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Electronic Effects on the Spectra and Carbon Monoxide Affinities of Heme and (Thiolato)heme Complexes. Cis and Trans Effects

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The absorption spectra of *n*-butanethiolate complexes of different iron porphyrins and their CO complexes were measured as models for the reported spectra of the corresponding heme-substituted cytochrome P_{450}. The CO affinities of solvent and *n*-butanethiolate-coordinated complexes of different iron porphyrins in dimethylacetamide were also measured for comparison to reported cis effects on the CO affinities of heme-substituted cytochrome P_{450} as well as (imidazole)heme complexes. CO affinities of (*n*-butanethiolato)heme complexes follow the order diacetyldeutero > spirographis > 2-acetyl-4-deutero > proto > meso, corresponding to the order of *increasing* porphyrin basicity. CO affinities of solvent-coordinated iron porphyrins follow the order proto > diacetyldeutero > dicyano, corresponding to the order of *decreasing* porphyrin basicity. The slopes of the Brønsted type plots (pK^{CO} vs. porphyrin pK_3) associated with these cis effects were shown to exhibit an apparent linear dependence on the trans ligand basicity. The absorption spectra and CO affinities of protohemes and diacetyldeuterohemes coordinated to thiolates of decreasing basicity were also measured to assess the possible trans effects. CO affinities of different (thiolato)diacetyldeuteroheme complexes followed the order 4-fluorothiophenolate > methyl thioglycolate > phenylmethanethiolate > *n*-butanethiolate, corresponding to the order of *increasing* thiolate basicity. The relationship between the spectroscopic and CO binding properties and the effective basicity of the thiolate ligand in the CO cytochrome P_{450} complex is discussed.

Introduction

The cytochromes P_{450} constitute a class of monooxygenase heme proteins that catalyze the hydroxylation of a wide variety of molecules including xenobiotics and steroids in eukaryotes and camphor, the growth substrate for *Pseudomonas putida*, in prokaryotes.[1,2] Numerous chemical and spectroscopic studies have identified heme protein intermediates in the reaction cycle and have attempted to characterize the heme iron coordination in order to understand the biochemical mechanism involved.[3,4] Studies of carbon monoxide binding to thiolate heme models[5-7] have indicated cysteine ligation to the heme iron in the protein. Resonance Raman studies[8] of ferric cytochrome P_{450} and EXAFS[9] studies of ferric and ferrous cytochromes P_{450} have confirmed these and other earlier spectroscopic[10-12] assignments.

Several investigators have noted significant differences between the carbon monoxide binding properties of cytochromes P_{450} from different sources (bacterial, liver microsomal, adrenal cortex microsomal)[13-18], from different isozymes (phenobarbitol-induced P_{450} LM-2 vs. benzoflavone-induced P_{450} LM-4),[16] and between cytochromes P_{450} that are substrate-free and those that have bound substrate.[13,18] Greater differences have been observed between the CO binding constants of cytochromes P_{450} and model heme complexes.[19,20] Several factors have been considered to contribute to the differences in ligand-binding properties of hemoproteins, including steric interactions between CO and the protein,[21-23] axial ligand strain,[23,24] solvent effects,[25,26] ligand stabilization through distal side effects,[27-32] and electronic effects associated with the porphyrin (cis effect)[33-43] or the axial ligand (trans effect).[35-37,44-49]

While extensive model heme studies have considered the importance of these parameters in studies of oxygen-carrying heme proteins, comparatively few studies have described the ligand-binding properties of model heme complexes for the cytochromes P_{450}. In the present study we have examined cis and trans electronic effects associated with varying basicities of 2,4-disubstituted porphyrins and thiolate ligands, respectively. The results provide a basis for assessing various factors contributing to the ligand-binding properties of cytochromes P_{450}.

Experimental Section

Materials. Gold Label grade *N,N*-dimethylacetamide (DMA), 1-butanethiol, and thiophenol were purchased from Aldrich. Dibenzo-18-crown-6 ether, benzyl mercaptan, methyl thioglycolate and 4-fluorothiophenol were obtained from Parish Chemical. Meso-, spirographis-(2-formyl-4-vinyl-), and 2,4-diacetyldeuteroheme free acids were obtained from Porphyrin Products as their chloride salts. Protohemin chloride was obtained from Sigma Chemical. 2,4-Dicyanodeuteroporphyrin was obtained from Midcentury-Man-Win Chemicals and 2-acetyl-4-deutero-porphyrin was obtained courtesy of Dr. Kevin Smith (U. of California—Davis), and the iron was inserted into the porphyrins ac-

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Table I. Soret Absorption Maxima[a] of Ferrous Heme Thiolates, Ferrous Cytochromes P_{450}, and Their CO Complexes

syst	meso	proto	2-acetyl-4-deutero	2-formyl-4-vinyl	diacetyl-deutero	dicyano-deutero	ref
heme thiolate[b]	397	411	413	414	432	420	this work
heme thiolate + CO[b]	372	383	381	380	391	391	this work
	450	464	465	474	480	473	this work
P_{450}-cam	402	408			431		56
P_{450}-cam + CO	358	364			368		56
	434	446			463		56

[a] nm. [b] In dimethylacetamide.

cording to the method of Chang et al.[52]

Carbon monoxide was obtained as Matheson Purity grade, and nitrogen as Linde oxygen-free grade. Matheson Model 8 and Model 19 (high purity) regulators used with CO and N_2 cylinders were connected via stainless-steel tubing and fittings to hypodermic needles (Hamilton point style #3).

