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Sulfur base ligation to iron(II) and cobalt(II) porphyrins

(equilibrium constants/cytochrome *P*-450 mechanism)

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ABSTRACT **Equilibrium constants for the binding of sulfur bases to cobalt(II) porphyrins were measured in toluene solution by a spectrophotometric method. The order of decreasing binding strength of sulfur ligands to cobalt(II) porphyrins was found to be mercaptide ions ≫ thioethers > mercaptans. It is suggested that a similar stability order of these sulfur ligands should exist towards iron(II) porphyrins, but formation constants could be obtained only for the mercaptide ions.**

The study of axial ligation to metalloporphyrins is relevant to our understanding of the mechanisms intimately involved with the biological activity of hemoproteins. In particular, sulfur base ligation to metalloporphyrins has been largely ignored. In fact, the binding of mercaptide ions, mercaptans, and thioethers to metalloporphyrins has not been directly compared before despite the importance of these ligands in cytochrome *c* and cytochrome *P*-450. Methionine is a known (1) axial ligand covalently bound through sulfur to the heme group in cytochrome *c*, an important electron transport agent in respiratory metabolism (2, 3). Cytochrome *P*-450 is a membrane-bound class of hemoproteins that functions as a catalyst for the hydroxylation of certain organic compounds by the activation of molecular oxygen. Although the structure of the active site of the cytochrome *P*-450 enzyme is not known, the absorption spectrum of the reduced-heme CO adduct has been modeled with simple ferrous porphyrins by means of an axial-bound mercaptide ion *trans* to CO (4–7). This information, combined with electron spin resonance experiments on both the native enzyme and appropriate model compounds (8–10), provides strong evidence for an axially coordinated mercaptide ion bound to the heme iron in cytochrome *P*-450. This mercaptide ion is believed to originate from a cysteine residue of the protein.

We now report the results of equilibrium studies of sulfur ligand binding to cobalt(II) and iron(II) porphyrins. Our studies include mercaptide ions, mercaptans, and thioethers, which contain sulfur ligand atoms as reasonable facsimilies to cysteine residues, undissociated cysteine, and methionine, respectively.

MATERIALS AND METHODS

The Fe-5,10,15,20-[pyromellitoyl(tetrakis-*o*-oxyethoxyphenyl)]porphyrin [Fe(Cap)], Co(Cap) (11), and Cotetra-*para*-methoxy *meso*-tetraphenylporphine [CoT(*p*-OCH_3)PP] (12) were synthesized by known procedures. *N,N*-Dimethylformamide (DMF) was purified by stirring over KOH for 48 hr and then distilled under reduced pressure. Toluene was reagent grade and was distilled under N_2 atmosphere from sodium benzophenone ketyl immediately prior to use. 1-Butanethiol was dried over calcium chloride and distilled under reduced pressure. Methylphenylsulfide and pentamethylene sulfide were distilled under reduced pressure prior to use. Dibenzo-18-crown-6 was recrystallized twice from dimethyl sulfoxide/H_2O and dried at 90°C at reduced pressure. Potassium butylmercaptide was prepared according to the synthesis of Chang and Dolphin (13). A stock base solution of butyl mercaptide K crown ether (BuSⓀ) in DMF was prepared by stirring a small amount of potassium butylmercaptide in DMF under N_2 and then adding an equivalent amount of weight of dibenzo-18-crown-6, also dissolved in DMF. Because the mercaptide solution is oxygen-sensitive all transfers were made under N_2. The concentration of a saturated solution of BuSⓀ in DMF is about 0.3 M. The base solution was titrated with HCl by using 1,2-dihydroxyanthraquinone (Alizarin) dissolved in DMF as an indicator to determine the exact mercaptide concentration.

The equilibrium constants were measured by a straightforward spectrophotometric titration procedure. Aliquots of deoxygenated base, either neat or dissolved in DMF, were added to a toluene or DMF solution of the metalloporphyrin under N_2 atmosphere in a 1-cm quartz cell with attached serum cap. The temperature of the solution was maintained at 23.1 ± 0.1°C. In general, the spectra were recorded in the 650- to 480-nm range on a Cary 14 spectrophotometer.

