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Received April 25, 1977

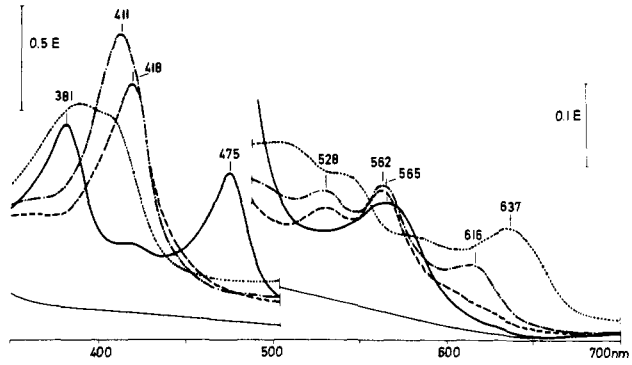

Figure 1. Electronic spectra of hemin-mercaptide complexes at 77 K. Chlorohemin (2 mM) in DMF (· · ·) was titrated with mercaptide (0.1 M *n*-BuSH with 0.1 M $(CH_3)_4NOH$)[10] at −60 °C. The mercaptide/heme ratios were 1 (· - ·),[8] 2 (- - -),[8] and 4.9 (—). The baseline (—) was recorded with DMF. The optical path length was $\sim 5 \times 10^{-3}$ cm.

Hyperporphyrin Spectra of Ferric Dimercaptide-Hemin Complexes. Models for Ferric Cytochrome P450-Thiol Complexes

Sir:

Cytochrome P450, catalyzing various monooxygenase reactions,[1] exhibits unusual spectroscopic properties, nearly all of which have been mimicked with heme complexes liganded with one mercaptide sulfur. Such model complexes have successfully been prepared for the ferric states[2,3] and for the ferrous carbonyl[4] and nitrosyl[5] complexes of cytochrome P450. Its prominant feature, the Soret band at 450 nm of the ferrous CO complex, has been identified by Hanson et al.[6] to be part of a "hyperporphyrin" spectrum. A common characteristic of a number of hyperporphyrin spectra are two intense "Soret bands", one in the 370-380-nm region and another in the 440-480-nm region. An interpretation was given using extended Hückel calculations and the very plausible assumption of mercaptide as a trans ligand. This mercaptide provided a lone-pair sulfur orbital, suitable for a charge-transfer transition to the porphyrin e_g (π^*), and the strong interaction of this transition with the porphyrin a_{1u} (π), a_{2u} (π) → e_g (π^*) transition could result in the intense UV and red-shifted Soret bands. This interpretation was given for ferrous heme mercaptide complexes, but our observation of Soret bands between 455 and 475 nm for complexes of ferric cytochrome P450 with organic thiols and phosphines[7] seemed to be a sign of hyperporphyrin spectra, also of ferric heme complexes. In this communication we present electronic and ESR spectra of ferric hemin-mercaptide models: hyperporphyrin spectra were found for dimercaptide-hemin (and mercaptide-phosphine-hemin) complexes which simulated the spectroscopic properties of the corresponding ferric cytochrome P450 complexes very closely.

For our model studies, hemin solutions were titrated with mercaptide below −60 °C. This temperature was sufficiently low to prevent the reduction of the hemin. The samples were frozen in liquid nitrogen, and electronic and ESR spectra were recorded from the same sample, using ESR quartz sample tubes with 2.8-mm i.d. with a glass insert to reduce the optical path length down to 0.05 mm. This procedure overcame the difficulty that ESR usually required a second, more concentrated sample compared to optical spectroscopy. The electronic spectra of such a titration are shown in Figure 1. At a mercaptide/heme ratio of >3, two Soret bands were observed at ~380 and 470 nm and a broad band at 565 nm, a spectrum very similar to that of cytochrome P450-thiol complexes.[8] Since both Soret peaks appear or disappear concomitantly always, we assigned one hyperporphyrin species to this spectrum. The corresponding ESR spectrum (Figure 2), with the determination of the spin concentration, clearly demonstrates the low spin ferric nature of this spectral species. Although the spectral data varied a little with the mercaptide and the solvent used (Table I), the hyperporphyrin type spectrum did not change.[9] The hyperporphyrin spectrum remained even at 30-fold excess of mercaptide or when an anaerobic procedure was used which maintained a concentration of O_2 of $<10^{-5}$ M corresponding to <1% of the concentration of hemin. The hyperporphyrin spectrum did not appear when a 10-fold excess of either mercaptane or $(CH_3)_4NOH$ was added to a solution with a mercaptide/heme ratio of 1. The addition of 1.5 equiv of imidazole to the hyperporphyrin species was sufficient to abolish the hyperporphyrin spectrum completely with concomitant shifts of the ESR values (Table I), suggesting ligand exchange of one mercaptide by imidazole.[10] From these findings we assign the hyperporphyrin spectrum to ferric heme with two axial mercaptide ligands.