Reagents were mixed in cuvettes made from 1 cm × 1 cm square glass tubing fitted to Ace #7663-06 glass sockets stoppered with two Alltech #6512 blue septa. All transfers were made via cannula or gas-tight syringes.

Reduced Heme. A 10-μL sample of a concentrated sodium dithionite solution in 0.05 M phosphate buffer at pH 7.0 was added to a 2.5-mL preevacuated solution of heme in DMA in the glass cuvette described above. The resulting solution was subjected to three freeze–pump–thaw cycles after which nitrogen was added to atmospheric pressure. Similar spectrophotometric titration results were obtained with heme that had been reduced with sodium dithionite–crown ether complex[53] in DMA, indicating that the trace amount of aqueous pH 7 buffer had no effect on the equilibria.

Potassium Thiolate–Crown Ether Complex. Equimolar additions of KOH, thiol, and crown ether were mixed according to the method of Chang and Dolphin.[19] Reagents were mixed as follows: deaerated DMA was transferred via cannula to a nitrogen-filled cuvette containing KOH pellets dissolved in a minimum amount of water (<5% final volume). Deaerated thiol was injected, and the solution of potassium thiolate was transferred via cannula to a preevacuated cuvette containing an equivalent amount of crown ether.

The mixture of crown ether and potassium thiolate was shaken until completely dissolved. The solution was then transferred via cannula to a deaerated cuvette containing a solution of heme in DMA. The resulting solution was subsequently subjected to five freeze–pump–thaw cycles with final addition of nitrogen. Spectrophotometric titrations were carried out with concentrations of 100 and 300 mM for butanethiol, benzyl mercaptan, and methyl thioglycolate and at 300 and 450 mM for thiophenol and 4-fluorothiophenol.

Carbon Monoxide Affinity Measurements. CO was added by injection of gas or gas-saturated DMA solutions to reduced hemes and heme thiolates. Equilibration was achieved by shaking for 30 min. Absorbance changes at or near the longer wavelength Soret maxima for CO complexes were monitored with a Cary 17 spectrophotometer. The concentration of CO was determined from the volume of the gas phase and the solubility of CO in DMA.[6,54] The equilibrium constant, K^{CO}, for CO binding was determined from the inverse slope of a plot of A vs. $(A - A_0)/[CO]$ according to the relation

$$A = -(A - A_0)/K[CO] + A_{100} \quad (1)$$

where A is the absorbance at a given CO concentration and A_0 and A_{100} are the limiting absorbances at zero and 100% complex formation. All spectroscopic titrations were carried out at 23 °C and exhibited no more than 10% deviation.

Infrared Spectra Measurements. Concentrated solutions of CO-bound and CO-free complexes of DMA and thiolate-coordinated heme were prepared as previously described. Each solution was transferred in a glovebag to a CaF_2 cell with a path length of 0.015 mm via gas-tight syringes. Difference infrared spectral data corresponding to the CO-

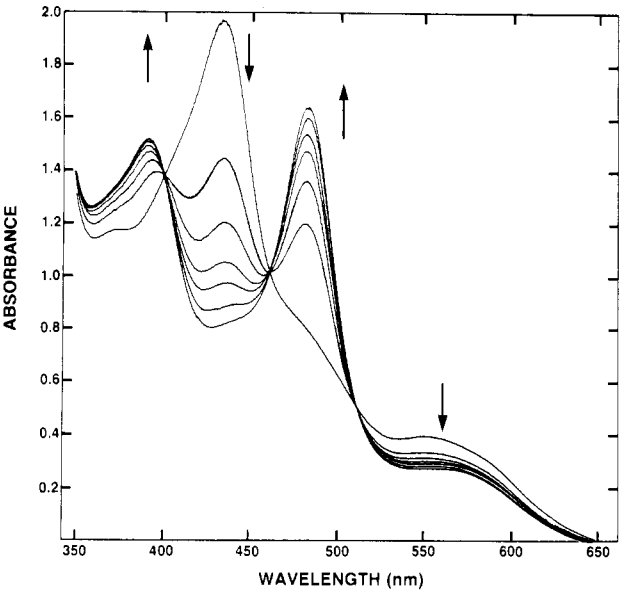

Figure 1. Spectrophotometric titration of diacetyldeuteroheme thiolate in dimethylacetamide with carbon monoxide.

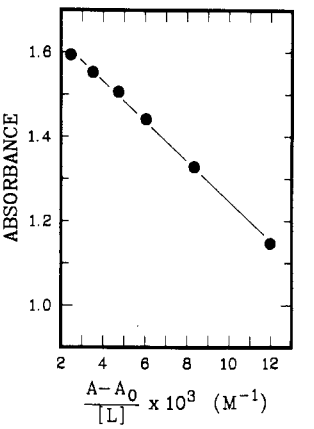

Figure 2. Plot of A vs. $(A - A_0)/[CO]$ according to eq 1 for a spectrophotometric titration of diacetyldeuteroheme thiolate in dimethylacetamide with carbon monoxide.

