bonds, exist between Fe–S cores and peptide bonds. The active site of rubredoxin consists of one iron and two specific sequences, Cys–X–Y–Cys, as shown in Fig. 1. Thus, Z–Cys–Thr–Val–Cys–OMe and

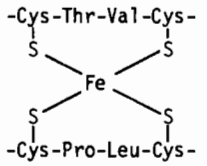

Fig. 1. Active site of rubredoxin.

Z–Cys–Pro–Leu–Cys–OMe were synthesized. Z–Cys–Ala–Ala–Cys–OMe was also examined as a chelating ligand for reference. A dipeptide, Z–Ala–Cys–OMe, was examined as non-chelating peptide. 1Fe and Fe_4S_4 complexes of t-Boc-(Gly–Cys–Gly)$_4NH_2$ were reported by Rydon [1] and Holm [2], respectively. However, the -Gly–Gly- sequence between the two Cys residues is not preferable for turn conformation which is essential for chelation and for formation of the NH···S hydrogen bonds. We reported that Fe(III)/Z–Cys–Ala–Ala–Cys–OMe is a good spectral model of rubredoxin in Me_2SO [3].

The electrochemical properties of 1Fe and Fe_4S_4 complexes are important in aqueous solutions. The redox potentials of ferredoxins are influenced by the core as well as the surrounding environments. In native proteins, the core is non-polar and surrounded by polar aqueous environments. Therefore, we examined these model complexes spectrally and electrochemically in micelle by using 2–10% solutions of Triton X-100. The redox potential values obtainable by cyclic voltammogram in micelle are compared with the values of native rubredoxin or iron–sulfur proteins in aqueous solution.

Fe(II)/Z–Cys–Pro–Leu–Cys–OMe (1:2) complex in aqueous micelle exhibited CD extrema at 309 nm ($\Delta\epsilon$: −24.2) and 332 nm ($\Delta\epsilon$: 10.9), similar to those of reduced rubredoxin [4]. A redox couple of Fe(II)/Fe(III) was observed for Fe(II)/Z–Cys–Pro–Leu–Cys–OMe (1:2) at −0.37 V(SCE) in aqueous micelle, which is very close to −0.30 V(SCE) reported for rubredoxin. Such a positive shift was observed for the first time in synthetic model complexes of rubredoxin. No redox couple was found for Fe(II)/Z–Cys–Thr–Val–OMe (1:2) or Fe(II)/Z–Cys–Ala–Ala–Cys–OMe (1:2) in aqueous micelle, whereas Fe(II)/Z–Ala–Cys–OMe (1:4) decomposed gradually in micelle. Observation of the redox couple of $[Fe(S_2\text{-}o\text{-}xyl)_2]^{2-}$ [5] at −0.64 V(SCE) in aqueous micelle reveals that the redox potentials of the Fe(II) complexes having two specific peptide ligands (Cys–X–Y–Cys) shift extraordinarily to the positive side.

CD and visible spectra of Fe_4S_4-type complexes of Cys-containing peptides, Z–Cys–Gly–OMe, Z–Cys–Gly–Ala–OMe, and Z–Cys–Gly–Ala–Cys–OMe, in aqueous micelle were found to be very similar to native 4Fe4S proteins. These complexes provided redox couples (2 − /3 −) except for the Fe_4S_4 complex of Z–Cys–Gly–OMe.

1 R. J. Burt, B. Ridge and H. N. Rydon, *J. Chem. Soc. Dalton Trans.*, 1228 (1980).
2 L. Que Jr., J. R. Anglin, M. A. Bobrick, A. Davison and R. H. Holm, *J. Am. Chem. Soc., 96*, 6042 (1976).
3 N. Ueyama, M. Nakata and A. Nakamura, *Bull. Chem. Soc. Jpn, 54*, 1727 (1981).
4 W. A. Eaton and W. Lovenberg 'Iron-Sulfur Proteins', Vol. II, Ed. W. Lovenberg, Academic Press, New York (1973).
5 R. W. Lane, J. A. Ibers, R. B. Frankel, G. C. Papaefthymiou and R. H. Holm, *J. Am. Chem. Soc., 99*, 84 (1977).

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Resonance Raman Studies of Models for the Reduced States of Cytochrome P_{450}

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The catalytic cycle of cytochrome P_{450} includes four stable states: a low spin ferric resting state A, a substrate bound high spin ferric state B, a high spin ferrous state C and a low spin ferrous oxy state D. When the system in state C is exposed to carbon monoxide instead of oxygen a low spin ferrous carboxy state D′ is generated. Several models have been proposed for states B, C, D′ and recently state D [1]. A large variety of spectroscopic techniques have been used to probe the similarities between the model compounds and the actual enzymatic states.

Resonance Raman spectroscopy (RR) is a very sensitive technique to investigate specifically the active site of hemoproteins. RR spectra have been obtained for Cyt P_{450} from various origins [2–4]: when compared to those of other hemoproteins, the frequencies of the so-called 'oxidation state' marker band [5] are unusually low in states C and D′; 'spin marker bands' frequencies [5] have been used to monitor the coordination of the iron atom in the ferric states A and B. Recently the Fe–S stretching mode has been detected at 351 cm^{-1} in oxidized Cyt $P_{450\ CAM}$ [6].

We report here the results of a RR study of $[Fe^{II}(T_{piv}PP)(X^-)(L)]18C6Na^+$* complexes, as models

*Abbreviations used: $T_{piv}PP$ = dianion of tetra kis (O-pivaloylamido)phenylporphyrin; TPP = dianion of tetraphenyl porphyrin; 2-MeIm = 2 methyl imidazole; py = pyridine.

for states C, D′ and D (an investigation of a state A model has already been published [7]). Using the nomenclature propsed in [8], the main RR frequencies are given in Table I.

