# Magnetic Circular Dichroism Studies of a Thiolate Complex of Zinc Protoporphyrin. A Non-iron Hyperporphyrin Model for Ferrous–CO Cytochrome P-450

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Nappa and Valentine [1] have reported that the UV–Vis absorption spectra of zinc porphyrin–thiolate complexes resemble that of the ferrous–CO complex of cytochrome P-450 [2]. Both are characterized by an unusually red-shifted Soret peak at about 450 nm and the appearance of a second, intense near-UV band. The observation of the second band in the near-UV region suggests that the thiolate-ligated zinc porphyrin complexes, like ferrous–CO P-450, exhibits a hyperporphyrin [3] spectrum. Molecular orbital calculations by Hanson *et al.* [4] for ferrous–CO P-450 have suggested that the extra band arises from an orbital mixing mechanism: a charge transfer absorption from the lone pair $p^{\dagger}$ orbital on the thiolate ligand to the porphyrin $\pi^*$ orbitals mixes with and splits the doubly degenerate $\pi$–$\pi^*$ Soret band to yield the characteristic 'split' Soret spectrum having two transitions region of equal integrated intensity in the Soret (300–500 nm) region. To investigate this further we have measured the magnetic circular dichroism (MCD)$^{\neq}$ spectra of zinc protoporphyrin IX dimethyl ester, Zn(PPIXDME)$^{\neq}$, and its ligand complexes with 1-propanethiolate, 1-methylimidazole and dimethyl sulfoxide. Protoporphyrin IX dimethyl ester is a derivative of the naturally occuring porphyrin found in P-450.

MCD spectroscopy [5, 6] has been shown repeatedly to be capable of providing more detailed information than UV–Vis absorption spectroscopy about the electronic structures of porphyrins and is therefore of greater diagnostic utility for comparing the properties of porphyrin complexes that display similar absorption spectra. This is, in part because MCD transitions can be of either positive or negative amplitude, thereby providing a more unique 'fingerprint' of a metalloporphyrin–ligand complex. In this work, our principal finding is that extensive similarities exist between the MCD spectra of the Zn(PPIXDME) thiolate adduct and that of ferrous–CO cytochrome P-450-CAM [7]. This clearly places the thiolate-ligated zinc porphyrin complexes in the ligand-type hyperporphyrin class.

## Experimental

Zn(PPIXDME) was used as purchased from Porphyrin Products. Reagent grade benzene was treated with sulfuric acid and distilled from sodium benzophenone ketyl. Reagent grade dimethyl sulfoxide and 1-methylimidazole were vacuum distilled. 1-Propanethiolate was prepared as previously described [8]. The dimethyl sulfoxide and 1-methylimidazole complexes were obtained by adding a concentrated benzene solution of Zn(PPIXDME) to the neat ligand. The thiolate complex was generated by the addition of Zn(PPIXDME) to a 0.5 M solution of 1-propanethiolate solubilized in benzene with dicyclohexyl-18-crown-6. Porphyrin concentrations did not exceed 40 $\mu$M. Anaerobic manipulations were carried out in a Vacuum Atmospheres glovebox under dinitrogen. MCD data were obtained at 24 °C as described previously [9] and are reported in units of molar magnetic absorptivity, $\Delta\epsilon/H$ (M cm T)$^{-1}$.

## Results and Discussion

The cytochrome P-450 isozymes are involved in drug and steroid metabolism, membrane detoxification and carcinogenesis [2, 10]. The name P-450 is derived from the occurrence of an unusually red-shifted Soret band at about 450 nm in the electronic absorption spectrum of the ferrous–CO complex. With the exception of chloroperoxidase [11], all other known CO-binding protoheme proteins have absorption maxima at ~420 nm in their ferrous–CO states. Ferrous–CO P-450 is also unusual in having an additional intense band in the near-UV region of its absorption spectrum [4]. Duplication of the hyperporphyrin or split Soret spectrum of ferrous–CO P-450 using thiolate–ligand model heme complexes has been of great importance in the characterization of the active site structure of the enzyme [2, 8, 11, 12].

