CH=C($p$-$C_6D_4Cl$)$_2$TPPH was prepared from chloral hydrate and chlorobenzene-$d_5$ in concentrated sulfuric acid by an established route.[43]

**Zinc Complexes.** Complexes **9** and **10** were prepared by treating a dichloromethane solution of the appropriate $N$-alkylporphyrin with a fivefold excess of $ZnCl_2$ in acetonitrile containing 100 μL of 2,2,6,6-tetramethylpiperidine. The solution immediately turned dark green. The solvent was removed and chromatographed on a silica gel column. Dichloromethane was used to elute $TPPH_2$ followed by 10:1 v/v dichloromethane–ethyl acetate to elute the desired zinc complex. The electronic and $^1$H NMR spectra for **9** were identical with those reported in the literature.[11,44]

**Iron(II) Complexes.** Complexes **2–7** were prepared by the previously reported method.[9] In a typical reaction dry, dioxygen-free THF (125 mL) was added to anhydrous iron(III) chloride (36.9 mg) and an excess of iron powder. The solution was heated under reflux in a nitrogen atmosphere for 2–3 h. A THF (25 mL) solution of the appropriate $N$-alkylporphyrin ($N$-$CH_3$OEPH, 53.6 mg) was added. The reaction mixture was removed from the heat, and a noncoordinating base (2,2,6,6-tetramethylpiperidine) was added until the solution was slightly basic. After 1 h the solution was filtered through Celite and the solvent was removed. The product was recrystallized from dichloromethane–$n$-hexane. The crystals were washed with $n$-hexane to yield 30 mg (48%) of red-green **7**. The electronic spectrum for **7** was similar to that reported for Fe($N$-methylprotoporphyrin IX dimethyl ester)Cl.[10] Complexes **2** and **6** give electronic spectra identical with those previously reported. The bromo complex **3** and the iodo complex **4** were obtained by the route described above starting with iron(II) bromide or iron(II) iodide, respectively.

**Instrumentation.** NMR spectra were obtained on Nicolet NT-360 FT and NMC-500 FT spectrometers operating in the quadrature mode ($^1$H frequencies are 360 and 500 MHz, respectively). Between 200 and 1000 transients were accumulated over a 40-kHz bandwidth with 16K data points for $^1$H (4–8K for $^2$H) and a 6-μs 90° pulse. The signal-to-noise ratio was improved by apodization of the free induction decay which introduced a negligible 3–10 Hz of line broadening. Line widths were determined for nonoverlapping peaks by using the Nicolet computer subroutine LF, which fit the peaks to a Lorenzian line. Overlapping peaks were fit by using the NTCCAP routine. The line broadening introduced by apodization was subtracted from the line widths. The peaks were referenced against tetramethylsilane. Electronic spectra were measured with a Hewlett-Packard 8450 A spectrophotometer.

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# Effect of N-Substituents and Axial Ligands on Reduction Potentials of N-Substituted Metalloporphyrins

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Cyclic voltammetry experiments have been carried out on Fe(II), Mn(II), and Cu(II) complexes of N-substituted porphyrins in order to test the effects of the overall coordination geometry, the N-substituent, and the axial ligand on reduction potentials. In all cases, the complexes exhibited reversible one-electron redox processes attributed to the metal center, specifically involving Fe(III)/Fe(II), Mn(III)/Mn(II), and Cu(II)/Cu(I) oxidation states. The N-substituted porphyrins are the first porphyrins to be reported that exhibit a well-defined Cu(II)/Cu(I) reduction. The half-wave potentials (vs. Ag/AgCl, in $CH_3CN$ with 0.1 M TBAP) for this process are as follows: −0.29 V ([Cu($N$-$CH_3$TPP)]$ClO_4$); −0.38 V ([Cu($N$-$CH_3$TPP)Cl]); −0.04 V ([Cu($N$-$CH_3$TPP)$PPh_3$]$^+$); −0.25 V ([Cu($N$-$C_2H_5$TPP)]$ClO_4$); −0.38 V ([Cu($N$-$C_2H_5$TPP)Cl]); −0.32 V ([Cu($N$-PhTPP)]$ClO_4$); −0.42 V ([Cu($N$-PhTPP)Cl]), demonstrating that the axial ligand exhibits a pronounced effect. For each series of complexes, the difference in potentials of the metal-centered process is similar for the N-substituents $-CH_3$, $-CH_2CH_3$, and phenyl (≤0.04 V). The exception to this small effect is the case of chloro($N$-$p$-nitrobenzyl-5,10,15,20-tetraphenylporphinato)manganese(II), which exhibits a Mn(III)/Mn(II) half-wave potential 0.09 V more positive than the corresponding $N$-methyl or $N$-ethyl complexes.

