bull. 36(2) 535—541 (1988)]

Reactivity of $[Fe(SAr)_4]^{n-}$ ($n=1$ or 2), Synthetic Model Complexes for Iron–Sulfur Protein, Rubredoxin

KAZUO YANADA, TETSUO NAGANO, and MASAAKI HIROBE*

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

(Received June 24, 1987)

Synthetic model complexes, $(Et_4N)[Fe(S\text{-}p\text{-}C_6H_4X)_4]$, for the oxidized form of rubredoxin (Rd) were prepared in CH_3CN solution by the ligand-substitution of $(Et_4N)[Fe(S_2\text{-}o\text{-}xyl)_2]$ with diaryl disulfides in the presence of *o*-xylene-α,α'-dithiol. The complex (X = H) exhibited an intense electron spin resonance (ESR) signal at $g=4.3$, due to the Fe(III) metal with the highly symmetrical ligand. The complex in the reduced state, $[Fe(S\text{-}p\text{-}tol)_4]^{2-}$, was found to reduce *tert*-butyl hydroperoxide to *tert*-butyl alcohol stoichiometrically. Rubredoxin is believed to act as an electron carrier in the reduction of alkyl hydroperoxides to the corresponding alcohols in the ω-hydroxylation of alkanes in *Pseudomonas olevorans*. Thus, the reaction could be considered as biomimetic reduction by a rubredoxin model complex. The reaction mechanisms are discussed in connection with the characterization of these novel complexes.

Keywords——rubredoxin; non-heme iron–sulfur protein; ω-hydroxylation; electron transfer; reduction; biomimetic reaction

Introduction

Rubredoxins (Rd) in bacteria are the simplest of the non-heme iron–sulfur proteins, having an active site that consists of an $[Fe(S\text{-}Cys)_4]$ tetrahedral complex.[2] The two redox states of the proteins, Rd_{ox} and Rd_{red}, contain coordinated Fe(III) and Fe(II), respectively.[3] Holm *et al.* reported the preparation of Fe(II)–S_4 complexes, which represent the most successful model complexes for Rd_{red},[4] and then Koch *et al.* succeeded in the preparation of Fe(III)–S_4 complexes with highly symmetrical ligands.[5] The steric and conformational properties of the thiolate ligands were crucial in accounting for the stability of Fe(III) tetrathiolate complexes.[4,6] We have prepared $[Fe(III)(SC_6H_4X)_4]^-$ complexes in CH_3CN solution by the ligand-substitution of $(Et_4N)[Fe(S_2\text{-}o\text{-}xyl)_2)]$ with diaryl disulfides in the presence of *o*-xylene-α,α'-dithiol[7] and have also examined the reactivity of these model complexes. In 1966, Coon *et al.* proposed that *Pseudomonas olevorans* Rd is involved in the ω-hydroxylation system of alkanes and long-chain fatty acids as an electron carrier with ω-hydroxylase and rubredoxin-NAD^+ reductase, according to the following equation: $RCH_3+O_2+NADH+H^+ \rightarrow RCH_2OH+H_2O+NAD^+$.[8] Studies on the mechanism of ω-hydroxylation suggest that Rd_{red} reduces hydroperoxide, which is one of the intermediates in the ω-hydroxylation, to the corresponding alcohol.[9] We have found that the $(Et_4N)_2[Fe(S\text{-}p\text{-}tol)_4]$ complex can reduce *tert*-butyl hydroperoxide to *tert*-butyl alcohol stoichiometrically. The reaction should closely resemble the reduction of alkyl hydroperoxides to the corresponding alcohols in the ω-hydroxylation system of alkanes in bacteria. In this paper, the reaction mechanisms are discussed in relation to the characterization of the novel oxidized model complexes of Rd. The reaction of the complexes with Fe-porphyrin or methylene blue has also been examined.