The possible base equilibria involving the monodentate sulfur base, B, are:

$$\mathrm{M(Por)} + \mathrm{B} \overset{K^{\mathrm{B}}}{\rightleftharpoons} \mathrm{M(Por)(B)} \qquad [1]$$

$$\mathrm{M(Por)B} + \mathrm{B} \overset{K_{\mathrm{B}}^{\mathrm{B}}}{\rightleftharpoons} \mathrm{M(Por)(B)_2}, \qquad [2]$$

where M is metal. For the complexes Co(II)(Cap) and Fe(II)(Cap), the addition of a second sulfur ligand is impossible due to the encumbrance of the capped porphyrin (11). For Co(II)[T(*p*-OCH_3)PP], $K_{\mathrm{B}}^{\mathrm{B}}$ is expected to be small because cobalt complexes of this type are known (14) to prefer five-coordination. Thus, assuming $K_{\mathrm{B}}^{\mathrm{B}} = 0$, the data can be fitted to the Hill equation

$$\log y/(1-y) = n \log [\mathrm{B}] + \log K^{\mathrm{B}} \qquad [3]$$

by using a nonweighted linear least-square method, where $y = (A_{obs} - A_0/A_\infty - A_0)$ and A_{obs} = absorbance at a specific [B], A_0 = initial absorbance where [B] = 0, and A_∞ = final absorbance when the fully ligated porphyrin is the only species present. Values for log K^{B} were obtained from the *y*-intercept of the regression line for a plot of log $y/1-y$ versus log [B]. If the equilibrium constant is small, and A_∞ is therefore difficult to achieve, the following derivation (15) of the Hill equation was employed to obtain K^{B}:

$$\frac{1}{(A_0 - A)} = \frac{1}{(A_0 - A_\infty)} + \frac{1}{(A_0 - A_\infty)(K^{\mathrm{B}})[\mathrm{B}]}. \qquad [4]$$

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Abbreviations: Cap, dianion of the capped porphyrin, 5,10,15,20-[pyromellitoyl(tetrakis-*o*-oxyethoxyphenyl)]porphyrin; T(*p*-OCH_3)PP, dianion of tetra-*para*-methoxy *meso*-tetraphenylporphine; BuSⓀ, butyl mercaptide potassium crown ether; DMF, *N,N*-dimethylformamide; B, any donor ligand; Por, dianion of any porphyrin.

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The K^B was then obtained from the y-intercept divided by the slope of the regression line for a plot of $1/A_0 - A$ versus $1/[B]$.

RESULTS AND DISCUSSION

Spectrophotometric titrations with sulfur ligands at 23.1°C of DMF or toluene solutions of Fe(II)(Cap), Co(II)(Cap), and Co(II)[T(p-OCH_3)PP] were performed. The results of typical titrations are shown in Figs. 1 and 2.

The titrations of Fe(Cap) with thioethers and mercaptans yielded anomalous results. The values of n (for Hill plots) varied from 0.5 to 0.75. This behavior of slope values (n) < 1 for Hill plots was noted for several thioethers and mercaptans. The behavior is consistent with the thioethers and mercaptans acting as bridging ligands between two Fe(Cap) moieties, although there is no direct evidence that bridging is occurring. For the equilibria listed in Table 1, plots of log [M(Por)(B)]/$[M(Por)]_{Total}$ versus log [B] gave straight lines with slopes equal to 1.00 ± 0.1. The observed equilibrium constants are given in Table 1. In addition, the equilibrium constants for a few nonsulfur bases are shown for comparative purposes. Differences between equilibria measured in DMF versus toluene should not be considered significant, because there is only a 2-fold increase in the binding of BuSⓀ to Fe(protoporphyrin IX dimethyl ester) (13) in toluene as compared to *N,N*-dimethylacetamide.

