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Analogues of Reduced Rubredoxin: Positive Shifts of Redox Potentials of Cysteine-Containing Peptide Iron(II) Complexes

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The Fe(II) complexes of Z-Cys-Thr-Val-Cys-OMe and Z-Cys-Pro-Leu-Cys-OMe as analogues of reduced rubredoxin from *Clostridium pasteurianum* were synthesized in solution and were characterized by using visible, CD, and MCD spectral methods. The Fe(II) complexes of Z-Cys-Ala-Ala-Cys-OMe, Z-Cys-Ala-Cys-OMe, and Z-Ala-Cys-OMe were also synthesized in solution for comparison. The tetrapeptides and the tripeptide provide macroring Fe(II) chelates similar to the Cys-X-Y-Cys sequences found in rubredoxin. The redox potentials of the Fe(II)/Fe(III) couple in these peptide complexes in Me_2SO were observed at ca. −0.5 V vs. SCE (saturated calomel electrode), which is substantially more positive than the value −0.99 V vs. SCE of $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$. The positive shifts of redox potentials of these peptide complexes are attributed to NH---S hydrogen bonding derived from the orientations of the chelating-peptide chains and protected by the hydrophobic side chains of the X and Y residues.

Introduction

Rubredoxin, which has one Fe ion at its active site in a single polypeptide chain (M_r 6000), belongs to the simplest class of iron–sulfur proteins.[1] Other rubredoxins are also known.[2,3] The Fe ion possesses a distorted-tetrahedral geometry chelated with two independent tetrapeptide units through four cysteine thiolates, e.g. Cys(6)-Thr-Val-Cys(9) and Cys(39)-Pro-Leu-Cys(42) in *Clostridium pasteurianum*.[4,5] Rubredoxins are found in many organisms but their biological roles have been ambiguous except for *Pseudomonas oleovoranes* rubredoxin, which participates in the fatty acid ω-hydroxylase system.[4,6]

In aqueous solution, the redox potential of the aquated Fe(II)/Fe(III) couple is +0.52 V vs. SCE (saturated calomel electrode) and the reaction of Fe(III) ion with thiolates gives the corresponding disulfides and Fe(II) species. Rubredoxin transfers one electron between its physiological redox partners utilizing the reversible Fe(II)/Fe(III) redox couple at −0.31 V vs. SCE.[1]

On the other hand, a model complex with simple organic thiolate, $[Fe(S_2\text{-}o\text{-}xyl)_2]^{-/2-}$ (o-xyl-S_2^{2-} = o-xylene-α,α'-dithiolate), has a redox potential (−0.99 V vs. SCE in Me_2SO) at a potential approximately 0.7 V more negative than that of the native protein.[7] In the case of rubredoxin, the shift is caused by the peptide units surrounding the $[FeS_4]$ core (S denotes cysteinyl sulfur atom) and the tetrapeptide units, e.g., Cys-Thr-Val-Cys and Cys-Pro-Leu-Cys, as well as the whole protein environments. Denatured rubredoxin in 80–90% aqueous dimethyl sulfoxide (Me_2SO) no longer has the specific tertiary structure but exhibits an absorption spectrum similar to that of the native protein, and the $[Fe^{III}S_4]$ core is stable under the conditions.[7,8] An X-ray structural analysis of rubredoxin revealed that hydrogen bonds from amide NH to cysteinyl S were found within each tetrapeptide chelate and they provide possible means for the fine tuning of the redox potential.[5,9,10]

The interposed amino acid residues (X and Y) of tetrapeptides (Cys-X-Y-Cys) around the active site are highly conservative among rubredoxins from various organisms.[6,7] Although many model studies on rubredoxin have been reported,[7,11-15] the roles of the protein or chelating ligands in the control of properties of the metal ion still remain ambiguous. Previous papers have described the effect of the side chains of the X and Y residues on the Fe(III) core stability in the case of Fe(III)/Z-Cys-X-Y-Cys-OMe complexes (Z = benzyloxycarbonyl).[16] In this paper, we present the spectral characterization of various Fe(II) peptide complexes, the relationship among their redox potentials, their stabilities, and the steric effects of the interposed amino acid residues.

In view of the importance of the tetrapeptide units in the active site of rubredoxin, spectral and electrochemical studies were carried out on the Fe(II) complexes of a series of cysteine-containing peptides such as Z-Cys-Thr-Val-Cys-OMe and Z-Cys-Pro-Leu-Cys-OMe, corresponding to the Fe binding peptide unints in *Cl. pasteurianum* rubredoxin, and also Z-Cys-Ala-Ala-Cys-OMe, Z-Cys-Ala-Cys-OMe, and Z-Ala-Cys-OMe.

Experimental Section

All operations were carried out under an argon atmosphere. Dimethyl sulfoxide for the present electrochemical work was purified by distillation according to the literature method.[17] Triethylamine was degassed and distilled before use. Iron(II) chloride dihydrate was prepared by the literature method.[18] Z-Ala-Cys-OMe, Z-Cys-Ala-Ala-Cys-OMe, and Z-Cys-Ala-Cys-OMe were prepared by a previously described method.[19] The synthesis of other cysteine-containing peptides will be described elsewhere.[20]

Synthesis of Fe(II) Complexes. The Fe(II) peptide complexes were prepared by the method reported by Anglin and Davison:[13] $FeCl_2{\cdot}2H_2O$ (1.0 mg, 6×10^{-6} mol) and the cysteine-containing dipeptides (3.0×10^{-5} mol) or tetrapeptides (1.5×10^{-5} mol) were mixed in Me_2SO (5 mL). The addition of triethylamine (9×10^{-3} mL, 6×10^{-5} mol) to the solution gave a solution of the corresponding peptide Fe(II) complex, which was used without further purifications. $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$[7] and $[Et_4N]_2[Fe(SPh)_4]$[14] were prepared according to the literature methods.