Results and Discussion

Formation of Mononuclear Complexes in the Oxidized State, Fe(III)–S_4

Mononuclear complexes $[Et_4N]_2[Fe(S\text{-}p\text{-}C_6H_4X)_4]$ (X=H: $\mathbf{1}_{red}$, X=CH_3: $\mathbf{2}_{red}$, X=Cl: $\mathbf{3}_{red}$ and X=OCH_3: $\mathbf{4}_{red}$) in the reduced state were prepared by the reaction of $FeCl_3$ with sodium thiolate by a modification of Holm *et al.*'s method.[10] The absorption spectral data of the complexes ($\mathbf{2}_{red}$ and $\mathbf{3}_{red}$) are shown in Table I; the spectrum of $\mathbf{1}_{red}$ is identical with that reported by Holm *et al.*[10] On the other hand, Fe(III)–S_4 complexes in the oxidized state were formed in CH_3CN solution by the ligand-substitution reaction of the $\mathbf{5}_{ox}$ complex with 10 eq of corresponding diaryl disulfides in the presence of 2 eq of *o*-xylene-α,α′-dithiol (Chart 1). The electronic spectra of these complexes in CH_3CN exhibited intense visible absorption at λ_{max}=530 or 540 nm (ε=7500, $\mathbf{1}_{ox}$; 6900, $\mathbf{2}_{ox}$ in Table I). From the spectral changes during the reaction of $\mathbf{5}_{ox}$ with diphenyl disulfide and *o*-xylene-α,α′-dithiol in CH_3CN solution at 25 °C under an Ar atmosphere, the absorbance at 530 nm reached its maximum in 200 min.

The electron spin resonance (ESR) spectrum of $\mathbf{1}_{ox}$ shows a sharp resonance at g=4.3 in CH_3CN glass at 77 K; this signal is characteristic of high-spin ferric ion in a rhombic field (Fig. 1a). The data are similar to the results reported for rubredoxin.[11] In comparison, the ESR spectrum of $[Fe(SC_{10}H_{13})_4]^-$ ($\mathbf{6}_{ox}$) closely resembles that of $\mathbf{1}_{ox}$,[6] while $\mathbf{5}_{ox}$ has a more complicated spectrum (g=8.4, 5.3 and 4.3) at 6 K. Conformational constraint of the bidentate ligand results in low symmetry of $[Fe(S_2\text{-}o\text{-xyl})_2]^-$, which should result in complicated resonances at g=8.4 and 5.3 with a very weak resonance at g=4.3, as shown in Fig. 1d. The addition of diphenyl disulfide and *o*-xylene-α,α′-dithiol enhanced the resonance at g=4.3, which is due to the symmetrical $[Fe(SC_6H_5)_4]^-$ unit (Fig. 1a, b and c). The ligand-substitution reaction of (Et_4N) $[Fe(S_2\text{-}o\text{-xyl})_2]$ ($\mathbf{5}_{ox}$) by benzenethiol alone without *o*-xylene-α,α′-dithiol produced not the oxidized form $[Fe(S\text{-}C_6H_5)_4]^-$ ($\mathbf{1}_{ox}$) but the reduced form $[Fe(S\text{-}C_6H_5)_4]^{2-}$ ($\mathbf{1}_{red}$), which exhibits no resonance at g=4.3. The $\mathbf{1}_{ox}$ complex should be readily reduced by C_6H_5SH to form the $\mathbf{1}_{red}$ complex.

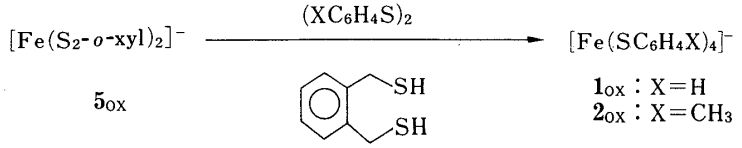


Chart 1. The Ligand-Substitution Reaction of the $\mathbf{5}_{ox}$ Complex with Diaryl Disulfides in the Presence of *o*-Xylene-α,α′-dithiol

TABLE I. Absorption Spectra of Mononuclear Complexes

Complex[a]	λ_{max} nm (ε)[b]	Reference
$[Fe(SPh)_4]^-$ ($\mathbf{1}_{ox}$)[c]	390 (sh, 8400), 530 (7500)	This work
$[Fe(S\text{-}p\text{-tol})_4]^-$ ($\mathbf{2}_{ox}$)[c]	464 (7100), 540 (sh, 6900)	This work
$[Fe(SC_{10}H_{13})_4]^-$ ($\mathbf{6}_{ox}$)[e]	295 (14300), 344 (6880), 450 (7230)	6)
$[Fe(S_2\text{-}o\text{-xyl})_2]^-$ ($\mathbf{5}_{ox}$)[e]	350 (8300), 450 (sh, 4200), 486 (6240), 640 (1600), 688 (1670)	4*b*)
$[Fe(SPh)_4]^{2-}$ ($\mathbf{1}_{red}$)[d]	337 (16300), 390 (sh, 2800)	10)
$[Fe(S\text{-}p\text{-tol})_4]^{2-}$ ($\mathbf{2}_{red}$)[e]	324 (10300), 340 (sh, 9600), 400 (sh, 2300), 480 (820)	This work
$[Fe(S\text{-}p\text{-}C_6H_4Cl)_4]^{2-}$ ($\mathbf{3}_{red}$)[e]	347 (10100), 415 (sh, 1200)	This work
$[Fe(S_2\text{-}o\text{-xyl})_2]^{2-}$ ($\mathbf{5}_{red}$)[e]	323 (7710), 355 (sh, 2660), 450 (sh, 390)	4*b*)

