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Stable Hyperporphyrin Spectra of Hemin-Dithiolate Complexes using Thioglycolic Acid Esters; a Model of Thiolate Binding Cytochrome P-450

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Buchler [1] has classified the absorption spectra of metalloporphyrins into three types such as *normal*, *hypso* and *hyper*. The hyperporphyrin spectra show strong absorption bands in the 300–800 nm region exhibiting 'two split Soret bands', one in the near-uv 350–380 nm region and the other in the 400–480 nm region [2]. Cytochrome P-450, a protoheme-containing monooxygenase system, exhibits anomalous Soret bands at 360 and 450 nm on reduction in the presence of CO [3], and these bands have been attributed to be a part of p-type hyperporphyrin spectrum due to the formation of thiolate carbon-monoxy ferrous porphyrin complex [2], demonstrating a CT transition from one lone-pair orbital of sulfur to the porphyrin $e_g(\pi^*)$ orbital [2]. On the other hand, based on the knowledge of dithiolate-hemin model complexes, two Soret bands at 378 and 471 nm observed for the complex of ferric cytochrome P-450 with organic thiol-compounds [4] have also been identified as a hyperporphyrin spectra in the ferric state [5, 6]. The model of hyperporphyrin complexes used in the work, however, were unstable at room temperature and detected only below –60 °C.

During the investigations on the model complexes for cytochrome P-450, we have found that ferric hyperporphyrin spectra could be obtained by thioglycolic acid esters as thiolate ligand at room temperature. This paper communicates the electronic and EPR spectra of hyperporphyrin complexes consisting of ferric hemin and thioglycolic acid esters.

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Abbreviations: EPR, electron paramagnetic resonance; TGE, thioglycolic acid ethyl ester; Fe(PPIXDME), Fe(III)-protoporphyrin IX dimethyl ester complex; Fe(TPP), Fe(III)-tetraphenylporphyrin complex; Im, imidazole.

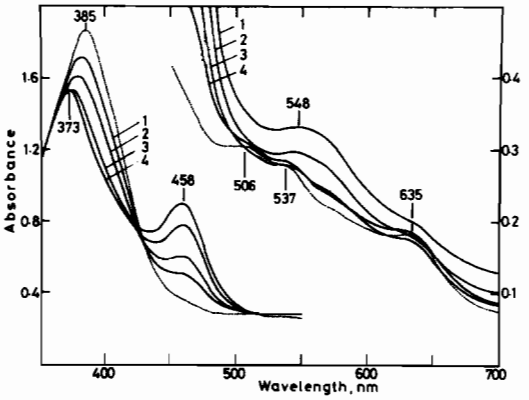


Fig. 1. Electronic Spectra of Hemin-Dithiolate Complex at Room Temperature (20 °C). The mixture of hemin (0.025 m*M* in 0.01 *N*–NaOH) and TGE (1.25 m*M* in acetone) was titrated with 1.10 *M* $(CH_3)_4NOH$ (in methanol) in acetone. Hemin:TGE:$(CH_3)_4NOH$ = (1) 1:500:73, (2)1:500:147, (3)1:500:293, (4)1:500:792.

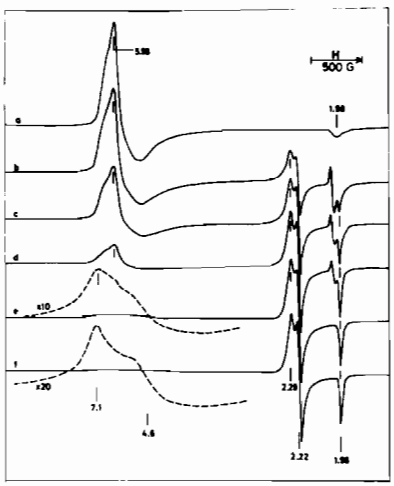


Fig. 2. EPR Spectra of Hemin-Dithiolate Complex at 77 K. Hemin (25 m*M* in 0.1 *N*-NaOH) was titrated with TGE in acetone. Hemin:TGE = (a) 1:0, (b) 1:0.25, (c) 1:0.5, (d)1:1, (e) 1:2, (f) 1:10.