(1) Lovenberg, W.; Sobel, B. E. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 193.
(2) Lode, E. T.; Coon, M. J. "Iron-Sulfur Proteins"; Lovenberg, W., Ed.; Academic Press: New York, 1973; Vol. I, pp 173–191.
(3) LeGall, J. *Ann. Inst. Pasteur, Paris* **1968**, *114*, 109.
(4) Orme-Johnson, W. . *Annu. Rev. Biochem.* **1973**, *42*, 159.
(5) Watenpaugh, K. D.; Sieker, L. C.; Jensen, L. H. *J. Mol. Biol.* **1979**, *131*, 509.
(6) Lovenberg, W.; Walker, M. N. *Methods Enzymol.* **1978**, *53*, 340.
(7) Lane, R. W.; Ibers, J. A.; Frankel, R. B.; Papaefthymiou, G. C.; Holm, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 84.
(8) Christou, G.; Ridge, B.; Rydon, H. N. *J. Chem. Soc., Chem. Commun.* **1979**, 20.
(9) Adman, E. T.; Watenpaugh, K. D.; Jensen, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4854.
(10) Adman, E. T. *Biochim. Biophys. Acta* **1979**, *549*, 107.
(11) (a) Sugiura, Y.; Kunishima, M.; Tanaka, H. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1400. (b) Ali, A.; Fahrenholz, F.; Faring, J. C.; Weinstein, B. *Int. J. Pept. Protein Res.* **1973**, *5*, 91. (c) Holah, D. G.; Coucouvanis, D. *J. Am. Chem. Soc.* **1975**, *97*, 6917. (d) Christou, G.; Ridge, B.; Rydon, H. N. *J. Chem. Soc., Chem. Commun.* **1977**, 908. (e) Muraoka, T.; Nozawa, T.; Hatano, M. *Chem. Lett.* **1976**, 1373.
(12) Millar, M.; Lee, J. F.; Koch, S. A.; Fikar, R. *Inorg. Chem.* **1982**, *21*, 4105.
(13) Anglin, J. R.; Davison, A. *Inorg. Chem.* **1975**, *14*, 234.
(14) Hagen, K. S.; Reynolds, J. G.; Holm, R. H. *J. Am. Chem. Soc.* **1981**, *103*, 4054.
(15) Hagen, K. S.; Holm, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 5496.
(16) (a) Ueyama, N.; Nakata, M.; Nakamura, A. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1727. (b) Nakata, M.; Ueyama, N.; Terakawa, T.; Nakamura, A. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3647.
(17) Mann, C. K. *Electroanal. Chem.* **1969**, *3*.
(18) Gayer, K. G.; Woontner, L. *Inorg. Synth.* **1957**, *5*, 179.
(19) Ueyama, N.; Nakata, M.; Nakamura, A. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 464.
(20) Ueyama, N.; Terakawa, T.; Fuji, M.; Nakamura, A., manuscript in prepartion.

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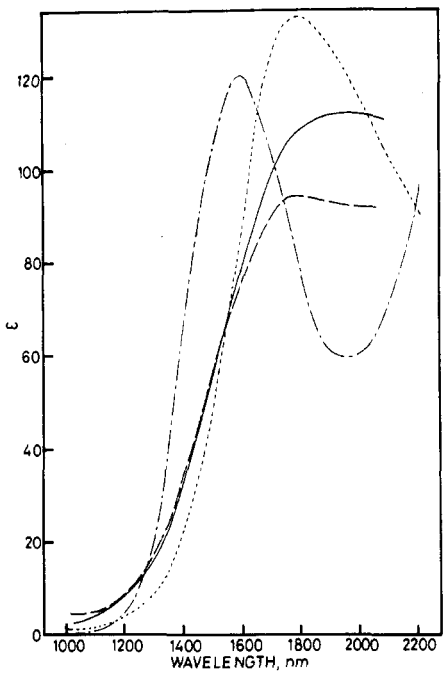

Figure 1. Near-IR absorption spectra of Fe(II) complexes of cysteine-containing peptides: (—) Z-Cys-Thr-Val-Cys-OMe complex in Me_2SO; (– – –) Z-Cys-Pro-Leu-Cys-OMe complex in Me_2SO; (- - -) $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$ in Me_2SO; (– · –) reduced rubredoxin in aqueous solution.[23]

The mixed solution of $FeCl_2 \cdot 2H_2O$ (1.0 mg, 6.0×10^{-6} mol), *o*-xylene-α,α'-dithiol (3.7 mg, 2.4×10^{-5} mol), and triethylamine (1.4×10^{-4} mol) was analyzed in situ. The results were compared with those of the isolated complex $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$ mentioned above.

Physical Measurements. Near-IR absorption spectra were recorded on a Hitachi 323 spectrophotometer. Absorption spectra were recorded on a JASCO UNIDEC-5A spectrophotometer. Circular dichroism (CD) and magnetic circular dichroism (MCD) spectra were measured with a JASCO J-40 spectropolarimeter equipped with an electromagnet. The magnetic field was calibrated with an aqueous $K_3[Fe(CN)_6]$ solution.[21] Calibration of the spectropolarimeter was performed with epiandrosterone in dioxane.[9] The values of ϵ and $\Delta\epsilon$ were given in the units of M^{-1} cm^{-1} and $\Delta\epsilon_M$ in M^{-1} cm^{-1} T^{-1}, where M expresses the molar concentration of Fe(II). The MCD spectra of the peptide complexes were corrected for zero-field circular dichroism.

Cyclic voltammograms were recorded on a Yanaco P8-CV equipped with a Yanaco Model FG-1218 function generator. Sample solutions were 10^{-3} M in Me_2SO containing 0.05 M $[(n\text{-}Bu)_4N][ClO_4]$ as a supporting electrolyte. The voltammograms were recorded with single-scanning and continuous-scanning methods using a glassy-carbon electrode at a scan rate of 100 mV s^{-1}. Potentials were determined at 25 °C vs. saturated calomel electrode (SCE) as reference. Both a directly dipped SCE and an SCE separated from a sample compartment by glass frits were employed. The redox potential of the ferrocenium ion/ferrocene couple in water-containing Me_2SO was used for the estimation of Me_2SO–water liquid junction potential before and after the measurement of redox potentials of the samples.[22] The redox potentials obtained were not corrected because of their constancies even in slightly water-containing Me_2SO.

Results

Spectral Properties. The absorption (Figures 1, 2, 3a, and 4a), MCD (Figures 3b and 4b), and CD spectra (Figures 3c and 4c) in Me_2SO were recorded for the Fe(II) complexes of a series of cysteine-containing peptides, Z-Cys-Thr-Val-Cys-OMe, Z-Cys-Pro-Leu-Cys-OMe, Z-Cys-Ala-Ala-Cys-OMe, Z-Cys-Ala-Cys-OMe, and Z-Ala-Cys-OMe, in their relevance to reduced rubredoxin. The former two tetrapeptides correspond to the residues 6–9 and 39–42, respectively, in the amino acid sequence of *Cl. pasteurianum* rubredoxin.[4,5] For the purpose of comparisons, the near-IR[23] and near-UV–visible absorption,[1] MCD, and CD

