Coordination of Alkanethiolate Anion to Ruthenium(II) Carbonyl Complex of Octaethylporphine: Model for Carbonyl Adduct of Cytochrome P-450

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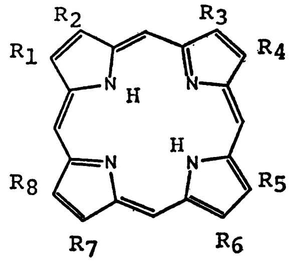
Absorption, infrared, and ^{1}H-NMR spectra of the ruthenium(II) octaethylporphine carbonyl complex [Ru(II)-OEP·CO] were studied in the presence of alkanethiolate anion ($^-$SR) as a model system for the reduced cytochrome P-450 carbonyl complex. The ^{1}H-NMR spectra reveal that alkanethiolate anions coordinte toa the central metal atom of Ru(II)OEP·CO as the sixth ligand. The hexaccordinated complex [Ru(II)OEP·CO·$^-$SR] has anomalous features as compared with the pentacoordinated complex [Ru(II)OEP·CO]: (1) the Soret band is splitted into two peaks, one in the near UV 363—365 nm region and the other in the 419—428 nm region, (2) the carbonyl stretching frequency is shifted by 25 cm^{-1} to lower wave number, (3) the proton signals of porphyrin ligand are shifted to higher magnetic field. Similarity of absorption and infrared spectra of the hexacoordinated complex to those of the reduced cytochrome P-450 carbonyl complex supports the axial ligation of the cysteinate to the central iron atom of the heme-proteins. The low wave number shifts of carbonyl stretching vibration and high field shifts of protons in the ^{1}H-NMR spectra suggest the charge transfer interaction from 3p lone pair electrons of sulfur atom of alkanethiolate anion to the central metal and/or the porphyrin ligand.

Cytochrome P-450 found in bacteria and lever microsomes are a kind of heme-enzymes which activate the molecular oxygen to catalyze the hydroxylation of the organic compounds in drug metabolism.1,2) Recent investigations have been focused on elucidation of the structure of the active site of the heme enzymes. Simulation to the moiety of the prothetic protoheme in the enzyme by use of model compounds has provided a plausible explanation for the properties of axial ligand to the heme in P-450.3–6) Spectroscopic studies showed similarity between the oxidized cytochrome P-450 and model compounds.7–11) Addition of CO to the reduced cytochrome P-450 shows an anomalous red-shifted Soret band at *ca.* 450 nm.12) This anomalous electronic spectrum has been successfully duplicated with synthetic heme in the presence of alkanethiolate anion and CO.3,4,6) This gives strong evidence of axial coordination of the cysteinate to the heme-CO complex in P-450.

Divalent and trivalent ruthenium porphyrin complexes have been prepared by Whitten and his coworkers.13) It is noted that the ruthenium(II) carbonyl porphyrin is isolable as a stable complex with or without the sixth ligand such as amine tetrahydrofuran and alcohol. These Ru(II)porphyrin·CO·L complexes are d^6-metal complexes of diamagnetic low spin state. Thus the electronic structure of the ruthenium(II) carbonyl complex is considered to be similar to that of the ferrous carbonyl porphyrin complex. The divalent metal ions of Ru(II)porphyrin·CO·(base) and Os(II)-porphyrin·CO·(base) are highly insensitive to oxygen as compared with the heme-CO adduct at ambient temperature.13,14) The advantage of high stability of the ruthenium(II) complexes enables us to study the axial ligation of the alkanethiolate anion to the metal carbonyl porphyrin. Replacement of the iron atom of ferrous carbonyl porphyrin with ruthenium atom might afford an alternative model for the carbonyl adduct of the reduced cytochrome P-450. We wish to report spectroscopic evidence of the interaction between the alkanethiolate anion and the Ru(II) porphyrin·CO.