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Table II. Equilibrium Constants for Binding of CO to Heme Thiolate Complexes: Cis Effects

heme syst (mM)	solvent	pK_3[a] of porphyrin	K,[b] M^{-1} (°C)	ref
		Heme Thioates		
mesoheme thiolate (100)	DMA	5.8	5.6×10^3	this work
protoheme thiolate (100)	DMA	4.8	6.6×10^3	this work
protoheme thiolate (100, 300)	DMA	4.8	1.0×10^4	6
protoheme thiolate (2)	toluene	4.8	2.0×10^4	6
chelated protoheme thiolate	$C_{16}H_{33}NMe_3Br$–H_2O	4.8	6×10^4	20
2-acetyl-4-deuteroheme thiolate	DMA	4.1[c]	1.0×10^4	this work
2-formyl-4-vinylheme thiolate	DMA	3.7	1.4×10^4	this work
diacetyldeuteroheme thiolate	DMA	3.3	2.1×10^4	this work
dicyanodeuteroheme thiolate	DMA	2.6[c]	1.7×10^4	this work
		Cytochromes P_{450}		
bacterial P_{450}, camphor free		4.8	2.2×10^6 (4)	13
bacterial P_{450}, with camphor		4.8	2.6×10^5 (4)	13
meso P_{450} bacterial[d]		5.8	1.7×10^5	41
deutero P_{450} bacterial[d]		5.5	1.8×10^5	41
proto P_{450} bacterial[d]		4.8	1.3×10^5	41
diacetyldeutero P_{450} bacterial[d]		3.3	5.3×10^4	41
dibromodeutero P_{450} bacterial[d]		3.0	4.6×10^4	41

[a] Reference 57. [b] 23 °C unless otherwise stated. [c] Reference 58; pK_3 estimated from the first reduction potential, $E_{1/2}^r$, where $pK_3 = -5.9\ E_{1/2}^r - 5.2$. [d] Reconstituted protein.

bound and CO-free solutions were collected between 4000 and 400 cm^{-1} on an IBM IR/32 spectrophotometer.

Results

To examine cis effects, the binding of carbon monoxide to the *n*-butanethiolate complexes of 2,4-disubstituted deuterohemes was observed. Figure 1 describes the Soret and visible absorption changes associated with the binding of carbon monoxide to the *n*-butanethiolate (BuSK) complex of diacetyldeuteroheme. Spectroscopic titrations for diacetyldeutero- as well as meso-, proto-, 2-acetyl-4-deutero-, 2-formyl-4-vinyl-, and dicyanodeuteroheme thiolates exhibited clean isosbestic points. Carbon monoxide binding to the heme thiolate complex is associated with the splitting of the single Soret peak observed in the unligated ferrous state into two peaks referred to as a "hyper"[55] type of spectrum with the longer wavelength maxima being red shifted 48 nm from that of the CO-free heme thiolate complex. CO complex formation of the other heme thiolates resulted in corresponding red shifts of 52–60 nm from the CO-free heme thiolate Soret maxima. These values may be compared to reported shifts of 32–38 nm for CO binding to cytochromes P_{450}–cam reconstituted with different hemes.[56] The absorption maxima of (*n*-butanethiolato)heme complexes together with those of reconstituted cytochromes P_{450} are recorded in Table I. The absorption maxima of the CO complexes of the models are consistently 16–18 nm longer than the corresponding heme-substituted cytochrome P_{450}. In contrast, the heme thiolates and the corresponding heme-substituted cytochrome P_{450} exhibit similar absorption maxima with no CO present.

Figure 2 shows a plot of absorbance vs. $(A - A_0)/[L]$ for carbon monoxide binding to diacetyldeuteroheme *n*-butanethiolate. The solid line corresponds to a linear least-squares fit of the data points. The inverse slope of the line corresponds to the binding constant for carbon monoxide, 2.1×10^4 M^{-1} in the case of diacetyldeuteroheme *n*-butanethiolate. Equilibrium constants for CO binding to each of the 2,4-disubstituted heme *n*-butanethiolate complexes studied are shown in Table II.

Titrations for all of the 2,4-disubstituted heme *n*-butanethiolate complexes were carried out with BuSK concentrations of 100 mM. Equilibrium constants were also determined at BuSK concentrations of 300 mM for meso-, proto-, and diacetyldeuteroheme thiolates and found to be equivalent to those values determined at 100 mM. This behavior agrees with earlier findings by Chang and Dolphin,[19] which indicated that CO binding to protoheme thiolate was independent of thiolate concentration at concentrations greater than or equal to 1 mM.

Table III. Soret Absorption Maxima[a] of Hemes and Their CO Complexes in DMA

syst	meso	proto	diacetyl-deutero	dicyano-deutero
heme	419	428	441	435
heme + CO	404	414	429	421

[a] nm.

Table IV. Equilibrium Constants for Binding of CO to Heme

heme syst	solvent	pK_3 of porphyrin	K,[b] M^{-1}	ref
deuteroheme	DMF	5.5	4.3×10^6	45
protoheme	DMA	4.8	8.6×10^5	this work
diacetyldeuteroheme	DMA	3.3	4.6×10^5	this work
dicyanodeuteroheme	DMA	2.6[a]	3.2×10^5	this work

[a] Reference 57. [b] 23 °C.

Table II also lists pK_3 values of the corresponding metal-free porphyrins as determined by previous investigators.[57] The pK_3 value for dicyanodeuteroporphyrin was estimated from its redox potential.[58] In general, the 2,4-disubstituted heme thiolate models that have greater porphyrin basicity (higher pK_3) exhibit a somewhat lower CO binding affinity. In contrast, the opposite trend was observed for CO binding to cytochromes P_{450} reconstituted with 2,4-disubstituted hemes,[56] as shown in Table II.