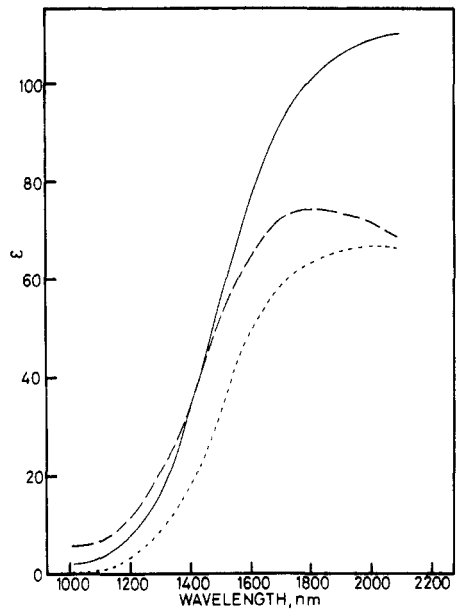

Figure 2. Near-IR absorption spectra (in Me_2SO) of Fe(II) complexes of cysteine-containing peptides: (—) Z-Cys-Ala-Ala-Cys-OMe complex; (– – –) Z-Cys-Ala-Cys-OMe complex; (- - -) Z-Ala-Cys-OMe complex.

spectra[24] of reduced rubredoxin from *Cl. pasteurianum* are reproduced in Figure 3. Those of a typical tetrahedral Fe(II) complex, $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$, are also represented in Figure 3. The spectral data are summarized in Table I.

In the near-IR absorption spectra of the Fe(II) peptide complexes, absorption maxima were found at 1770–1950 nm (ϵ = 100) (Figures 1 and 2, Table I). Tetrahedral Fe(II) complexes with high spin have spin-allowed $^5E \rightarrow {}^5T_2$ transitions with broad absorptions at 1700–2500 nm (ϵ = 30–200). Variation of the cysteine-containing peptide ligands alters the ligand field parameters, Δ_t, from 500 to 5600 cm^{-1} (Table I). These values agree with those previously reported for the tetrahedral Fe(II) complexes with thiolate ligands.[7,14,15] However, the less resolved d–d bands (Figures 1 and 2) left some uncertainties for the precise determinations of the Δ_t values.

The absorption and MCD spectra in the near-UV region of the Fe(II) peptide complexes confirm the cysteine thiolate coordinations to the Fe(II) ion in comparison with those of native protein (Figures 3 and 4, Table I). The bands at 300–340 nm were tentatively assigned to the $S^- \rightarrow$ Fe(II) charge transfer.[25] The Fe(II) complexes of Z-Cys-Thr-Val-Cys-OMe, Z-Cys-Pro-Leu-Cys-OMe, and Z-Cys-Ala-Ala-Cys-OMe exhibit characteristic absorption maxima at 313–314 nm (ϵ = 4400–5500), whereas the Fe(II) complex of Z-Ala-Cys-OMe has a shoulder at 320 nm (ϵ = 2100). An additional maximum was observed at 335 nm (ϵ = 3000–3400) for Fe(II) complexes of Z-Cys-Thr-Val-Cys-OMe or Z-Cys-Pro-Leu-Cys-OMe, although the Fe(II) complex of Z-Cys-Ala-Ala-Cys-OMe clearly does not absorb around 335 nm. No absorption maximum of the Fe(II)/Z-Ala-Cys-OMe complex was observed in the region of 330–340 nm. Additional much weaker bands observed at ~500 nm were assigned to the spin-forbidden quintet–triplet d–d transition according to the MO calculations by Bair and Goddard.[26] In the absorption spectra, the charge-transfer bands (322 nm) of $[Fe(S_2\text{-}o\text{-}xyl)_2]^{2-}$ in Me_2SO exhibited small red shifts compared with those of reduced rubredoxin as previously mentioned by Lane et al.[7]

The MCD spectra of $[Fe(S_2\text{-}o\text{-}xyl)_2]^{2-}$ of native protein revealed the differences more definitely as shown in Figure 3b. The Fe(II)

(21) Bair, R. A.; Goddard, W. A., III. *J. Am. Chem. Soc.* **1977**, *99*, 3505.
(22) DePamphilis, B. V.; Averill, B. A.; Herskovitz, T.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159.
(23) Eaton, W. A.; Palmer, G.; Fee, J. A.; Kimura, T.; Lovenberg, W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 3015.
(24) Eaton, W. A.; Lovenberg, W. "Iron-Sulfur Proteins"; Lovenberg, W., Ed.; Academic Press: New York, 1973; Vol. II, p 131.
(25) Palmer, G. "Iron-Sulfur Proteins"; Lovenberg, W., Ed.; Academic Press: New York, 1973; Vol. II, p 285.
(26) Bair, R. A.; Goddard, W. A., III. *J. Am. Chem. Soc.* **1978**, *100*, 5669.

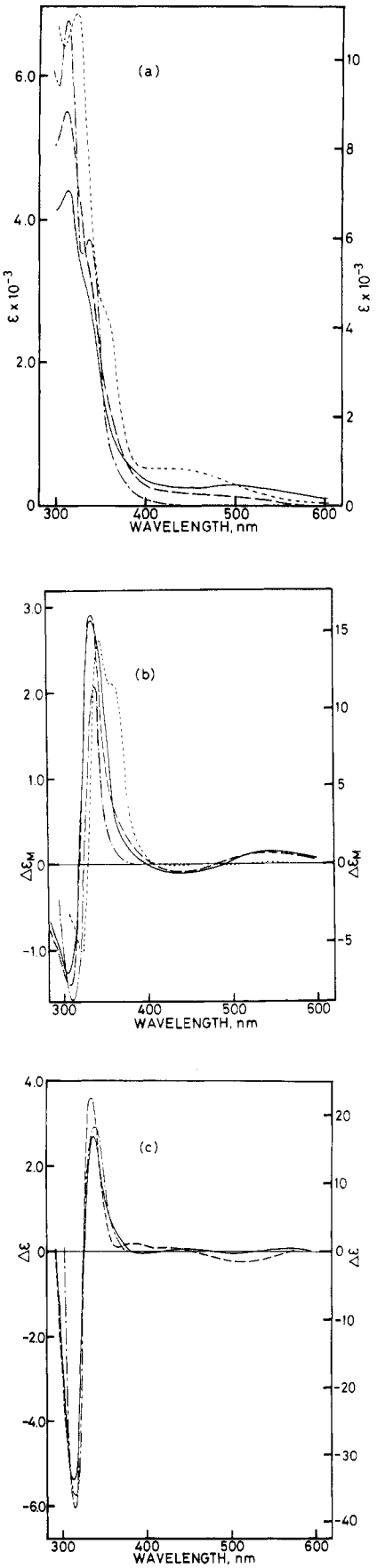

Figure 3. Near-UV–visible (a) absorption, (b) MCD, and (c) CD spectra of Fe(II) complexes of cysteine-containing peptides: (—) Z-Cys-Thr-Val-Cys-OMe complex in Me_2SO (left-hand scale); (– – –) Z-Cys-Pro-Leu-Cys-OMe complex in Me_2SO (left-hand scale); (- - -) $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$ in Me_2SO (left-hand scale); (–·–·) reduced rubredoxin in aqueous solution (right-hand scale).[1,24]