Experimental

Ruthenium(II) carbonyl octaethylporphine [Ru(II)OEP·CO] and ruthenium(II) carbonyl mesoporphyrin IX dimethyl ester were obtained by the reaction of octaethylporphine and mesoporphyrin IX dimethyl ester with $Ru_3(CO)_{12}$ in dry benzene, respectively.13) The other chemicals and solvents were purified. Solutions of sodium alkanethiolates (RS^-Na^+, $R=-CH_2CH_2CO_2CH_3$, $-CH_2CH_3$, and $-C(CH_3)_3$) were prepared by the following procedure. Pulverized sodium hydride was washed with pentane several times and dried in a vacuum. Mercaptan (RSH, $R=-CH_2CH_2CO_2CH_3$, $-CH_2CH_3$, and $-C(CH_3)_3$) was added to NaH under argon atmosphere. After stirring for 15 min, the mixture was evacuated in order to remove excess mercaptan. To the deaerated solvents (benzene, ether, acetonitrile, *N,N*-dimethylformamide, dimethyl sulfoxide; 1 ml containing dibenzo-18-crown-6 (12 mg, 0.032 mmol) was added an equimolar amount of sodium alkanethiolate. The mixture was stirred until the sodium alkanethiolate dissolved completely. To the solution was added Ru(II)OEP·CO (20 mg, 0.032 mmol) and the solution was stirred for 1 h. The red solution turned to yellowish orange gradually. Disappearance of the Soret band due to Ru(II)-OEP·CO was monitored by measurement of the visible



$R_1=R_2=R_3=R_4=R_5=R_6=R_7=R_8=CH_2CH_3$ octaethylporphine

$R_1=R_3=R_5=R_8=CH_3$
$R_2=R_4=$vinyl — protoporphyrin IX dimethyl ester
$R_6=R_7=CH_2CH_2CO_2CH_3$

$R_1=R_3=R_5=R_8=CH_3$
$R_2=R_4=CH_2CH_2$ — mesoporphyrin IX dimethyl ester
$R_6=R_7=CH_2CH_2CO_2CH_3$

spectra. Spectral measurements were carried out under argon atmosphere. Infrared spectra were measured on a Hitachi Model G-3 spectrophotometer in NaCl liquid cells with 0.1 mm optical path. Absorption spectra were measured with a Hitachi EPS-3T spectrophotometer in 0.1 mm quartz cells. Proton NMR were obtained with JEOL FX-60 and Varian HA-100 D spectrometers in CD_3CN and referred to tetramethylsilane (TMS) or hexamethyldisiloxane (HMDS).

Results and Discussion

Absorption Spectra. The visible spectra of Ru(II)-OEP·CO in various solvents are summarized in Table 1. The ruthenium complex shows characteristic absorptions of the divalent metalloporphyrin at *ca.* 550 and 520 nm (the α-, β-bands) assigned to the first $\pi \rightarrow \pi^*$ transitions splitted by vibronic interaction and the Soret band at 395 nm due to the second $\pi \rightarrow \pi^*$ transitions.[15] The transition energy for the absorption shows small dependency on polarity of solvent molecule. Slight red shifts of these absorptions were observed in DMF. Nucleophilicities of alkanethiolate anion can be enhanced by the incorporation of alkaline metal ion to crown ether. Addition of an equimolar amount of alkanethiolate anion thus generated (*e.g.* RS^-, R=$-CH_2CH_2CO_2CH_3$, $-CH_2CH_3$, and $-C(CH_3)_3$) to Ru(II)OEP·CO caused the splitting of the Soret band into two bands at 363—366 and 419—428 nm. The absorption strength of both α- and β-bands decreased considerably, a new band appearing at 540 nm. On the other hand, addition of an equimolar amount of mercaptan (*e.g.* RSH, R=$-CH_2CH_2CO_2CH_3$, $-CH_2CH_3$, and $-C(CH_3)_3$) gave no marked change in the transition energies and the absorption strength.