The aerobic addition of thioglycolic acid ethyl ester (TGE), greater than 100-fold molar excess to hemin in alkaline acetone solvent, cause the formation of two new split Soret bands at 373 and 458 nm with a broad absorption at 548 nm at room temperature (Fig. 1). This spectrum is very similar to those of the ferric hemin-dithiolate complex which has been proposed as hyperporphyrin spectrum [5, 6]. The corresponding EPR spectra (Fig. 2) clearly showed the formation of ferric low-spin species (g = 2.29, 2.22, 1.96) with disappearance of high-

TABLE I. Electronic and EPR Spectroscopic Properties of Hyperporphyrin Complexes.[a]

Complex	Electronic Spectrum λ_{max} (nm) at 20 °C			EPR Spectrum g-value at 77 K		
Hemin + $^{-}SCH_2COOC_2H_5$ (TG-Ethyl)	373	458	548	2.285	2.223	1.959
Hemin + $^{-}SCH_2COO(CH_2)_3CH_3$ (TG-Buthyl)	375	457	550	2.289	2.222	1.958
Hemin + $^{-}SCH_2COC_8H_{17}$ (TG-2-Ethyl-Hexyl)	377	457	547	2.29	2.23	1.95
Hemin + $^{-}SCH_2COOCH_2(CH_2)_6CH_3$ (TG-n-Octyl)	376	457	547	2.283	2.223	1.957
Hemin + $^{-}SCH_2COOC_8H_{17}$ (TG-iso-Octyl)	375	459	550	2.283	2.223	1.957
Hemin + $^{-}SCH_2COOC_{18}H_{37}$ (TG-Octadecyl)	374	458	541	2.3	2.23	1.95
Fe(PPIXDME) + $^{-}SCH_2COOC_2H_5$	371	466	562	2.299	2.234	1.954
Fe(PPIXDME) + $^{-}SC_4H_9$[b]	377	475	562	2.310	2.227	1.958
Cytochrome P-450$_{CAP}$ + $C_6H_5CH_2SH$[c]	377	465	557	2.37	2.25	1.94

[a]Experimental conditions are shown in figures. [b]Reference 5. [c]Reference 4, P-450 from *Pseudomonas putida*.

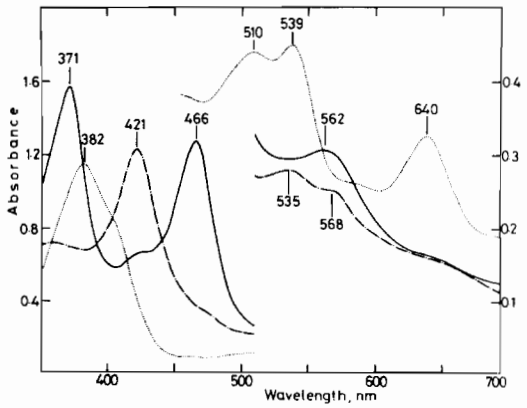


Fig. 3. Electronic Spectra of Hemin-Dithiolate Complex at Room Temperature (20 °C). The mixture (·····) of Fe-(PPIXDME) (0.025 m*M*) and TGE (12.5 m*M*) was added 0.73 m*M* of $(CH_3)_4NOH$ (——) and was followed with addition of 0.67 m*M* of Im (–·–) in acetone solvent.

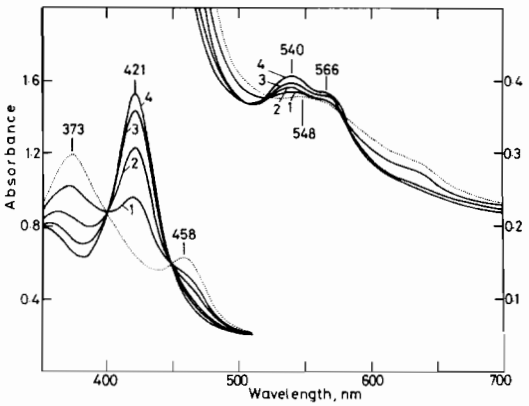


Fig. 4. Spectrophotometric Titration of Hemin-Dithiolate Complex with Imidazole at Room Temperature (20 °C). The hyperporphyrin complex consisting of hemin (0.025 m*M* in 0.1 *N*-NaOH) and TGE (12.5 m*M* in acetone) was titrated with Im (1 *M* in acetone). Hemin:TGE:Im = (1)1:500:27, (2) 1:500:80, (3) 1:500:267, (4) 1:500:1292.

spin species (g = 5.98) by adding TGE. The anisotropic EPR g-values were also very similar to those of hyperporphyrin complexes [5, 6]. It is interesting to note that another high-spin species was detected with EPR spectrometry, probably due to the formation of monothiolatehemin complex (g = 7.1, 4.6).

The hyperporphyrin-like spectra were stable at room temperature (20 °C) for at least 60 minutes in air. Intensity of the Soret band at 458 nm depended on the solvent water content; decreasing water content increased the absorption intensity at 458 nm, while increasing water–acetone ratio over 50% brought about the formation of high-spin species due to the monothiolate–hemin complex.

