of the inhibition and oxygen bubbling studies support the hypothesis that hydroxylation by the NADH/PMS/P-450-CAM system occurs by an oxygen-dependent enzymatic pathway. The role of the PMS in the system appears to be as a mediator of electrons from NADH to P-450. This system provides the first protocol for achieving molecular oxygen dependent multiple turnovers of P-450 in the absence of the fully reconstituted three protein system.

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The Active Sites of Chloroperoxidase and Cytochrome P-450-CAM: Comparative Spectroscopic and Ligand Binding Properties

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Spectral similarities between chloroperoxidase (CPO) and cytochrome P-450 (P-450) have previously been used to suggest endogenous thiolate axial ligation to the CPO heme iron [1–6] as has been established for P-450. Both enzymes exhibit unique hyperporphyrin ('split Soret') spectra in their ferrous CO forms [1]. However, no free sulfhydryl groups available for ligation to the heme iron have been found in chemical studies of CPO [7]. The MCD [4] and EPR [6] spectroscopic properties of analogous CPO and P-450 derivatives are generally similar, although some differences do exist. To obtain additional information about the active site structure of CPO, we have carried out extensive optical

Abbreviations: CPO, chloroperoxidase; P-450, cytochrome P-450; CAM, camphor; MCD, magnetic circular dichroism; EPR, electron paramagnetic resonance.

†Recipient of a Henry and Camille Dreyfuss Tescher-Scholar Award.

TABLE I. Soret Absorption Maxima (nm) and Spin States of the Ligand Complexes of Ferric CPO and P-450-CAM.[a]

Ligand	CPO[b]		P-450-CAM[c]	
	$\lambda(\epsilon_{mM})$	Spin[d] State	$\lambda(\epsilon_{mM})$	Spin[d] State
SH^-	449(e)	ls	467(43)[f]	ls
CH_3COS^-	446(62)[g]	ls	459[e]	ls
CN^-	439(92)	ls	439(78)	ls
Pyridine	439(82)	ls	421(111)	ls
NO	437(114)	ls	430.5(105)	ls
$CH_3(CH_2)_3NC$	435(105)	ls	429.5(98)	ls
N_3^-	432(110)	ls	427(81)	ls
$SeCN^-$	432(80)	ls	425(90)	ls
SCN^-	429(112)	ls	425(98)	ls
Imidazole	429(100)	ls	425(98)	ls
NO_2^-	427.5(101)	ls	[e]	
OCN^-	~427[e]	ls	418.5(98)	ls
$HCOO^-$	425(121)	ls	419(106)	ls
$HOCH_2CH_2SH$	418(69)	ms	464(58)[f]	ls
$CH_3CH_2CH_2SH$	416(74)	ms	417(~60) 464(~33)[f]	ls
CH_3SCH_3	416(93)	ms	424(91)	ls
Thioxane	416(88)	ms	418(97)	ls
CH_3SSCH_3	416(93)	ms	418(98)	ls
CH_3COO^-	413(97)	ms	420(98)	ls
F^-	409(98)[g]	hs	no binding	

[a]0.1 *M* K^+Phosphate, 4 °C. [b]pH 6.0. [c]pH 7.0, (Refs. 9–11). [d]ls, low spin; ms, high-low mixed spin; hs, high spin. [e]Unstable. [f]Red Soret of hyperporphyrin spectrum (Ref. 11). [g]pH 3.0.

TABLE II. Soret Absorption Maxima (nm) of the Ligand Complexes of Ferrous CPO and P-450-CAM.

Ligand	CPO[a] $\lambda(\epsilon_{mM})$	P-450-CAM[b] $\lambda(\epsilon_{mM})$
Me_2PhP[c]	459(d)	460(98)
CN^-	454(~140)	Not observed
$CH_3(CH_2)_3NC$	452(141)	452(106)
CO	444(167)	446(120)
NO	440(114)	438(83)

[a]pH 6.0. [b]pH 7.0. [c]Dimethylphenylphosphine. [d]Not determined.