Table 1. Absorption maxima of Ru(II)OEP·CO·L

Solvent	L	Soret	β	α (nm)
Benzene	none	396	517	549
CH_3CN	none	393	518	549
DMF	none	407	529	560
DMSO	none	396	520	551
Benzene	^-SR[a]	363, 419	535	
CH_3CN	^-SR	364, 423	538	
DMF	^-SR	365, 428	540	
DMSO	^-SR	366, 428	540	
	Absorption maxima of Fe(II)OEP·CO·L			
Benzene	^-SR[b]	357, 436	540	
DMF	^-SR	370, 447	540	

a) R=$-CH_2CH_2CO_2CH_3$, alkanethiolate anion was solubilized by dibenzo-18-crown-6. [Ru(II)OEP·CO]=0.032 mM, [^-SR]=0.032 mM. b) [Fe(II)-OEP·CO]=0.032 mM, [^-SR]=0.32 mM. The 408 nm peak assignable to the pentacoordinated complex [Fe(II)OEP·CO] did not completely disappear even in the presence of the 100 eq. amount of alkanethiolate anion.

The hexacoordinated complex of ferrous carbonyl porphyrin[Fe(II)OEP·CO·(^-SR)] was obtained by treatment of the ferric porphyrin OEP·Fe(III)Cl with a large excess of alkanethiolate anion in the presence of carbon monoxide.[5,6] Coordination of alkanethiolate anion to Fe(II)OEP·CO in benzene solution caused the appearance of new bands at 357 and 436 nm. However, the absorption at 408 nm due to the pentacoordinated complex [Fe(II)OEP·CO] was observed even in the presence of ten times molar amounts of alkanethiolate anion.[5,6,16] Spectral change due to ligation of alkanethiolate anion was quite similar to that of the ferrous carbonyl complex of protoporphyrin IX dimethyl ester.[5,6] Two vinyl groups at the peripheral positions of protoheme may cause bathochromic shifts of those absorption maxima as compared with the complex of octaethylporphine. Complete formation of the hexacoordinated complex [Fe(II)OEP·CO·(^-SR)] requires large excess amounts (10^5—10^6 eq.) of the alkanethiolate anion.[5,6,16] Splitting of the Soret band of these hexacoordinated complexes depends a great deal on polarity of solvent molecule. In more polar solvent such as DMF the absorptions shift to longer wave length.

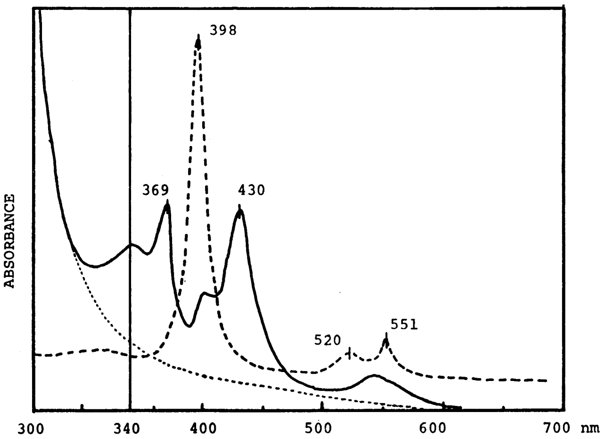


Fig. 1. Absorption spectra of Ru(II) carbonyl mesoporphyrin IX dimethyl ester, (----) with an equimolar amount of $HSCH_2CH_2CO_2CH_3$ in DMF; (——) with equimolar amount of $^-SCH_2CH_2CO_2CH_3$ solubilized by dibenzo-18-crown-6 in DMF; (····) base line.

Similar anomalous absorption spectra can be duplicated by using the ruthenium(II) carbonyl complex of mesoporphyrin IX dimethyl ester with an equimolar amount of the alkanethiolate anion as shown in Fig. 1. Axial ligation of alkanethiolate anion to ruthenium(II) carbonyl porphyrin causes clear splitting of the Soret band to two peaks as has been found for ferrous carbonyl porphyrin. Recently, Hanson and his co-workers have reported that[17] splitting of the Soret band into two bands of the reduced cytochrome P-450 carbonyl adduct and its model compounds can be attributed to the charge transfer transition from the 3p lone pair electrons of the sulfur atom to the lowest vacant π^* orbitals of the porphyrin ring and that the transition may cause strong interaction with the $\pi \rightarrow \pi^*$ (a_{1u}, $a_{2u} \rightarrow e_g^*$) transitions. Recent MCD studies of the cytochrome P-450 and its model compounds seem to support their theoretical interpretation.[11]

