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

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Synthetic model complexes, (Et_4N)[Fe(S_p-C_6H_5X)_4], for the oxidized form of rubredoxin (Rd) were prepared in CH_3CN solution by the ligand-substitution of (Et_4N)[Fe(S_2-o-xylyl)_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_p-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 oleovorans. 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-Cys)_4] tetrahedral complex. The two redox states of the proteins, Rd_{ox} and Rd_{red}, contain coordinated Fe(III) and Fe(II), respectively. Holm et al. reported the preparation of Fe(II)–S_4 complexes, which represent the most successful model complexes for Rd_{red}, and then Koch et al. succeeded in the preparation of Fe(III)–S_4 complexes with highly symmetrical ligands. The steric and conformational properties of the thiolate ligands were crucial in accounting for the stability of Fe(III) tetrathiolate complexes. We have prepared [Fe(III)(SC_6H_5X)_4] complexes in CH_3CN solution by the ligand-substitution of (Et_4N)[Fe(S_2-o-xylyl)_2] with diaryl disulfides in the presence of o-xylene-α,α'-dithiol and have also examined the reactivity of these model complexes. In 1966, Coon et al. proposed that Pseudomonas oleovorans 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^+ → RCH_2OH + H_2O + NAD^+. 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. We have found that the (Et_4N)[Fe(S_p-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)–S₄

Mononuclear complexes \([\text{Et}_4\text{N}]_2[\text{Fe}(S-p\text{-C}_6\text{H}_4\text{X})_4] (X=\text{H: } 1_{\text{red}}, X=\text{CH}_3: 2_{\text{red}}, X=\text{Cl: } 3_{\text{red}} \text{ and } X=\text{OCH}_3: 4_{\text{red}})\) in the reduced state were prepared by the reaction of \(\text{FeCl}_3\) with sodium thiolate by a modification of Holm et al.’s method. The absorption spectral data of the complexes \((2_{\text{red}} \text{ and } 3_{\text{red}})\) are shown in Table I; the spectrum of \(1_{\text{red}}\) is identical with that reported by Holm et al. On the other hand, Fe(III)–S₄ complexes in the oxidized state were formed in CH₃CN solution by the ligand-substitution reaction of the 5ox complex with 10 eq of corresponding diaryl disulfides in the presence of 2 eq of o-xylene-\(\alpha,\alpha’\)-dithiol (Chart 1). The electronic spectra of these complexes in CH₃CN exhibited intense visible absorption at \(\lambda_{\text{max}}=530 \text{ or } 540 \text{ nm} (\epsilon=7500, 1_{\text{ox; } 6900, 2_{\text{ox}} \text{ in Table I}). From the spectral changes during the reaction of 5ox with diphenyl disulfide and o-xylene-\(\alpha,\alpha’\)-dithiol in CH₃CN 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 \(1_{\text{ox}}\) shows a sharp resonance at \(g=4.3\) in CH₃CN 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. In comparison, the ESR spectrum of \([\text{Fe}(\text{SC}_6\text{H}_4\text{H}_3)_4]^{-}\) (6ox) closely resembles that of \(1_{\text{ox}}\), while 5ox has a more complicated spectrum \((g=8.4, 5.3 \text{ and } 4.3)\) at 6 K. Conformational constraint of the bidentate ligand results in low symmetry of \([\text{Fe}(\text{S}_2\text{-o-xy})_2]^{-}\), 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-\(\alpha,\alpha’\)-dithiol enhanced the resonance at \(g=4.3\), which is due to the symmetrical \([\text{Fe}(\text{SC}_6\text{H}_4\text{H}_3)_4]^{-}\) unit (Fig. 1a, b and c). The ligand-substitution reaction of \((\text{Et}_4\text{N}) [\text{Fe}(\text{S}_2\text{-o-xy})_2] (\text{5ox})\) by benzenethiol alone without o-xylene-\(\alpha,\alpha’\)-dithiol produced not the oxidized form \([\text{Fe}(\text{SC}_6\text{H}_4\text{H}_3)_4]^{-}\) (1ox) but the reduced form \([\text{Fe}(\text{S}_2\text{-C}_6\text{H}_5\text{H}_3)_4]^{-}\) (1red), which exhibits no resonance at \(g=4.3\). The 1ox complex should be readily reduced by \(\text{C}_6\text{H}_5\text{SH}\) to form the 1red complex.

