Spectroscopic Models for Cytochrome P-450 Derivatives: Hyperporphyrin Spectra in Thiolatoiron(III)–Porphyrin Complexes

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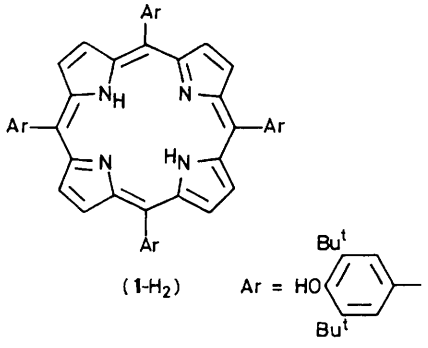
The iron(III) complex of the porphyrin *meso*-tetrakis(3,5-di-t-butyl-4-hydroxyphenyl)porphyrin, (**1**-H_2), reacts with certain thiols in basic dimethyl sulphoxide solution at 25 °C to give products having hyperporphyrin spectra similar to those of thiolate complexes of iron(III)–cytochrome P-450.

Cytochrome P-450 represents a class of enzymes responsible for the hydroxylation of a wide variety of organic compounds. These haem proteins have aroused considerable interest not only because of their biochemical importance (*e.g.* in detoxification, drug metabolism, carcinogenesis, and steroid biosynthesis[1,2]) but also because of the unique spectroscopic properties, apparently arising from the presence of thiolate ligands, shown by some of their derivatives. For example the CO adducts of the reduced enzymes possess 'hyperporphyrin' spectra characterised by intense Soret absorptions at ~363 and ~450 nm.[3]† These spectra, which have proved invaluable in biochemical assays of the protein, have been successfully mimicked in the CO adducts of some simple iron(II)–porphyrin complexes containing axial thiolate ligands.[4,5] Hyperporphyrin spectra have also been obtained for complexes of iron(III)–cytochrome P-450 with organic thiols at room temperature,[6] and spectroscopic studies on iron(III)–protoporphyrin IX dimethyl ester (PPIXDME)–thiolate model systems have established that the species responsible for the Soret splitting are low-spin bis(thiolato)iron(III) haem complexes.[7] However the susceptibility of iron(III)–porphyrin complexes towards reduction in the presence of thiolate ligands necessitated the use of low temperatures in these studies and the hyperporphyrin spectra could only be obtained below −60 °C for these model haemin–thiolate complexes. This communication describes spectroscopic models for the protein–thiolate complexes which, by contrast, are remarkably stable in the presence of air at room temperature.

Addition of Et_3N to a solution containing $Fe^{III}Cl$–(**1**)[8] and $HSCH_2CO_2Me$ in dimethyl sulphoxide (DMSO) at 25 °C caused an immediate colour change from yellow to green and the Soret band at 433 nm in the spectrum of the original solution to be replaced by split Soret bands at 379 and 460 nm (Figure 1).‡ As this spectrum is very similar to those reported for the protein–thiolate complexes at room temperature and for low-spin bis(thiolato)iron(III)–PPIXDME complexes at −196 °C it may be concluded that the product is low-spin Fe^{III}–(**1**)($^-SCH_2CO_2Me$)$_2$.§ Hyperporphyrin spectra were also obtained in dimethylformamide (DMF) and MeCN (but not toluene) as solvents and in solutions of other thiols containing electron-withdrawing groups (Table 1). By contrast more strongly reducing thiols, *e.g.* simple alkyl thiols



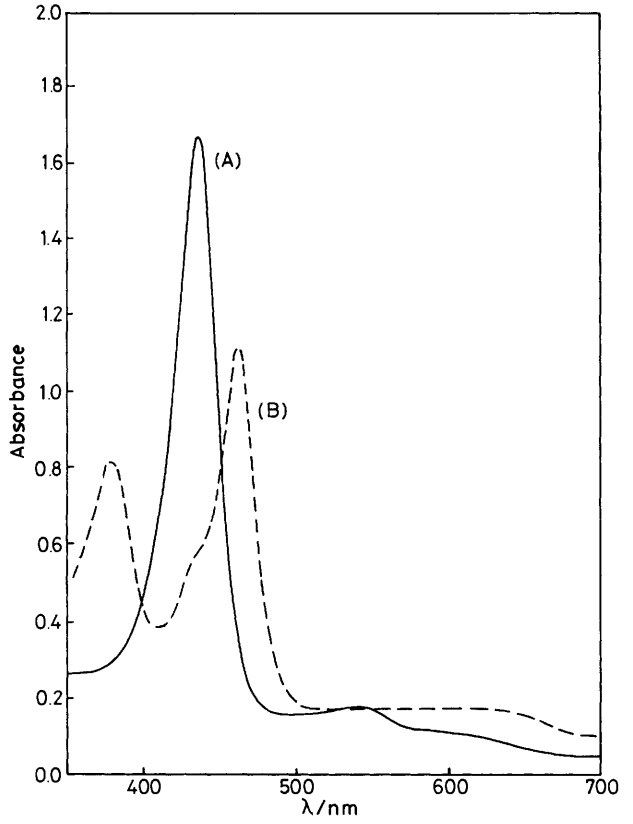


Figure 1. (A) Spectrum of a solution of $Fe^{III}Cl$–(**1**) (~1.3×10^{-5} M) in DMSO containing $HSCH_2CO_2Me$ (0.2 M) at 25 °C; (B) above solution also containing Et_3N (0.2 M).