Infrared Spectra. The carbonyl stretching vibrations[14] of Ru(II)OEP·CO were observed in various solvents (Table 2). Figure 2 shows the infrared spectra

Table 2. Carbonyl stretching vibrations of Ru(II)OEP·CO·L

Solvent	L	ν_{co}(cm^{-1})
Ether	PPh_3	1966
Ether	none	1942
THF	none	1938
Pyridine	none	1931
Benzene	none	1946
Benzene	$^-$SR[a]	1917
DMF	none	1917
DMF	$^-$SR	1889
	Carbonyl stretching vibrations of Fe(II)OEP·CO·L	
Benzene	none	1975
Benzene	$^-$SR[b]	1950
DMF	none	1948
DMF	$^-$SR	1923

a) R = $-CH_2CH_2CO_2CH_3$, alkanethiolate anion was solubilized by dibenzo-18-crown-6. [Ru(II)OEP·CO] = 0.032 mM, [$^-$SR] = 0.032 mM. b) [Fe(II)-OEP·CO] = 0.032 mM, [$^-$SR] = 0.32 mM.

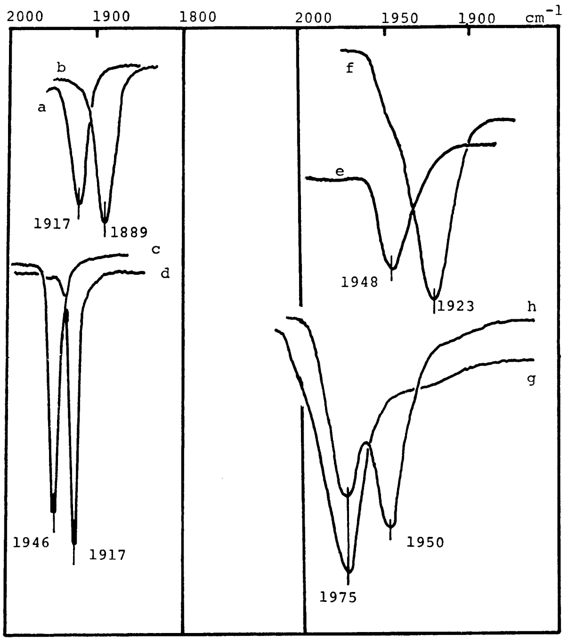


Fig. 2. Infrared spectra of M(II)OEP·CO·L in the carbonyl stretcfling region of; (a) M=Ru, L=none in DMF, (b) M=Ru, L=$^-$SR in DMF, (c) M=Ru, L=none in benzene, (d) M=Ru, L=$^-$SR in benzene, (e) M=Fe, L=none in DMF, (f) M=Fe, L=$^-$SR in DMF, (g) M=Fe, L=none in benzene, (h) M=Fe, L=$^-$SR in benzene. R=$-CH_2CH_2CO_2CH_3$. The conditions of measurements are described in Table 2.

of these complexes in the region of carbonyl stretching. Addition of alkanethiolate anion reduced the wave number of the carbonyl stretching by 28—29 cm^{-1} for the ruthenium complex and 25 cm^{-1} for a ferrous complex.[16] In the case of Fe(II)OEP·CO with excess amounts of alkanethiolate anion in benzene, two carbonyl stretching vibrations were observed at 1975 and 1950 cm^{-1}. This provides further evidence of the coexistence of the pentacoordinated complex (**2**) (1975 cm^{-1}) and the hexacoordinated complex (**4**) (1950 cm^{-1}) for the iron complex as has been found for the absorption spectra. These trends can be explained in terms of

$$\text{M(II)OEP·CO} + {}^-\text{SR} \rightleftarrows \text{M(II)OEP·CO·}({}^-\text{SR})$$