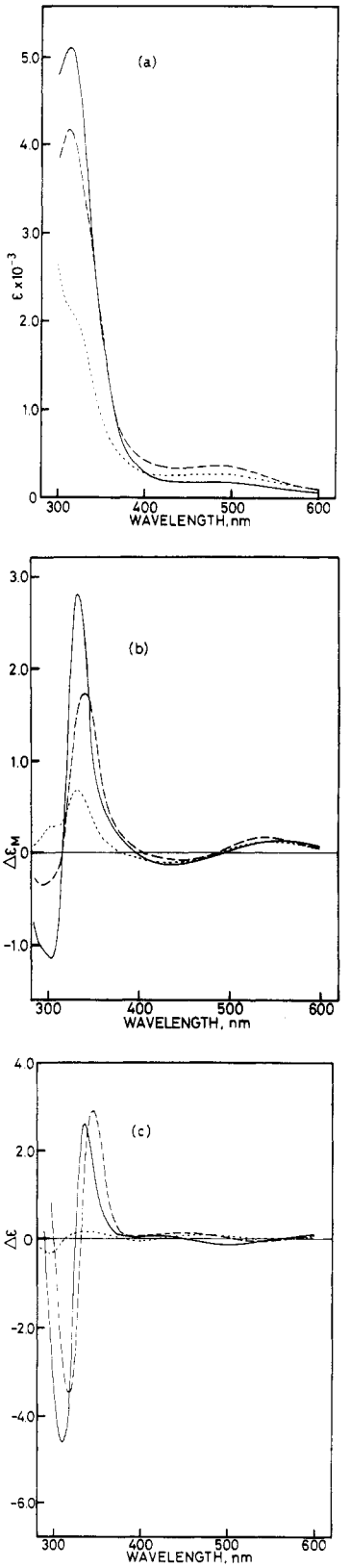

Figure 4. Near-UV–visible (a) absorption, (b) MCD, and (c) CD spectra (in Me_2SO) of Fe(II) complexes of cysteine-containing peptides: (—) Z-Cys-Ala-Ala-Cys-OMe complex; (– – –) Z-Cys-Ala-Cys-OMe complex; (- - -) Z-Ala-Cys-OMe complex.

complexes of Z-Cys-Thr-Val-Cys-OMe, Z-Cys-Pro-Leu-Cys-OMe, and Z-Cys-Ala-Ala-Cys-OMe show extrema at 303–307 nm, 315–317 nm, and 332–336 nm, exhibiting absorption and MCD spectral features similar to those of the native protein even in Me_2SO. The MCD maximum in the Fe(II)/2-Cys-Ala-Cys-OMe complex was shifted to 294 nm. The Fe(II)/Z-Ala-Cys-

Table I. Spectral Data of Fe(II) Complexes of Cysteine-Containing Peptides

ligands		$S^- \rightarrow Fe(II)$ charge transfer									$^5E \rightarrow {}^5T_2$	
Z-Cys-Thr-Val-Cys-OMe[a]	abs[a]		314 (4400)	335 sh (3000)				485 (300)			1950 (120)	
	MCD[c]	303 (−1.3)	317 (0)	336 (2.8)		430 (−1.0)			523 (0.2)			
	CD[d]		313 (−5.4)	336 (2.7)	398 (−0.04)	440 (0.03)		495 (−0.07)		555 (0.03)		
Z-Cys-Pro-Leu-Cys-OMe[a]	abs		313 (5500)	335 sh (3400)			450 (180)				1770 (100)	
	MCD	307 (−1.4)	317 (0)	333 (2.9)		430 (−0.1)			530 (0.1)			
	CD		314 (−6.1)	333 (3.6)	376 (0.2)	440 (0.1)		502 (−0.3)				
Z-Cys-Ala-Ala-Cys-OMe[a]	abs		314 (5200)					480 sh (180)			2000 (110)	
	MCD	304 (−1.1)	315 (0)	332 (2.8)		425 (−0.1)			546 (0.1)			
	CD		310 (−4.6)	335 (2.6)		425 (0.1)				555 (0.03)		
Z-Cys-Ala-Cys-OMe[a]	abs		313 (4200)	340 sh (2800)				490 (360)			1770 (80)	
	MCD	294 (−0.6)	316 (0)	339 (1.7)				460 (−0.1)	530 (0.2)			
	CD		319 (−3.5)	346 (2.9)				450 (0.2)	540 (0.04)			
Z-Ala-Cys-OMe[a]	abs		320 sh (2100)					450 sh (280)			1800 (60)	
	MCD	305 (0.3)		332 (0.7)		425 (−0.1)			550 (0.1)			
	CD	298 (−0.3)		334 (0.2)	398 (−0.03)			494 (0.1)				
$[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$[a]	abs		322 (6900)		355 sh (2700)			440 sh (530)			1800 (130)	2200 sh (120)
	MCD		320 (−1.2)	328 (0)	343 (2.6)	358 (2.1)	416 (−0.01)	480 (−0.01)	540 (0.02)			
reduced rubredoxin[e]	abs		311[f] (10900)	333[f] (6000)							1600[g] (130)	2700[g]
	MCD[c,h]		313 (−9.1)	324 (0)	330 (11.3)							
	CD[d,h]		314 (−36)	334 (18)								

[a] In Me_2SO. [b] In nm (ϵ, M^{-1} cm^{-1}). [c] In nm ($\Delta\epsilon_M$, M^{-1} cm^{-1} T^{-1}). [d] In nm ($\Delta\epsilon$, M^{-1} cm^{-1}). [e] In aqueous solution. [f] From ref 1. [g] From ref 19. [h] From ref 20.