## Introduction

The N-substituted metalloporphyrins differ markedly from non-N-substituted metalloporphyrins in a number of important respects. While the structural[3] and many of the spectroscopic features (UV–visible absorption and fluorescence,[4] NMR,[4,5] and IR[6] spectra) of these complexes have received considerable attention, there have been few reports of their electrochemical properties.[7] An aspect that we introduce herein is the effect of the N-substituent on reduction potentials. Several other features are also discussed, including identification of a Cu(II)/Cu(I) reduction and measurement of potentials for copper complexes of N-substituted porphyrins with different axial ligands. While it has been shown that reduced states of metal atoms are stabilized in N-substituted metalloporphyrins relative to non-N-substituted analogues, no new oxidation states have been reported. Although many studies have involved the effects of axial ligation on potentials of non-N-substituted metalloporphyrins,[8] there have been no comparable studies for N-substituted metalloporphyrins.

We have previously demonstrated that the stability of N-substituted metalloporphyrins with respect to loss of the N-substituent via nucleophilic attack is highly dependent on the nature of the N-substituent. It appears that the rate of dissociation of the

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Table I. Half-Wave Potentials (V vs. SCE) of Chloro(*N*-substituted 5,10,15,20-tetraphenylporphinato)iron(II) Complexes

| complexes | Fe(III)/Fe(II) $E_{1/2}$ ($\Delta E_{pa-pc}$)[a] | ligand | | | $\Delta E_{1/2}$ | |
|---|---|---|---|---|---|---|
| | | $Ox_1$[b] | $Red_1$[b] | $Red_2$ | Ox–Red | Red |
| Fe(*N*-$CH_3$TPP)Cl[c] | 0.50 (0.070) | 1.52 | −0.85 | −1.34 | 2.37 | 0.49 |
| Fe(*N*-$C_2H_5$TPP)Cl[d] | 0.51 (0.075) | | −0.86 | −1.09 | | 0.23 |
| Fe(*N*-$C_6H_5$TPP)Cl[d] | 0.54 (0.075) | | −0.83 | | | |
| Fe(*N*-$C_6H_5$TPP)$^{2+}$ [e] | −0.06 | | −0.97 | | | |

[a] Under these conditions, $\Delta E_{pa-pc}$ for ferrocenium ion/ferrocene is 0.075 ± 0.005 V. [b] This reduction and oxidation could have considerable effect at the metal center and may be better represented as M(IV)/M(III) and M(II)/M(I) processes, respectively. [c] Solvent $CH_2Cl_2$, supporting electrolyte 0.1 M TBAP, taken from: Kopelove, A. B. M.S. Thesis, Colorado State University, 1981; p 38. [d] Solvent DMF or DMF/THF, supporting electrolyte 0.1 M TEAP. [e] Reference 7d.