The observation by Nappa and Valentine [1] of similarities between the absorption spectra of zinc porphyrin thiolate complexes and of ferrous–CO P-450 prompted us to examine the two species with MCD spectroscopy (Table I). For comparison, we have also investigated non-thiolate complexes of Zn(PPIXDME) with dimethyl sulfoxide and with

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≠Abbreviations: MCD, magnetic circular dichroism; Zn(PPIXDME), zinc protoporphyrin IX dimethyl ester.

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TABLE I. MCD Data for Ligand Complexes of Zinc Protoporphyrin IX Dimethyl Ester[a]

| Axial ligand | Soret | | | Beta | | | Alpha | | |
|---|---|---|---|---|---|---|---|---|---|
| | Peak | Cross-over | Trough | Peak | Cross-over | Trough | Peak | Cross-over | Trough |
| No ligand[b] | 407 (28.6) | 415 | 423 (−37.7) | 530 (13.7) | 538 | 546 (−14.4) | 574 (79.3) | 579 | 585 (−80.7) |
| Dimethyl sulfoxide[c] | 416 (49.2) | 423 | 429 (−61.5) | 538 (14.5) | 548 | 558 (−19.0) | 581 (67.1) | 586 | 591 (−77.8) |
| 1-Methylimidazole[d] | 421 (70.1) | 428 | 433 (−79.4) | 544 (14.0) | 553 | 563 (−21.6) | 586 (60.0) | 591 | 596 (−62.2) |
| 1-Propanethiolate[e] | 444 (113.0) | 453 | 461 (−87.6) | 565 (22.9) | 573 | 582 (−28.8) | 598 (9.5) | 606 | 614 (−23.5) |

[a]The wavelengths are given in nanometers. The MCD intensities are given in parentheses in units of $\Delta\epsilon/H$, molar magnetic absorptivity $(M\ cm\ T)^{-1}$. [b]Ligand free Zn(PPIXDME) in benzene. [c]Spectrum recorded in neat dimethyl sulfoxide. [d]Spectrum recorded in neat 1-methylimidazole. [e]Solubilized as the potassium salt using dicyclohexyl-18-crown-6 in benzene.

1-methylimidazole. We have previously found that zinc porphyrins form five-coordinate fully saturated complexes when added to these latter two liquid ligands [13, 14].

The Soret region MCD spectrum of the Zn(PPIXDME) thiolate complex is distinct from that of unligated Zn(PPIXDME) (Fig. 1A). The crossover point for the thiolate complex shifts 38 nm to 453 nm and the peak-trough intensity triples. The crossover points of the non-thiolate adducts also red-shift and the signal intensities increase but to a much smaller extent. In addition, the spectrum of the thiolate complex has a broad, moderately intense [−28 $(M\ cm\ T)^{-1}$], trough at 380 nm that is not seen in the other spectra.

In the visible (500–700 nm) region (Fig. 1B), the MCD spectrum of the thiolate complex again stands out relative to the other spectra. The $\beta$ crossover at 538 nm for Zn(PPIXDME) red-shifts by 35 nm for the thiolate complex while those of the non-thiolate adducts shift much less (~12 nm). In addition, the $\beta$ band peak-trough intensity almost doubles upon thiolate binding but hardly changes in the other cases. Somewhat different behavior is seen for the $\alpha$ band. As above, the $\alpha$ band crossover point at 579 nm for Zn(PPIXDME) red-shifts more substantially (27 nm) upon thiolate ligation than with the other ligands (~9 nm). However, the $\alpha$ band peak-trough intensity of the thiolate adduct decreases to less than twenty percent of the value for the unligated species while relatively minor decreases in signal intensity are seen for the other complexes. The $\alpha$ band intensity also decreases substantially in the absorption spectrum of the thiolate complex [1]. The magnitude of the changes seen in the MCD spectra upon thiolate binding to Zn(PPIXDME) are substantially greater than those seen upon addition of any other neutral or anionic ligand [14].

