A Model Complex for the Active Site of Cytochrome P-450

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Cytochromes P-450 catalyze hydroxylation of a variety of organic substrates with dioxygen in the presence of reductants such as NADPH, and much attention has been paid to elucidating the structure of the active site [1]. Spectroscopic studies have provided strong evidence that these enzymes contain iron protoporphyrin-IX as a prosthetic group coordinated with the mercaptide anion of a cysteine residue at the axial position [1b, 2]. The proposed structure has been recently confirmed by an X-ray crystal structure of cytochrome P-450$_{cam}$ [3]. It has also been suggested that the mercaptide bond plays an important role in the catalytic cycle and that it is responsible for some spectroscopic characteristics [2, 4]. The role of the mercaptide in the enzymic reaction is not, however, fully understood.

Model systems for the enzymes so far proposed are either those prepared from the mixture of heme and a large excess of a mercaptide [5] or iron mercaptan–tail porphyrin complexes [6]. In both systems the mercaptide–iron bonds are weak and easily cleaved, although the bond in the native enzyme is strong and not broken throughout the catalytic cycle. Therefore these systems are not suitable as models for studying the more detailed features or the reactivities of the active site of the enzyme. Battersby *et al.* [7] reported recently a Fe(II)–porphyrin carrying a strapped thiolate ligand as a model of the active site of cytochrome P-450, which is expected to firmly hold a mercaptide anion at the axial position of the heme iron. This model has provided much information on a heme–CO complex but little information about other states, such as the resting state. We have also designed another iron–strapped porphyrin complex **6** containing a mercaptide and a methyl group connected to the center of a strap which binds to the porphyrin nucleus through two covalent bonds. The methyl group is expected to force the mercaptide in the direction of the porphyrin plane. Here we wish to report the preparation of the model system by a different approach and the results of spectroscopic studies by which we could detect several steps included in the catalytic cycle.

Synthesis of **6** is summarized in Scheme 1. Monotetrahydropyranyl ether of propylene glycol (**1**) (b.p. 112–3 °C/2 mmHg) was condensed with methylmalonic acid chloride in ether to give the diester **2**#* as a yellow oil in 66% yield. Dropwise addition of chloromethyl thiobenzoate at −30 °C to an anion generated *in situ* from **2** yielded **3**# as a colorless oil (78% yield) after hydrolysis by TsOH in MeOH, which is used as a strap. Condensation of the acid chloride of mesoporphyrin II with the diol **3** carried out in CH_2Cl_2 under high dilution conditions (performed by a very slow simultaneous addition of each 1.7×10^{-3} M solution of both reagents) at room temperature and subsequent purification by column chromatography on silica gel (EtOAc:$CHCl_3$ = 1:1 as an eluent) and recrystallization from $CHCl_3$–hexane afforded the desired strapped porphyrin **4**#, carrying a protected thiol group at the center of the strap, as a purple–red powder (m.p. 80–82 °C, 17% yield). The ^{1}H NMR of **4** showed remarkable high-field shifts ($\Delta\delta$ 2.72 ~ 0.37) for the methyl and methylene protons of the strap compared to those of **3** due to the porphyrin ring current, which certifies the presence of a strap bridging the porphyrin nuclei, as expected. Iron insertion to **4** ($FeSO_4$ method) gave the hemin chloride **5**# as a dark purple powder (m.p. 223–226 °C, 73% yield). Reduction of complex **5** with $Na_2S_2O_4$–dicyclohexyl 18-crown-6-ether in DMSO and subsequent deprotection of the S-benzoyl group by addition of a slight excess (about 8-fold excess) of dimsyl sodium produced the ferrous strapped-porphyrinato complex **6**, which easily combined carbon monoxide on introduction of gaseous CO to give the CO adduct **7**. The electronic spectrum of **7** exhibited three major absorptions at 370, 446 and 548 nm, accompanied by a weak absorption band at 406 nm (Fig. 1). The unusual 'hyper' Soret bands (370, 446 nm) are characteristic of the absorption spectrum of the CO adduct of the reduced cytochrome P-450, indicating that a mercaptide coordinates to a heme iron as an axial ligand [4]. A weak absorption at 406 nm may arise from the CO adduct of ferrous porphyrin lacking mercaptide coordination (*vide infra*). In contrast to the previous models, the mercaptide in this model complex is firmly fixed in a position suitable for feasible ligation to the heme iron, just as in the native enzymes, leading to the enhanced stability of

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*All new compounds indicated by # showed satisfactory values for elemental analyses and reasonable spectroscopic data.