N-substituent is related to its ability to stabilize incipient carbocation formation. We would like to determine whether such reactivity is correlated with ground-state properties of the system. Another reaction where ground-state properties of various N-substituted metalloporphyrins is of interest is the reversible migration of aryl and alkyl groups from the nitrogen atom of an Fe(II) N-substituted metalloporphyrin to the iron atom to form an Fe(III) $\sigma$-aryl or $\sigma$-alkyl non-N-substituted metalloporphyrin.[9] Such reactions may occur in vivo when cytochrome P-450 is decomposed by certain drugs[10] and have been demonstrated for reactions of aryl- and alkylhydrazines with myoglobin, hemoglobin, and catalase.[11]

## Experimental Section

**Reagents.** Dichloromethane and acetonitrile were purified by literature methods.[12] Spectrograde DMF was used without further purification. The supporting electrolyte tetrabutylammonium perchlorate (TBAP) was recrystallized from ethanol several times. Preparation of $Cu(CF_3SO_3)_2 \cdot 6H_2O$ has been described elsewhere.[13] THF was freshly distilled under Ar over metallic potassium. The noncoordinating bases 2,6-lutidine and 2,2,6,6-tetramethylpiperidine (Aldrich) were used as such without further purification. In the synthesis of iron complexes, anhydrous ferric chloride (Aldrich) and iron wire (Mallinckrodt) were used as received.

**Synthesis of Fe(II) Complexes of N-Substituted Porphyrins.** The synthesis, characterization, cyclic voltammetry, and crystal structure of chloro(*N*-methyl-5,10,15,20-tetraphenylporphinato)iron(II) has been reported.[5b] Chloro(*N*-ethyl-5,10,15,20-tetraphenylporphinato)iron(II) and chloro(*N*-phenyl-5,10,15,20-tetraphenylporphinato)iron(II) were prepared in the manner described in previous reports, but on a smaller scale. In a typical experiment, 15–25 mg of *N*-$C_2H_5$HTPP or *N*-PhHTPP were used. THF was deaerated with Ar for 15 min. About 15 mg of anhydrous $FeCl_3$ (0.092 mol) and excess iron wire were refluxed in a two-neck flask, one neck of which had the addition funnel (20 mL) containing the solution of *N*-$C_2H_5$HTPP or *N*-PhHTPP in THF under argon or nitrogen. Refluxing was continued until an amber color developed. Then, the solution of *N*-$C_2H_5$HTPP (violet) or *N*-PhHTPP (green) was added drop by drop, and refluxing was continued for about another 0.5 h. A stoichiometric amount of noncoordinating base, 2,2,6,6-tetramethylpiperidine, was added to the mixture after it was cooled. The mixture was then filtered and evaporated.

**Synthesis of Mn(II) Complexes.** Mn(*N*-PhTPP)Cl was synthesized in the same way as Mn(*N*-$CH_3$TPP)Cl.[14] In a typical experiment, 48 mg (0.07 mmol) of *N*-PhHTPP was dissolved in about 10 mL of dry dichloromethane and mixed with about 70 mg (0.35 mmol) of $MnCl_2 \cdot 4H_2O$ (Fisher) dissolved in 10 mL of absolute ethanol. Acetonitrile (15 mL) and 55 $\mu$L of 2,6-lutidine were added, and the solution was stirred overnight. The visible absorption spectrum slowly changed from that of free base to that of the complex. The residue was washed with water, extracted with dry $CH_2Cl_2$, and evaporated to dryness. The Mn(*N*-PhTPP)Cl was purified by flash chromatography. Anal. (Analytische Laboratorien, Engelskrichen, West Germany) Calcd for $MnC_5H_{33}N_4Cl$: C, 76.94; H, 4.27; N, 7.18. Found: C, 75.65; H, 4.94; N, 7.06. Visible spectrum: 457, 469 (sh), 572, 638, and 689 nm. Mn(*N*-*p*-$CH_2C_6H_4NO_2$TPP)Cl was synthesized as above. The elemental analysis (Engelskrichen, West Germany) was not as satisfactory. Anal. Calcd for $MnC_{51}H_{34}N_5O_2Cl$: C, 72.97; H, 4.09; N, 8.35. Found: C, 69.05; H, 4.73; N, 7.02. But, the visible absorption spectrum was similar to that of Mn(*N*-$CH_3$TPP)Cl. The spectrum did not change on addition of excess noncoordinating base (2,6-lutidine) so there was little protonated *N*-alkylporphyrin present. The N-substituted porphyrin complexes of Mn(II) appear to lose Mn(II) very readily.