† The splitting of the Soret band results from interaction of a thiolate sulphur → porphyrin $e_g(\pi^*)$ charge-transfer transition with the porphyrin $a_{1u}(\pi)$, $a_{2u}(\pi) \rightarrow e_g(\pi^*)$ transitions. A recent *X*-ray crystallographic study has established that Cys-357 provides the thiolate ligand to the haem in iron(III)–cytochrome $P\text{-}450_{cam}$: T. L. Poulos, B. C. Finzel, I. C. Gunsalus, G. C. Wagner, and J. Kraut, *J. Biol. Chem.*, 1985, **260**, 16122.

‡ Addition of thiol or Et_3N alone did not give the hyperporphyrin spectrum. In the latter case aerial oxidation of the porphyrin ligand in the complex occurred (L. R. Milgrom, *Tetrahedron*, 1983, **39**, 3895) but addition of thiol to the product caused immediate reduction and gave the hyperporphyrin spectrum.

§ Under the reaction conditions one or more of the phenolic groups may be ionized. Delocalisation of the phenolate charge on to the porphyrin would strengthen the ligand field around the metal and may account for the ready formation of low-spin bis(thiolate) complexes at room temperature.

Table 1. Spectroscopic data for haemin–bis(thiolate) complexes.

Complex	$\lambda_{max.}$/nm
Cytochrome P-450$_{cam}$–($^-SCH_2Ph$)[a]	377, 465[b]
Cytochrome P-450$_{RLM}$–($^-SC_8H_{17}{}^n$)[a]	378, 471[b]
Fe^{III}–PPIXDME–($^-SBu^n$)$_2$	377, 475[c]
Fe^{III}–(**1**)–($^-SCH_2CO_2Me$)$_2$	379, 460[d]
Fe^{III}–(**1**)–($^-SCH_2CO_2H$)$_2$[e]	379, 458[d]
Fe^{III}–(**1**)–(^-SPh)$_2$	398, 468[d]
Fe^{III}–(**1**)–($^-SC_6F_5$)$_2$	–, 483[d,f]

[a] In these complexes the second thiolate ligand is from the Cys-357 residue of the protein. [b] In H_2O, pH 7.3 at room temperature. [c] In CH_2Cl_2 at −196 °C. [d] In DMSO at 25 °C. [e] Although the CO_2H group is much more acidic than the SH group in H_2O (pK_a values, 3.82 and 9.30 respectively in 3 M $NaClO_4$ solution at 20 °C; M. Aguilar, M. Valiente, and E. Casassas, *An. Quim.*, 1978, **74**, 253) their acidities are much closer in DMSO (estimated pK_a values are 12—13 for both; A. Williams, personal communication; F. G. Bordwell and D. L. Hughes, *J. Org. Chem.*, 1982, **47**, 3224; D. Martin in 'Dimethyl Sulfoxide,' eds. D. Martin and H. G. Hauthal, Van Nostrand Reinhold, U.K., 1976, p. 81). As the thiolate conjugate base is stabilised by complexation, $^-SCH_2CO_2H$ is the stable form of the ligand under the reaction conditions. [f] In this case the shorter wavelength band could not be observed because of the strong background absorption of a solution of the thiol in DMSO containing Et_3N.

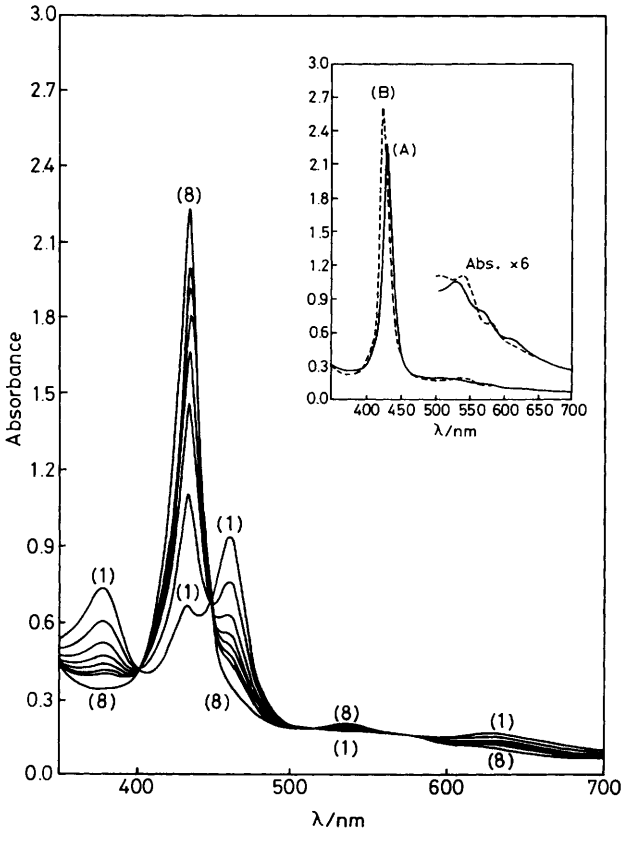
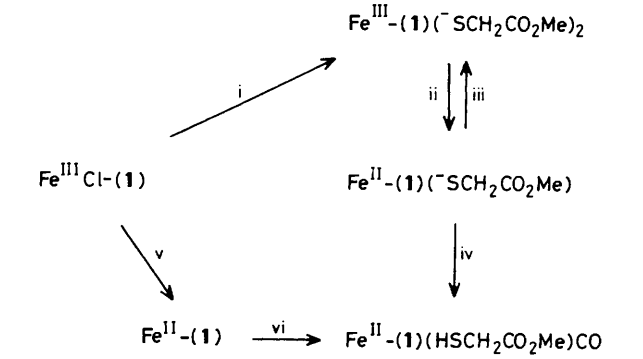