Table II. Cyclic Voltammetric Data of Fe(II) Complexes of Cysteine-Containing Peptides[a]

	$E_{p,a}$[b]	$E_{p,c}$[c]	E_p[d]	ΔE_p[e]		$E_{p,a}$[b]	$E_{p,c}$[c]	E_p[d]	ΔE_p[e]
Z-Cys-Thr-Val-Cys-OMe	−0.26	−0.80	−0.53	0.54	Z-Cys-Ala-Cys-OMe	−0.35	−0.75	−0.55	0.40
Z-Cys-Pro-Leu-Cys-OMe	−0.43	−0.65	−0.54	0.22	o-xylene-α,α'-dithiolate[f]	−0.94	−1.02	−0.98	0.08
Z-Cys-Ala-Ala-Cys-OMe	−0.32	−0.83	−0.58	0.51	$[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$[g]	−0.91	−1.06	−0.99	0.15

[a] In Me_2SO, in V vs. SCE. [b] Anodic peak potential. [c] Cathodic peak potential. [d] $E_p = (E_{p,a} + E_{p,c})/2$. [e] $\Delta E_p = E_{p,a} - E_{p,c}$. [f] Prepared in situ. [g] Isolated.[14]

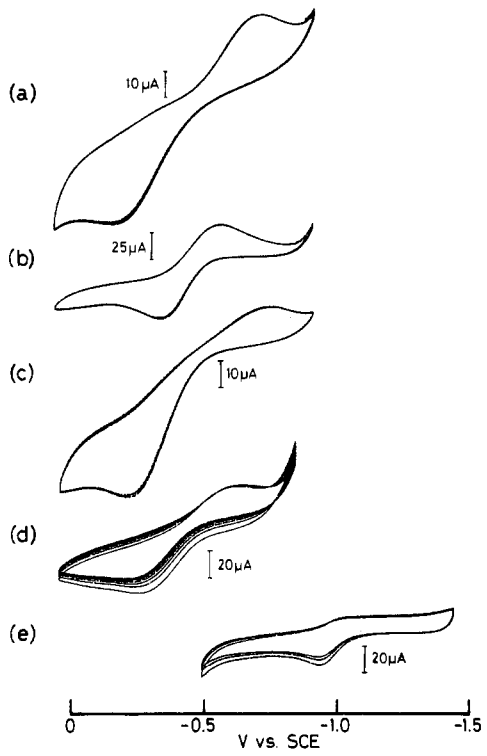

Figure 5. Cyclic voltammograms of Fe(II) complexes of cysteine-containing peptides: (a) Z-Cys-Thr-Val-Cys-OMe complex; (b) Z-Cys-Pro-Leu-Cys-OMe complex; (c) Z-Cys-Ala-Ala-Cys-OMe complex; (d) Z-Cys-Ala-Cys-OMe complex; (e) *o*-xylene-α,α'-dithiolate complex prepared in situ (see Results).

OMe complex exhibited two weak extrema at 305 and 332 nm, and no MCD maximum between them was observed, different from those of the other Fe(II) peptide complexes.

The CD spectra of the Fe(II) complexes of the tetrapeptides Z-Cys-Thr-Val-Cys-OMe, Z-Cys-Pro-Leu-Cys-OMe, and Z-Cys-Ala-Ala-Cys-OMe were composed of two large negative and positive Cotton effects at 312 and 335 nm, respectively, and of additional much weaker bands at the longer wavelength as shown in Figures 3b and 4b and in Table I. The main characteristics of the CD spectra of the tetrapeptide complexes were very similar to those of reduced rubredoxin although the magnitude of the Cotton effects of the peptide complexes was ca. $^1/_5$ of that in the native protein.

The CD spectrum of the Fe(II) complex of Z-Cys-Ala-Cys-OMe showed the small red shift at the main charge-transfer bands. Only much weaker Cotton effects in the near-UV–visible region were observed for the Fe(II) complex of Z-Ala-Cys-OMe.

Redox Properties of Model Complexes. The electrochemical properties of Fe(II) complexes of the Cys-containing peptides were investigated by cyclic voltammetry. Figure 5 shows the cyclic voltammograms of these complexes in Me_2SO. The redox potentials ($E_{pa}/2 + E_{pc}/2$) obtainable from the quasi-reversible peaks are listed in Table II. The redox potentials were unchanged with the scanning rates of 20–200 mV s^{-1}. In order to ensure the validity of these redox potentials for the various peptide complexes prepared in solution, the cyclic voltammograms of isolated $[Et_4N][FeS_2$-*o*-xyl$)_2]$ and $FeCl_2{\cdot}2H_2O/o$-xylene-α,α'-dithiol/NEt_3 (1:4:23) prepared in situ were examined. Fairly identical redox potentials at −0.99 and −0.98 V (vs. SCE) were respectively observed. The preparation in situ generally requires excess reagents, e.g. NEt_3 or thiol. The above results indicate that excess thiol or NEt_3 does not affect the redox potential of $[Fe(SR)_4]^-$ in Me_2SO.

Recently, many electrochemical studies have been reported for the effect of additives, e.g. bipyridyl,[27] 6-mercaptopurine,[28] and methylviologen,[29] on cytochrome *c* and spinach ferredoxin. These additives do not change the redox potentials even if they have redox potentials close to those of the metalloproteins. In our case, the in situ complex gave a small ΔE_p (0.08 V) compared to the value for the isolated $[Et_4N][Fe(S_2$-*o*-xyl$)_2]$ (ΔE_p = 0.15 V). Therefore, an excess of NEt_3 or thiol eases the reversible electron transfer of the iron–thiolate complexes.

All the Fe(II) peptide complexes except for the Fe(II) complex of Z-Ala-Cys-OMe exhibited quasi-reversible redox couples at 0.54–0.58 V (vs. SCE) as shown in Figure 5 and Table II. Only the Fe(II) complex of Z-Cys-Pro-Leu-Cys-OMe showed a small ΔE_p value (0.22 V). The dipeptide complex had an irreversible redox process under the experimental conditions employed.

Discussion

Structures of Fe(II) Complexes of Cysteine-Containing Peptides. From the absorption and MCD spectra of the Fe(II) peptide complexes, mononuclear tetrahedral coordinations around the Fe(II) ion with four cysteine thiolates were ensured by the $^5E \rightarrow {}^5T_2$ transitions at the near-IR region and by the $S^- \rightarrow$ Fe(II) charge-transfer transitions at the near-UV region. Absence of any absorption of the Fe(II) peptide complexes around 330–350 nm except for that of the Fe(II)/Z-Ala-Cys-OMe complex suggests that the peptide sequence of Cys-X-Y-Cys or Cys-X-Cys serves to prefer a chelating structure to Fe(II) ion in Me_2SO. On the other hand, a mononuclear complex, $[Fe(S_2$-*o*-xyl$)_2]^{2-}$, and a binuclear complex, $[Fe_2(S_2$-*o*-xyl$)_3]^{2-}$, were reported to exhibit a maximum at 355 nm (sh, ϵ = 2700) and at 360 nm (ϵ = 4290), respectively.[7] The latter maximum with some red shift was also reported for $[(FeSPh)_4(\mu$-SPh$)_6]^{2-}$ (430 nm (sh), ϵ = 2300) compared with that of $[Fe(SPh)_4]^{2-}$.[14,30] The bridging thiolate causes a red shift or broad tailing of the charge-transfer transitions compared with the terminal ligations. Absence of polynuclear complexes is thus due to the solvent effect of Me_2SO, while DMF does not have such an effect.[31] A slightly red-shifted absorption of Z-Cys-Ala-Cys-OMe at 340 nm (ϵ = 2800) may indicate the formation of polynuclear species, when compared with the absorption of the Fe(II)/Cys-X-Y-Cys complex at 335 nm.