The good simulation of the spectra of cytochrome P450-

Table I. Spectroscopic Data of Hemin-Mercaptide Complexes

Complex	Ligand/heme	Electronic spectrum max, nm	ESR spectrum (at 100 K), *g*
FePPIXDME + *n*-$C_4H_9S^-$ [a]	10	377, 475, 565	2.310, 2.227, 1.958
FePPIXDME + $C_6H_5CH_2S^-$ [a]	3.4	376, 470, 561	2.302, 2.228, 1.959
FePPIXDME + $C_6H_5S^-$ [a]	3.4	387, 473, 565	2.385, 2.262, 1.936
FePPIXDME + p-$NO_2C_5H_4S^-$ [a]	2.5	376, 455, 555, 611	2.405, 2.274, 1.925
FePPIXDME + $C_6H_5CH_2S^-$ + Im[a]	3.4, 1.5	428, 538, 568	2.363, 2.241, 1.937
FePPIXDME + *N*-MeIm[c]	2	409, 522, 558[d]	2.90, 2.29, 1.57
FePPIXDME + $C_6H_5CH_2S^-$ + Et_2PhP	3.4, 10	374, 458, 556 (585)	2.391, 2.264, 1.924
Cytochrome $P450_{CAM}$ + $C_6H_5CH_2SH$[b]		377, 465, 557[d]	(2.43), 2.37, 2.25, 1.94 (1.92)
Cytochrome $P450_{RLM}$ + *n*- $C_8H_{17}SH$[b]		378, 471, 552, 592[d,e]	(2.42, 2.26, 1.91)
Cytochrome $P450_{CAM}$ + Et_2PhP[b]		377, 454, 556[d]	2.50, 2.28, 1.88

[a] Solvent CH_2Cl_2, mixing temperature −78 °C, electronic spectra at 77 K. [b] Reference 7, CAM from *Pseudomonas putida*, RLM rat liver microsomes. [c] Reference 2b, no mercaptide complex. [d] Ambient temperature. [e] Difference spectrum.

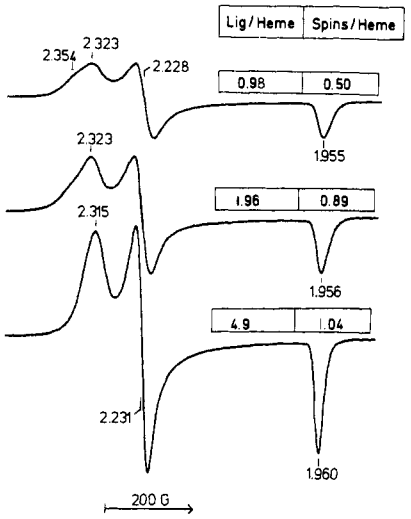

Figure 2. ESR spectra of hemin-mercaptide complexes at 100 K. The same samples of Figure 1 were used. The ESR spectrometer (Varian E-9) was connected to a computer (Data General NOVA 820) for the double integration of the spectra. ESR was recorded at a microwave frequency of 9.35 GHz, at a microwave power of 20 mW, and with a modulation amplitude of 10 G; 1 mM copper(II)-EDTA was used as standard for the determination of the spin concentration by double integration.

thiol complexes by our dimercaptide-heme complexes (Table I) increases the evidence for mercaptide sulfur as one native ligand of ferric cytochroms P450. Also the data for the corresponding phosphine complexes[11] contribute to this evidence.