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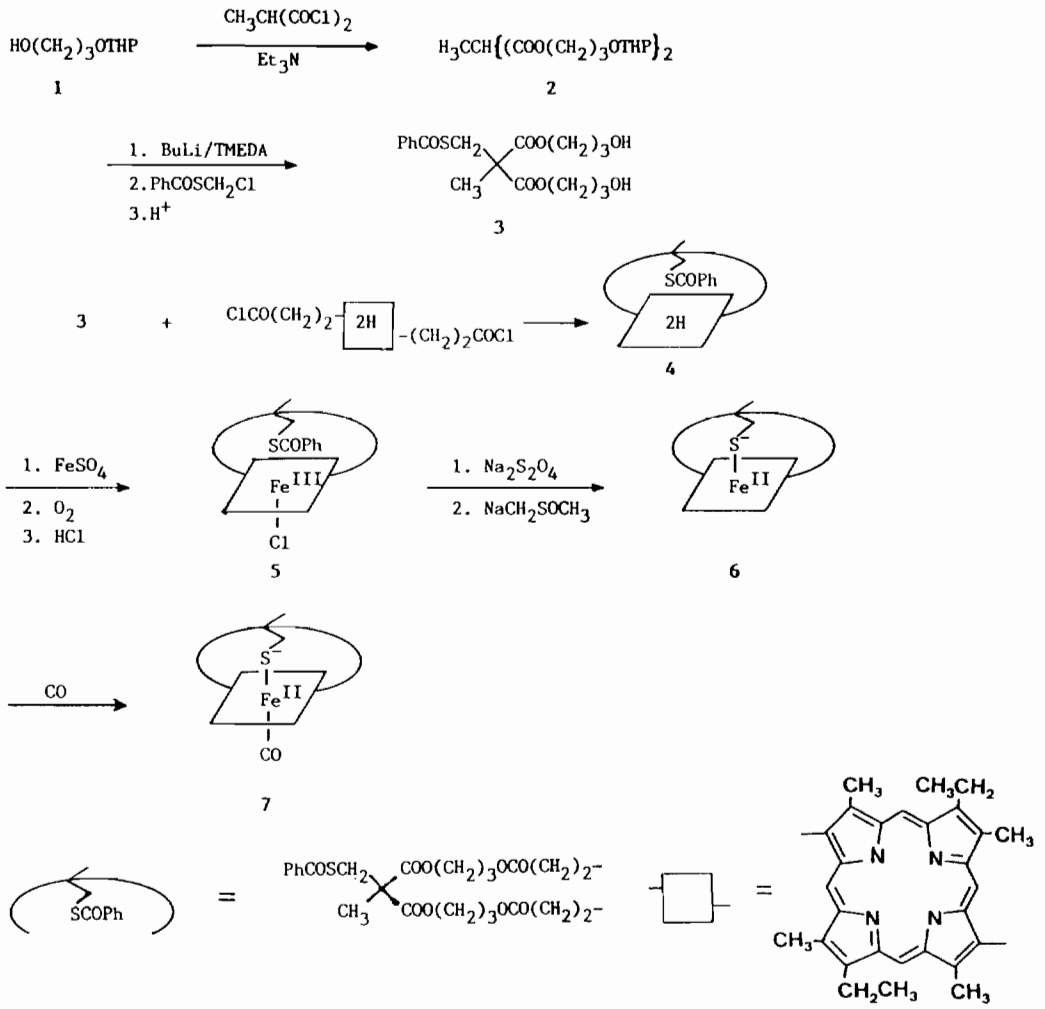


Scheme 1.