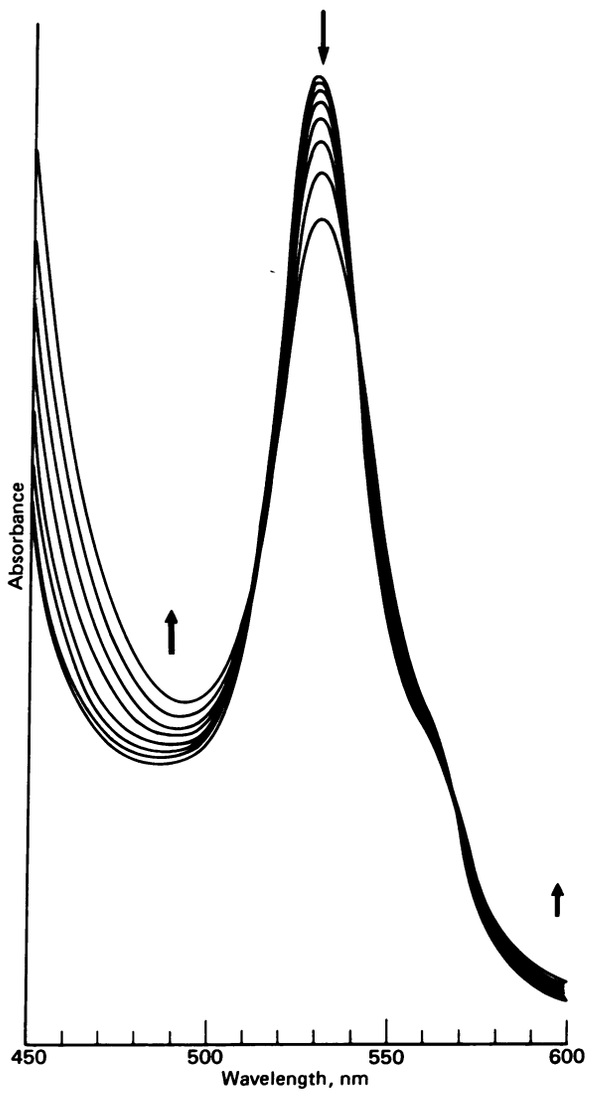

FIG. 1. Spectral changes that occur upon titration of a ≈0.1 mM toluene solution of Co(Cap) with neat methylphenylsulfide.

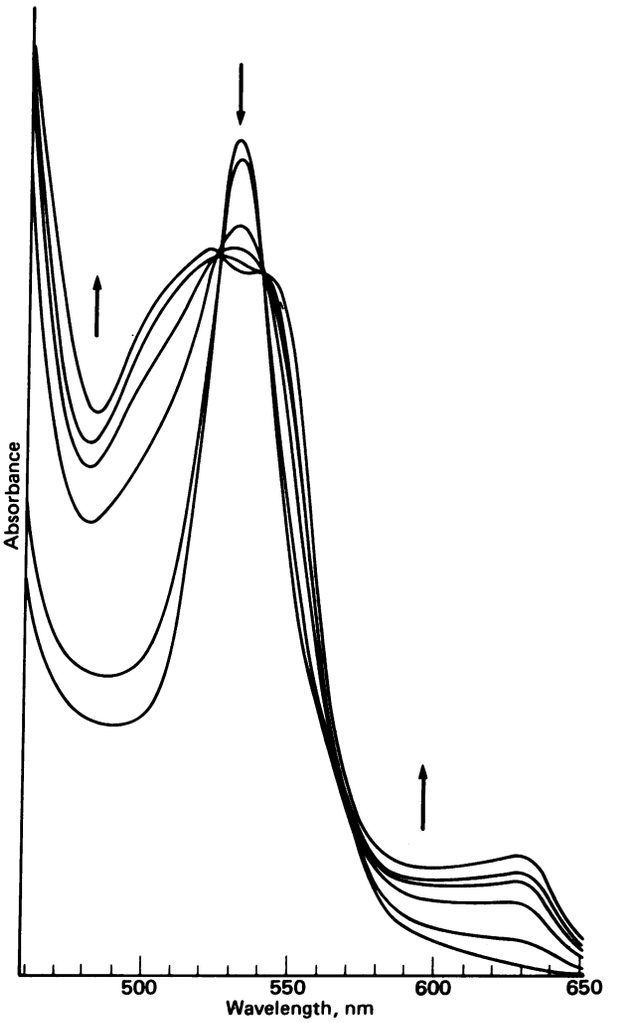

FIG. 2. Spectral changes that occur upon titration of a ≈0.1 mM toluene solution of Co(Cap) with ≈0.3 M BuSⓀ in DMF.