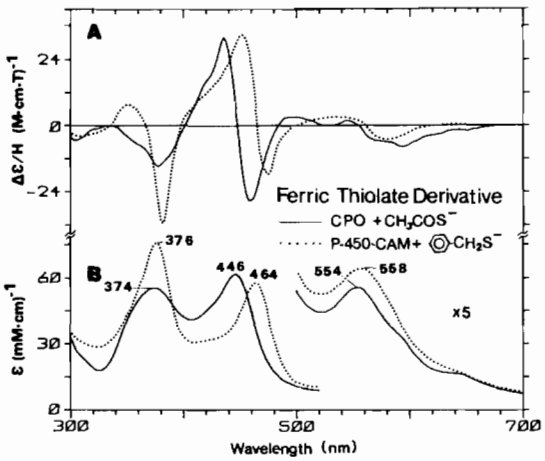

Fig. 1. MCD (A) and Optical Absorption (B) Spectra of Thiolate Complexes of Ferric CPO (——) and P-450-CAM (·····). CPO (103.5 μM) and P-450-CAM (20.2 μM, plus 55 μM CAM) were mixed with 80 mM thioacetic acid at pH 3.0 and 4.0 mM benzylmercaptan at pH 7.0, respectively, in 0.1 M K^+ phosphate buffer at 4 °C.

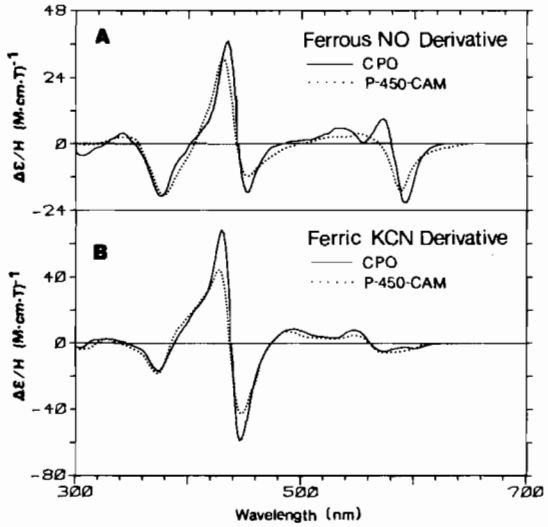

Fig. 2. MCD (A) and Optical Absorption (B) Spectra of Isocyanide Complexes of Ferrous CPO (——) and P-450-CAM (·····). CPO (5.82 μM) and P-450-CAM (6.13 μM, plus 100 μM CAM) were mixed with 10.3 mM n-butyl isocyanide at pH 6.0 and 1.06 mM benzyl isocyanide at pH 7.0, respectively, in 0.1 M K^+ phosphate buffer at 4 °C.

and MCD spectroscopic studies of both ferric and ferrous CPO in the presence of various exogenous ligands. The results obtained with CPO have been compared with those obtained with P-450 [9 - 11, this work] in order to obtain a better understanding of the electronic structure of the heme iron in both proteins.

CPO was isolated and purified ($A_{399}/A_{280} > 1.4$) from *C. fumago* as reported previously [8]. The purification of P-450-CAM and the procedure and instrumentation used for the ligand binding experiments and spectroscopic measurements have been previously reported [9–11].

A wide variety of exogenous anionic and neutral ligands, including several not previously known to bind to ferric or ferrous CPO, form complexes with the enzyme having K_d values ranging from 10^{-5} to 10^{-1} M (pH 3–6, 4 °C). The Soret region optical absorption maxima and absorption coefficients of the ferric and ferrous CPO and P-450 derivatives are summarized in Tables I and II, respectively. For ferric CPO, the absorption maxima vary from 409–449 nm. A similar range is found for the analogous ferric P-450-CAM adducts (Table I). All ferrous CPO and P-450 ligand complexes have Soret maxima in a narrow range from 438–460 nm (Table II).

Addition of a strongly acidic (pKa ~ 3.5) thiol, thioacetic acid, to ferric CPO yields a complex that has a hyperporphyrin absorption spectrum that is very similar to that of the ferric P-450-CAM·benzylmercaptan adduct (Fig. 1), a well characterized bisthiolate heme complex [11]. Furthermore, MCD spectrum of the ferric CPO·thioacetic acid adduct also exhibits a band pattern that is typical of hyper-