a) Et_4N^+ salt. *b*) At 25 °C under Ar. *c*) Prepared by the ligand substitution reaction of 0.10 mM (Et_4N) $[Fe(S_2\text{-}o\text{-xyl})_2]$ with 1.0 mM diphenyl or di-*p*-tolyl disulfide in the presence of 0.2 mM *o*-xylene-α,α′-dithiol in CH_3CN. *d*) In CH_3CN. *e*) In dimethylformamide (DMF).

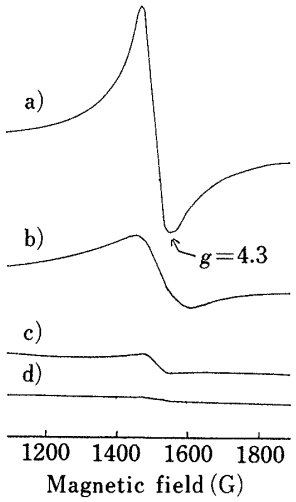
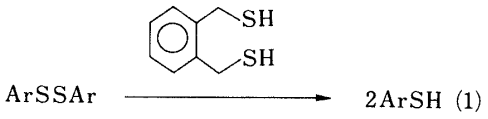
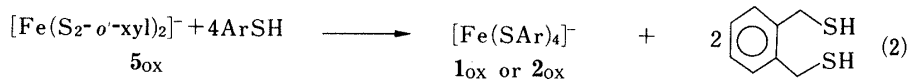


Fig. 1. ESR Spectra of $[Fe(SC_6H_5)_4]^-$ Prepared by the Ligand-Substitution Reaction

a) The complete system contained 5.0 μmol of $\mathbf{5}_{ox}$, 10 μmol of *o*-xylene-α,α'-dithiol and 50 μmol of PhSSPh in 0.57 ml of CH_3CN. The ESR spectrum was recorded at 77 K under Ar at 3 h after the start of the reaction. b) The complete system minus PhSSPh. c) The complete system minus *o*-xylene-α,α'-dithiol. d) 5.0 μmol of $\mathbf{5}_{ox}$ in 0.57 ml of CH_3CN.





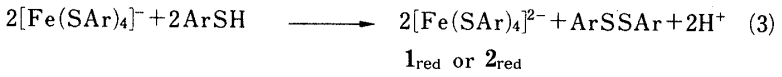


Chart 2. The Reaction Mechanism of the Ligand-Substitution of $\mathbf{5}_{ox}$ Complex with Diaryl Disulfides in the Presence of *o*-Xylene-α,α'-dithiol

The proposed reaction mechanism is shown in Chart 2. The first step should be the reaction of *o*-xylene-α,α'-dithiol with diaryl disulfide to form arylthiol. The arylthiol reacts with the $\mathbf{5}_{ox}$ complex in the ligand-substitution reaction to produce the complex in the oxidized state ($\mathbf{1}_{ox}$ or $\mathbf{2}_{ox}$). Excess amounts of arylthiol could reduce the $\mathbf{1}_{ox}$ or $\mathbf{2}_{ox}$ complex to the $\mathbf{1}_{red}$ or $\mathbf{2}_{red}$ complex as shown in Chart 2, scheme 3. The differences of visible absorption or ESR properties between Fe(II) and Fe(III) complexes make it easy to examine the reduction process by reduced mononuclear complexes.