**Synthesis of Cu(II) Complexes.** The synthesis and complete characterization of (*N*-ethyl-5,10,15,20-tetraphenylporphinato)copper(II) trifluoromethanesulfonate and its *N*-phenyl analogue have been described.[15] Solid samples were dissolved in $CH_3CN$ for cyclic voltammetry. (*N*-methyl-5,10,15,20-tetraphenylporphinato)copper(II) trifluoromethanesulfonate was prepared in situ by mixing 15.7 mg of *N*-$CH_3$HTPP and 12.3 mg of $Cu(CF_3SO_3) \cdot 2.6H_2O$ in $CH_3CN$ and stirring the solution for about 2 h. Then, 2.5 mL of a 0.005 M solution of 2,2,6,6-tetramethylpiperidine in $CH_3CN$ (total volume) was added drop by drop until complex formation was complete as indicated by its characteristic spectrum. The total volume at this stage was about 17 mL. It was converted into its chloride salt by the addition of tetraethylammonium chloride. The chloro complexes of Cu(*N*-PhTPP)$^+$ and Cu(*N*-$C_2H_5$TPP)$^+$ were prepared similarly. The visible absorption spectra of the chloro complexes are different from those of their cations: for Cu(*N*-$CH_3$TPP)$^+$, 430, 442, 546, 597, 656 nm; for Cu(*N*-$CH_3$TPP)Cl, 371, 451, 564, 617, 672 nm. There is no change in the visible spectra or in the cyclic voltammograms of Cu(*N*-RTPP)Cl complexes upon addition of excess chloride.

**Cyclic Voltammetry.** Cyclic voltammetric experiments employed a Bioanalytical Systems CV-1A istrument equipped with a three-electrode system. A Pt-wire electrode and Pt-wire coil were used as the working and auxiliary electrodes, respectively. The reference electrodes used were either a commercial aqueous saturated calomel electrod (SCE) or Ag/AgCl electrode with a porous Vycor bridge. Except in the case of Fe(III)/Fe(II) system, all other potentials have been measured in acetonitrile. Tetraethylammonium perchlorate (TEAP, Fisher grade CV only), 0.1 M in DMF, was used as the supporting electrolyte in Fe(III)/Fe(II) systems. Tetrabutylammonium perchlorate recrystallized several times from ethanol was used in all experiments performed in dry $CH_3CN$. In a typical experiment, about 3 mg of an N-substituted metalloporphyrin was dissolved in 5–6 mL of $CH_3CN$ containing ca. 0.1 M (0.5 M in the case of ClMn(*N*-PhTPP)) TBAP and deaerated for 15 min with Ar prior to the measurements. In most cases, a blanket of Ar over the solution was maintained during the experiments. The Mn(*N*-$CH_3$TPP)Cl complex is nearly insoluble in $CH_3CN$, so it was first dissolved in 0.2 mL of spectrograde benzonitrile and then diluted to 5 mL with 0.1 M TBAP in $CH_3CN$. There is no effect of additional benzonitrile on the cyclic voltammogram of Mn(*N*-PhTPP)Cl.

## Results and Discussion

Previous reports of the electrochemistry of N-substituted metalloporphyrins have focused on comparisons with corresponding non-N-substituted complexes[7a-c] or with identification of the mechanism of electrochemically induced $\sigma$-alkyl (or aryl) migration.[7d] This is the initial attempt to investigate the effects of the N-substituent in a systematic way.