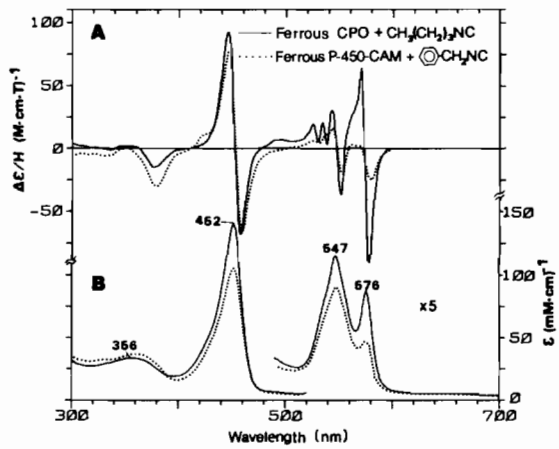

Fig. 3. MCD Spectra of Ferrous NO (A) and Ferric KCN (B) Complexes of CPO (——) and P-450-CAM (·····). In (A), ferrous CPO (99 μM) at pH 6.0 and P-450-CAM (14.5 μM, plus 13 μM CAM) at pH 7.0 were mixed with 8 mM and 5 mM $NaNO_2$, respectively, in the presence of small amounts of $Na_2S_2O_4$ under N_2. In (B), ferric CPO (7.25 μM) at pH 6.0 and P-450-CAM (13.4 μM, minus CAM) at pH 7.0 were mixed with 3.3 mM and 200 mM KCN, respectively. All measurements were done in 0.1 M K^+ phosphate buffer at 4 °C.

porphyrin complexes [11]. Together, these data suggest that the CPO–thiolate complex has a bisthiolate heme coordination structure; clearly, such a deduction requires an endogenous thiolate ligand in the native enzyme. The UV-visible absorption and

MCD spectra of ferrous CPO and P-450-CAM adducts with isocyanides are presented in Fig. 2. While very similar overall, some spectral differences are observed, especially in the more intense visible region MCD features of the CPO adduct. The MCD spectra of the ferrous NO and ferric KCN derivatives of CPO and P-450-CAM are displayed in Fig. 3. As has been observed for native ferric, ferrous and ferrous-CO CPO and P-450-CAM [4], the MCD spectral features of the NO or KCN adducts of both enzymes are similar except for some minor intensity and line shape differences.

In contrast to the above spectral similarities observed between analogous CPO and P-450-CAM derivatives, we have observed significant differences between the two enzymes in the spin state distribution of certain ferric ligand adducts. Although most anionic ligands listed in Table I and some neutral ligands (pyridine, isocyanide, imidazole and NO) form exclusively low spin complexes with ferric CPO, the adducts with other ligands such as neutral sulfur donors are instead a mixture of high and low spin. In addition, formate (low spin) and acetate (mixed spin) behave differently even though both are carboxylate anions at pH 6. Finally, among the heme ligands studied, only fluoride forms a high spin complex with ferric CPO. These diverse spin state properties of the ferric CPO·ligand complexes stand in contrast to the uniformly low spin nature of all ferric P-450-CAM ligand complexes [10].

In conclusion, the hyperporphyrin spectra that are observed for the complex obtained through ligation of a strongly acidic thiol to ferric CPO provide compelling evidence for the presence of an endogenous thiolate ligand to the heme iron of the *ferric* enzyme. The additional spectral similarities observed between analogous ligand complexes of CPO and P-450-CAM add further support to this conclusion for both the ferric and ferrous CPO cases. However, since ligand complexes of P-450-CAM and thiolate-ligated heme models are exclusively low spin, the spin state distribution distinctions seen between some weak field ligand complexes of ferric CPO and P-450 suggests that the heme-iron: thiolate-sulfur interaction in chloroperoxidase is somewhat different from that in P-450 and model complexes. Additional work is in progress to try to identify the cause of these differences.

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Q20

Electron Transfer at Crystallographically Known Long Distances (25 Å) in [Zn^{II}, Fe^{III}] Hybrid Hemoglobin

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In [Zn, Fe] hybrid hemoglobins, a zinc protoporphyrin (ZnP) and heme are held rigidly at known orientation, with Zn—Fe distance of 25 Å in the functional α_1-β_2 electron transfer entity. Room temperature electron transfer from flash photolytically generated ^{3}ZnP to the partner aquoferriheme occurs with rate, $k_t = 60 \pm 25\ s^{-1}$. Oxidation of ferroheme by the partner $(ZnP)^+$ π-cation radical occurs with rate $k_e = 3.3(\pm 0.7) \times 10^3\ s^{-1}$.

Q21

Gold-induced Spin-state Changes in Haem Proteins

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During the course of our work on anti-arthritic gold drugs, we discovered that Et_3PAuCl converted