Reduction of *tert*-Butyl Hydroperoxide by $\mathbf{2}_{red}$

In this experiment $\mathbf{2}_{red}$ was used in order to examine the reduction of *tert*-BuOOH, since the redox potentials of **1** (-0.56 V $1-/2-E_{1/2}$ *vs.* saturated calomel electrode (SCE)), **2** (-0.62 V) and **3** (-0.44 V) suggest $\mathbf{2}_{red}$ to be the most powerful reducing catalyst. Nine 20 μl portions of CH_3CN (180 μl), containing 0.279 μmol of *tert*-BuOOH, were added to $(Et_4N)_2[Fe(S\text{-}p\text{-}tol)_4]$ ($\mathbf{2}_{red}$, 2.24 μmol) in 3.0 ml of 0.1% (v/v) H_2O–CH_3CN. The reaction solution of $\mathbf{2}_{red}$ with *tert*-BuOOH exhibited the resonance at $g=4.3$, which should be assigned to $\mathbf{2}_{ox}$. Furthermore, the formation of $\mathbf{2}_{ox}$ was followed by an increase of absorbance at 540 nm (A_{540}) stoichiometrically with addition of *tert*-BuOOH (Fig. 2). These results indicate that $\mathbf{2}_{red}$ can readily reduce *tert*-BuOOH to *tert*-BuOH. The reaction should be considered as a biomimetic reduction of alkyl hydroperoxides to the corresponding alcohols, as seen in the ω-hydroxylation of alkanes. The reaction required a small amount of H_2O, as would be

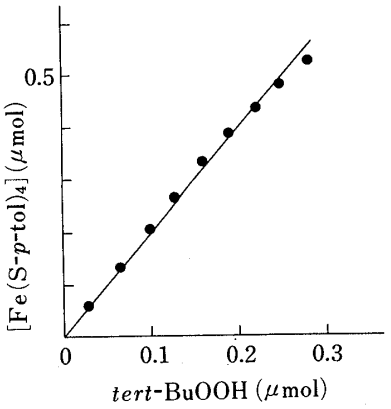


Fig. 2. Reduction of *tert*-BuOOH by $\mathbf{2}_{red}$

Nine 20 μl portions of CH_3CN (180 μl) containing 0.279 μmol of *tert*-BuOOH were added to $\mathbf{2}_{red}$ (2.24 μmol) in 3.0 ml of 0.1% (v/v) H_2O/CH_3CN. The absorbance at 540 nm increased on addition of *tert*-BuOOH.

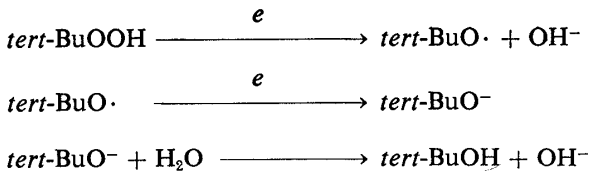
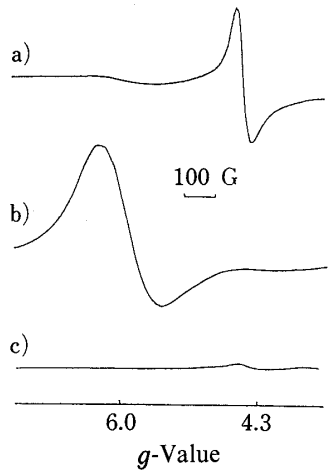


Chart 3. The Proposed Reaction Mechanism of the Reduction of *tert*-BuOOH by the $\mathbf{2}_{red}$ Complex



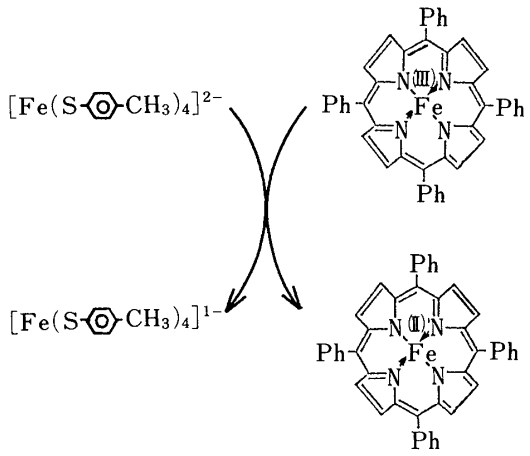


Fig. 3. ESR Spectra during the Reaction of $\mathbf{2}_{red}$ with Fe(III)TPPCl

a) The complete reaction system contained 10.3 μmol of $\mathbf{2}_{red}$ and 1.9 μmol of Fe(III)TPPCl in 1.03 ml of CH_3CN and 0.05 ml of CH_2Cl_2. b) The complete system minus $\mathbf{2}_{red}$. c) The complete system minus Fe(III)TPPCl.

expected from the reaction mechanism shown in Chart 3, though larger amounts of H_2O decomposed $\mathbf{2}_{ox}$.

