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Iron–Sulfur Vibrations in Model Compounds of Cytochromes P-450 and c

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Abstract

Five model compounds of cytochrome P-450, [Fe(III)(porphyrin)(thiolate)] (high spin) and two model compounds of cytochrome c, [Fe(III)(TPP)-(thioether)$_2$]ClO$_4$ (low spin) have been prepared, and their far-infrared spectra measured to assign the axial Fe–S stretching vibrations. Based on ^{54}Fe–^{56}Fe isotope shift data, the Fe–S$^-$ (thiolate) and Fe–S^0 (thioether) stretching vibrations have been assigned at 345–335 and 333–323 cm^{-1}, respectively. The [Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$ ion (low spin) exhibits the antisymmetric S$^-$–Fe–S$^-$ stretching band at 345 cm^{-1} which is about 20 ~ 16 cm^{-1} higher than the S^0–Fe–S^0 stretching vibration of the [Fe(TPP)-(thioether)$_2$]$^+$ ion. This result is interpreted as indicating that the Fe–S$^-$ bond is stronger than the Fe–S^0 bond if other conditions are equal. Possible origins of frequency discrepancy between cytochrome P-450$_{\mathrm{cam}}$ (351 cm^{-1}) and its model compounds have been discussed, and the failure to observe the Fe–S vibrations in Raman spectra has been attributed to the small oscillator strength of the Fe–S CT transition in the UV region.

Introduction

Cytochromes P-450 are monooxygenase enzymes which catalyze the necessary functions of substrate hydroxylation in drug metabolism, steroid synthesis and carcinogenesis [1]. Although X-ray crystal structures have not been reported on cytochromes P-450, the majority of workers agree that its active site consists of an iron protoporphyrin with the iron center axially bonded to the thiolate sulfur of a cysteinyl residue throughout its reaction cycle [2]. An EXAFS study on cytochrome P-450-LM-2 estimates its Fe–S distance to be 2.19 Å in its resting state [3].

Cytochromes c transfer electrons from cytochromes c reductase to cytochromes c oxidase in the mitochondrial respiratory chain [4]. The structures of cytochromes c from various sources have been determined by X-ray crystallography [4]. According to these studies, the prosthetic group of cytochrome c is a heme in which the vinyl side chains of protoporphyrin are replaced by cysteinyl thioether bonds and to which the imidazole (histidine) nitrogen and the methionine (thioether) sulfur atoms are axially bonded.

Thus far, X-ray analyses have been reported on several model compounds of cytochrome P-450 [5–7] and cytochrome c [8, 9]. These studies show that the axial Fe–S distances are confined in a rather narrow range from 2.27 to 2.43 Å and that no apparent relationships exist between the Fe–S distance and the nature of the sulfur ligand (thiolate/thioether) or the spin and/or oxidation state of the iron atom. This is somewhat surprising since the thiolate ligand binds to Co(II) and Fe(II) porphyrins much more strongly than the thioether [10] and since the metal–ligand distance is generally sensitive to the change in the oxidation [11] and spin states [12] of the metal.

Thus far, no vibrational assignments have been made on Fe–S stretching vibrations of these cytochromes except for that of Champion *et al.* [13, 14] who observed the ν(Fe–S) (ν: stretching) of cytochrome P-450$_{\mathrm{cam}}$ at 351 cm^{-1} in the oxidized, substrate-bound state *via* UV excitation at 363.8 nm. This serious lack of information about axial ν(Fe–S) of iron porphyrins prompted us to undertake the present IR investigation.

In this study, we prepared four model compounds of cytochrome P-450 with axial Fe–S$^-$ (thiolate) bonds (five-coordinate, Fe(III) and high spin) and two model compounds of cytochrome c with axial Fe–S^0 (thioether) bonds (six-coordinate, Fe(III) and low spin). In addition, we prepared the crown ether salt of the [Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$ ion (low spin) [15]. The IR spectra of all these compounds have been measured, and their ν(Fe–S) vibrations assigned based on the observed ^{54}Fe–^{56}Fe isotope shifts.