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Table II. Half-Wave Potentials (V vs. Ag/AgCl) for Chloro(N-substituted 5,10,15,20-tetraphenylporphinato)manganese(II) Complexes

| complex | Mn(III)/Mn(II) $E_{1/2}$ ($\Delta E_{pa-pc}$)[a] | ligand $Ox_1$[b] | ligand $Ox_2$ | ligand $Red_1$[b] | ligand $Red_2$ | $\Delta E_{1/2}$ Ox-Red | $\Delta E_{1/2}$ Ox | $\Delta E_{1/2}$ Red |
|---|---|---|---|---|---|---|---|---|
| Mn(*N*-$CH_3$TPP)Cl[c] | 0.815 (0.081) | 1.19 | 1.40 | −1.10 | −1.31 | 2.29 | 0.21 | 0.21 |
| Mn(*N*-PhTPP)Cl[d] | 0.82 (0.082) | 1.12 | 1.32 | −1.00 | −1.28 | 2.12 | 0.20 | 0.28 |
| Mn(*N*-*p*-$CH_2C_6H_4NO_2$TPP)Cl[d] | 0.91 (0.084) | 1.24 | | −0.85 | −1.14 | 2.09 | | 0.29 |

[a] Under these conditions, $\Delta E_{pa-pc}$ for ferrocenium ion/ferrocene is 0.075 ± 0.005 mV. [b] This reduction and oxidation could have considerable effect at the metal center and may be better represented as M(IV)/M(III) and M(II)/M(I) processes, respectively. [c] Solvent $CH_3CN/C_6H_5CN$, supporting electrolyte 0.4 M TBAP. [d] Solvent $CH_3CN$, supporting electrolyte 0.1 M TBAP.

Table III. Comparison of the Half-Wave Potentials (V vs. Ag/AgCl) of (N-Substituted 5,10,15,20-tetraphenylporphyrinato)copper(II) Complexes[a]

| copper complexes | Cu(II)/Cu(I) $E_{1/2}$ ($\Delta E_{pa-pc}$)[b] | ligand $Ox_1$ | ligand $Ox_2$ | ligand $Red_1$ | ligand $Red_2$ | $\Delta E_{1/2}$ Ox-Red | $\Delta E_{1/2}$ Ox | $\Delta E_{1/2}$ Red |
|---|---|---|---|---|---|---|---|---|
| Cu(*N*-$CH_3$TPP)$^+ClO_4^-$ | −0.29 (0.075) | 1.29 | 1.53 | −1.15 | −1.36 | 2.44 | 0.24 | 0.21 |
| Cu(*N*-$CH_3$TPP)Cl | −0.38 (0.090) | | | −1.51 | −1.70 | | | 0.19 |
| [Cu(*N*-$CH_3$TPP)($PPh_3$)]$^+ClO_4^-$ | −0.04 (0.085) | | | | | | | |
| Cu(*N*-$C_2H_5$TPP)$^+ClO_4^-$ | −0.25 (0.075) | 1.31 | 1.52 | −1.14 | −1.41 | 2.45 | 0.21 | 0.27 |
| Cu(*N*-$C_2H_5$TPP)Cl | −0.38 (0.090) | | | −1.28 | −1.50 | | | 0.22 |
| Cu(*N*-PhTPP)$^+ClO_4^-$ | −0.32 (0.085) | 1.24 | 1.46 | −1.01 | −1.27 | 2.25 | 0.22 | 0.26 |
| Cu(*N*-PhTPP)Cl | −0.415 (0.090) | | | | −1.46 | | | |

[a] Solvent $CH_3CN$, supporting electrolyte 0.1 M tetrabutylammonium perchlorate. [b] Under these conditions, $\Delta E_{pa-pc}$ for ferrocenium ion/ferrocene is 0.075 ± 0.005 V.