Eaton and Lovenberg proposed that the MCD bands of reduced rubredoxin at 313 and 330 nm mainly arise from *B* or *C* terms although a contribution of the *A* term to the band at 313 nm is not ruled out.[24] Comparison of the MCD spectra among the tri- and tetrapeptide complexes, native rubredoxin, and $[Fe(S_2$-*o*-xyl$)_2]^{2-}$ revealed a significantly different spectral pattern for the last one with a red shift of the main Faraday effects in the charge-transfer region and an additional positive MCD band at 358 nm corresponding to the shoulder at 355 nm in the absorption spectrum. According to Jørgensen's "optical electronegativity" theory, higher reducing ability of the coordinating atom lowers the energy of the charge-transfer bands.[32] On the basis of the criteria, the electron density on the S atoms of $[Fe(S_2$-*o*-xyl$)_2]^{2-}$ is higher than the density on those of the peptide complexes and of rubredoxin. Chemical environments at the $[Fe^{II}S_4]$ core of the peptide complexes are different from those of the simple alkanethiolate complexes. This will affect the redox properties of the complexes as discussed later.

Comparison of the CD spectra of the tetrapeptide Fe(II) complexes with native rubredoxin serves to infer the solution structure (Figures 3c and 4c, Table I). The tetrapeptide complexes have $[Fe^{II}S_4]$ core environments essentially the same as that of reduced rubredoxin as judged from the CD spectral characteristics. The CD spectral evidences indicate macroring chelate structure for the tetrapeptide complexes. In the native protein, the active

(27) Schreurs, J.; Veugelers, P.; Wonders, A.; Barendrecht, E. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 263.
(28) Taniguchi, I.; Murakami, T.; Toyosawa, K.; Yamaguchi, H.; Yasukouchi, K. *J. Electroanal. Chem. Interfacial Electrochem.* **1982**, *131*, 397.
(29) Van Dijk, C.; Van Eijs, T.; Van Leeuwen, J. W.; Veeger, C. *FEBS Lett.* **1984**, *166*, 76.
(30) Coucouvanis, S.; Swenson, D.; Baenziger, N. C.; Murphy, C.; Holah, D. G.; Sfarnas, N.; Simopoulos, A.; Kostikas, A. *J. Am. Chem. Soc.* **1981**, *103*, 3350.
(31) Dance, I. A. *J. Am. Chem. Soc.* **1979**, *101*, 6264.
(32) Jørgensen, C. K. *Prog. Inorg. Chem.* **1970**, *12*, 101.

site consists of two independent tetrapeptide units, Cys-Thr-Val-Cys and Cys-Pro-Leu-Cys.[4] The synthetic tetrapeptide units of the Cys-X-Y-Cys type are very important for the chemical simulations of the active site environments found in rubredoxin. The spectral profiles of the Z-Ala-Cys-OMe complex in the charge-transfer region are entirely different from those of the tetrapeptide complexes and of the native protein. The $[Fe^{II}S_4]$ core in the dipeptide complex is probably placed in an environment distinct from that of others.

The absorption and MCD spectra of the Fe(II) complex of Z-Cys-Ala-Cys-OMe revealed that the tripeptide complex had a core structure similar to those of the tetrapeptide complexes. A small red shift of the Cotton effect at 294 nm of the tripeptide complex in the charge-transfer region (Figure 4c and Table I) indicated somewhat different arrangement of the tripeptide around the $[Fe^{II}S_4]$ core. The Cys-X-Cys sequence is thus not very suitable, but possible, for chelation to the Fe(II) ion.

Redox Properties of Fe(II) Complexes of Cysteine-Containing Peptides. As shown in Table II, the tetra- and tripeptide complexes had redox potentials (E_p) at ~−0.5 V vs. SCE. These values were substantially more positive than those of $[Et_4N]_2[Fe(S_2\text{-}o\text{-}xyl)_2]$ (−0.99 V vs. SCE in Me_2SO), $[Et_4N][Fe(SEt)_4]$ (−1.08 V vs. SCE),[15] and $[Et_4N][Fe(2,3,5,6\text{-tetramethylbenzenethioate})_4]$ (−0.85 V vs. SCE)[12] and were were similar to that of $[Et_4N]_2[Fe(SPh)_4]$ (−0.54 V vs. SCE).[14]

Several reasons may be considered to explain the differences in the redox potentials among the peptide complexes and the simple alkanethiolate complexes. First, as expected by Bair and Goddard,[26] the redox potentials are varied by the difference in the levels of the Fe d orbitals finely tuned by the orientation of the S lone pair pointing toward the Fe ion. However, the contributions from the Fe d-orbital energies are trivial when the ligand field parameters (Δ_t) are compared: peptide complexes, Δ_t = 5000–5650 cm^{-1}; $[Fe(S_2\text{-}o\text{-}xyl)_2]^{2-}$, Δ_t = 5560 cm^{-1}; $[Fe(SPh)_4]^{2-}$, Δ_t = 4810 cm^{-1}.[15]

Construction of CPK models of the peptide and simple alkanethiolate complexes revealed the remarkable diferences in molecular crowding around the $[FeS_4]$ cores. Thus, the $[FeS_4]$ core in $[Fe(S_2\text{-}o\text{-}xyl)_2]^{-/2-}$ is exposed to the solvent but that in the Z-Cys-Pro-Leu-Cys-OMe complex is wrapped by the peptide ligand and shielded from the solvent. The S,S chelation by Z-Cys-Pro-Leu-Cys-OMe ligand forces the complex to take a conformation in which the S atom of the Z-Cys residue is hydrogen bonded with the two amide NH groups of the Leu and Cys-OMe residues. The structure, containing two NH---S hydrogen bonds (3.55 and 3.71 Å) in the Cys-Pro-Leu-Cys sequence, was actually found in the X-ray structural analysis of native rubredoxin.[5] Such NH---S hydrogen bonds have been thought to be crucial for determining the redox potentials of iron–sulfur proteins.[9] The hydrogen bonds are expected to decrease electron density on the S atom of the peptide ligand, and the positive shift of the redox potentials results.