Experimental

Compounds

All the five thiolate complexes of iron(III) porphyrins were prepared by the literature methods [5, 15, 16]. The crown ether salt of the [Fe(TPP)-(SC$_6$H$_5$)$_2$]$^-$ ion gave g values which are almost

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identical to that reported in the literature [15]. The two thioether complexes containing THT (tetrahydrothiophene) and PMS (pentamethylene sulfide) were prepared by the method reported by Mashiko *et al.* [9]. The ^{54}Fe complexes were prepared on a milligram scale by using ^{54}FeO (99% pure) purchased from Oak Ridge National Laboratory. Since iron in natural abundance (^{NA}Fe) consists of 91.5% pure ^{56}Fe, the complexes containing ^{NA}Fe were used as the substitutes for the ^{56}Fe analogs.

Octaethylporphyrin (OEP), tetraphenylporphyrin (TPP) and protoporphyrin IX dimethyl ester (PPIXDME) were purchased from Alfa Inorganics, Danvers, MA. All the axial ligands and the crown ether were purchased from Aldrich Chemical Co. Milwaukee, WI.

Spectral Measurements

IR spectra were measured on a Beckman 4260 infrared spectrophotometer. The spectra between 4000 and 500 cm^{-1} were obtained in KBr pellets and those below 500 cm^{-1} as Nujol mulls on a CsI window. To determine the ^{54}Fe–^{NA}Fe isotope shifts, the IR spectra below 500 cm^{-1} were measured with a 10 cm^{-1}/in. chart expansion and 5 cm^{-1}/min chart speed. Rotation-vibration bands of standard molecules and polystyrene film bands were used for calibration of frequency reading. The accuracy of frequency reading below 500 cm^{-1} was ±0.5 cm^{-1}. The magnitudes of metal isotope shifts were confirmed by multiple scans over the desired frequency range. To avoid thermal decomposition, the IR spectra of all the thioether complexes and the crown ether salt of the $[Fe(TPP)(SC_6H_5)_2]^-$ were measured as Nujol mulls on a CsI window which was cooled to ~170 K by a CTI Model 21 closed cycle helium refrigerator. The IR spectra (500–100 cm^{-1}) of the $Fe(OEP)(SC_6H_5)$, $Fe(TPP)(SC_6H_5)$ and their ^{54}Fe analogs were also measured on an IBM IR/98 FTIR spectrometer at Loyola University, Chicago. The nujol mull technique with polyethyene windows was used.

The UV-Visible spectra were measured on a Perkin-Elmer Model 320 spectrophotometer. The solutions (~10^{-5} M/L) were prepared in an oxygen-free atmosphere at –68 °C using dry methylene chloride as the solvent, and the measurements made by using a Dewar-cell at the same temperature.

Results

$Fe(OEP)(SC_6H_5)$

The upper traces of Fig. 1 show the IR spectra of $^{NA}Fe(OEP)(SC_6H_5)$ (high spin) and its ^{54}Fe analog in the 500–300 cm^{-1} region. The former exhibits seven bands at 475.5(m), 420.5(m), 347(sh), 341.0(s), 325(sh), 276(w) and 258(w) cm^{-1}. (The spectra

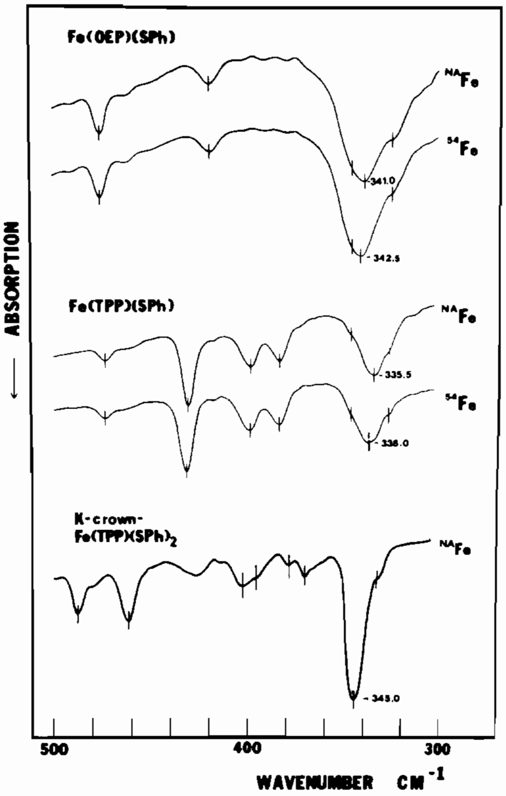

Fig. 1. Far-infrared spectra of $Fe(OEP)(SC_6H_5)$ (^{NA}Fe and ^{54}Fe), $Fe(TPP)(SC_6H_5)$ (^{NA}Fe and ^{54}Fe) and K-crown $Fe(TPP)(SC_6H_5)_2$. The spectrum of the last compound was measured at ~170 K.