$$\text{M=Ru}\ (\mathbf{1}) \qquad \text{M=Ru}\ (\mathbf{3})$$

$$\text{M=Fe}\ (\mathbf{2}) \qquad \text{M=Fe}\ (\mathbf{4})$$

π-back donation from dπ-orbitals of metal to the *anti*-bonding π^*-orbitals of the carbonyl. Divalent ruthenium in the pentacoordinated complex (**1**) is considered to be a stronger π-base than ferrous ion in the pentacoordinated complex (**2**) on the basis of the shift of the carbonyl stretching vibration. Enhanced back donation from the dπ-orbitals of ruthenium to the anti-bonding π^*-orbitals of the carbonyl decreases electron density on the ruthenium atom. On the other hand, addition of a strong π-acceptor ligand such as PPh_3 shows appreciable increase of the carbonyl stretching frequency. A similar trend has been found for the osmium(II) carbonyl porphyrin complexes by Buchler and Rohback.[14] These results suggest that ligation of alkanethiolate anion to central metal facilitates charge transfer to the central metal from sulfur atom.[11d] Furthermore, it is noted that carbonyl stretching frequencies are very sensitive to polarity of solvent molecule. Increasing of polarity of solvent from benzene to DMF reduces the frequencies of the carbonyl stretching by 28—29 cm^{-1} for the ruthenium complex and 17 cm^{-1} for the ferrous complex. The carbonyl stretching frequency (1923 cm^{-1}) of the hexacoordinated complex (**4**) in DMF is the same as that (1923 cm^{-1}) of Fe(II) protoporphyrin IX dimethyl ester in *N*,*N*-dimethylacetamide in the presence of both carbon monoxide and 1-butanethiolate.[6] Polar solvent molecules may stabilize the polarization along the axial direction due to the charge transfer from alkanethiolate

Table 3. Proton chemical shifts (60 MHz, ppm from TMS) of Ru(II)OEP·CO·L

	Assignment			
L	meso	α-CH_2-	β-CH_3	Alkanethiolate
none[a]	0.06 (s, 4H)	6.16 (q, 16H)	8.03 (t, 24H)	
$^-SCH_2CH_3$[b]	0.35 (s, 4H)	6.03 (q, 16H)	8.05 (t, 24H)	11.59 (t, 3H, $-CH_2\underline{CH_3}$) 13.00 (q, 2H, $-\underline{CH_2}CH_3$)
$^-SC(CH_3)_3$	0.36 (s, 4H)	6.04 (q, 16H)	8.03 (t, 24H)	11.99 (s, 9H, $-C(\underline{CH_3})_3$

a) Measured in $CDCl_3$ at 31.5 °C. b) Measured in CD_3CN at 31.5 °C, [Ru(II)OEP·CO] = 0.032 mM, [$^-$SR] = 0.032 mM.

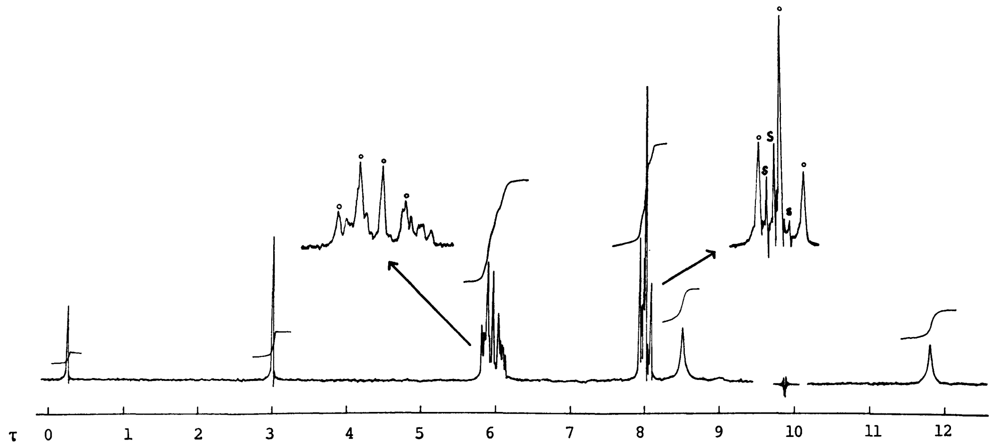


Fig. 3. 100 MHz NMR spectrum of Ru(II)OEP·CO with twice molar amounts of $^{-}SC(CH_3)_3$ solubilized by dibenzo-18-crown-6 in CD_3CN at 31.5 °C. Symbols (o) and (s) denote signals due to ethyl protons of porphyrin ligand and solvent, respectively. Singlet at τ 3 and multiplet at τ 6 are assigned to protons of crown ether. The chemical shifts are calculated from the outernal rock signal of hexamethyldisiloxane (HMDS) to τ values.