The Soret region MCD spectrum of the Zn(PPIXDME) propanethiolate complex is compared

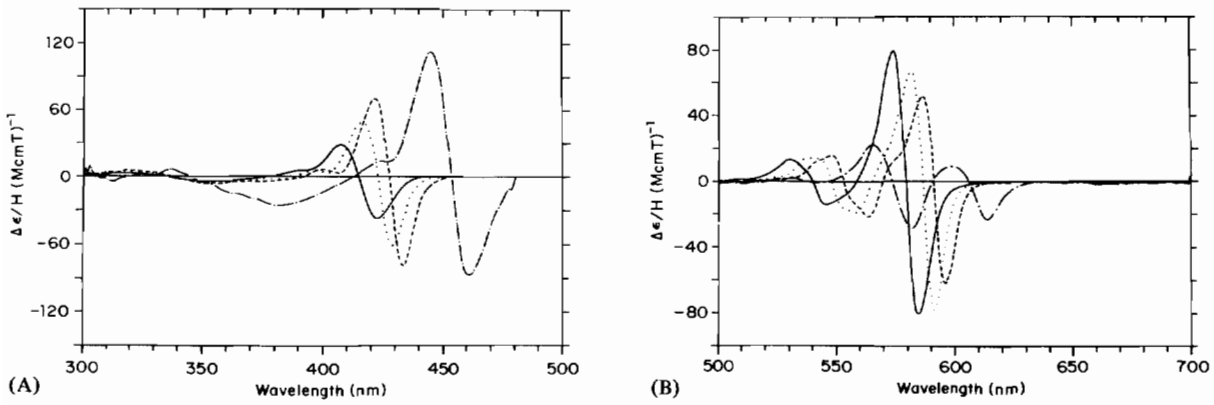


Fig. 1. (A) MCD spectra from 300–500 nm of unligated Zn(PPIXDME) (solid line) and its complexes with dimethyl sulfoxide (dotted line), 1-methylimidazole (dashed line) and 1-propanethiolate (dot-dashed line). (B) MCD spectra of the same species from 500–700 nm.

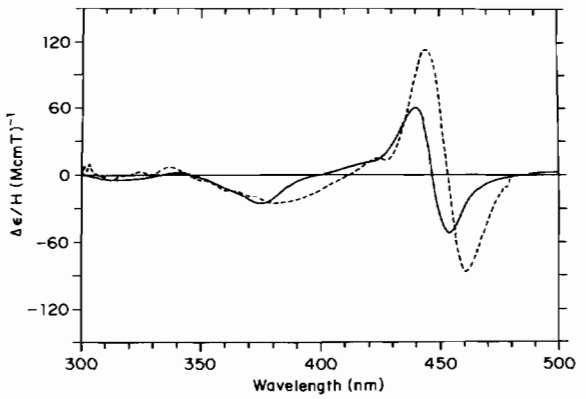


Fig. 2. MCD spectra from 300–500 nm of ferrous–CO cytochrome P-450-CAM (solid line, previously reported [7]) and of the Zn(PPIXDME) 1-propanethiolate complex (dashed line).

to that of ferrous-CO P-450-CAM [7] in Fig. 2. Both species have considerably red-shifted Soret crossover points and display the extra trough at about 380 nm having comparable amplitudes. For ferrous-CO P-450, the near-UV MCD band corresponds to the near-UV portion of the split Soret observed in the absorption spectrum [4, 7]. The close similarity between the MCD spectra of the zinc porphyrin thiolate complex and ferrous-CO P-450-CAM provides substantial support for the assignment of the zinc complex as a ligand-type hyperporphyrin [1]. In addition, the significant decrease in the α band peak-trough intensity seen for the zinc thiolate adduct (Fig. 1B) is reminiscent of the complete lack of an α band in the absorption or MCD spectra of ferrous–CO P-450 [7]. Ferrous–CO chloroperoxidase, which also has an axial thiolate ligand *trans* to CO, has weak but observable α band intensities [11]. The unique spectral features of the thiolate adduct of Zn(PPIXDME), like those of ferrous–CO P-450 [7], result from thiolate ligation. As stated above, Hanson *et al.* [4] have invoked an orbital mixing mechanism involving the sulfur $p^{\pi}$ orbital to explain the unique spectral properties of ferrous–CO P-450. The orbital mixing mechanism also applies to the the Zn(PPIXDME) thiolate adduct.

In summary, the close similarity between the MCD spectra of the thiolate complex of Zn(PPIXDME) and ferrous–CO P-450 provides strong support for the assignment made by Nappa and Valentine [1] that the zinc complex is a hyperporphyrin and therefore can serve as a model for ferrous–CO P-450.

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