Communications to the Editor

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Optical and Electron Paramagnetic Resonance Studies on the Model for Cytochrome P-450

The model system, which showed previously the hydroxylations to aniline and *p*-toluidine, consisting of hemin, cysteine and pyridine exhibited very similar absorption and EPR spectra to cytochrome P-450 at 293 and 77°K. From the spectral properties the axial cysteinate S–Fe–N (pyridine) coordination is necessary to show a low-spin state of porphyrin at 293°K. In the presence of trace amount of pyridine, a transition of the high-spin state observed at 293°K to the low-spin state of porphyrin was detected at 77 °K.

Keywords——cytochrome P-450; cysteine; model system; optical spectra; electron paramagnetic resonance spectra; spin transition

The cytochrome P-450 class of the heme proteins is an important hydroxylating enzyme involved in detoxification, drug metabolism, carcinogenesis and steroid biosynthesis.[1–3] The possibility of an axial sulfur ligation to the protoheme in cytochrome P-450 has been shown by the recent extensive studies on the physico-chemical properties of both natural and synthetic porphyrin complexes.[4–6] However, little has been studied on the enzymatic hydroxylation using these synthetic model complexes.

We have previously demonstrated the hydroxylations of aniline and *p*-toluidine in the model systems consisting of iron-thiol and hemin-thiol complexes as a possible chemical model for cytochrome P-450.[7–9] In a recent paper, the optical studies on hemin-thiol complexes strongly suggested that these model systems reflect the properties of intact cytochrome P-450 systems.[10] This communication deals with the electron paramagnetic resonance (EPR) and optical studies at low temperature in order to obtain more precise informations about the states of the heme-iron in the model systems.

We found the necessity of a pyridine as an axial ligand to the heme-iron to produce a low-spin species of porphyrin, and also found the transition of the high-spin observed at 293°K to the low-spin state in the model system consisting of cysteine, hemin and pyridine at 77°K.

Optical and EPR spectral properties of the model complexes consisting of cysteine, hemin and pyridine are summarized in Table I. In the system consisting of hemin and cysteine without pyridine, the absorption maxima were observed at 510 and 650$^{(sh.)}$ nm at 293°K, corresponding to the typical spectrum of ferric high-spin species. When the EPR spectrum was measured at 77 °K, the mixed-spin state was identified for this system, though the absorption was not clearly resoluted in the visible region (Fig. 1-a, Fig. 2-B).

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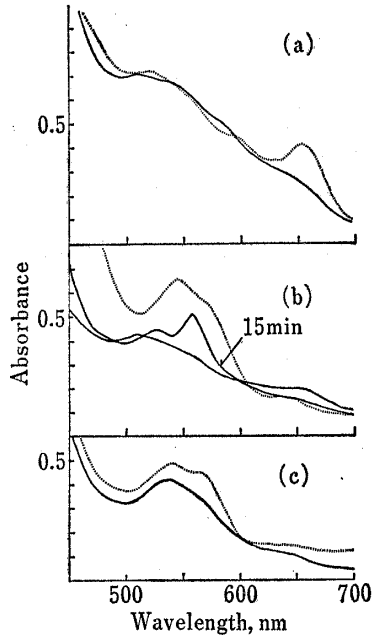

Fig. 1. Absorption Spectra of Model Complexes at pH 7.3

hemin: (a) 10^{-4}M, (b) and (c) 5×10^{-6}M, cysteine: 1.65×10^{-2}M, pyridine: (a) 0M, (b) 2.25×10^{-3}M, (c) 4.40×10^{-1}M
temperature: ——— 293°K, 30 sec. after mixing
-------- 77°K, 40 sec. after mixing