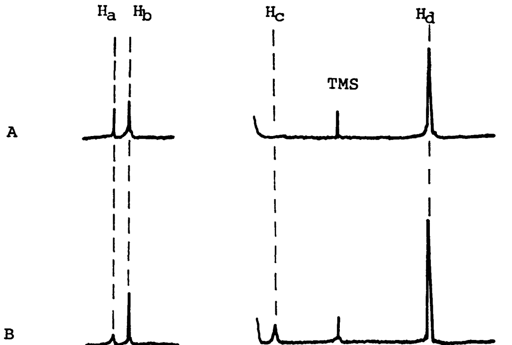


Fig. 4. 60 MHz NMR spectra of Ru(II)OEP·CO at various concentrations of alkanethiolate anion in CD_3CN in the low and high magnetic field regions. [Ru(II)OEP·CO]=0.032 mM, A [$^{-}$SR]=0.016 mM, B [$^{-}$SR]=0.032 mM, and C [$^{-}$SR]=0.045 mM. R=$-C(CH_3)_3$. H_a, H_b, H_c, and H_d are assignable to meso protons of Ru(II)OEP·CO, meso protons of Ru(II)OEP·CO·($^{-}$SR), *tert*-butyl protons of the free alkanethiolate anion, and *tert*-butyl protons of the coordinated alkanethiolate anion, respectively.

anion to the carbonyl group.

¹H-NMR Spectra. ¹H-NMR spectroscopic study on diamagnetic ruthenium(II) carbonyl porphyrin enables us to obtain insight into the interaction between the divalent metalloporphyrin and the alkanethiolate anion. The ¹H-NMR spectra of Ru(II)OEP·CO and the complexes with an equimolar amount of alkanethiolate anions are given in Table 3. In the presence of alkanethiolate anion the signals of the meso protons appear at higher magnetic field than those of Ru(II)OEP·CO. The up-field shifts might be explained in terms of the increased electron density at the ruthenium atom because of the charge transfer of the negatively charged alkanethiolate anion to the central metal.[11,17] Axial ligation of alkanethiolate anion was rationalized by marked up-field shifts of alkyl protons of alkanethiolate anion due to the diamagnetic ring current of porphyrin ring. For ethanethiolate anion, the signals of the α-methylene and β-methyl group were found at τ 13.00 (quartet 2H) and 11.59 (triplet 3H), respectively. Figure 3 shows 100 MHz ¹H-NMR spectrum of Ru(II)OEP·CO with twice molar amounts of 2-methyl-2-propanethiolate anion ($^{-}SC(CH_3)_3$) in CD_3CN. Two signals at τ 8.68 and 11.94 are assignable to the methyl protons of the free $^{-}SC(CH_3)_3$ and those of the coordinated $^{-}SC(CH_3)_3$ to Ru(II)OEP·CO, respectively. Strong affinity of ruthenium atom for alkanethiolate anion was confirmed by ¹H-NMR spectra. Figure 4 shows 60 MHz ¹H-NMR spectra of Ru(II)OEP·CO with various concentrations of alkanethiolate anion. When a half molar amount of the alkanethiolate anion was added, no signal (H_c) of the free $^{-}S(CH_3)_3$ could be observed. Two meso proton signals appear at τ 0.06 (H_a) and 0.30 (H_b) (Fig. 4A). The former signal appearing at lower magnetic field is due to the meso protons of the Ru(II)OEP·CO. The assignment was confirmed by the measurement in the absence of alkanethiolate anion. The NMR spectrum of the Ru(II)OEP·CO (**1**) with an equimolar amount of the alkanethiolate anion indicates predominant existence of the hexacoordinated complex [Ru(II)OEP·CO·($^{-}$SR)] (**3**) (Fig. 4B). Thus, the affinity of Ru(II)OEP·CO (**1**) to the alkanethiolate anion is much higher than that of the ferrous carbonyl complex (**2**). Figure 5 shows the 100 MHz ¹H-NMR spectra of Ru(II)carbonyl mesoporphyrin IX dimethyl ester and its alkanethiolate complex.