By the extensive studies of the ligand effects on the redox potentials of $[Fe(SR)_4]^{-/2-}$,[7,12,14,15] $[Fe_2S^*_2(SR)_4]^{2-}$,[33] and $[Fe_4S^*_4(SR)_2]^{2-}$[34] (S* denotes inorganic sulfide) among a series of R groups from *tert*-butyl to aryl, a positive shift has been found with electron-attracting substituents. Thus, the redox potentials of $[Fe_4S_4(SPh)_4]^{2-}$ are the most positive. E_p values of the Fe(II) peptide complexes, −0.53 to −0.58 V vs. SCE, nearly identical with that of $[Fe(SPh)_4]^{2-}$, −0.54 V vs. SCE, indicate that the decrease in donation from sulfur atom to Fe(II) ion is due to NH---S hydrogen bonding.

This conclusion is also supported by the red shift (322 nm) of the charge-transfer transitions for the latter. In particular, the Fe(II) complexes of the tetrapeptide provide a significant blue shift (313–314 nm) from the transition of $[Fe(S_2\text{-}o\text{-}xyl)_2]^{2-}$. Such blue shifts are explainable by the decrease of electron density on the S atom with the NH---S hydrogen bond.

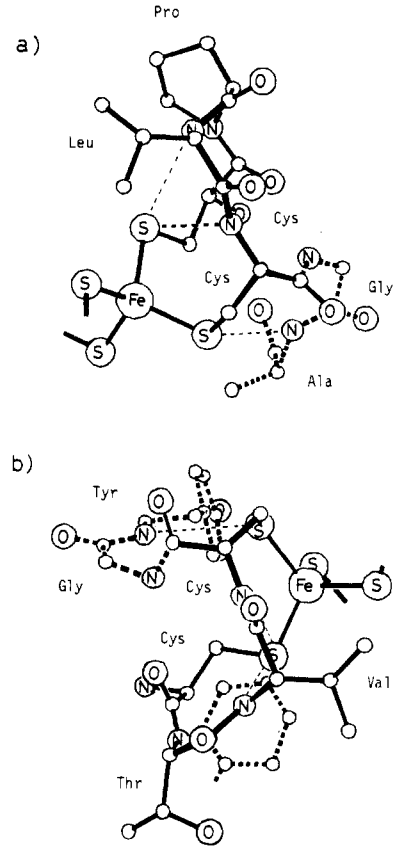

Figure 6. Graphical representations of the structural data determined by X-ray analysis of rubredoxin: (a) Cys(39)-Pro-Leu-Cys(42) and (b) Cys(6)-Thr-Val-Cys(9) sequences.[5,35]

The Fe(II)/Z-Cys-Pro-Leu-Cys-OMe complex showed a fairly good reversibility (ΔE_p = 0.22 V) as evidenced by the cyclic voltammograms. The cyclic voltammetric behavior is not correlated with the thermal stabilities of the peptide complexes in the oxidized state (Fe(III)). For example, the Z-Cys-Ala-Cys-OMe complex is more thermally unstable than the Z-Ala-Cys-OMe complex in the Fe(III) state but exhibits a quasi-reversible voltammogram. Leussing and co-workers reported high formation constants for the Fe(III) complexes of 2,3-dimercapto-1-propanaol (dmp) and 1,2-ethanedithiol under basic conditions (pH 11), in contrast to the low thermal stability of Fe(III) complexes of monothiols.[36] However, the complexes of these dithiols were unstable with rapid oxidation–reduction reactions under neutral conditions in water. They also determined the formation constants of Fe(II) complexes of dmp, which were about 10^{28} for $[Fe_2(dmp)_3]^{2-}$ and 6×10^{15} for $[Fe(dmp)_4]^{2-}$ in 0.10 M aqueous KCl at 30 °C.[37] In the case of Fe(II) peptide complexes, formation of a mononuclear complex, $[Fe(S_2R)_2]^{2-}$, is preferred except for the Fe(II) complex of Z-Cys-Ala-Cys-OMe. For the Z-Cys-Pro-Leu-Cys-OMe complex, the oxidized species is considered to be stabilized by the hydrophobic side chains of the Pro and Leu residues, especially in a nonpolar solvent.

Reversibility of the Fe(II)/Fe(III) couples of the Fe(II) complexes depends on the identity of the amino acid residues interposed between the two Cys residues (see Figure 5). The Fe(II)/Z-Cys-Pro-Leu-Cys-OMe complex is the best among others for keeping reversible redox cycles. Figure 6 illustrates the structures of Cys(39)-Pro(40)-Leu(41)-Cys(42) and Cys(6)-Thr(7)-Val-(8)-Cys(9) sequences of rubredoxin with ball and stick models.[5,35] The hydrophobic side chains of Pro and Leu residues wrap the hydrogen bonds of Leu(41)–NH---S(39) and Cys(42)–NH---

(33) Reynolds, J. G.; Holm, R. H. *Inorg. Chem.* **1980**, *19*, 3257.
(34) Mayerle, J. J.; Denmark, S. E.; Depamphilis, B. V.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 1032.
(35) Taketani, M.; Iga, Y.; Matsuura, Y.; Yasuoka, N.; Kakudo, M.; Isomoto, Y. *Proc. Int. CODATA Conf.* **1980**, *7*, 84.
(36) Leussing, D. L.; Mislan, J. P. *J. Phys. Chem.* **1960**, *64*, 1908.
(37) Leussing, D. L.; Jayne, J. *J. Phys. Chem.* **1962**, *66*, 426.

S(39). In the case of the Cys-Thr-Val-Cys sequence, the NH---S bondings in Cys(9)-NH---S(6) and Val(8)-NH---S(6) are protected by the hydrophobic side chains such as phenyl groups of Tyr and Phe residues which do not belong to the Cys(6)-X-Y-Cys(9) part.

In the Fe(II)/Z-Cys-Thr-Val-Cys-OMe complex, the NH---S bonds are protected by the alkyl side chain of the Val residue only. Our results indicate that reversibility of the redox couple for the Fe(II) complex of Z-Cys-Pro-Leu-Cys-OMe is primarily due to the Cys-NH---S and Leu-NH---S hydrogen bonds surrounded by the side chains of the Pro and Leu residues. On the other hand, NH---S hydrogen bonds in the Fe(II)/Z-Cys-Thr-Val-Cys-OMe complex are exposed to solvents without being shielded by hydrophobic groups. The side chain of the Val residue is not enough to protect the NH---S hydrogen bond as illustrated in Figure 6b.

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The Pentachlorooxotechnetate(VI) Anion, $[TcOCl_5]^-$: An EPR Study

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The anion pentachlorooxotechnetate(VI), $TcOCl_5^-$, has been prepared by reduction of TcO_4^- with HCl in concentrated H_2SO_4 and investigated by means of the EPR technique. The axial symmetric spectrum suggests an "in-plane π-type" ground state of the MO of the unpaired electron. The Tc-Cl_{eq} bonding properties have been discussed in terms of MO theory, and a comparison has been given to the bonding properties in likely complexes of neighboring elements of Tc.