below 300 cm^{-1} are not shown in Fig. 1). Here, s, m, w and sh denote strong, medium, weak and shoulder, respectively. No bands of measurable intensity were observed between 250 and 100 cm^{-1}. Previously, we [17] have assigned the far-IR spectra of Fe(OEP)X type complexes (X = Cl, Br, I, NCS and N_3) based on ^{54}Fe–^{56}Fe isotope shifts; two bands in the 357–346 and 347–310 cm^{-1} were not metal-isotope sensitive and assigned to OEP vibrations, whereas doublet bands in the 280–255 cm^{-1} were metal-isotope sensitive and attributed to ν(Fe–N(porphyrin)) which are split by the lowering of symmetry from D_{4h} (Fe(OEP)) to C_{4v}(Fe(OEP)X). $Fe(OEP)(SC_6H_5)$ exhibits all these bands. In addition, it shows a strong band at 341.0 cm^{-1} which is shifted by +1.5 cm^{-1} by $^{NA}Fe \rightarrow {}^{54}Fe$ substitution. Thus, it is most reasonable to assign this band to the ν(Fe–S$^-$) of $Fe(OEP)(SC_6H_5)$. The remaining two bands at 475.5 and 420.5 cm^{-1} are insensitive to metal isotope substitution; the former is assigned to the SC_6H_5 ligand since it appears only when the axial ligand is SC_6H_5, whereas the latter is attributed to an OEP vibration since it appears in all OEP complexes.

Fe(TPP)(SC$_6$H$_5$)

The middle traces of Fig. 1 show the IR spectra of $^{\text{NA}}$Fe(TPP)(SC$_6$H$_5$) (high spin) and its ^{54}Fe analog. The former exhibits seven bands at 437.0(w), 431.0(s), 399.0(m), 384.0(m), 347(sh), 335.5(s) and 328(sh) cm^{-1}. In addition, it shows four weak bands (277, 258, 247, and 220 cm^{-1}) below 300 cm^{-1}. (The spectra below 300 cm^{-1} are not shown in Fig. 1). As stated above, the band at 473.0 cm^{-1} is due to the axial ligand, SC$_6$H$_5$. Among the remaining six bands, the strong band at 335.5 cm^{-1} shows a large isotope shift (+2.5 cm^{-1}) by $^{\text{NA}}$Fe → ^{54}Fe substitution while other bands show only small shifts of 0.5 ~ 1 cm^{-1}. Thus the former is assigned to the ν(Fe–S$^-$) of Fe(TPP)(SC$_6$H$_5$) while the latter are attributed to TPP vibrations coupled with ν(Fe–N (porphyrin)) below 300 cm^{-1} [17]. These assignments are also supported by the observation that [Fe(TPP)]$_2$O (high spin) exhibits IR bands at 433(s), 400(m), 381.5(m), 350(vw) and 326(vw) cm^{-1} [18]. As shown in our previous work [18], the appearance of a strong band in the 435–432 cm^{-1} region is common to all high-spin Fe(II/III) TPP complexes.

[Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$ Ion

The bottom trace of Fig. 1 shows the IR spectrum of the potassium 18-crown-6 salt of the [Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$ ion which is known to be low-spin [15]. The general feature of the spectrum is similar to that of Fe(TPP)(SC$_6$H$_5$) except that weak bands due to the crown ether appear in the region from 426 to 400 cm^{-1} and that the strong band at 431 cm^{-1} of Fe(TPP)(SC$_6$H$_5$) is replaced by a medium intensity band at 461.5 cm^{-1} which is characteristic of low spin complexes [18]. The strong band at 345.0 cm^{-1} can be assigned to the antisymmetric stretching mode of the S$^-$–Fe–S$^-$ linkage (ν_a(Fe–S$^-$)) based on similarity of the spectra between the mono and di-benzene-thiolate complexes.