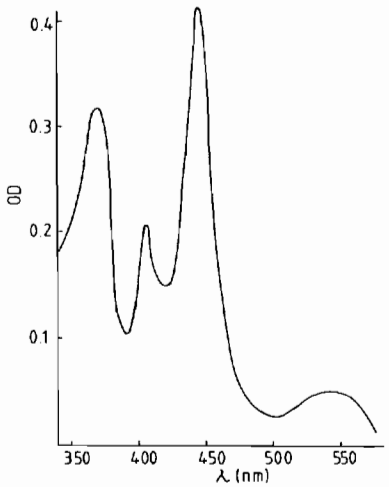
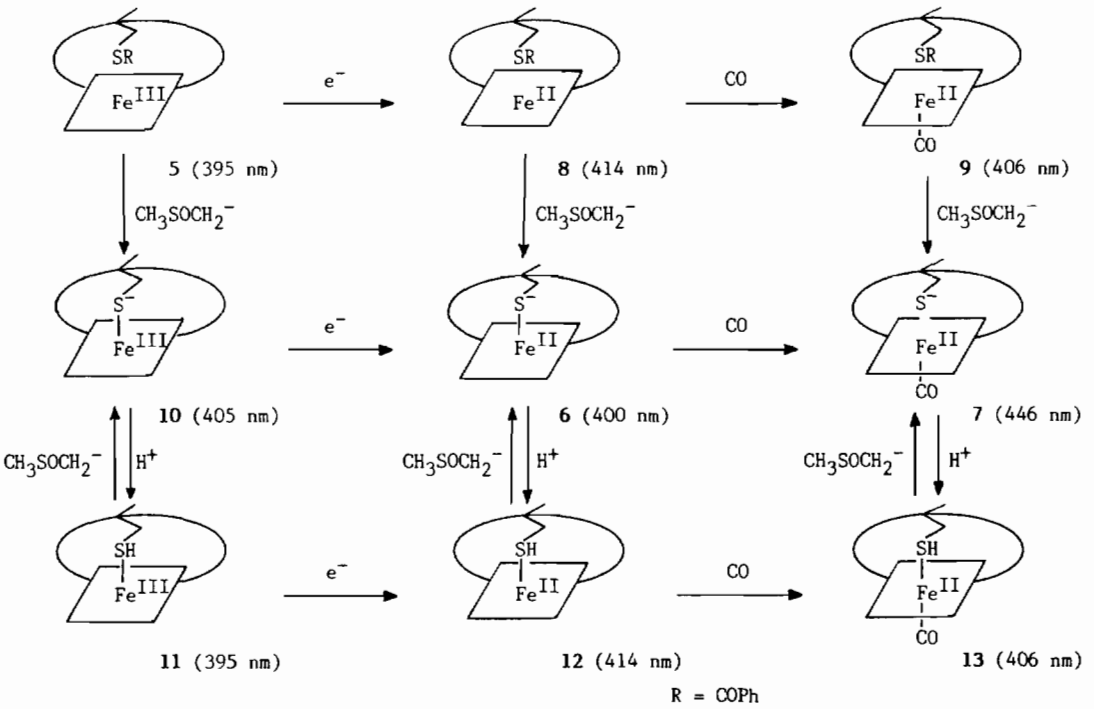


Fig. 1. Visible spectrum of 7 generated *in situ* in DMSO saturated with CO from 5 by reduction with $Na_2S_2O_4$–dicyclohexyl 18-crown-6-ether followed by deblocking with dimsyl sodium ([5] = 3.67 M; cell length = 2 mm).

the Fe–S bond. Owing to this stability, several states involved in the P-450 catalytic cycle could be detected by electronic spectra and these are depicted in Scheme 2 with their characteristic Soret bands.

Treatment of **5** with dimsyl sodium in the absence of $Na_2S_2O_4$ produced a ferric complex (**10**) with the coordinated mercaptide ligand. Complex **10** may be considered to correspond to the resting state of the enzyme where it has a ferric low-spin hexa-coordinate structure and shows ESR signals at $g = 2.46$, 2.26, 1.91 [8]; **10** was found to be a ferric low-spin hexa-coordinate complex from its ESR spectrum ($g = 2.31$, 2.27, 1.96). DMSO probably coordinates to the iron on an opposite site to the mercaptide ligand.

Either by reduction of **5** and the subsequent elimination of the benzoyl group, or by the reverse order of the above procedures a ferrous mercaptide complex (**6**) was obtained. Similarly, **7** was formed either by deprotection of **9** or by carbonylation of **6**.



Scheme 2.

Protonation of **6**, **7** or **10** gave the corresponding mercaptan complexes **12**, **13** or **11** respectively, and deprotonation of these mercaptan complexes was easily accomplished by addition of dimsyl sodium to obtain the initial mercaptide complexes. These reversible processes were easily traced by the electronic spectra. It is believed that when Fe(III) and R–SH are present in the same system the former oxidizes the latter to disulfide [7]. In our system, however, we could detect Fe(III)–RSH systems (**11**, **12**, **13**); this implies that the steric congestion of the present system, due to the presence of a methyl group at the center of the strap, may prevent intramolecular coupling resulting in the formation of the disulfide. Such a cyclic diagram containing several steps corresponding to those in the P-450 catalytic process has not been reported so far. Coordination of the mercaptide/mercaptan to iron in the ferrous deoxy form of the enzymes or a proton shuttle between the mercaptide/mercaptan during catalysis is intriguing. Ligation of mercaptide in **6**, **7** or **10** showed a significantly different Soret band from that of the mercaptan complexes **12**, **13** or **11**, or the benzoyl-protecting mercaptan ones **8**, **9** or **5**. The diagram obtained in this study may serve to elucidate the states operating in the enzymatic reaction. Owing to their stability, the present model complexes are expected to be catalysts for hydroxylation of organic substrates, just as the real enzyme. Catalytic reactions using the present model complex are the subject of further study.

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