Figure 3 shows the spectroscopic titration of reduced protoheme in dimethylacetamide with carbon monoxide. Clean isosbestic points were also observed for all titrations of heme in DMA. Previous investigators have observed that substituted formamides weakly coordinate to deuteroheme[45,59] and protoheme,[50,51] binding one molecule resulting in five-coordinate complexes. The visible absorption spectra of proto-, diacetyldeutero-, and dicyanodeuterohemes in DMA each exhibit a single broad visible band with a shoulder on the red side similar to those of deutero-[59] and protoheme[51] in DMF, indicating that the former hemes also form five-coordinate heme complexes. Carbon monoxide binding to

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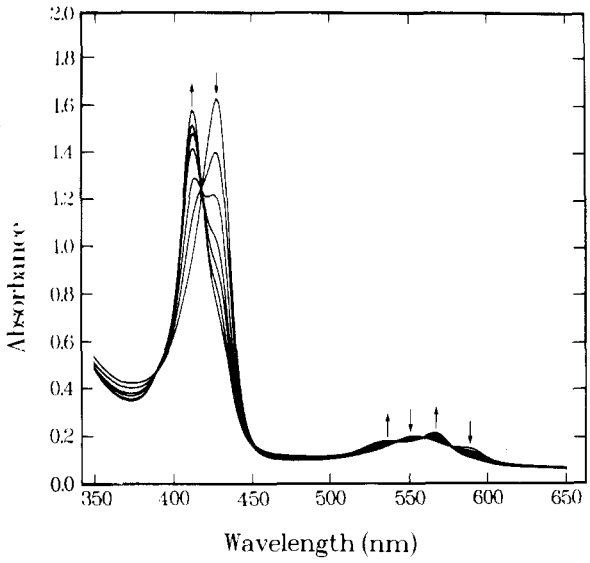

Figure 3. Spectrophotometric titration of protoheme in dimethylacetamide with carbon monoxide.

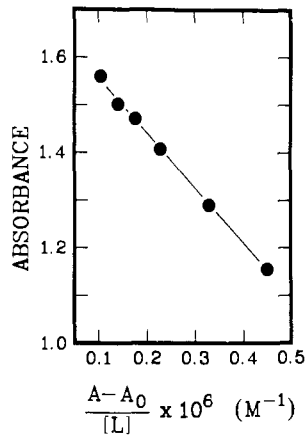

Figure 4. Plot of A vs. $(A - A_0)/[CO]$ according to eq 1 for a spectrophotometric titration of protoheme in dimethylacetamide with carbon monoxide.

the reduced heme results in a 14-nm blue shift of the single Soret maximum as well as changes in the visible region. The blue shift that occurred upon CO binding ranged from 12 nm for diacetyldeuteroheme to 15 nm for mesoheme. The absorption maxima of reduced hemes coordinated to dimethylacetamide and their corresponding CO complexes are summarized in Table III.

Figure 4 shows the corresponding plot of absorbance vs. $(A - A_0)/[L]$ for protoheme in dimethylacetamide. Table IV summarizes the equilibrium constants for CO binding to reduced hemes as well as the pK_3 values of the corresponding porphyrins.

Figure 5 compares plots of pK^{CO} vs. pK_3 for model heme *n*-butanethiolates, imidazole-chelated hemes,[42] and heme coordinated to dimethylacetamide. Thiolate CO studies show that cis effects that remove electron density (lower pK_3) result in a small but significant increase in carbon monoxide binding affinity (lower pK^{CO}). Dimethylacetamide-coordinated heme complexes show that cis effects that remove electron density result in a small decrease in CO binding, in contrast to that observed for thiolate CO studies.

Table V expresses the cis effects of the above three models as well as that for the reconstituted cytochromes P_{450}–cam[41] in terms of their Brønsted constants for carbon monoxide binding. Brønsted constants were obtained from the slope of the least-squares line resulting from plots of pK^{CO} vs. pK_3 for each heme system. Heme thiolate models exhibit a more positive slope for CO binding than the reconstituted cytochromes P_{450} cam, which actually exhibit a Brønsted slope more similar to that of the heme coordinated to dimethylacetamide.

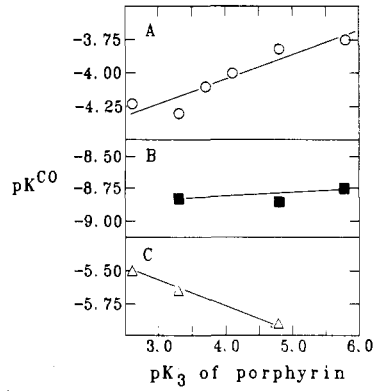

Figure 5. Plot of pK^{CO} vs. pK_3 of the corresponding porphyrins. A: Heme thiolate in DMA (○) (Table II). B: Imidazole-chelated heme in phosphate buffer pH 7.3 containing 2% myristoyltrimethylammonium bromide (MTAB) (■).[42] C: Heme in DMA (△) (Table IV).

Table V. Ratio $\Delta pK^{CO}/\Delta pK_3$ for CO Binding to Heme Lig nd Complexes

heme ligand complex	$\Delta pK^{CO}/\Delta pK_3$	ref	ligand pK_a	ref
ferrous heme thiolate	+0.19	this work	10.66	60
ferrous heme imidazole	+0.03[a]	42	6.95	61[a]
ferrous heme dimethylacetamide	−0.19	this work	−0.19	61
ferrous cytochrome P_{450}	−0.22[a]	41		

[a] Determined from data reported in reference cited.