Fe(Porphyrin)(SC$_6$H$_4$-p-NO$_2$)

To further confirm our assignments of the ν(Fe–S$^-$) vibrations, we have measured the far-infrared spectra of two five-coordinate, high-spin Fe(III) porphyrins containing *p*-nitrobenzenethiolate as the axial ligand [5]. Fe(OEP)(SC$_6$H$_4$-*p*-NO$_2$) exhibits a strong band at 338.0 cm^{-1} which is lower by 3 cm^{-1} than the ν(Fe–S$^-$) of Fe(OEP)(SC$_6$H$_5$). Fe(PPIXDME)(SC$_6$H$_4$-*p*-NO$_2$) also exhibits a strong ν(Fe–S$^-$) band at 333.5 cm^{-1}.

[Fe(TPP)(THT)$_2$]ClO$_4$

The IR spectra of [$^{\text{NA}}$Fe(TPP)(THT)$_2$]ClO$_4$ and its ^{54}Fe analog (low spin) are shown in the upper traces of Fig. 2. These spectra were obtained at ~170 K to prevent decomposition during the measurements. The former exhibits bands at 468.5(m), 463.0(sh), 433.5(m), 406.5(m), 328.0(s) and 322.5(sh) cm^{-1}. Among these bands, only the strong band at 328.0 cm^{-1} showed a distinct shift of +1.5 cm^{-1} by $^{\text{NA}}$Fe → ^{54}Fe substitution. This band is assigned to the antisymmetric stretching mode of the axial ^{0}S–Fe–S^0 linkage, ν_a(Fe–S^0) since neither TPP nor THT exhibits strong bands in this region. The bands at 468.5 and 433.5 cm^{-1} are assigned to the THT ligand because free THT shows the IR bands at 468 and 422 cm^{-1}. The remaining bands at 463.0, 406.5 and 322.5 cm^{-1} are attributed to TPP vibrations.

[Fe(TPP)(PMS)$_2$]ClO$_4$

The lower traces of Fig. 2 show the IR spectra of [$^{\text{NA}}$Fe(TPP)(PMS)$_2$]ClO$_4$ and its ^{54}Fe analog (low spin) obtained at ~170 K. The former shows bands at 466.5(m), 402.0(m), 386.5(m), 359.5(m), 330(sh) and 323.5(s) cm^{-1}. The bands at 466.5, 386.5 and 323.5 cm^{-1} are shifted by +1.0, +1.0 and +2.0 cm^{-1}, respectively, by $^{\text{NA}}$Fe → ^{54}Fe substitution. The strong band at 323.5 cm^{-1} with the largest isotopic shift is assigned to the ν_a(Fe–S^0). The bands at 386.5 and 359.5 cm^{-1} are attributed to the PMS ligand since free PMS shows the IR bands at 392 and 350 cm^{-1}. The remaining bands at 466.5, 402.0 and 330 cm^{-1} are assigned to the TPP vibrations. Apparently, the bands at 466.5 and 386.5 cm^{-1} are coupled with core vibrations involving the motion of the Fe atom.

Discussion

Table I lists all the ν(Fe–S) frequencies determined by this work. It is seen that the ν(Fe–S$^-$) appears between 345 and 333 cm^{-1} whereas the ν(Fe–S^0) is in the range from 328 to 323 cm^{-1}. Thus, the former is higher than the latter by 5–10 cm^{-1}. A better comparison may be made between [Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$ (344 cm^{-1}) and [Fe(TPP)(THT)$_2$]$^+$ (328 cm^{-1}) or [Fe(TPP)(PMS)$_2$]$^+$ (323.5 cm^{-1}), all of which are six-coordinate, low-spin complexes of Fe(III). In this case, the ν_a(Fe–S$^-$) of the former is higher than the ν_a(Fe–S^0) of the latter by 6 to 10.5 cm^{-1}. Since the masses of SC$_6$H$_5$ and PMS are similar, this result may indicate that the Fe–S$^-$ (thio-

TABLE I. Fe–S Stretching Frequencies of Fe(III) Porphyrins (cm^{-1}).