Reaction of $\mathbf{2}_{red}$ with Metalloporphyrin or Methylene Blue

Cytochrome c (cyt. c) reduction is used to assay the activity of Rd, because Rd can transfer one electron from reduced flavoenzyme (ferredoxin–NADP reductase: FNR) to cyt. c. Thus, much interest has been focused on the reaction of $\mathbf{2}_{red}$ with metalloporphyrin complexes. When a CH_3CN solution of $\mathbf{2}_{red}$ was added to a CH_2Cl_2 solution of Fe(III)TPPCl, which exhibits the resonance at $g=6.0$, the resonance of Fe(III)TPPCl disappeared with the concomitant appearance of the resonance at $g=4.3$, as shown in Fig. 3. The result indicates that $\mathbf{2}_{red}$ can reduce Fe(III)TPPCl to Fe(II)TPP, which is inert to ESR. Methylene blue (MB) is often used as an indicator in redox reactions. In order to examine the reducing activity of the complexes, we tried the reaction of synthetic model complexes of reduced Rd with MB. Compound $\mathbf{2}_{red}$ reduced MB_{ox} to MB_{red} completely. Addition of MB_{ox} to the $\mathbf{2}_{red}$ solution under anaerobic conditions increased the absorption at 540 nm. The maximal absorption was

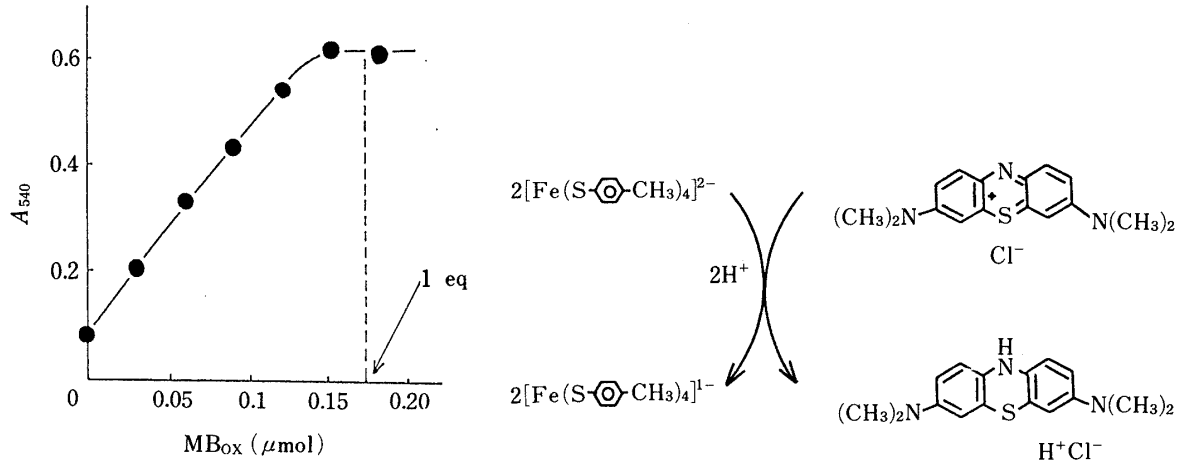


Fig. 4. The Absorbance at 540 nm in the Reaction of $\mathbf{2}_{red}$ with MB_{ox}
Conditions of measurement are described in Experimental.

obtained by addition of 1 eq of MB_{ox} (Fig. 4). The molar extinction coefficient ($\varepsilon = 7000$) of $\mathbf{2}_{ox}$ at 540 nm calculated from the slope in Fig. 4 had the same value as that obtained from the ligand-substitution. These results show that $\mathbf{2}_{red}$ was oxidized quantitatively by MB_{ox} to form $\mathbf{2}_{ox}$ and MB_{red}.

In summary, synthetic model complexes of oxidized Rd were prepared by the ligand-substitution of $[Fe(S_2\text{-}o\text{-}xyl)_2]^-$ with diaryl disulfide in the presence of *o*-xylene-α,α'-dithiol. The reduced complex $[Fe(S\text{-}p\text{-}tol)_4]^{2-}$ was found to reduce *tert*-butyl hydroperoxide to *tert*-butyl alcohol. The reaction may be considered as a biomimetic reduction by a rubredoxin model complex, because rubredoxin has been suggested to act as an electron carrier in the reduction of alkyl hydroperoxides to the corresponding alcohols in the ω-hydroxylation of alkanes in *Pseudomonas olevorans*.