Table VI. Soret Absorption Maxima[a] of Heme Thiolates and Their CO[b] Complexes

syst	*n*-butane-thiolate	phenyl-methane-thiolate	methyl thio-glycolate	4-fluoro-thio-phenolate
protoheme	411	410		
protoheme + CO	465	464		
diacetyldeuteroheme	432	432	429	428
diacetyldeuteroheme + CO	480	478	470	475

[a] nm. [b] Red shifted Soret maxima only.

To examine trans effects, the binding of carbon monoxide to proto- and diacetyldeuteroheme complexes of alkane- and arenethiolates of differing basicities was observed. The absorption maxima of protoheme thiolates prepared from the potassium salts of butane- and phenylmethanethiols and their CO complexes are reported in Table VI. We were unable to observe satisfactory CO titrations with protoheme complexed to thiolates of pK_a's lower than that of benzyl mercaptan due to weak binding of the less basic thiolates. Previous investigators have noted that protoheme does not bind thiophenolate.[6] Absorption maxima indicative of DMA-coordinated heme carbonyl complexes (λ_{max} 414 nm) resulted when carbon monoxide was added to protoheme solutions containing methyl thioglycolate and 4-fluorothiophenolate. Complexes of diacetyldeuteroheme bound to methyl thioglycolate and 4-fluorothiophenolate (pK_a's = 7.8 and 6.4, respectively) exhibited typical hyperspectra characteristic of thiolate-coordinated heme carbonyl complexes. The absorption maxima of the alkyl- and aryldiacetyldeuteroheme complexes are summarized in Table VI. The longer wavelength Soret absorption band of the (alkanethiolato)heme CO complexes exhibited as much as a 10-nm difference as the basicity of the thiolates was varied.

Equilibrium constants obtained for carbon monoxide binding to proto- and diacetyldeuterohemes coordinated to thiolates of varying basicity are summarized in Table VII, which also lists the pK_a values of the corresponding thiols as determined by previous investigators.[60] The pK_a for 4-fluorothiophenol was

(60) *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC: Cleveland, 1976; Vol. I, p 346.

Table VII. Equilibrium Constants for Binding of CO to Heme Thiolate Complexes: Trans Effects

heme syst[b]	corresponding thiol	K,[a] M^{-1}	thiol pK_a
protoheme thiolate	*n*-butanethiol	6.6×10^3	10.66[c]
protoheme thiolate	benzyl mercaptan	1.1×10^4	9.43[c]
diacetyldeuteroheme thiolate	*n*-butanethiol	2.1×10^4	10.66[c]
diacetyldeuteroheme thiolate	benzyl mercaptan	2.5×10^4	9.43[c]
diacetyldeuteroheme thiolate	methylthioglycolate	3.2×10^4	7.8[c]
diacetyldeuteroheme thiolate	4-fluorothiophenol	4.1×10^4	6.42[d]

[a] 23 °C. This work. [b] DMA solvent, 300 mM thiolate. [c] Reference 60. [d] Reference 62; pK_a estimated from the Hammet constant, σ, where $pK_a = -1.88\sigma + 6.54$. σ for 4-fluorothiophenol is from ref 63.

Table VIII. Infrared Stretching Frequencies of Carbon Monoxide in Heme Complexes

compd/ligand (solvent)	ν_{CO}, cm^{-1}	ref
protoheme (DMA)	1946	this work
protoheme butane thiolate (DMA)	1918	this work
diacetyldeuteroheme butane thiolate (DMA)	1923	this work
diacetyldeuteroheme 4-fluorothiophenolate (DMA)	1932	this work
protoheme butane thiolate (DMA)	1923	19
protoheme methane thiolate (benzene)	1945	7
protoheme *N*-methylimidazolate (Me_2SO)	1935	49b
protoheme *N*-methylimidazole (Me_2SO)	1951	49b
bacterial cytochrome P_{450}, with camphor	1940	64
bacterial cytochrome P_{450}, without camphor	1963, 1942	64
microsomal cytochrome P_{450}	1948	64
microsomal cytochrome P_{450}	1954	64

estimated from its Hammet constant[62,63] (see Table VII note). The hemes coordinated to thiolate ligands of lower basicity (lower pK_a) exhibit greater CO binding affinity.

The stretching frequencies, ν_{CO}, of the carbon monoxide complexes of thiolate- and DMA-coordinated hemes were measured to determine the extent of π-back-bonding for these axial ligand complexes. The stretching frequencies are shown in Table VIII together with those of the carbonyl complexes of imidazole-coordinated hemes[49b] and various cytochromes P_{450}.[64]

Discussion

Soret Maxima. A comparison of the absorption maxima of the heme thiolate models and their CO complexes to the corresponding maxima of the cytochromes P_{450} and their CO complexes indicates similarities as well as significant differences. The heme thiolate models have Soret maxima that are also similar to those of the corresponding proteins, indicating that the electronic properties of these model complexes are very similar to the heme coordination centers in the proteins. In contrast, the longer wavelength Soret maxima of the CO complexes of the heme thiolate models observed in this study are consistently red shifted 16–18 nm relative to those of the CO complexes of the cytochromes P_{450}, indicating a significant difference between the electronic properties of these complexes.