Compound	Spin State	ν(Fe–S)
Fe(OEP)(SC$_6$H$_5$)	high spin	341.0
Fe(TPP)(SC$_6$H$_5$)	high spin	335.5
[Fe(TPP)(SC$_6$H$_5$)$_2$]$^-$	low spin	345.0
Fe(OEP)(SC$_6$H$_4$-*p*-NO$_2$)	high spin	338.0
Fe(PPIXDME)(SC$_6$H$_4$-*p*-NO$_2$)	high spin	333.5
[Fe(TPP)(THT)$_2$]ClO$_4$	low spin	328.0
[Fe(TPP)(PMS)$_2$]ClO$_4$	low spin	323.5

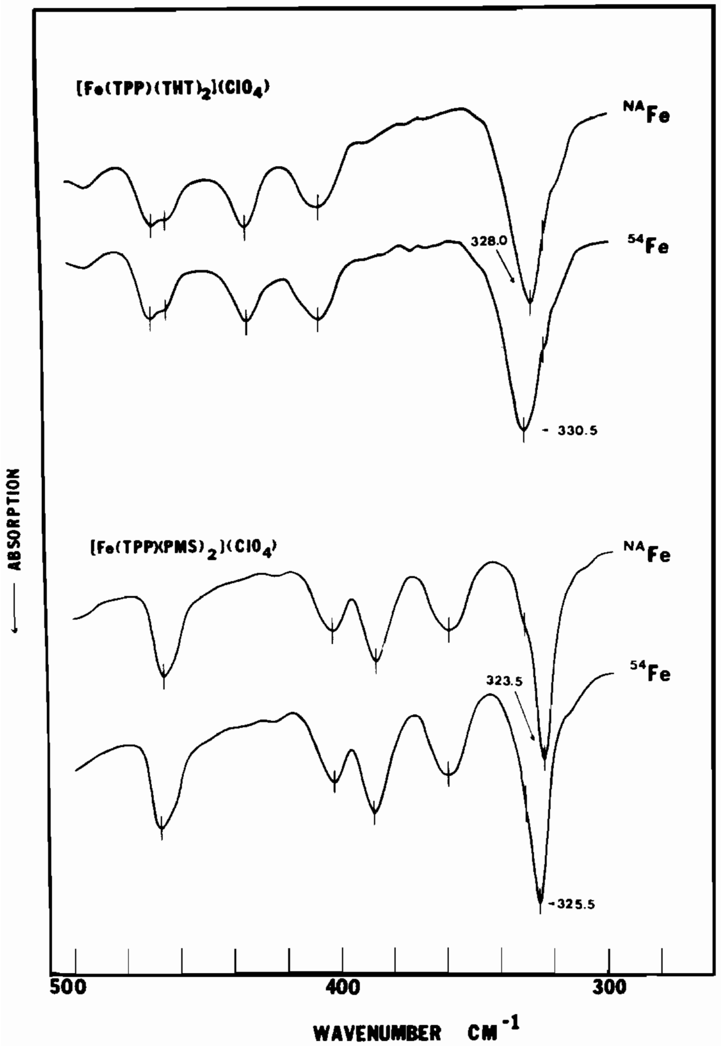

Fig. 2. Far-infrared spectra of [Fe(TPP)(THT)$_2$](ClO$_4$)(^{NA}Fe and ^{54}Fe) and [Fe(TPP)(PMS)$_2$](ClO$_4$) (^{NA}Fe and ^{54}Fe). All the spectra were measured at ~170 K.

late) bond is stronger than the Fe–S^0 (thioether) bond. A more rigorous comparison must be made in terms of Fe–S stretching force constants obtained from normal coordinate calculations. Unfortunately, this is not possible since Raman data on symmetric S–Fe–S vibrations are lacking (*vide infra*) and since normal coordinate analyses on such large molecules inevitably involve drastic approximations.

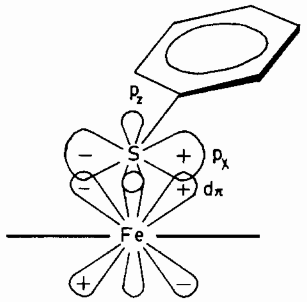

Fig. 3. Bonding scheme of benzenethiolate ligand to iron porphyrin.