Experimental

$(Et_4N)[Fe(S_2\text{-}o\text{-}xyl)_2]$ ($\mathbf{5}_{ox}$),[4a] chloro(5,10,15,20-tetra-phenylporphyrinato)iron(III) (Fe(III)TPPCl),[12] and *o*-xylene-α,α'-dithiol[13] were prepared by the cited procedures. Di-*p*-tolyl disulfide was prepared by the controlled air oxidation of *p*-toluenethiol (6.0 mmol) with a catalytic amount (0.128 mmol) of $(n\text{-}Bu_4N)_2[Fe_4S_4(S\text{-}p\text{-}tol)_4]$ in 20 ml of CH_3CN with stirring for 30 min.[14] The product was purified by silica gel column chromatography, followed by recrystallization from MeOH. Di-*p*-tolyl disulfide was obtained as pale yellow needles, mp 43—44 °C, in quantitative yield. Methylene blue trihydrate, diphenyl disulfide and *tert*-butyl hydroperoxide (70% in water) were purchased from Nakarai Chemicals Ltd. The concentration of *tert*-butyl hydroperoxide was measured by iodometry.[15] All solvents were purified by distillation. Acetonitrile was dried over 5 Å molecular sieves for several days prior to use. The reagents used for the synthesis of $(Et_4N)_2[Fe(SAr)_4]$ were commercial grade products. Mononuclear complexes were prepared under a pure Ar atmosphere and all solvents were bubbled through with Ar over 30 min to remove dioxygen. Melting points were determined on Yanagimoto micro-melting point apparatus in a capillary under Ar. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-FX-100 spectrometer and chemical shifts are given as values (ppm) from tetramethylsilane as an internal standard. Redox potentials $E_{1/2}$ were measured with a Yanaco P-1000 voltammetric analyzer in 0.1 M $n\text{-}Bu_4NClO_4$ solution.

$(Et_4N)_2[Fe(S\text{-}C_6H_5)_4]$ ($\mathbf{1}_{red}$)——Bis(tetraethylammonium) tetrakis(benzenethiolato)ferrate(II) was prepared by a modification of Holm *et al.*'s method.[10] A solution of 120 mmol of NaSPh prepared from 12.7 ml of benzene-thiol and 2.8 g of sodium in 100 ml of ethanol was added to a stirred solution of 5.4 g (20 mmol) of $FeCl_3 \cdot 6H_2O$ in 30 ml of ethanol over a 30 min period. The mixture was stirred for 1 h, and sodium chloride was removed by filtration. A solution of 9.3 g (44 mmol) of Et_4NBr in 30 ml of ethanol was then added to the black filtrate and the reaction mixture was stirred for 1.5 h, causing separation of a microcrystalline solid, which was collected by filtration. The complex thus obtained was washed with ethanol and then ether, and dried *in vacuo*. The product (13.5 g) was recrystallized from 100 ml of warm acetonitrile initially at 60 °C, yielding 9.1 g (61%) of $\mathbf{1}_{red}$, mp 149—151 °C (dec.), as light green prisms. *Anal.* Calcd for $C_{40}H_{60}FeN_2S_4$: C, 63.80; H, 8.03; N, 3.72. Found: C, 63.99; H, 8.12; N, 3.71. IR (KBr): 3036, 2972, 1568, 1460, 1430, 1395, 1385, 1370, 1302, 1260, 1179, 1145, 1120, 1079, 1061, 1020, 1000, 991, 959, 894, 785,

747, 736, 692, 478 cm^{-1}. ^{1}H-NMR (CD_3CN) δ: −25.3 (*p*-H), −18.6 (*o*-H), 24.2 (*m*-H), 1.8 ($C\underline{H}_3CH_2N^+$), 3.7 ($CH_3C\underline{H}_2N^+$). All NMR peaks were observed as broad singlets due to core paramagnetism. Halfwave potential ($E_{1/2}$) *vs.* SCE: −0.56 V.

$(Et_4N)_2[Fe(S\text{-}p\text{-}C_6H_4X)_4]$——Bis(tetraethylammonium) tetrakis(*p*-toluenethiolato)ferrate(II) ($\mathbf{2}_{red}$), bis(tetraethylammonium) tetrakis(*p*-chlorobenzenethiolato)ferrate(II) ($\mathbf{3}_{red}$) and bis(tetraethylammonium) tetrakis(*p*-methoxybenzenethiolato)ferrate(II) ($\mathbf{4}_{red}$) were prepared from $FeCl_3 \cdot 6H_2O$ and the corresponding thiolates by a method similar to that used for the preparation of $\mathbf{1}_{red}$.