The hyperspectra of the CO complexes of the heme thiolate models and cytochromes P_{450} have been interpreted as being due to an orbital mixing of the normal porphyrin Soret π–π^* transition from either the lone-pair sulfur orbital[65] or the axial π system of sulfur π, CO π, and iron dπ orbitals.[66] One explanation for the 16–18-nm red shift for the CO complexes of heme thiolate models as compared to that of cytochromes P_{450} is the difference in the heme environment in the protein compared to the heme environment of the model in DMA. Previous investigators have noted the effect of solvent[5,19,53] on the Soret maxima, which could alter the electron distribution in the complex. It has been suggested that the absorption maxima in proteins can be accounted for by a more nonpolar heme environment.[5,19]

The present results suggest that a second contribution to the differences in the absorption maxima could be due to a greater effective thiolate ligand basicity in the models, which is consistent with either of the mechanisms involving charge-transfer transitions. This explanation is supported by the observed relative position of the longer wavelength Soret maxima for the (alkanethiolato)diacetyldeuteroheme complexes of different thiolate basicities reported in this work (Table VI) as well as for the (alkanedithiolato)hemin complexes of different thiolate basicities.[12] As previously observed for hemin complexes, no correlation exists between the position of the Soret absorption maxima and the basicities of alkane- and arenethiolates. The lack of correlation between alkane- and arenethiolate complexes could be due to possible charge-transfer interactions between the aromatic ring of the ligand and the porphyrin.[12]

IR Stretching Frequencies. The CO stretching frequencies for the thiolate, imidazole, and DMA heme model complexes shown in Table VIII follow the order thiolate < DMA < imidazole. ν_{CO} provides a measure of the extent of back-bonding from the iron to the carbon monoxide and has recently been shown to be correlated with the redox potentials of the heme complex.[67] A lower stretching frequency is associated with an increase in electron density at the heme iron, which results in a lower redox potential.[67] The lower ν_{CO} observed for DMA-coordinated vs. imidazole-coordinated hemes is consistent with the reported π-donor properties of this ligand.[68] Similarly, the lower IR stretching frequency observed for heme thiolate models relative to those of the other models is indicative of greater π-donor ability of the thiolate ligand. As observed in Table VIII, a decrease in the stretching frequency is also observed on deprotonation of imidazole, suggesting that an increase in back-bonding from iron to the CO may be associated with an increase in electron density at the iron resulting from both an increase in σ and π donation from the trans ligand.

The stretching frequencies for the various cytochromes P_{450} (Table VIII) are significantly greater than those of the protoheme *n*-butanethiolate models in DMA. Several factors that have been proposed to affect the ν_{CO} of CO bound to heme have been categorized as those interacting with the carbonyl ligand directly and those modifying the electron density at the iron.[69] Factors that influence the carbonyl ligand directly include solvent[30] and hydrogen-bonding[69,70] interactions. A linear relationship was observed for a plot of the redox potentials vs. ν_{CO}'s for heme proteins including cytochrome P-450–cam, indicating that the IR stretching frequencies for these heme proteins are a function of electronic effects at the heme iron rather than direct protein interactions.[69] Factors that influence the electron density at the iron include cis[67] and trans[71] effects. The present results show an increase (5 cm^{-1}) in observed CO stretching frequency associated with changing protoheme to diacetyldeuteroheme (cis effect) in the thiolate model complex. Changing the axial ligand from *n*-butanethiolate to 4-fluorothiophenolate (trans effect) resulted in a very significant increase (9 cm^{-1}) in the CO stretching frequency that was closer to the frequencies observed for cytochromes P_{450} (Table VIII). These results suggest that the higher ν_{CO}'s observed for the cytochromes P_{450} could also be accounted for by a lower trans axial ligand basicity.

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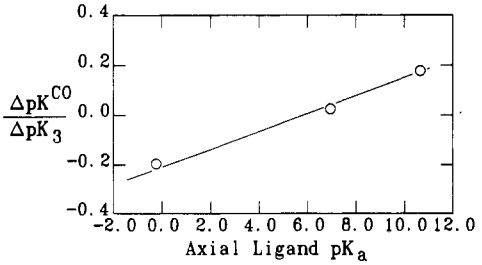

Figure 6. Relationship between the Brønsted constant, $\Delta pK^{CO}/\Delta pK_3$ (Table V), and axial ligand basicity.

CO Binding Affinities. Cis Effects. A comparison of the carbon monoxide affinities of thiolate complexes of various 2,4-disubstituted hemes in Table II shows that cis effects can contribute a small but significant effect on CO bonding, as indicated by the threefold greater CO affinity of diacetyldeuteroheme vs. protoheme. Thus, for the thiolate heme complexes, cis effects that remove electron density (lower pK_3) from the heme iron increase CO binding, as shown in Figure 5A.

