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Anion Complexes of Ferrous Porphyrins[1]

Sir:

Although anionic ligands such as carboxylate, phenoxide,[2] imidazolate,[3-7] and mercaptide[8,9] have been considered as proximal iron ligands in hemoproteins, detailed model studies have so far been confined to the ferric and ferrous porphyrin complexes with mercaptide and certain ferric derivatives of other anions.[3,4,6,7] Ferrous porphyrin complexes with hydroxide[10] and alkoxide[11] have been described but the authors were unable to obtain carbon monoxide complexes of the anion heme.[11,12] The interesting suggestion that hydrogen bonding or deprotonation of proximal imidazole might provide a means by which hemoproteins can control heme reactivity[4,5] prompted as to prepare this unknown imidazolate-heme-CO and other anionic complexes.

The reported difficulties in forming both five- and six-coordinated hydroxy heme complexes and in producing anion-heme-CO complexes[10,11] were overcome by careful exclusion of water or other protic solvents from dimethyl sulfoxide (Me_2SO) solvent. Anionic bases were prepared as ~0.01 M solutions by treating the conjugate acid with sodium hydride in the presence of a slight excess of 18-crown-6 ether. In a typical example, dry argon purged Me_2SO (10 mL), in a 1-cm^2 cuvette, was treated with 6 mg of 2-methylimidazole and 2 mg of sodium hydride and the solution heated to make the imidazolate ion. Successive additions of 1 mg of a sodium dithionite-18-crown-6 complex[13] (as reducing agent) and 2 μL of a 3 mM solution of protohemin dimethyl ester (or its *N,N*-dimethyl diamide) produced a 5 μM solution of the heme-2-methylimidazolate complex, entry 13 in Table I. In a similar manner it was possible to prepare heme dialkoxides or, in some cases, five-coordinated heme-anion complexes. Either ferric[7] or ferrous complexes of imidazolate could be prepared if the Me_2SO anion (which could reduce ferric porphyrins) was carefully excluded. Table I lists the absorption maxima of the anion and other complexes. These complexes, and their carbon monoxide derivatives, provide references for comparison with hemoproteins.

Table I. Spectra of Complexes of Protoheme Dimethyl Ester with Anions and Carbon Monoxide in Me_2SO at 25 °C at ~5 μM Concentration[a]

		λ_{max}, nm		
entry	ligand	five coordinated	six coordinated	CO complex
1	Me_2SO		424, 524, 552[b]	414, 532, 562[b]
2	H_2O ($CetMe_3NBr$)[c]	probably four coordinated		414, 532, 562[b]
3	HO^- (dry)	444, 561,[b] 595[d]	433, 532, 562[b]	438, 545
4	HO^- (wet)	444, 405, 561,[b] 595		414, 532, 562[b,c]
5	HO^- (wet)[e]	444, 405, 561, 595		no change with CO
6	HO^- ($CetMe_3NBr$)	444, 561, 595		414, 532, 562[b,c]
7	MeO^-		433, 532, 562[b]	360,[f] 437, 545
8	*t*-BuO^-		427, 532, 560[b]	414,[c] 437, 545
9	PhO^-		434, 530 (sh), 558, 595 (sh)[g]	438, 545
10			430, 530, 560[b]	431, 539,[b] 570
11			429, 529, 559[b]	430, 542,[b] 573 (sh)
12			425, 525, 556[b]	420, 540,[b] 569
13		440, 560,[b] 595		430.5, 545,[b] 575[h] (sh)
14		430, 558		419, 537,[b] 563
15	$CH_3SOCH_2^-$	443, 560, 595		414, 532, 562[b,c]

[a] The solvent was Me_2SO except in entries 2 and 6 where the solvent was 2% aqueous cetyltrimethylammonium bromide ($CetMe_3NBr$). The assignment of the spectra as corresponding to five or six coordination is tentative until definite structure proofs are available. They are reasonably based on the typical visible band extinctions for hexacoordinated hemes and on the fact that in some cases the presumed five-coordinated species are converted to the six-coordinated species upon addition of more base. Where no spectra are listed, we were unable to observe the indicated species. Only with hydroxide ion in dry Me_2SO could we observe both five- and six-coordinated heme. [b] The indicated peak is larger than the other visible bands. [c] Complexes of protoheme DME with CO in Me_2SO, alcohols, and water (in cetyltrimethylammonium bromide ($CetMe_3NBr$) suspension) all have essentially the spectrum indicated here. The 414-nm peak is diagnostic for an oxygen ligand in the sixth position. Where the 414-, 532-, and 562-nm spectrum occurs in the presence of anions, we assume that the anion is displaced giving the neutral base-heme-CO complex. [d] At low concentration (10^{-4} M) of hydroxide the five-coordinated heme is obtained. Further addition of OH^- (~10^{-3} M) produces the hexacoordinated species. Both forms bind carbon monoxide to give HO^-HmCO. [e] Using benzyltrimethylammonium hydroxide to increase the hydroxide concentration to 0.2 M in wet Me_2SO produced only the five-coordinated type spectrum. Addition of 1 atm of carbon monoxide had no effect on this spectrum, a result similar to that of ref 11. [f] A trace of a possible hyperporphyrin band at ~360 nm appeared upon addition of carbon monoxide. This band is under investigation. [g] This spectrum appears to be a mixture of five- and six-coordinated hemes. [h] NMR of this complex confirms the anion-Hm-CO structure: A. Berzinis, unpublished results.