Although coordination compounds of technetium have attracted a growing interest in view of their relevance in the field of nuclear medicine,[1-3] their chemistry is much less known than that of their corresponding neighboring elements. This holds true especially for compounds in which Tc possesses the formal oxidation state "+6". To our knowledge there are only very few well-characterized compounds, namely TcF_6,[4] $TcOF_4$,[5] $(NO)_2TcF_8$,[6] $TcOCl_4$,[7] $[(C_3H_3)_4N]_2TcO_4$,[8-10] and the trigonal-prismatic coordinated complexes tris(toluenedithiolato)technetium(VI), $Tc(tdt)_3$,[11] and tris(*o*-aminobenzenethiolato)technetium(VI), $Tc(abt)_3$.[12]

Up to now, only two EPR studies on Tc(VI) compounds have been reported, made on the trigonal-prismatic complexes $Tc(tdt)_3$[11] and $Tc(abt)_3$.[12] In the former case only the liquid-solution EPR spectrum was reported; for $Tc(abt)_3$ studies in frozen solution have been made. Therefore, the EPR behavior of Tc(VI) compounds-excluding the studies on the trigonal-prismatic ones for which, according to the symmetry, only very small ^{99}Tc hfs (hfs = hyperfine splitting) is observed—is unknown up to now. However, considering the radioactivity of technetium, EPR appears to be a very suitable method for investigating paramagnetic Tc complexes because only very small amounts of the compounds are needed.

The reduction of pertechnetate with HCl has been studied by several authors.[13-19] The formation of Tc(VI) species earlier proposed for this reaction[13] was disputed later when $[TcOCl_4]^-$ and $[TcOCl_5]^{2-}$ containing the metal in the oxidation state "+5" were isolated. In this paper we report an EPR spectroscopic investigation of the reaction of $KTcO_4$ dissolved in concentrated H_2SO_4 with a concentrated aqueous solution of HCl. After mixing of the reactants, immediately a deep blue solution was obtained, giving very intense EPR spectra that can be attributed unambiguously to a Tc(VI) complex species, most likely to $[TcOCl_5]^-$. The formation of $TcOCl_4$ cannot be excluded completely but appears not to be favored because of the conditions applied for the reduction of TcO_4^-.

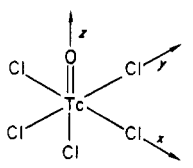

Table I. EPR Parameters for $[TcOCl_5]^-$ (Hyperfine Coupling Constants Given in 10^{-4} cm^{-1})

$\tilde{g}$	$\tilde{A}^{Tc}$
$g_\parallel = 2.057 \pm 0.003$	$A_\parallel = 230.1 \pm 2.0$
$g_\perp = 1.938 \pm 0.005$	$A_\perp = 95.8 \pm 5.0$
$\langle g_{av}\rangle^a = 1.978$	$\langle A_{av}\rangle^a = 140.6$

$^a \langle g_{av}\rangle = (g_\parallel + 2g_\perp)/3$; $\langle A_{av}\rangle = (A_\parallel + 2A_\perp)/3$.

Lower oxidation states can be ruled out. Except for Tc compounds in the formal oxidation state "+2",[20-24] no resolved EPR signals

(1) Jones, A. G.; Davison, A. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 867. Davison, A.; Jones, A. G. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 881.
(2) Schwochau, K. *Radiochim. Acta* **1983**, *32*, 139. Münze, R. *Isotopenpraxis* **1983**, *19*, 401.
(3) Subramanian, S. G.; Rhodes, B. A.; Cooper, J. F.; Sodd, V. J. "Radiopharmaceuticals"; The Society of Nuclear Medicine: New York, 1975.
(4) Selig, H.; Chernick, C. L.; Malm, J. G. *J. Inorg. Nucl. Chem.* **1961**, *19*, 377.
(5) Edwards, A. J.; Hugill, D.; Peacock, R. D. *Nature (London)* **1963**, *200*, 672.
(6) Holloway, J. A.; Selig, H. *J. Inorg. Nucle. Chem.* **1968**, *30*, 473.
(7) Guest, A.; Lock, C. J. L. *Can. J. Chem.* **1972**, *50*, 1807.
(8) Schwochau, K.; Astheimer, L.; Hauck, J.; Schenk, H.-J. *Angew. Chem.* **1974**, *63*, 1988.
(9) Astheimer, L.; Hauck, J.; Schenk, H.-J.; Schwochau, K. *J. Chem. Phys.* **1975**, *63*, 350.
(10) Astheimer, L.; Schwochau, K. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1131.
(11) Kakashima, M.; Koyama, M.; Fujinaga, T. *J. Inorg. Nucl. Chem.* **1976**, *38*, 801.
(12) Kirmse, R.; Stach, J.; Spies, H. *Inorg. Chim. Acta* **1980**, *45*, L 251.
(13) Ryabchikov, D. I.; Podzuyakov, A. A. *Dokl. Akad. Nauk SSSR* **1964**, *155*, 153.
(14) Münze, R.; Noll, B. *Isotopenpraxis* **1975**, *11*, 190.
(15) Spitsyn, V. I.; Glinkina, M. I.; Kusina, A. P. *Dokl. Akad. Nauk SSSR* **1971**, *200*, 1372.
(16) Dalziel, J.; Gill, N. S.; Nyholm, R. S.; Peacock, R. D. *J. Chem. Soc.* **1958**, 4012.
(17) Cotton, F. A.; Davison, A.; Day, V. W.; Gage, L. D.; Trop, H. S. *Inorg. Chem.* **1979**, *18*, 3024.
(18) Jezowska-Trzebiatowska, B.; Baluka, M. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **1965**, *13*, 1.
(19) Baluka, M.; Hanuza, J.; Jezowska-Trzebiatowska, B. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **1972**, *20*, 271.
(20) Orvig, C.; Davison, A.; Jones, A. G. *J. Labelled Compd. Radiopharm.* **1981**, *18*, 148.
(21) Heitzmann, M. W.; Yang, G. C.; Ford, L. A.; Benson, W. R. *Inorg. Chem.* **1982**, *21*, 3242.
(22) Yang, G. C.; Heitzmann, M. W.; Ford, L. A.; Benson, W. R. *J. Labelled Compd. Radiopharm.* **1981**, *18*, 535.
(23) Kirmse, R.; Lorenz, B.; Schmidt, K. *Polyhedron* **1983**, *2*, 935.

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0020-1669/85/1324-2196$01.50/0