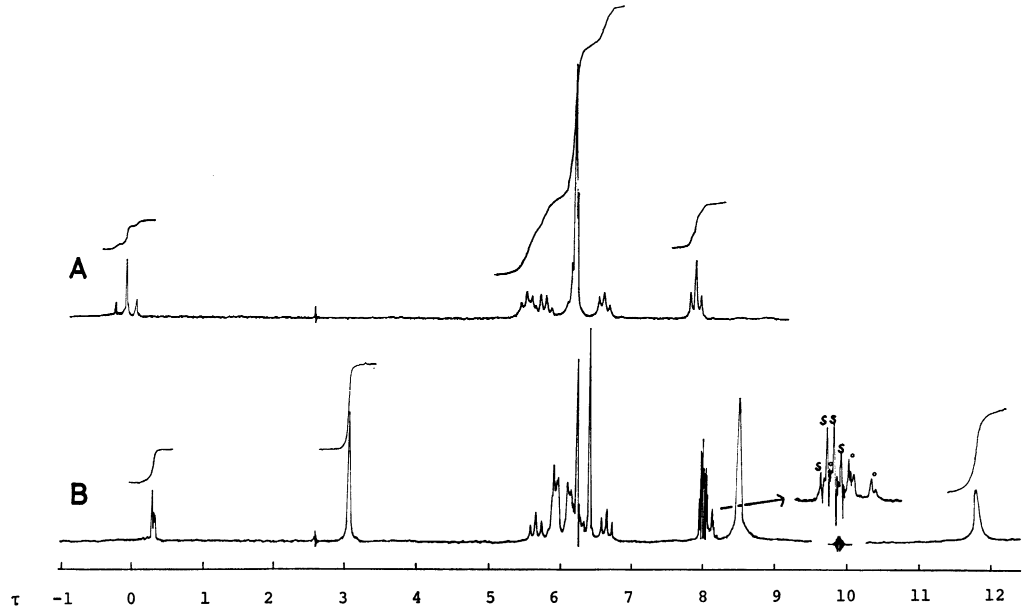


Fig. 5. 100 MHz NMR spectra of CD_3CN solution of (A) Ru(II) carbonyl mesoporphyrin IX dimethyl ester, and (B) Ru(II) carbonyl mesoporphyrin IX dimethyl ester with three equivalents of $^-SC(CH_3)_3$ solubilized by dibenzo-18-crown-6. Symbol (o) denotes the methyl protons of ethyl groups of porphyrin ligand. Singlet at τ 3 and multiplet at τ 6 are assigned to crown ether. The chemical shifts are calculated from the outernal rock signal of hexamethyldisiloxane (HMDS) to τ values.

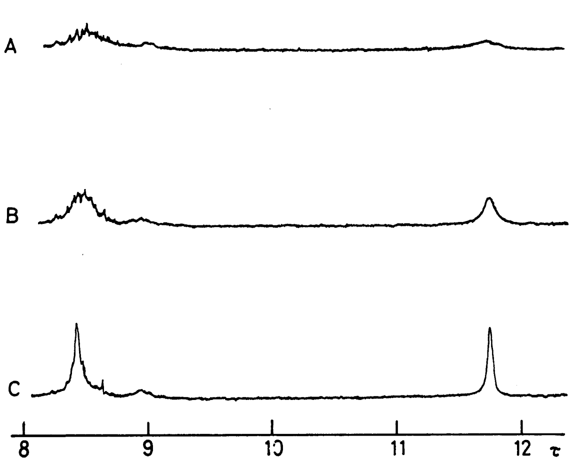


Fig. 6. The high field part of 100 MHz NMR spectra for Ru(II)OEP·CO with twice molar amounts of $^-SC(CH_3)_3$ solubilized by dibenzo-18-crown-6 in CD_3CN; (A) at 80 °C, (B) at 50 °C, and (C) at 32 °C.