**Iron and Manganese Complexes.** We have previously discussed the cyclic voltammetry of Fe(*N*-$CH_3$TPP)Cl[7b] and Fe(*N*-$CH_3$TPP)Cl,[7c] and Kadish and co-workers have reported potentials for the complexes [Fe(*N*-$C_6H_5$TPP)]$^{2+}$ and [Fe(*N*-$C_6H_5$OEP)]$^{2+}$.[7d] In Table I are shown potentials measured for Fe(*N*-RTPP)Cl complexes where R = $-CH_3$, $-CH_2CH_3$, and $-C_6H_5$. Despite the fact that the visible absorption spectrum of the *N*-phenyl complex is significantly shifted from those for the *N*-methyl and *N*-ethyl complexes, we find little effect in the Fe(III)/Fe(II) potentials. From the cyclic voltammetry results in Table I, the effect appears to be small. The difference in potentials between the cationic complex Fe(*N*-$C_6H_5$TPP)$^{2+}$ (with $ClO_4^-$ as counterion) and the complex with chloride-bound Fe(*N*-$C_6H_5$TPP)Cl is remarkable. In our hands, the iron complexes of N-substituted porphyrins were not sufficiently stable to perform extensive studies of the effects of axial ligand substitution. The Cu(II) complexes, however, are more tractable and the effect of different axial ligands will be discussed below.

The potentials for Mn(II) complexes of N-substituted porphyrins shown in Table II indicate that the *p*-nitrobenzyl substituent gives rise to easier reduction. This result is likely to be due to differences in the electron distributions of the Mn(III) and/or reduced ligand species since the visible absorption spectrum of the *N*-benzyl complex of Mn(II) is essentially identical with those of the *N*-methyl and *N*-ethyl analogues.

**Copper Complexes.** To determine the effects of axial ligands on the Cu(II)/Cu(I) potentials of N-substituted porphyrins, it is important to ascertain the stoichiometry of the complexes. The clear difference of visible absorption spectra for Cu(II) N-substituted porphyrin complexes with weakly binding counterions (e.g., $CF_3SO_3^-$ or $ClO_4^-$) and complexes with $Cl^-$ or $PPh_3$ establishes that in $CH_3CN$ at the concentrations used for the cyclic voltammetry experiments (about $10^{-3}$ M) formation of the 1:1 complexes with $Cl^-$ or $PPh_3$ is complete. Cyclic voltammograms were recorded in the presence of excess ligand (several different concentrations up to a 20-fold excess) without evident change in potential. As in the case of the Mn(II) and Fe(II) complexes of N-substituted porphyrins described above, for each voltammogram of these copper complexes the peak heights of the "metal-centered" process in the cathodic and anodic directions matched and the difference in potentials for the cathodic and anodic maxima was close to that for ferrocene, indicating a reversible process. The process involves one electron, as indicated by the fact that in each case the peak height matched (within 10%) that of the next subsequent reduction (assigned as ligand reduction I in Tables I–III) and next subsequent oxidation (assigned as ligand oxidation I in Tables I–III), as is evident in the voltammogram shown in Figure 1.

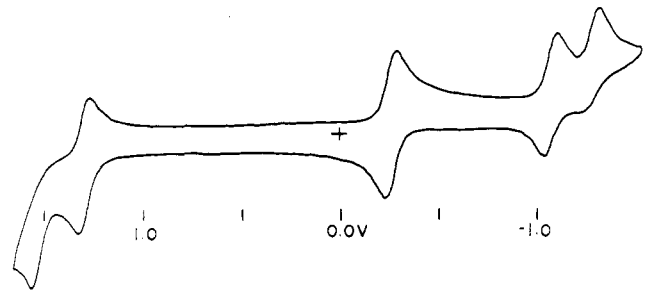


**Figure 1.** Cyclic voltammogram of Cu(*N*-$CH_3$TPP)Cl in $CH_3CN$ with 0.1 M tetrabutylammonium perchlorate as supporting electrolyte and a Ag/AgCl electrode for reference.