\[
\begin{align*}
\text{Chart 1. The Ligand-Substitution Reaction of the 5ox Complex with Diaryl Disulfides in the Presence of o-Xylene-\(\alpha,\alpha’\)-dithiol}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Complex⁸</th>
<th>(\lambda_{\text{max}} \text{ nm} (\epsilon)⁹</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Fe}(\text{SPh})<em>4]^{-} (1</em>{\text{ox}})³)</td>
<td>390 (sh), 8400, 530 (7500)</td>
<td>This work</td>
</tr>
<tr>
<td>([\text{Fe}(\text{S}-\text{p-tol})<em>4]^{-} (2</em>{\text{ox}})³)</td>
<td>464 (7100), 540 (sh), 6900</td>
<td>This work</td>
</tr>
<tr>
<td>([\text{Fe}(\text{SC}<em>{10}\text{H}</em>{13})<em>4]^{-} (6</em>{\text{ox}})⁶)</td>
<td>295 (14300), 344 (6880), 450 (7230)</td>
<td>6</td>
</tr>
<tr>
<td>([\text{Fe}(\text{S}_2\text{-o-xy})<em>2]^{-} (5</em>{\text{ox}})⁶)</td>
<td>350 (8300), 450 (sh), 4200, 486 (6240), 640 (1600), 688 (1670)</td>
<td>4b</td>
</tr>
<tr>
<td>([\text{Fe}(\text{SPh})<em>4]^2^{-} (1</em>{\text{ox}})³)</td>
<td>337 (16300), 390 (sh), 2800</td>
<td>10</td>
</tr>
<tr>
<td>([\text{Fe}(\text{S}-\text{p-tol})<em>4]^2^{-} (2</em>{\text{ox}})³)</td>
<td>324 (10300), 340 (sh), 9600, 400 (sh), 2300, 480 (820)</td>
<td>This work</td>
</tr>
<tr>
<td>([\text{Fe}(\text{S}-\text{p-C}_6\text{H}_5\text{Cl})<em>4]^2^{-} (3</em>{\text{red}})⁹)</td>
<td>347 (10100), 415 (sh), 1200</td>
<td>This work</td>
</tr>
<tr>
<td>([\text{Fe}(\text{S}_2\text{-o-xy})<em>2]^2^{-} (5</em>{\text{red}})⁹)</td>
<td>323 (7710), 355 (sh), 2660, 450 (sh), 390</td>
<td>4b</td>
</tr>
</tbody>
</table>

\(^{a}\) Et₄N⁺ salt. \(^{b}\) At 25°C under Ar. \(^{c}\) Prepared by the ligand substitution reaction of 0.10 mm \((\text{Et}_4\text{N}) [\text{Fe}(\text{S}_2\text{-o-xy})_2]\) with 1.0 mm diphenyl or di-p-tolyl disulfide in the presence of 0.2 mm o-xylene-\(\alpha,\alpha’\)-dithiol in CH₃CN. \(^{d}\) In CH₃CN. \(^{e}\) In dimethylformamide (DMF).
Fig. 1. ESR Spectra of [Fe(SC\(_2\)H\(_2\))\(_6\)]\(^-\) Prepared by the Ligand-Substitution Reaction

a) The complete system contained 5.0 \(\mu\)mol of \(5_{\text{ox}}\), 10 \(\mu\)mol of \(\alpha\)-xylene-\(\alpha,\alpha\)'-dithiol and 50 \(\mu\)mol of PhSSPh in 0.57 ml of CH\(_2\)CN. 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 \(\alpha\)-xylene-\(\alpha,\alpha\)'-dithiol. d) 5.0 \(\mu\)mol of \(5_{\text{ox}}\) in 0.57 ml of CH\(_2\)CN.

![](chart)

**Chart 2.** The Reaction Mechanism of the Ligand-Substitution of \(5_{\text{ox}}\) Complex with Diaryl Disulfides in the Presence of \(\alpha\)-Xylene-\(\alpha,\alpha\)'-dithiol

The proposed reaction mechanism is shown in Chart 2. The first step should be the reaction of \(\alpha\)-xylene-\(\alpha,\alpha\)'-dithiol with diaryl disulfide to form arylthiol. The arylthiol reacts with the \(5_{\text{ox}}\) complex in the ligand-substitution reaction to produce the complex in the oxidized state (\(1_{\text{ox}}\) or \(2_{\text{ox}}\)). Excess amounts of arylthiol could reduce the \(1_{\text{ox}}\) or \(2_{\text{ox}}\) complex to the \(1_{\text{red}}\) or \(2_{\text{red}}\) 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 \(2_{\text{red}}\)**