A comparison of the CO affinities of 2,4-disubstituted hemes in dimethylacetamide in Table IV also shows that cis effects have a small but significant effect on CO binding, though the effect is opposite that observed for the (thiolato)heme models. Cis effects that remove electron density from the iron decrease CO bonding, as shown in Figure 5C for hemes in dimethylacetamide. The CO binding constant of dicyanodeuteroheme is fourfold smaller than that of protoheme. This effect is consistent with the reported decrease in CO association rate constants for porphyrins of decreasing basicity observed for hemes coordinated to Me_2SO and ethylene glycol.[38]

It is interesting to compare the above results to previous model studies of CO binding to various imidazole-chelated hemes.[42] While imidazole-chelated hemes exhibit a minimal cis effect (Figure 5B), thiolate- and DMA-coordinated hemes exhibit opposite cis effects. The cis effects associated with these ligands appear to be correlated with the relative basicities of these axial ligands, as indicated by a comparison of the Brønsted constants ($\Delta pK^{CO}/\Delta pK_3$) and the pK_a values for the ligands, as shown in Table V. The Brønsted constants increase from −0.19 to +0.19 as the axial ligand basicity increases from DMA to imidazole to thiolate. This relationship is depicted in Figure 6, which shows a plot of the Brønsted constants determined for CO binding to 2,4-disubstituted hemes with different axial ligands vs. the axial ligand pK_a. The above relationship can be expressed by

$$\Delta pK_L{}^{CO}/\Delta pK_3 = 0.035pK_a - 0.19 \quad (2)$$

The present results indicate then that the CO binding constants for these heme ligand complexes can be expressed empirically as

$$pK_L{}^{CO} = (0.035pK_a - 0.19)pK_3 + C_L \quad (3)$$

where the first term corresponds to the observed cis effects and C_L is a constant characteristic of each ligand.

These relationships indicate that the magnitude and direction of the cis effects are in part dependent on the trans ligand basicity such that CO affinity can be enhanced by an increase in porphyrin basicity when the axial ligand basicity is relatively small, as for DMA, and can be enhanced by a decrease in porphyrin basicity when the axial ligand basicity is relatively large, as in the case of the thiolate ligand. This behavior can be understood in part in terms of the σ-donor and π-acceptor interactions associated with CO bond formation. A decrease in electron donation from Fe to CO due to weak σ donation from a trans ligand such as DMA can be compensated to some extent by an increase in porphyrin basicity, which may stabilize the net positive charge on the carbon.[72] Correspondingly, a decrease in σ donation from CO to Fe due to strong σ donation from the trans thiolate ligand can be partially compensated by a decrease in porphyrin basicity,

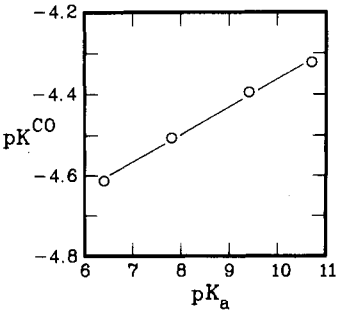

Figure 7. Plot of pK^{CO} vs. pK_a of the corresponding thiols for diacetyldeuteroheme thiolates in DMA.

which reduces the large electron density at Fe.

These observations may be relevant to an understanding of the greater CO affinity of cytochrome P_{450} vs. the thiolate models. Table V shows that cytochrome P_{450} reconstituted with different iron porphyrins exhibits a CO Brønsted constant of −0.22, which compares to +0.19 observed for that of the thiolate models. The Brønsted constant for cytochrome P_{450} is in fact more similar to that of the less basic ligand dimethylacetamide (−0.19), suggesting that the effective basicity of the thiolate cytochromes P_{450} might be significantly lower than that of the thiolate models. The larger CO binding constant observed for these proteins is thus consistent with a smaller effective charge on the thiolate sulfur compared to the models, as earlier suggested.[20] It is further interesting to observe that the cytochromes P_{450} have CO binding constants similar to those of the less basic DMA ligand.

It has been suggested that the observed protein stabilization of CO relative to the models might be due to the presence of a proton donor that acts to reduce the effective electron density of the cysteine sulfur.[20] Alternatively, we suggest that the effective ligand basicity in the proteins may be reduced through restricted axial ligand coordination resulting in a bond length or ligand orientation that is different from the models.

Trans Effects. A comparison of the carbon monoxide affinities of various thiolate complexes of proto- and diacetyldeuterohemes in Table VII shows that trans effects that remove electron density from the heme iron through a reduction in thiolate ligand basicity increase CO binding affinity. This relationship is depicted in Figure 7, which shows a linear plot of pK^{CO} values determined for diacetyldeuteroheme coordinated to various thiolates vs. the pK_a values of the corresponding thiols. The above relationship can be expressed by

$$pK^{CO} = 0.07\ pK_a - 5.04 \quad (4)$$

As shown in Table VII, the CO affinity of diacetyldeuteroheme coordinated to 4-fluorothiophenolate more closely approaches the CO affinities of the cytochromes P_{450} than the corresponding *n*-butanethiolate model.

The differences in trans axial ligand basicity could also contribute significantly to the observed differences in CO binding between the (*n*-butanethiolato)protoheme model and cytochrome P_{450}. As shown in Table VII differences between CO binding constants for (phenylmethanethiolato)- and (*n*-butanethiolato)-protoheme complexes indicate that the trans effect appears to have a greater influence on carbon monoxide affinity for protoheme than for diacetyldeuteroheme. On the basis of CO binding constants for *n*-butane- and phenylmethanethiolates, the magnitude of the trans effect ($\Delta pK^{CO}/\Delta pK_a$) is 0.18 for protoheme, which is comparable to that observed for imidazole- and pyridine-coordinated hemes[45,73] and could lead to larger differences in carbon monoxide binding.