The results in Table 1 indicate that the binding of sulfur ligands to cobalt(II) porphyrins decreases in the order mercaptide ions ≫ thioethers > mercaptans. Although all of the equilibrium constants measured for Co(Cap) were not possible for Fe(Cap), the binding order of sulfur ligands found for cobalt should be identical to the order for iron. It might even be safe to assume that the semiquantitative differences between the binding constants of the three types of sulfur bases are about the same for iron(II) as for cobalt(II). Thus, this allows us to make a rough estimate of equilibrium constants for the addition of thioethers and mercaptans to iron(II) porphyrins, for which there is, to our knowledge, no quantitative data available. Mercaptide ions, which are strong bases and highly polarizable, are known (20) to form strong bonds to metal complexes. The most obvious reasons for the strong binding of mercaptides compared with thioethers and mercaptans is the negative charge on the mercaptide ion and its extra lone pair of electrons. The fact that thioethers bind with larger K^B values than do the mercaptans is due to the greater electron donor ability of an alkyl group relative to hydrogen, and this corresponds well with their proton base strengths as measured both in solution (16) and in the gas phase (21). Attempts were made to obtain the electron paramagnetic resonance spectra of mercaptide adducts of

Table 1. Equilibrium constants for the binding of an axial ligand to cobalt(II) and iron(II) porphyrins at 23.1°C

Metalloporphyrin	Ligand	Solvent	$pK_a(BH^+)$	$\log K^B$	Ref.
Co(Cap)	1-Butanethiol	Toluene	(−6)*	< -1	This work
	Methylphenylsulfide	Toluene	(−4)*	0.52 ± 0.05	This work
	Pentamethylenesulfide	Toluene	(−4)*	1.00 ± 0.05	This work
	BuS⊗	DMF	10.6*	3.0 ± 0.2	This work
Co[T(*p*-OCH_3)PP]	1-Butanethiol	Toluene	(−6)*	−0.35 ± 0.10	This work
	Methylphenylsulfide	Toluene	(−4)*	0.94 ± 0.06	This work
	BuS⊗	DMF	10.6*	3.4 ± 0.2	This work
Co(Cap)	1-Methylimidazole	Toluene	7.25†	2.32 ± 0.05	18
Co(mesoporphyrin IX dimethyl ester)	Dimethylsulfide	Toluene	(−4)*	1.35‡	19
Fe(Cap)	Bus⊗	DMF	10.6*	4.2 ± 0.2	This work
Fe(protoporphyrin IX dimethyl ester)	Bus⊗	*N,N*-Dimethyl-acetamide	10.6*	4.0	13
	Bus⊗	Toluene	10.6*	4.4	13
Fe(Cap)	1-Methylimidazole	Toluene	7.25†	2.90 ± 0.05	18

See Eq. 1.
* As estimated from data in ref. 16.
† See ref. 17.
‡ At 2.6°C.

Co(Cap) and Co[T(*p*-OCH_3)PP] in several different solvents at 77 K. Satisfactory spectra were not obtainable due to poor glasses, solubility problems, or the presence of radical species, which did not affect the base titrations.

Peisach and coworkers (22, 23) and others (10) have proposed that in the low spin ferric cytochrome *P*-450 the axial ligands are an imidazole from a histidine residue and a mercaptide sulfur, probably due to a cysteine residue. Further, Peisach (22) suggests that upon addition of CO to the reduced cytochrome *P*-450 the imidazole is displayed by the CO to give a complex similar to the model systems (4–7) that contain a mercaptide ion and CO bound to the ferrous porphyrin. However, in the O_2 adduct of ferrous cytochrome *P*-450, Peisach believes that the O_2 binds *trans* to the imidazole group displacing the mercaptide ion. This view is supported by the fact that the optical spectrum of oxygenated cytochrome *P*-450 is similar to that of oxyMb or oxyHb.

Our results show the binding of mercaptide ion to Fe(II) porphyrins to be quite strong. Because the binding of mercaptide ion, as indicated by K^B (Eq. 1), to Fe(Cap) is greater than the binding of 1-methylimidazole, the contention (23) that CO would preferentially replace imidazole over mercaptide ion in ferrous cytochrome *P*-450 seems valid. However, the fact that mercaptide ion binds more strongly to Fe(II) porphyrins than does imidazole does not explain the postulated (23) replacement of mercaptide ion upon oxygenation of ferrous cytochrome *P*-450.

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