In this experiment \(2_{\text{red}}\) was used in order to examine the reduction of tert-BuOOH, since the redox potentials of 1 (-0.56 V \(1/2\)) and 2 (-0.62 V) and 3 (-0.44 V) suggest \(2_{\text{red}}\) to be the most powerful reducing catalyst. Nine 20 \(\mu\)l portions of CH\(_2\)CN (180 \(\mu\)l), containing 0.279 \(\mu\)mol of tert-BuOOH, were added to (Et\(_4\))\(_2\)[Fe(S-p-tol)\(_2\)] \(2_{\text{red}}\) (2.24 \(\mu\)mol) in 3.0 ml of 0.1% (v/v) H\(_2\)O-CH\(_2\)CN. The reaction solution of \(2_{\text{red}}\) with tert-BuOOH exhibited the resonance at \(g=4.3\), which should be assigned to \(2_{\text{ox}}\). Furthermore, the formation of \(2_{\text{ox}}\) was followed by an increase of absorbance at 540 nm \((A_{540})\) stoichiometrically with addition of tert-BuOOH (Fig. 2). These results indicate that \(2_{\text{red}}\) 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 \(\omega\)-hydroxylation of alkanes. The reaction required a small amount of H\(_2\)O as would be
Fig. 2. Reduction of tert-BuOOH by $2_{\text{red}}$
Nine 20 μl portions of CH$_3$CN (180 μl) containing 0.279 μmol of tert-BuOOH were added to $2_{\text{red}}$ (2.24 μmol) in 3.0 ml of 0.1% (v/v) H$_2$O/CH$_3$CN. 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 $2_{\text{red}}$ Complex

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

a) The complete reaction system contained 10.3 μmol of $2_{\text{red}}$ and 1.9 μmol of Fe(III)TPPCl in 1.03 ml of CH$_3$CN and 0.05 ml of CH$_2$Cl$_2$. b) The complete system minus $2_{\text{red}}$. c) The complete system minus Fe(III)TPPCl.

expected from the reaction mechanism shown in Chart 3, though larger amounts of H$_2$O decomposed 2$_{\text{ox}}$.

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

Cytochrome c (crt. 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 $2_{\text{red}}$ with metalloporphyrin complexes. When a CH$_3$CN solution of 2$_{\text{red}}$ was added to a CH$_2$Cl$_2$ 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 $2_{\text{red}}$ 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 2$_{\text{red}}$ reduced MB$_{\text{ox}}$ to MB$_{\text{red}}$ completely. Addition of MB$_{\text{ox}}$ to the 2$_{\text{red}}$ 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 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 (ε = 7000) of 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 2_red was oxidized quantitatively by MB_ox to form 2_ox and MB_red.

In summary, synthetic model complexes of oxidized Rd were prepared by the ligand-substitution of [Fe(S_2-o-xyl)_2]^- with diaryl disulfide in the presence of o-xylene-α,α'-dithiol. The reduced complex [Fe(S-p-tol)_4]^- 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 o-hydroxylation of alkanes in *Pseudomonas oleovorans*.

**Experimental**

$\left(\text{Et_3N}\right)_2\left[\text{Fe(S_2-o-xyl)_2}\right] \cdot (\text{S_2NCH}_2)_2$ chloro(5,10,15,20-tetra-phenylporphyrinato)iron(III) (Fe(III)TPPCl), and o-xylene-α,α'-dithiol were prepared by the cited procedures. Di-p-tolyl disulfide was prepared by the controlled air oxidation of p-toluene dihydrochloride (6.0 mmol) with a catalytic amount (0.128 mmol) of (n-Bu_4N)_2[Fe(S_2S_p-tol)_4] in 20 ml of CH_3CN with stirring for 30 min. 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. 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 $\left(\text{Et_3N}\right)_2[\text{Fe(SAr)_4}]$ 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 voltametric analyzer in 0.1 M n-Bu_4NCIO_4 solution.

$\left(\text{Et_3N}\right)_2\left[\text{Fe(S-C_6H_4Ar)_4}\right]$ (1_red) — Bis(tetraethylammonium) tetrakis(benzenthiolato)ferrate(II) was prepared by a modification of Holm et al.'s method. 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_3·6H_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_3NBBr 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 1_red, mp 149–151 °C (dec.), as light green prisms. Anal. Calcd for C_44H_48Fe_8N_8S_8: 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, 1062, 1000, 991, 959, 894, 785.
747, 736, 692, 478 cm
-1. 1H-NMR (CD3CN) δ: -25.3 (p-H), -18.6 (o-H), 24.2 (m-H), 1.8 (CH3CH2N+). All NMR peaks were observed as broad singlets due to core paramagnetism. Halfwave potential (E1/2) vs. SCE: -0.56 V.

**Formula**: 

(Et4N)2[Fe(S-p-C6H4CH3)2] Bis(tetraethylammonium) tetrakis(p-tolueneethiolato)ferrocene(II) (2εt), bis(tetraethylammonium) tetrakis(p-chlorobenzenethiolato)ferrocene(II) (3εt) and bis(tetraethylammonium) tetrakis(p-methoxybenzenethiolato)ferrocene(II) (4εt) were prepared from FeCl3·6H2O and the corresponding thiolates by a method similar to that used for the preparation of 1εt.

X = CH3 (2εt): Yield 73%, mp 190–202°C (dec.), as green columns. Anal. Calcd for C63H60Fe4N4S6: 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. 1H-NMR (CD3CN) δ: -17.4 (o-H), 22.8 (m-H), 33.8 (p-H), 1.5 (CH3CH2N+), 3.3 (CH3</p>