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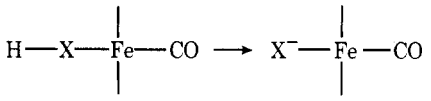
A comparison of the spectra of heme complexes of oxygen and nitrogen anions with those of the biologically important sulfur anions reveals some interesting trends. Thus deprotonation of proximal bases in the carbon monoxide complexes



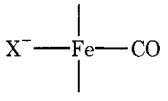
leads to red shifts in the Soret band of 414 → 438 for X = $^-$OR, 420 → 430 for X = imidazolate, compared with 420 → 460 for X = RS$^-$.[9] The deoxy forms of the RO$^-$ and imidazolate complexes also display a rather large red shift compared with complexes of their conjugate acids, e.g., 423 → 429 for the diimiazolate heme complex and 430 to 440 for the 2-methylimidazolate complex.

The reactivity of heme complexes of nitrogen and oxygen proximal bases is also greatly altered by deprotonation, an effect also seen in the RS$^-$–heme complexes.[9] We find that the diimidazolate heme (at 0.1 M imidazole concentration) has a pressure for carbon monoxide half-saturation ($P_{1/2}^{CO}$) of ~0.3 Torr compared with ~0.004 Torr for a similar solution of diimidazole–protoheme complex, both in $CetMe_3NBr$ suspension,[14] and ~18 Torr for an RS$^-$–heme complex in dimethylacetamide[11] or in $CetMe_3NBr$ suspension.[13b]

These results suggest that *red-shifted Soret bands* and *lowered CO affinities,* formerly observed for RS$^-$ complexes, are general properties of *heme–anion complexes.*

Comparisons of these spectra with those of hemoproteins affords some conclusions and suggests some speculations concerning deprotonation of proximal bases as a general phenomenon in hemoproteins.[4] The typical deoxy and carbonmonoxy bands for RO$^-$ complexes at 444 and 438 nm, respectively, are not observed in hemoproteins, making it unlikely that serine or tyrosine anions are proximal iron ligands.[15] This leaves mercaptide as the only ligand which gives the spectra recorded for cytochrome P-450. However, the 440, 560, and 595 bands for the 2-methylimidazolate complex and 430, 545, and 575 for its CO complex compare well with the 440, 560, and 595 bands and 424, 542, and 573 bands of deoxy and CO complexes of peroxidases[16,17] and catalase[18] and are quite different from those of hemoglobin or its chelated protoheme model.[13]

The suggestion of Peisach[4] and of Morrison and Schonbaum[5] that deprotonation or strong hydrogen bonding of the proximal imidazole constitutes a means of controlling hemoprotein reactivity seems to be supported by these data. This conclusion is further strengthened by our finding that heme–anion complexes are much more sensitive to oxidation by dioxygen than are their neutral complexes, just as peroxidases[16-18] and cytochrome P-450[19] are more easily oxidized by dioxygen than are hemoglobin or myoglobin. Additionally, the low affinity of the heme–anion complexes for CO is shared by peroxidases and P-450.



Further comparisons of heme–anion complexes with hemoproteins by other physical and chemical methods are in progress to test these speculations.[20]

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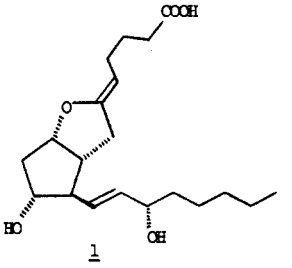
6,9-Pyridazaprostacyclin and Derivatives: the First "Aromatic" Prostacyclins[1]

Sir:

Prostacyclin (PGI_2, **1**),[2] owing to its remarkable biological properties and its chemical instability, has prompted an intense search for biological mimics with improved properties as potential therapeutic agents.[3] One possible solution to the



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problem of retaining biological activity and increasing stability at the same time is to substitute the second ring of prostacyclin with an aromatic nucleus. This concept of the "aromatic" prostacyclins retains the sp^2 character of C-6, a seemingly important feature for biological activity,[4] and, therefore, appeared to be an attractive proposition. In order to test this hypothesis we initiated a program directed toward the synthesis of such molecules and in this communication we report the synthesis of the first aromatic prostacyclin, namely 6,9-pyridazaprostacyclin (**16**), and its derivatives, dihydropyridazaprostacyclin (**14**) and *N*-oxides **18a** and **18b**, and describe our findings on their biological and chemical properties.

Model studies with the diketone **2** indicated that the desired bicyclic pyridaza system **3** could be constructed quite efficiently and under very mild conditions by the action of hydrazine in ethanol, THF, or aqueous THF followed by treatment with PtO_2. This methodology led to the isolation of **3**[5] in

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