For the formation of a ferrous hyperporphyrin spectrum, obviously only one mercaptide ligand was necessary, while the sixth ligand was CO (or pyridine, isocyanide, or presumably carbene[12] according to ligand spectra with ferrous cytochrome P450). In the ferric state, however, two mercaptides or one mercaptide and one phosphine were necessary ligands for the hyperporphyrin spectrum. Mercaptide-hemin complexes with isocyanide, thioether, or nitrogeneous bases[10] exhibited normal porphyrin spectra with one Soret band, although red shifted to ~430 nm. Hemin with two negative mercaptide ligands is remarkably stable: the irreversible electron transfer from sulfur to iron is quenched below −50 °C even with alkyl mercaptides.[13]

It is interesting to note the only slight shift of the ESR spectra when the second mercaptide ligand was bound, while the electronic spectra changed rather drastically to the hyperporphyrin type. This finding supports the interpretation[6] of the hyperporphyrin spectrum by a charge-transfer interaction between mercaptide and porphyrin which does not directly involve the iron d orbitals responsible for the g values.

Recently Chang and Dolphin[14] described a spectral species, with peaks at 378, 476, and ~563 nm which was formed after the addition of O_2 to a ferrous protoheme-mercaptide complex at −45 °C, and interpreted it as a mercaptide-heme (II)-oxygen complex. This species would differ from the described oxy complex of cytochrome P450.[15] In view of the striking spectral similarity with our dimercaptide-hemin complex, one should consider the possibility that dioxygen may cause the oxidation to a ferric protoheme-dimercaptide complex which can be reduced by mercaptide in the presence of CO.

Acknowledgments. We thank Professors V. Ullrich, Homburg/Saar, and T. Traylor, San Diego, Calif., for encouraging and stimulating discussions. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 38, Project L and Grant U1 36/11).

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(8) The structures of the intermediate spectral species at mercaptide/heme ratio of 1 and 2 were not examined in detail; possible ligands were DMF, chloride, and mercaptide.
(9) We obtained identical data for the hyperporphyrin spectra when we prepared the mercaptides either from mercaptans with equimolar base ($(CH_3)_4NOH$) or from sodium mercaptides with dibenzo-18-crown-6.
(10) The comparison of the data from the mercaptide/imidazole/heme system with the data for a diimidazole-heme complex[2b] show that imidazole, even at 10-fold excess, exchanges only one mercaptide. By this ligand exchange method, we were able to obtain spectroscopic data for the complexes $[RS^-Fe^{III}L]$ where L was imidazole, primary amine, and thioether. Since their Soret bands were found between 426 and 434 nm, compared to 418 nm for low spin ferric cytochrome P450, none of these ligands appear to be the native sixth ligand in cytochrome P450.
(11) When 10 equiv of diethylphenylphosphine was added to a dimercaptide-hemin complex, a new spectral species was observed (Table I) showing also a hyperporphyrin spectrum, although the bands were blue shifted. The ESR spectrum indicated also low spin ferric heme. The relative shifts of the g values compare favorably with those of the thiol and phosphine complexes of cytochrome P450.[7]
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(13) Measuring the concentration of ferric heme by double integration of the ESR spectrum, we found in the system hemin/n-BuS^- in DMF that the reduction of the heme proceeds above −40 °C. With CO the reduction was facilitated: it began at −45 °C.
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Received December 22, 1976

Metal–Nitroxyl Interactions. 2. A Resolved Weak Exchange Electron Paramagnetic Resonance Spectrum in Solution[1]

Sir:

Interaction of paramagnetic transition metals with nitroxyl radicals is being used to provide structural information about biological systems.[2] Transition metal-nitroxyl radical species also provide an entree to the study of interactions between nonequivalent unpaired electrons, i.e., electrons with different g values, coupling constants, and/or relaxation times.[3]

Following a theory put forth by Leigh,[4] the analysis of EPR spectra of spin-labeled biomolecules to ascertain the distance between the metal and the nitroxyl spin label assuming dipolar interaction is quite common.[2] In some cases in which a nitroxyl radical containing moiety functions as a ligand to a paramagnetic metal or encounters the metal in collisions in solution, the EPR spectra of the nitroxyl radical differs from the spectrum of the nitroxyl in the absence of the metal. In some of the papers the investigators assumed that the spectral changes were due to exchange interactions.[3b,5] In other papers it was assumed that the effects were dipolar in origin.[6] Molin and coworkers have explored collisions of nitroxyl radicals with