Using Fe(PPIXDME) or Fe(TPP) in place of hemin, virtually the same hyperporphyrin spectra were obtained showing more strong and slightly shifted bands (Fig. 3) and with high stability at room temperature in air.

From these findings, we assigned these characteristic spectra to be a part of hyperporphyrin spectra due to the ferric hemin complex with two axial thiolate ligands.

This assignment could be successfully substantiated by ligand exchange reactions. When the hyperporphyrin complex was titrated with imidazole at room temperature, a new Soret band at 421 nm and four isosbestic points were observed indicating the imidazole-induced spectral change is reversible and the two chemical species are in equilibrium (Fig. 4). EPR spectrometry clearly revealed this new species; when the hyperporphyrin complex was titrated with imidazole or bisimidazole-hemin complex was titrated with TGE, the same complex was formed by both the methods and its g-values are different from those of the hyperporphyrin and bisimidazole-hemin complex (Fig. 5). (Under the alkaline conditions shown in Fig. 5 imidazole is deprotonated

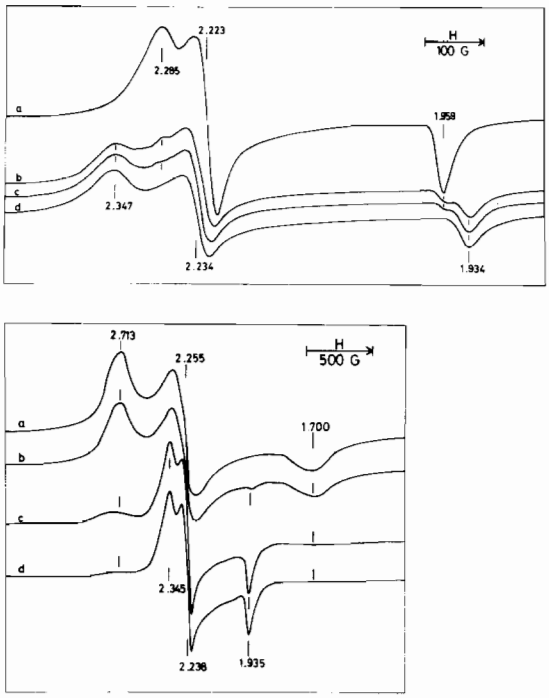
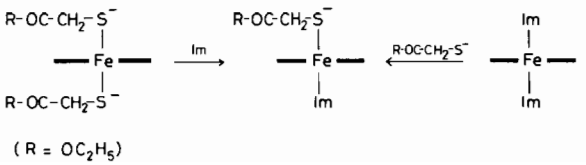


Fig. 5. EPR Titration of Hyperporphyrin Complex with Imidazole (A) and of Bis-imidazole-Hemin Complex with Thioglycolic Acid Ethyl Ester (B). (A): The mixture of hemin (25 m*M* in 0.1 *N*-NaOH, 0.15 ml) and TGE (250 m*M* in acetone, 0.15 ml) was titrated with Im (25 m*M* in acetone). Hemin:TGE:Im = (a) 1:10:0, (b) 1:10:0.33, (c) 1:10:0.67, (d) 1:10:1. (B): The mixture of hemin (25 m*M* in 0.1 *N*-NaOH, 0.15 ml) and Im (250 m*M* in acetone, 0.33 ml) was titrated with TGE (50 m*M* in acetone). Hemin:Im:TGE = (a) 1:2:0, (b) 1:2:0.4, (c) 1:2:0.8, (d) 1:2:2.8.

to imidazole anion [7].). The new complexes with a Soret band at 421 nm and an anisotropic low-spin EPR signal, therefore, have a thiolate at fifth position and an imidazole at sixth position (the bis-imidazole-hemin complex has absorption bands at 396, 414 and 576 sh nm), and the reaction sequence can be represented as follows:



When thioglycolic acid was used in place of TGE, the hyperporphyrin spectrum was not detected probably due to its high pK_{SH}-value (10.40) of acid, compared with that (7.97) of ester [8]. Electronic and EPR spectral properties of hyperporphyrin complexes with several thioglycolic acid esters are summarized in Table I.

Our findings are similar to the observation made by Ruf *et al.* [5, 6] that by varying the sixth ligand of hexa-coordinated monothiolate complexes with a thiolate ligand produces only minor variations of EPR g-values, while it results into dramatic changes of the electronic spectrum. Hence, the interpretation for occurrence of hyperporphyrin spectrum by both charge-transfer interaction between thiolate and porphyrin and that between porphyrin and iron(III) should be supported in the ferric hemin hyperporphyrin complexes ligated with dithiolate ligands. Details will be published in the future.

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