As is shown in Fig. 3, the thiolate sulfur has two lone pair electrons (p$_z$ and p$_x$) whereas the thioether or mercaptan sulfur has only one lone pair electrons (p$_z$) for coordination. Thus, the thiolate sulfur can form the second bond *via* the p$_x$(S)–dπ(Fe) overlap in addition to the coordinate bond (*via* the p$_z$(S)–d$_{z^2}$(Fe) overlap) formed by thioether or mercaptan. In the case of Fe(II) complexes, this extra bond in thiolate complexes causes a low frequency shift of the oxidation-state marker band (1370–1340 cm^{-1}, polarized) in resonance Raman spectra. For example, this band shifts from 1360 cm^{-1} (cytochrome P-420, mercaptan co-ordination) to 1346 cm^{-1} (cytochrome

P-450, thiolate coordination) [10] because extra electrons are drifted from the thiolate sulfur to the $\pi^*(e_g)$ orbital of the porphyrin *via* the p_x(S)–$d\pi$(Fe)–$\pi^*(e_g$(por)) overlap [19]. However, this does not occur in the case of low-spin Fe(III) complexes due to their high oxidation states. As a result, their oxidation-state marker bands are not sensitive to the nature of the axial ligand [20]. We have also confirmed this trend in our resonance Raman study of Fe(TPP) complexes [18].

It is noted in Table I that the ν(Fe–S$^-$) of Fe(TPP)(SC_6H_5) (335.5 cm^{-1}) is by 5.5 cm^{-1} lower than that of Fe(OEP)(SC_6H_5). According to X-ray analysis on Fe(PPIXDME)(SC_6H_4-*p*-NO_2) [5], the Fe–S–C angle is 100.4° and the benzene ring is almost parallel to the porphyrin plane (Fig. 3). In the case of the TPP complex, the Fe–S–C angle may increase due to steric repulsion between the SC_6H_5 ligand and the four phenyl groups of TPP. This would weaken the Fe–S bond and decrease the ν(Fe–S$^-$). It is also noted that the introduction of the *p*-NO_2 group to Fe(OEP)(SC_6H_5) reduces its ν(Fe–S$^-$) from 341.0 to 338.0 cm^{-1}. This may be due to the lower basicity of the SC_6H_4-*p*-NO_2 ion (pK_a = 4.49) relative to the SC_6H_5 ion (pK_a = 7.78) as a result of the π-withdrawing effect of the nitro group.

Thus far, the ν(Fe–S) vibrations of the model compounds listed in Table I have not been observed in Raman spectra in spite of our extensive search utilizing all available laser lines in the 351 ~ 647 nm region. Previous resonance Raman studies of cytochromes P-450 [19, 21] and cytochrome c [22] were focused on the porphyrin core vibrations. The only report on the ν(Fe–S) vibration is that of Champion *et al.* [13, 14] who observed the ν(Fe–S$^-$) of cytochrome P-450$_{cam}$ at 351 cm^{-1} for the Fe(III), high-spin, camphor-bound state using the 363.8 nm excitation of an Ar-ion laser. In the cytochrome P-450 reaction cycle, this state is modeled by a five-coordinate Fe(III), high-spin complex such as Fe(PPIXDME)(SC_6H_4-*p*-NO_2) [5] which exhibits the ν(Fe–S$^-$) at 333.5 cm^{-1} (Table I). Thus, the ν(Fe–S$^-$) of this model compound is ~17 cm^{-1} lower than that of cytochrome P-450$_{cam}$ in the corresponding state. This difference may originate in one or a combination of the following factors:

(1) In cytochrome P-450$_{cam}$, the axial sulfur is presumably a cysteinyl (aliphatic) sulfur where as it is a benzenethiolate (aromatic) sulfur in the model system.

(2) The Fe–S–C geometry may be different between the natural and model systems. The observed ν(Fe–S) should be strongly influenced by these factors since they change the Fe–S bond strength as well as vibrational coupling between ν(Fe–S) and other modes.

It is interesting to note that the ν(Fe–S) of cytochrome P-450$_{cam}$ was not observed for the substrate-free, Fe(III), low-spin state in which the Fe atom is presumably bonded to a cysteinyl sulfur and an imidazole nitrogen [13]. Champion *et al.* [13] interpret this as indicating that the substrate binding has shifted the Fe–S CT transition near the Soret region (~360 nm) or increased its oscillator strength in the Soret region. In any case, further work (such as an excitation profile study) is necessary to locate the Fe–S CT band since only a single, symmetric Soret band (λ_{max}, ~380 nm) was observed for cytochrome P-450$_{cam}$.