Conclusions

The direction and magnitude of cis effects on carbon monoxide binding to model heme complexes is dependent on the trans axial ligand basicity. Likewise, the magnitude of trans effects on carbon monoxide binding to model heme complexes appears to be de-

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pendent on the cis porphyrin basicity. Differences in properties observed between cytochromes P_{450} and (*n*-butanethiolato)heme models could, in part, be accounted for in terms of trans ligand basicity.

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Registry No. Mesoheme thiolate, 102940-33-0; protoheme thiolate, 102940-34-1; 2-formyl-4-vinyldeuteroheme thiolate, 102940-35-2; diacetyldeuteroheme thiolate, 102940-36-3; dicyanodeuteroheme thiolate, 102940-37-4; mesoheme thiolate, CO complex, 102940-38-5; protoheme thiolate, CO complex, 102940-39-6; 2-formyl-4-vinyldeuteroheme thiolate, CO complex, 102940-40-9; diacetyldeuteroheme thiolate, CO complex, 102940-41-0; dicyanodeuteroheme thiolate, CO complex, 102940-42-1; CO, 630-08-0.

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Magnetic and Spectroscopic Study of Pyrazine-Bridged Iron(II) Halide Complexes

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The low-dimensional compounds $Fe(pyz)_2X_2$ (X = Cl, Br, I; pyz = pyrazine) and $Fe(pyz)Cl_2$ have been prepared and characterized by vibrational, electronic, and Mössbauer spectroscopies, by differential scanning calorimetry, and by magnetic susceptibility measurements between 300 and 4.2 K [and to 1.9 K for the mono(pyrazine) complex]. Although bidentate bridging pyrazine ligands are known to support magnetic exchange interactions in a number of other systems, including iron(II) complexes, no conclusive evidence for exchange coupling is found here.

Introduction

Pyrazine-bridged transition-metal complexes represent examples of materials that may exist as one-dimensional linear chains or two-dimensional lattices, with the possibility of magnetic exchange interactions being propagated through the bridging pyrazine ligand. The most extensive series of pyrazine complexes investigated have been derivatives of copper(II). Chloride and bromide complexes of copper(II) have been synthesized with a series of mono- and dimethyl-substituted pyrazine ligands,[1] and all exhibit a maximum in the magnetic susceptibility vs. temperature curve, indicative of antiferromagnetic interactions. Using a variety of substituted pyrazine ligands L in complexes of the type $CuL(NO_3)_2$, Richardson and Hatfield[2] elegantly demonstrated that the variation in the antiferromagnetic coupling constant J correlates with the energy of the $\pi-\pi^*$ transition of the pyrazine ligand in the complex. Thus, the pyrazine π-system is implicated in the exchange mechanism. Another important factor in determining the nature of the magnetic exchange interactions in copper(II) pyrazine complexes has been found to be the overlap between the copper d orbitals and the pyrazine π-system.[3-5]

Investigations of the nickel(II) pyrazine halides, $Ni(pyz)_2X_2$ (X = Cl, Br, I),[6] revealed temperature-independent magnetic moments between 90 and 300 K. Similarly, the cobalt(II) derivatives $Co(pyz)_2X_2$ (X = Cl, Br)[7] showed no evidence of magnetic interactions over the temperature range 1.8-300 K, although subsequent heat capacity[8] and susceptibility[9] measurements to substantially lower temperatures show both these cobalt(II) complexes to be *xy* antiferromagnets with critical temperatures of 0.85 and 0.66 K, respectively.

Relatively little research has been undertaken on iron(II) complexes of pyrazine, a fact that may be due to the problems associated with the relative ease of oxidation of iron(II) to iron(III), and to the difficulties in analyzing magnetic susceptibility data for d^6 systems. Previous work on iron(II) pyrazine halides includes the preparation of $Fe(pyz)Cl_2$,[10] $Fe(pyz)_2Cl_2$,[11] and $Fe(pyz)_2Br_2$[12] and a hydrate, $Fe(pyz)_2Cl_2 \cdot H_2O$.[12] Characterization of these complexes has included thermal studies,[11] infrared spectroscopy,[10,12,13] and room-temperature magnetic susceptibility measurements.[12] The pseudohalide complex, $Fe(pyz)_2(NCS)_2$,[14] has been reported, but no synthetic procedure was given and characterization was limited to susceptibility measurements in the 80-300 K temperature region.

Very recently we have reported, inter alia, Mössbauer spectra of $Fe(pyz)_2(NCS)_2$ in the range 1.8-10 K.[15] The magnetic susceptibility of this complex exhibited a maxiumum at about 8 K, indicative of antiferromagnetic exchange coupling,[16] and Mössbauer spectroscopy revealed a magnetic phase transition at $T_N = 9.1$ K with substantial line broadening, due to relaxation effects, in the vicinity of T_N. Reiff et al.[17] have also reported briefly their low-temperature Mössbauer measurements on $Fe(pyz)_2Cl_2$ and $Fe(pyz)Cl_2$. Neither complex was found to show any sign of magnetic ordering in the zero field down to 0.5 K. No other information on these derivatives seems to be available.

We report here the synthesis and characterization of the bis(pyrazine)halide complexes $Fe(pyz)_2X_2$ (X = Cl, Br, I) and also the mono(pyrazine) complex $Fe(pyz)Cl_2$. The bis(pyrazine) derivatives are proposed to have polymeric structures as found by X-ray crystallography for $Co(pyz)_2Cl_2$.[18] In this cobalt(II)

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