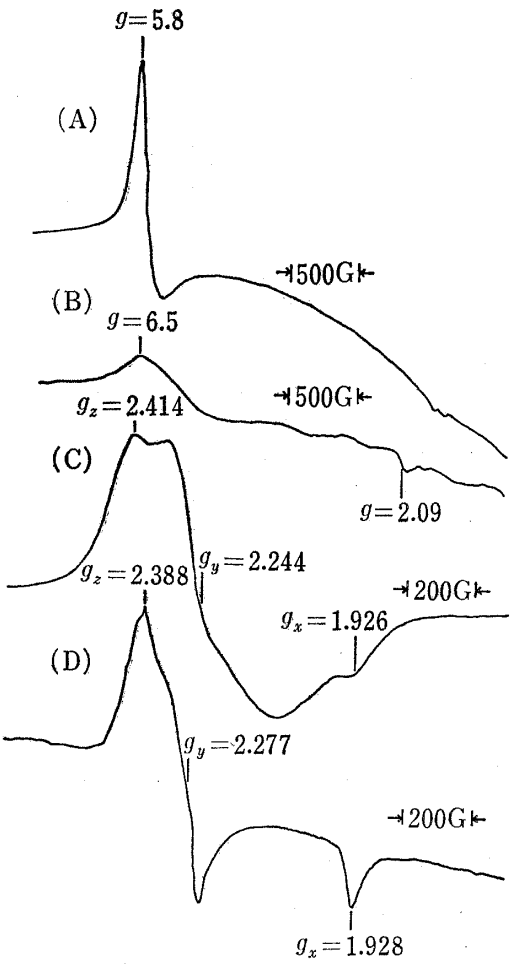

Fig. 2. EPR Spectra of Model Complexes at pH 7.3

(A): hemin 5×10^{-3}M, (B): (A)+cysteine 2.0×10^{-1}M, (C): (B)+pyridine 5×10^{-2}M, (D): (B)+pyridine 6.0M
All samples were freezed (77°K) within one minute after mixing.

When small amount of pyridine to be a final concentration of 2.25×10^{-3} M was added to the mixture of hemin (final conc. $=5\times10^{-5}$ M) and cysteine (final conc. $=1.65\times10^{-2}$ M) in phosphate buffer of pH 7.3 at 293°K, the spectrum exhibited the absorption maxima at 413, 518, and 650(sh.) nm within 3 minutes, which corresponds to the spectrum of typical ferric high-spin species of cytochrome P-450, and after 5 to 30 minutes of the reaction the spectrum changed to show the absorption maxima at 415, 528, and 560 nm, corresponding to the spectrum of the reduced cytochrome P-450.[11] However, at 77°K this complex showed the absorption maximun at 543 nm, corresponding to the low-spin species of cytochrome P-450 as shown in Fig. 1-b. The spin state transition observed at low temperature was corroborated with the EPR spectrum (Fig. 2-C). The well-defined three g-values were very similar to those of oxidized cytochrome P-450 in purely low-spin state.[12] The spin state transition phenomenon depending on the temperature has already been identified by EPR and absorption spectra of the complexation of azide with ferric horse erythrocyte catalase.[13]

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The addition of large amount of pyridine (final conc. $=4.40\times10^{-1}$ M) gave the absorption maximum at 538 nm, corresponding to the spectrum of typical ferric low-spin species of cytochrome P-450 at both 293 and 77°K (Fig. 1-c). The EPR spectrum also showed the low-spin state of ferric porphyrin, though the *g*-values of this complex are different slightly from those in the presence of small amount of pyridine (Fig. 2-D).

It is apparent from these results that the spin-state of iron in porphyrin is dependent on the concentration of pyridine at both 293 and 77°K.

TABLE I. Optical and EPR Spectral Properties of Model Systems and Cytochrome P-450[a)]

System	Absorption maxima (nm)							EPR spectra				
	293°K			77°K				*g*-Value (77°K)			Oxidation state of Fe	Spin (s)
Hemin		570^{sh}						5.8			+3	high (5/2)
Hemin–cysteine	510		650^{sh}	518	550^{sh}	590^{sh}	654	6.5	2.1		+3	high (5/2) + low (1/2)
Hemin–cysteine–pyridine[b)]	518	550^{sh}	650^{sh}	543		570^{sh}	640^{sh}	2.414	2.244	1.926	+3	low (1/2)
Hemin–cysteine–pyridine[c)]	538		640^{sh}	538		563	640	2.388	2.277	1.928	+3	low (1/2)
Cytochrome P-450[d)]												
Oxidized high-spin	505		650					6.6	2.0		+3	high (5/2)
Oxidized low-spin	535	570	650	535		566	646[e)]	2.41	2.25	1.91	+3	low (1/2)

a) All samples were measured within one minute after mixing.
b) pyridine: 2.25×10^{-3}M
c) pyridine: 4.40×10^{-1}M
d) C.R.E. Jefcoate and J.L. Gayler, *Biochemistry*, 8, 3464 (1969); H.A.O. Hill, A. Röder, and R.J.P. Williams, *Structure and Bonding*, 8, 123 (1970)
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The conclusions drawn from these results on the model complexes are in the following;

(1) The high-spin species observed in the absence of pyridine or in the presence of small amount of pyridine at 293°K would be due to the formation of five-coordinated ferric porphyrin by complexation of an axial cysteine with ferric porphyrin.

(2) The addition of small amount of pyridine facillitates the transition of the high-spin state observed at 293°K to the low-spin state of porphyrin in the hemin-cysteine-pyridine model complex at 77°K.

(3) The addition of large amount of pyridine produces the axial cysteinate S–Fe–N (pyridine) coordination in low-spin state.

In order to design a physiological model complex closer to cytochrome P-450, further investigations are under way and the detailed results will be reported in the near future.

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