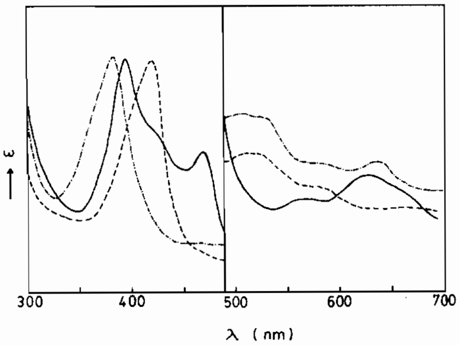

Fig. 4. UV-visible spectra of: Fe(OEP)(SC_6H_5) (—·—·-), [Fe(TPP)(THT)$_2$](ClO$_4$) (- - - -) and K-crown Fe(TPP)(SC_6H_5)$_2$ (———). All the spectra were measured in methylene chloride at –68 °C.

TABLE II. Electronic Spectra of Fe(III) Porphyrins Containing Axial Fe–S Bonds (nm) in CH_2Cl_2 at –53 °C.[a]

Compound	α	β	Soret
Fe(OEP)(SC_6H_5)	636	525, 502	382
Fe(OEP)(SC_6H_4-*p*-NO_2)	641	532, 505	375
Fe(PPIXDME)(SC_6H_4-*p*-NO_2)	650	541, 513	386
[Fe(TPP)(SC_6H_5)$_2$]$^-$ [b]	625	566	469 394
[Fe(TPP)(THT)$_2$]$^+$	580	516	419
[Fe(TPP)(PMS)$_2$]$^+$	566		426

[a] Band assignments are based on Ref. 5. [b] The shoulder near 420 nm (see Fig. 2) is due to impurity which resulted from decomposition products caused by trace oxygen contamination.

Figure 4 shows the electronic spectra of three typical compounds and Table II lists the λ_{max} values of all the compounds studied in this work. It was not possible to obtain the electronic spectrum of Fe(TPP)(SC_6H_5) due to decomposition in solution during the measurement [16]. Previously, Tang *et al.* [5] measured the electronic spectra of the first three compounds listed in Table II. Our spectra are in good agreement with theirs.

Perhaps, the most significant observation is the splitting of the Soret band (469 and 394 nm) in the case of the $[Fe(TPP)(SC_6H_5)_2]^-$ ion. Similar splittings have been observed previously for bis(tertiary-butylthiolate) (475 and 377 nm) [23] and bis-(thioglycolato acid ester) complexes (458 and 373 nm) [24] of Fe(III)–hemin. According to Hanson [25], such 'split-Soret' bands (hyper spectra) are observed for the CO adducts of mono-thiolate Fe(II) porphyrins, and the splitting is due to the interaction between the $p\pi(S)$–$\pi^*(e_g$, por) CT transition and the $\pi(a_{1u}$, a_{2u}, por)–$\pi^*(e_g$, por) transition. Since the $p\pi(S)$ lone pair orbital of the thioether sulfur atom is not present in the vicinity of the porphyrin valence region, no such interactions are expected for thioether complexes. In fact, the electronic spectra of the THT and PMS complexes are normal (single Soret band near 420 nm).

In the case of five-coordinate, high-spin Fe(III) mono-thiolate complexes, no 'split-Soret' bands are observed as shown in Fig. 4 and Table I. The electronic spectrum of cytochrome P-450_{cam} Fe(III), high-spin, five-coordinate) also exhibits a single Soret band near 380 nm [25]. According to Hanson *et al.* [25, 26] this Soret band involves the Fe–S CT character which may be responsible for the observation of its ν(Fe–S) vibration by Champion *et al.* [13, 14]. However, attempts to resonance-enhance the $\nu(Fe–S^-)$ of our model compounds have not been successful in spite of laser excitations involving UV lines (360 nm). Although more experiments must be made before final conclusions are drawn, it is probable that the Fe–S CT component in the Soret transition is too small to cause resonance-enhancement of ν(Fe–S) vibrations by presently available techniques.