X = CH_3 ($\mathbf{2}_{red}$): Yield 73%, mp 199—202 °C (dec.), as green columns. *Anal.* Calcd for $C_{44}H_{68}FeN_2S_4$: C, 65.31; H, 8.47; N, 3.46. Found: C, 65.41, H, 8.65, N, 3.51. IR (KBr): 2968, 2908, 2848, 1620, 1589, 1480, 1455, 1392, 1300, 1248, 1178, 1080, 1028, 802, 785, 625, 485 cm^{-1}. ^{1}H-NMR (CD_3CN) δ: −17.4 (*o*-H), 22.8 (*m*-H), 33.8 (*p*-CH_3), 1.5 ($C\underline{H}_3CH_2N^+$), 3.3 ($CH_3C\underline{H}_2N^+$). NMR peaks were broadened, like those of $\mathbf{1}_{red}$. $E_{1/2}$ *vs.* SCE: −0.62 V.

X = Cl ($\mathbf{3}_{red}$): Yield 40%, mp 195—196 °C (dec.), as light green prisms. *Anal.* Calcd for $C_{40}H_{56}Cl_4FeN_2S_4$: C, 53.93; H, 6.34; N, 3.14. Found: C, 53.80; H, 6.36; N, 3.23. IR (KBr): 3020, 2968, 2944, 1620, 1560, 1466, 1397, 1299, 1245, 1178, 1122, 1085, 1057, 1028, 1001, 842, 820, 780, 534, 489 cm^{-1}. $E_{1/2}$ *vs.* SCE: −0.44 V.

X = OCH_3 ($\mathbf{4}_{red}$): Yield 17%, mp 131—138 °C (dec.), as dark green columns. *Anal.* Calcd for $C_{44}H_{68}FeN_2O_4S_4$: C, 60.53; H, 7.83; N, 3.21. Found: C, 59.82; H, 7.88; N, 3.43. IR (KBr): 2980, 2928, 2828, 1625, 1581, 1480, 1440, 1389, 1362, 1298, 1264, 1223, 1170, 1099, 1080, 1028, 999, 828, 812, 783, 631, 618, 510 cm^{-1}.

Formation of Mononuclear Complexes in the Oxidized State——The ligand-substitution of $\mathbf{5}_{ox}$ with diphenyl or di-*p*-tolyl disulfide in the presence of *o*-xylene-α,α′-dithiol to form $\mathbf{1}_{ox}$ or $\mathbf{2}_{ox}$ was monitored by measuring the absorption and/or ESR spectra. Diaryl disulfide (10 eq) and *o*-xylene-α,α′-dithiol (2 eq) were added to a CH_3CN solution of the complex $\mathbf{5}_{ox}$ at room temperature. The reaction system was maintained under an O_2-free atmosphere by passage of Ar through pyrogallol–NaOH. All solvents and solutions were bubbled through with Ar for 30 min prior to use. Absorption spectra were recorded on a Hitachi 557 dual-wavelength double-beam spectrophotometer at 25 °C using a quartz cell plugged by a rubber serum stopper. ESR spectra of $\mathbf{1}_{ox}$ were measured on a JEOL JES-FX 3X spectrometer (X band, 100 kHz) with the following instrumental settings: power, 1 mW; modulation amplitude, 5.0 G; scan rate, 250 G/min; response, 0.1 s; temperature, 77 K. Samples were put in quartz tubes (5 mm o.d.) plugged by rubber serum stoppers and frozen by immersion in liquid N_2. MnO was used as an external standard.

Reduction of *tert*-BuOOH by $\mathbf{2}_{red}$——Nine 20 μl portions of CH_3CN (180 μl) containing 0.279 μmol of *tert*-BuOOH were added to 2.24 μmol of $\mathbf{2}_{red}$ in 3.0 ml of 0.1% (v/v) H_2O–CH_3CN. The ESR and absorption spectra of the reaction mixture were measured under Ar. On the other hand, the reaction of *tert*-BuOOH (6.5 μl, 50 μmol) with $\mathbf{2}_{red}$ (407 mg, 503 μmol) at room temperature under Ar gave *tert*-BuOH in 80% yield, as determined by gas-liquid chromatography (GLC). GLC conditions: column, 10% polyethylene glycol (PEG) 6000 3 mm × 300 cm; column temp., 90 °C; inj. temp., 120 °C; N_2 flow rate, 40 ml/min; internal standard, benzene. Under the GLC conditions, *tert*-BuOOH was not converted into *tert*-BuOH.

Reaction of $[Fe(S\text{-}p\text{-}tol)_4]^{2-}$ with Fe(III) TPPCl——A solution of 10.3 μmol of $\mathbf{2}_{red}$ in CH_3CN (1.03 ml) was added to a CH_2Cl_2 (0.05 ml) solution of Fe(III)TPPCl (1.9 μmol). The reaction was monitored by measuring the ESR spectra as shown in Fig. 3.