The potentials for copper complexes (Table III) are of interest in two respects. Typical non-N-substituted porphyrin complexes of copper show no peak in the region intermediate between the two peaks generally attributed to ligand oxidation process and the two peaks attributed to ligand reduction,[15] whereas the N-substituted complexes show a well-defined reversible peak (Figure 1) that we attribute to a Cu(II)/Cu(I) process. The Cu(I) state is evidently greatly stabilized in the coordination site of a N-substituted porphyrins relative to the site of a non-N-substituted porphyrin.

From spectral similarities of Cu(II) N-substituted porphyrin complexes to the complexes with other metal ions and the close structural resemblance of all structures of mono-N-substituted porphyrins reported to date (which include Fe(II), Mn(II), Co(II), and Zn(II) complexes), it seems very likely that the coordination geometry of the Cu(II) complexes studied herein is typical. This geometry affords only three strong metal-to-nitrogen bonds and, therefore, a smaller ligand field than that of the more highly planar non-N-substituted porphyrins that form four strong metal-to-nitrogen bonds. The stabilization of a reduced form with considerable Cu(I) character is consistent with the positive shift of reduction potentials of Fe(III)/Fe(II), Mn(III)/Mn(II), and Co(III)/Co(II) processes upon N-substitution of porphyrin ligands.[5] The appearance of an additional peak, separate from the peaks for the predominantly ligand process, however, is remarkable. No additional peak is evident, for example, for a separate Co(III)/Co(II) process in the cyclic voltammograms of Co(II) complexes of N-substituted porphyrins.[7a] (In that case it is quite reasonable that $d^8$ Co(I) would be stabilized in a planar coor-

dination geometry, but not in the domed coordination site of an N-substituted porphyrin).

The reduction potentials for copper complexes of N-substituted porphyrins (Table III) clearly show two trends: (1) a lack of a large difference between potentials for *N*-alkyl and *N*-aryl substituents and (2) a significant dependence of potentials in the nature of the axial ligand. A relatively hard ligand that binds well to Cu(II), $Cl^-$, gives rise to the least favorable potential shown. A poorly coordinating ligand, $ClO_4^-$, is more favorable for reduction, and a soft ligand expected to stabilize Cu(I), triphenylphosphine, provides the most favorable reduction potential. The visible absorption spectra of Cu(II) complexes with $Cl^-$ and $PPh_3$ as axial ligands and the lack of any shift of potentials on addition of excess ligand shows the stoichiometry of these complexes to be 1:1. All of the cyclic voltammograms show reversible processes (i.e., the difference between cathodic and anodic peak potentials was within 10 mV of that for ferrocene under the same conditions), indicating that the coordination site geometry (and, hence, the presence or absence of covalently bound axial ligand) remains the same when the copper atom is reduced or oxidized.

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# Kinetics and Mechanisms of Reduction of Rusticyanin, a Blue Copper Protein from *Thiobacillus ferrooxidans*, by Inorganic Cations

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Oxidation-reduction reactions of the blue copper protein rusticyanin from *Thiobacillus ferrooxidans* have been investigated. Cyclic voltammetry using a 4,4'-bipyridyl-modified carbon-paste electrode gives a reduction potential of 0.67 V (vs. NHE) for the protein, independent of pH in the range 1-3. Kinetic studies of the reduction of the copper(II) form of rusticyanin by iron(II) show anion effects. The dependence of reaction rate on iron(II) concentration is first order in chloride media but shows limiting zero-order kinetics in the presence of sulfate ion. The absence of inhibition by cobalt(II) ion suggests that this is due to a rate-limiting protein conformational change. Although the reaction with iron(II) is slow, it is not inconsistent with an interaction of physiological importance. In contrast, reduction by chromium(II) is rapid and comparable with reactions of other blue copper proteins.