Similar up-field shifts were observed for the meso protons, the methyl protons of 1, 3, 5, and 8-positions and the α-methylene protons of 6 and 7-positions of the complex. As shown in Figs. 3 and 5, the broad signals due to the free and coordinated alkanethiolate anion suggest existence of exchange between them. Figure 6 shows the signals of $^-SC(CH_3)_3$ at elevated temperature. The methyl proton resonance is broadened with rise in temperature owing to rapid exchange of ligand. The coalescence temperature could not be determined.

^{1}H-NMR studies of the hexacoordinated complex [Ru(II)OEP·CO·($^-$SR)] show unequivocally that the ligation of the alkanethiolate anion to the ruthenium increases electron density at the central metal. The effect is in line with the observation of anomalous splitting of the Soret band and reduction in the carbonyl stretching vibrations.[11d)]

On the other hand, addition of the alkanolate anion to Ru(II)OEP·CO shows no significant changes in absorption and infrared spectra as has been reported for ferrous complexes.[6)] The trend was also confirmed by ^{1}H-NMR measurement. Figure 7 shows the 60 MHz ^{1}H-NMR spectrum of Ru(II)OEP·CO in the presence of three equimolar amounts of 2-methyl-2-propanolate anion. Signals of *t*-butyl protons of the free alcoholate anion were observed at τ 8.70.

Strong ligation of alkanethiolate anion might be ascribed to more polarizable 3p lone pair electrons of sulfur atom of alkanethiolate anion than 2p lone pair electrons of oxygen of alkanolate anion. Energy levels of lone pair electrons of the alkanethiolate anion seem to be closer to the anti-bonding orbitals $\pi^*(e_g^*)$ of the porphyrin ring than those of the alkanolate anion.[17)] Consequently the lone pair electrons of alkanethiolate anion may be allowed to interact strongly with $\pi^*(e_g^*)$ orbitals of porphyrin ring. In conclusion, NMR, infrared, and absorption studies on the present model systems provide strong evidence to support the axial ligation of the alkanethiolate (cysteinate) to the central iron metal of the heme in the cytochrome P-450, which causes considerable charge transfer from cysteinate to iron and/or porphyrin ring.

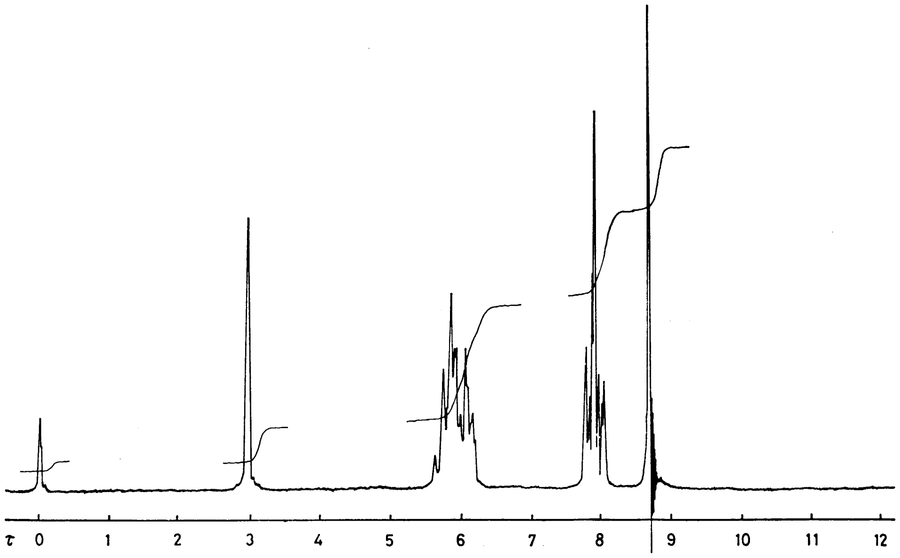


Fig. 7. 60 MHz NMR spectrum of Ru(II)OEP•CO with three amounts of $^{-}OC(CH_3)_3$ solubilized by dibenzo-18-crown-6 in CD_3CN. Singlet at τ 3 and multiplet at τ 6 are assigned to crown ether.

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