Reaction of $[Fe(S\text{-}p\text{-}tol)_4]^{2-}$ with Methylene Blue——Six 20 μl portions of CH_3CN containing 0.03 μmol of MB_{ox} were added to $\mathbf{2}_{red}$ (0.345 μmol) in 3.0 ml of 0.005% (v/v) H_2O/CH_3CN. Addition of 1 eq of MB_{ox} gave the maximal absorption at 540 nm. The molar extinction coefficient ($\varepsilon = 7000$) of $\mathbf{2}_{ox}$ at 540 nm was calculated from the slope of the curve shown in Fig. 4.

Acknowledgement This work was supported by research grants from the Ministry of Education, Science and Culture of Japan.

References and Notes

1) Abbreviations used: S-*p*-tol, *p*-toluenethiolate; S_2-*o*-xyl, *o*-xylene-α,α′-dithiolate; $SC_{10}H_{13}$, 2,3,5,6-tetramethylbenzenethiolate; NAD^+, oxidized form of nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide.

2) *a*) W. A. Eaton and W. Lovenberg, "Iron–Sulfur Proteins," Vol. II, ed. by W. Lovenberg, Academic Press, New York, 1973, Chapter 3; *b*) W. Lovenberg, "Microbial Iron Metabolism," ed. by J. B. Neilands, Academic Press, New York, 1974, Chapter 8; *c*) L. H. Jensen, *Annu. Rev. Biochem.*, **43**, 461 (1974); *d*) W. H. Orme-Johnson, *ibid.*, **42**, 159 (1973); *e*) G. Palmer, "The Enzymes," Vol. XII, Part B, 3rd ed. by P. D. Boyer, Academic Press, New York, 1975, Chapter 1; *f*) J. A. Ibers and R. H. Holm, *Science*, **209**, 223 (1980).

3) *a*) W. Lovenberg and W. M. Williams, *Biochemistry*, **8**, 141 (1969); *b*) W. A. Eaton, G. Palmer, J. A. Fee, T. Kimura and W. Lovenberg, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 3015 (1971); *c*) D. D. Ulmer, B. Holmquist and B. L. Vallee, *Biochem. Biophys. Res. Commun.*, **51**, 1054 (1973); *d*) R. Cammack, D. P. E. Dickson and C. E. Johnson, "Iron–Sulfur Proteins," Vol. III, ed. by W. Lovenberg, Academic Press, New York, 1977, Chapter 8.

4) *a*) R. W. Lane, J. A. Ibers, R. B. Frankel and R. H. Holm, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2868 (1975); *b*) R.

W. Lane, J. A. Ibers, R. B. Frankel, G. C. Papaefthymiou and R. H. Holm, *J. Am. Chem. Soc.*, **99**, 84 (1977).
5) S. A. Koch, L. E. Maelia and M. Millar, *J. Am. Chem. Soc.*, **105**, 5944 (1983).
6) M. Millar, J. F. Lee, S. A. Koch and R. Fikar, *Inorg. Chem.*, **21**, 4105 (1982).
7) K. Yanada, T. Nagano and M. Hirobe, *Chem. Pharm. Bull.*, **31**, 4589 (1983).
8) *a*) J. A. Peterson, D. Basu and M. J. Coon, *J. Biol. Chem.*, **241**, 5162 (1966); *b*) E. T. Lode and M. J. Coon, *ibid.*, **246**, 791 (1971).
9) R. F. Boyer, E. T. Lode and M. J. Coon, *Biochem. Biophys. Res. Commun.*, **44**, 925 (1971).
10) K. S. Hagen, J. G. Reynolds and R. H. Holm, *J. Am. Chem. Soc.*, **103**, 4054 (1981).
11) J. Peisach, W. E. Blumberg, E. T. Lode and M. J. Coon, *J. Biol. Chem.*, **246**, 5877 (1971).
12) E. B. Fleischer, J. M. Palmer, T. S. Srivastava and A. Chatterjee, *J. Am. Chem. Soc.*, **93**, 3162 (1971).
13) J. J. Mayerle, S. E. Denmark, B. V. DePamphilis, J. A. Ibers and R. H. Holm, *J. Am. Chem. Soc.*, **97**, 1032 (1975).
14) T. Nagano, K. Yoshikawa and M. Hirobe, *Tetrahedron Lett.*, **1980**, 297.
15) The Chemical Society of Japan (ed.), "Shin Jikken Kagaku Koza," Vol. 15-I-2, Maruzen, Tokyo, 1976, p. 685.