TABLE I. RR Frequencies (cm^{-1}) of $[Fe^{II}(T_{piv}PP)(X^-)(L)]Na^+18C6$ Complexes.

Complex		Porphyrin vibrations					Fe–L vibr.
X^-	L	A	B	C	D		
$C_6HF_4S^-$		1341	a	a	a	369	
Cl^-		1343	1355	1494	1545	369	
OH^-		1344	1355	a	a	371	
$C_6HF_4O^-$		1343	1354	a	1545	369	
$C_6HF_4S^-$	CO	1364		a	1567	380	479
$C_6HF_4S^-$	O_2	1366		a	a	379	

[a]Not observed.

All the pentacoordinated ferrous species exhibit very similar porphyrinic frequencies. They compare well with the frequencies of the typical high spin ferrous complex Fe(TPP)(2-Me Im) (A = 1345, B = 1361, C = 1500 and D = 1538 [8]). Moreover the A frequency of the carboxy adduct is very close to that of Fe(TPP)(py)(CO) [12], whereas that of the oxy adduct is the same as that of $Fe(T_{piv}PP)$(1-Me Im)-(O_2) [9]. Therefore our RR data do not stress any special π donor properties of the RS^- ligand that would induce an extra lowering of the oxidation state marker band frequencies.

Soret excitation of the low frequency RR spectrum is readily accessible for the carboxy adduct λ_{max} Soret 448 nm, λ_{exc} 454,5 nm: it reveals a new strong polarized band at 479 cm^{-1}. The intensity of this band decreases with partial photodissociation of the CO ligand. An isotopic substitution experiment, using ^{13}CO, induces a 5 cm^{-1} lowering of its frequency. This is in good agreement with a calculated shift of -5 cm^{-1} for the stretching vibration of the Fe–CO moiety, using the harmonic oscillator approximation. This leads to the assignment of this band to the Fe–CO stretching vibration. This value is to be compared to those observed for MbCO, HbCO [10] and P_{450}–CO (work in progress).

1 M. Sappacher, L. Ricard, R. Weiss, R. Montiel-Montoya, E. Bill, U. Gonser and A. Trautwein, *J. Am. Chem. Soc., 103*, 7646 (1981) and references therein.
2 P. M. Champion, I. C. Gunsalus and G. C. Wagner, *J. Am. Chem. Soc., 100*, 3743 (1978).
3 Y. Ozaki, T. Kitagawa, Y. Kyogoku, Y. Imai, C. Hashimoto-Yutsudo and R. Sato, *Biochem., 17*, 5827 (1978).
4 T. Shimizu, T. Kitagawa, F. Mitani, T. Ilzuka and Y. Ishimura, *Biochim. Biophys. Acta, 670*, 236 (1981).
5 T. G. Spiro and J. M. Burke, *J. Am. Chem. Soc., 98*, 5482 (1976).
6 P. M. Champion, B. R. Stallard, G. C. Wagner and I. C. Gunsalus, *J. Am. Chem. Soc., 104*, 5469 (1982).
7 P. Anzenbacher, Z. Sipal, B. Strauch, J. Twardowski and L. M. Proniewicz, *J. Am. Chem. Soc., 103*, 5928 (1981).
8 G. Chottard, P. Battioni, J. P. Battioni, M. Lange and D. Mansuy, *Inorg. Chem., 20*, 1718 (1981).
9 J. M. Burke, J. R. Kincaid, S. Peters, R. R. Gagne, J. P. Collman and T. G. Spiro, *J. Am. Chem. Soc., 100*, 6083 (1978).
10 M. Tsubaki, P. B. Srivastava and N. T. Yu, *Biochem., 21*, 1132 (1982).

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Vanadium Catalyzed Oxygenation of 4,6-Di-*tert*-butylpyrogallol. A Model Reaction for Intradiol Dioxygenase

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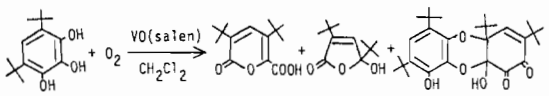
Recently, we have reported the intradiol cleavage of 3,5-di-*tert*-butylcatechol with molecular oxygen catalyzed by several vanadium complexes as a model reaction for intradiol dioxygenase [1]. Although the enzymatic [2] or the base catalyzed [3] cleavage of pyrogallol are known, metal catalyzed oxygenations of pyrogallol have not been reported yet. Here we wish to report vanadium catalyzed oxygenation of 4,6-di-*tert*-butylpyrogallol (*1*) and discuss the reaction mechanism based on the isotopic labelling experiment and the structure of the isolated reaction intermediate complex.

Oxidation of *1* (0.1 *M* in CH_2Cl_2) in the presence of a catalytic amount of VO(salen) (1 mol%) with molecular oxygen at room temperature for 20 h produced 3,5-di-*tert*-butyl-2-pyrone-6-carboxylic acid (*2*) (41%), 3,5-di-*tert*-butyl-5-hydroxy-2-furanone (*3*) (8%) besides a quinone dimer (*4*) (24%) [see eqn. (1)]. These products were characterized from elemental analyses, IR, ^1H NMR and mass spectra.



1 *2* *3* *4* (1)

^{18}O isotopic labelling experiments indicated that ^{18}O atoms were incorporated into *2* (one atom) and *3* (two atoms) and that an ^{18}O atom in *2* was located in the carboxylic acid moiety, but not in the lactone moiety. These facts suggest that the main product *2* is formed by rearrangement of an intermediate (*5*) arising from the intradiol ring cleavage of *1* just as in the enzymatic reaction [see eqn. (2)]. As the compound *5* corresponds to the seven membered lactone intermediate proposed by Hamilton [4] in the enzyme reaction, the vanadium catalyzed