Figure 2. Spectra during the reduction of Fe^{III}–(**1**)($^-SCH_2CO_2Me$)$_2$ (~1.3 × 10^{-5} M) in deoxygenated DMSO solution also containing $HSCH_2CO_2Me$ (0.2 M) and Et_3N (0.2 M) at 25 °C. Time interval between successive spectra (1)—(7) is 3.2 min; the spectrum (8) of the product was recorded after 60 min. The inset shows the spectrum (A) of the reduction product and (B) its CO adduct with the α,β regions of both expanded 6-fold.



Scheme 1. *Conditions:* i, in DMSO containing excess of $HSCH_2CO_2Me$ and Et_3N at 25 °C; ii, deoxygenation of solution in i; iii, aeration of solution in ii; iv, CO added to solution in ii; v, in deoxygenated toluene containing excess of $HSCH_2CO_2Me$ and Et_3N at 25 °C; vi, CO added to solution in v.

such as EtSH, did not give hyperporphyrin spectra under the above conditions but instead caused gradual haemin decomposition.¶

When the above experiments were carried out under anaerobic conditions gradual reduction of Fe^{III}–(**1**)($^-SCH_2CO_2Me$)$_2$ by thiolate occurred (Figure 2) to give a product solution having a spectrum characteristic of high-spin 5-co-ordinate thiolatoiron(II)–tetra-arylporphyrin complexes (Figure 2, inset).[5,9] The formation of this product is consistent with the previously reported chemistry of iron(II)–porphyrin complexes in the presence of thiolate ligands.[9,10] Aeration of this solution immediately regenerated the iron(III) complex with the hyperporphyrin spectrum and the removal of excess of air from the resulting oxidised solution again caused the reduction shown in Figure 2. This redox cycle could be carried out indefinitely with little or no haem decomposition. The reduction product obtained in deoxygenated toluene differs from that in DMSO and has a spectrum consistent with the formation of the 4-co-ordinate complex Fe^{II}–(**1**).** Addition of CO to either of the reduced solutions however gave the same adduct suggesting the formation of Fe^{II}–(**1**)($HSCH_2CO_2Me$)CO in both solvents (Figure 2, inset).‖ These results, which are included in the overall reaction in Scheme 1, substantiate previous conclusions regarding the inability of neutral thiols on their own to form complexes with 4-co-ordinate iron(II)–tetra-arylporphyrins but their tendency to do so in the presence of π-acid ligands such as CO.[4]

¶ In the absence of air these thiols caused immediate reduction to stable iron(II)–porphyrin complexes which decomposed when air was admitted.

** This spectrum has bands of similar intensity at 415 and 435 nm, each of which is roughly 1/3 the intensity of the Soret band in the 6-co-ordinate adduct Fe^{II}–(**1**)($HSCH_2CO_2Me$)CO (see later in text and Figure 2, inset). These spectroscopic properties characterise the product as a 4-co-ordinate iron(II)–tetra-arylporphyrin: J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, E. Bunnenberg, R. E. Linder, G. N. LaMar, J. D. Gaudio, G. Lang, and K. Spartalian, *J. Am. Chem. Soc.*, 1980, **102**, 4182.

‖ The CO adduct of Fe^{II}–(**1**)($^-SCH_2CO_2Me$) has a spectrum characteristic of low-spin Fe^{II}(porphyrin)(thiol)CO complexes, rather than the hyperporphyrin spectrum generally associated with Fe^{II}(porphyrin)(thiolate)CO species (see refs. 4, 5, and 9). The increased basicity of the thiolate ligand when CO is added to Fe^{II}–(**1**)($^-SCH_2CO_2Me$) may be a consequence of back bonding from CO to the metal in the low-spin 6-co-ordinate adduct. This reaction is accompanied by protonation (H^+ from the medium) of the thiolate ligand (Scheme 1).

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