## Introduction

*Thiobacillus ferrooxidans*[3] is a bacterium capable of growth solely on the energy available from the oxidation of iron(II) to iron(III) by $O_2$ at pH 2 and in doing so fixes its own $CO_2$ and $N_2$ and also produces a blue copper protein, rusticyanin, $RCu^{II}$, that can constitute up to 5% of the total cell protein.[4] Rusticyanin comprises a single polypeptide chain with 159 residues ($M_r$ 16300) and a single copper ion as prosthetic group. The oxidized form of the protein shows visible[5] ($\epsilon_{597}$ 2240 $M^{-1}$ $cm^{-1}$) and EPR[6] spectroscopic parameters consistent with type 1 copper and has a relatively high reduction potential of 0.68 V (vs. NHE).[7] The protein has an isoelectric point at pH 9.1[8] and is unusually stable at low pH. It has been proposed[9] that rusticyanin is the initial electron acceptor from iron(II) in the bacterial electron-transport chain. An examination of the interactions and electron-transfer mechanisms between rusticyanin and iron(II) in solution is of considerable interest.

Electron-transfer reactions of blue copper electron-transfer proteins with low molecular weight inorganic complexes have been studied extensively.[10,11] Although these reactions are not physiologically important, they reveal a great deal about the mechanisms of electron transfer available to the proteins. No previous reactions of rusticyanin have been reported, though there are studies[12,13] of the reduction of a number of blue copper proteins by the chromium(II) ion that, like iron(II), is a labile metal cation. It would seem to be important to compare the reactivity of rusticyanin, a protein where the physiological partner is an inorganic complex, with similar proteins where the physiological redox partners are other metalloproteins.

## Experimental Details

*T. ferrooxidans* (strain T.f.3), obtained from the Microbiological Research Establishment, Porton Down, Salisbury, Wilts, U.K., was grown and harvested as described by Cobley and Haddock.[4] Rusticyanin was isolated and purified by the method of Cox and Boxer[5] and yielded a single protein band by poly(acrylamide) gel electrophoresis. The protein was stored at -70 °C in $5 \times 10^{-2}$ M β-alanine buffer, pH 3.5, until use. Protein that had not been covalently modified was recycled by reoxidation ($Na_2IrCl_6$, Aldrich) followed by dialysis.

After removal from storage, protein solutions were diluted and dialyzed for 4-12 h at 0 °C against the appropriate medium required for kinetic studies. In the chromium(II) experiments, air-free conditions were achieved by bubbling the dialyzing solution with $N_2$ gas.

Reduced protein was reoxidized with small amounts of $Na_2IrCl_6$ followed by dialysis. Attempts to modify the protein with chromium(III) were carried out by reduction of the protein with a small excess of chromium(II) in sulfate media, pH 2 and 0.5 M ionic strength, followed immediately by reoxidation using $IrCl_6^{2-}$ and dialysis (3 h) against $1 \times 10^{-2}$ M $H_2SO_4$ at 0.5 M ionic strength ($Na_2SO_4$) or against $2 \times 10^{-2}$ M HCl at 0.5 M ionic strength (NaCl).

The reagents $H_2SO_4$, HCl, $Na_2SO_4$, NaCl, $CoSO_4 \cdot 7H_2O$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $FeCl_2 \cdot 4H_2O$, $FeNH_4(SO_4)_2 \cdot 12H_2O$, $Fe_2(SO_4)_3$, and $FeCl_3 \cdot 6H_2O$ (Fisher ACS and Baker "Analyzed") were used without further purification. Solutions containing iron(II) were stored under $N_2$ and were standarized by titration with $KMnO_4$.

Chromium(II) stock solutions were made by zinc amalgam reduction of chromium(III) chloride (Baker, "Analyzed") in 0.5 M HCl, under an $N_2$ atmosphere. Dilute solutions were prepared by adding aliquots of the stock to flasks containing deionized and $N_2$-purged water and sufficient HCl/NaCl or $H_2SO_4/Na_2SO_4$ to give the required pH and 0.5 M ionic strength. Transfers were achieved by syringes fitted with Teflon or

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