Acknowledgments

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References

1 B. W. Griffin, J. A. Peterson and R. W. Estabrook, 'Cytochrome P-450' in 'The Porphyrins' (D. Dolphin, ed.), vol. VII, Biochemistry, Part B, Academic Press, New York (1979).

2 For example, see L. S. Alexander and H. M. Goff, *J. Chem. Educ., 59,* 179 (1982).

3 S. P. Cramer, J. H. Dawson, K. O. Hodgson and L. P. Hager, *J. Am. Chem. Soc., 100,* 7282 (1978).

4 R. Timkovich, 'Cytochromes c' in 'The Porphyrins' (D. Dolphin, ed.), vol. VII, Biochemistry, Part B, Academic Press, New York (1979).

5 S. C. Tang, S. Koch, G. C. Papaefthymiou, S. Foner, R. B. Frankel, J. A. Ibers and R. H. Holm, *J. Am. Chem. Soc., 98,* 2414 (1976).

6 S. W. McCann, F. V. Wells, H. H. Wickman, T. N. Sorrell and J. P. Collman, *Inorg. Chem., 19,* 621 (1980); J. P. Collman, T. N. Sorrell, K. O. Hodgson, A. K. Kulshrestha and C. E. Strouse, *J. Am. Chem. Soc., 99,* 5180 (1977).

7 C. Caron, A. Mitschler, G. Rivière, L. Ricard, M. Schappacher and R. Weiss, *J. Am. Chem. Soc., 101,* 7401 (1979).

8 T. Mashiko, J.-C. Marchon, D. T. Musser, C. A. Reed, M. E. Kastner and W. R. Scheidt, *J. Am. Chem. Soc., 101,* 3653 (1979).

9 T. Mashiko, C. A. Reed, K. J. Haller, M. E. Kastner and W. R. Scheidt, *J. Am. Chem. Soc., 103,* 5758 (1981).

10 P. E. Ellis, R. D. Jones and F. Basolo, *Proc. Nat. Acad. Sci. USA, 76,* 5418 (1979).

11 N. E. Kime and J. A. Ibers, *Acta Cryst., B25,* 168 (1969).

12 E. König and K. J. Watson, *Chem. Phys. Lett., 6,* 457 (1970).

13 P. M. Champion, B. R. Stallard, G. C. Wagner and I. C. Gunsalus, *J. Am. Chem. Soc., 104,* 5469 (1982).

14 P. M. Champion, I. C. Gunsalus and G. C. Wagner, *J. Am. Chem. Soc., 100,* 3743 (1978).

15 M. P. Byrn and C. E. Strouse, *J. Am. Chem. Soc., 103,* 2633 (1981).

16 J. P. Collman, T. N. Sorrell and B. M. Hoffman, *J. Am. Chem. Soc. 97,* 913 (1975).

17 H. Ogoshi, E. Watanabe, Z. Yoshida, J. Kincaid and K. Nakamoto, *J. Am. Chem. Soc., 95,* 2845 (1973).

18 H. Oshio, T. Ama, T. Watanabe, J. Kincaid and K. Nakamoto, *Spectrochim. Acta,* submitted for publication.

19 Y. Ozaki, T. Kitagawa, Y. Kyogoku, H. Shimada, T. Iizuka and Y. Ishimura, *J. Biochem., 80,* 1447 (1976).

20 G. Chottard, P. Battioni, J. P. Battioni, M. Lange and D. Mansuy, *Inorg. Chem., 20,* 1718 (1981).

21 Y. Ozaki, T. Kitagawa, Y. Kyogoku, Y. Imai, C. Hashimoto-Yatsudo and R. Sato, *Biochem., 17,* 5826 (1978).

22 W. G. Valance and T. C. Strekas, *J. Phys. Chem., 86,* 1804 (1982).

23 H. H. Ruf and P. Wende, *J. Am. Chem. Soc., 99,* 5499 (1977).

24 H. Sakurai and T. Yoshimura, *Inorg. Chim. Acta, 56,* L49 (1981).

25 L. K. Hanson, Int. J. Quantum Chem., *Quantum Biology Symposium, 6,* 73 (1979).

26 L. K. Hanson, S. G. Sligar and I. C. Gunsalus, *Croatica Chem. Acta, 49,* 237 (1977).