Table I. Thermochemical Data

ref	substance (phase)	$\Delta H_f^\circ_{298}$, kcal/mol	S°_{298}, cal/(mol K)
7	H_2O_2(g)	−32.6	55.6
7	H_2O_2(l)	−44.9	26.2
7	H_2O_2(aq)	−45.7	34.5
7	H_2O(g)	−57.8	45.1
7	H_2O(l)	−68.3	16.7
8	HO_2(g)	3.0 ± 0.5	54.1[a] (55.1)[b]
a	HO_2(aq)	−8.6 ± 1	33 ± 1
7	O_2(g)	0	49.0
7	O_2(aq)	−2.8	26.5
7	OH(g)	9.3	43.9
a	OH(aq)	−1.7 ± 0.5	23 ± 1
a	HO_2^-(g)	−40.3 ± 2.5[c]	53 ± 1
4, 7	HO_2^-(aq)	−38.3	6.5
3, 7	HO^-(g)	−33.7	41.2[a]
7	HO^-(aq)	−55.0	−2.6
3, *a*	O_2^-(g)	−10.1	50.4[a]
6, *a*	O_2^-(aq)	−5.9 ± 1	19

[a] Values estimated in this work. [b] Value estimated in ref 5 from independent data. [c] $EA(HO_2)$ = 1.88 ± 0.11 eV (43 kcal).

O_2^- (aqueous).[6] These values together with related values on structurally similar species make it possible to make new estimates of the electron affinity of $HO_2\cdot$ with much greater reliability.

From known data on $HO_2\cdot$(g) and HO_2^-(aq) (Table I) one can estimate the sum of $EA(HO_2\cdot)$ and $H_{aq}[HO_2^-(g)]$:

$$e^- + HO_2(g) \overset{1}{\rightleftharpoons} HO_2^-(g) \qquad -EA(HO_2) \equiv \Delta H_1$$

$$HO_2^-(g) \overset{2}{\rightleftharpoons} HO_2^-(aq) \qquad \Delta H_{aq}[HO_2^-(g)] = \Delta H_2$$

$$e^- + HO_2(g) \overset{1,2}{\rightleftharpoons} HO_2^-(aq)$$

$$\Delta H_1 + \Delta H_2 = -EA(HO_2) + \Delta H_{aq}[HO_2^-(g)] \quad (I)$$

From the data in Table I, $\Delta H_1 + \Delta H_2 = -41.3$ kcal/mol so that we can write

$$EA[HO_2(g)] = 41.3 + H_{aq}[HO_2^-(g)] \quad (II)$$

To estimate $H_{aq}[HO_2^-(g)]$ we make use of the empirical relation, that the heats of solvation of negative ions seem to be monotonically related to their diameters.[9] Table II lists some of the relevant values. If we use O_2^- as the closest species in dimensions to HO_2^-, we could estimate a value for $\Delta H_{aq}[HO_2^-(g)] = 2 \pm 2.5$ kcal/mol. The argument for a more exothermic value than that for O_2^- would be based on the observation that OH^-(g) is more strongly solvated than F^- by ~3 kcal (Table II) and HS^- is more strongly solvated than Cl^- by ~7 kcal. The actual value of $\Delta H_{aq}(O_2^-)$ suggests a value closer to that for Cl^- than to F^- for the difference but we have chosen to be conservative and allow the greater difference to appear in the uncertainty. This leads then to the value for $EA[HO_2(g)] = 43.3 \pm 2.5$ kcal/mol (1.88 ± 0.11 eV).

The value for the $\Delta H_{aq}[HO_2(g)] = -11.6 \pm 1$ kcal/mol which appears in Table I is estimated by assuming that it is approximately the average of the values for H_2O of −10.5 and for H_2O_2 of −13.1 kcal/mol. The value for $\Delta H_f^\circ[HO_2(aq)]$ turns out to be in excellent agreement with the value of 8.0 ± 1 kcal/mol estimated from kinetic measurements on the Fe^{3+}/H_2O_2 system.[5]

If we compare the value of $EA[HO_2(g)]$ with measured values for related species shown in Table III, we see that it is quite reasonable. Based on these values we feel that it is also reasonable to estimate the electron affinities for species such as $RO_2\cdot$, $RO_3\cdot$, and $HO_3\cdot$ to be the same as $HO_2\cdot$, namely 1.85 ± 0.12 eV. Support for such an assumption is to be found in the pK_{ion} values of hydroperoxides[11] which tend to fall within ±0.5 pK units of $pK_{ion}(H_2O_2)$. This suggests that the differences in solvation enthalpies of $RO_2\cdot$ and RO_2H must be within ~0.7 kcal of that for $HO_2\cdot$ and HO_2H. It is a situation which finds a striking parallel in the pK_{ion} values of the carboxylic acids.[12]

Table II. Enthalpies of Aquation of Some Gas-Phase Anions

anion	ΔH°_{aq}, kcal/mol	anion	ΔH°_{aq}, kcal/mol
OH^-	−21.3	HS^-	12
F^-	−18.0	NO_2^-	
H^-	−16.0[a]	CN^-	9
Cl^-	18.9	O_2^-	4
Br^-	27	HO_2^-	2 ± 2.5[b]
I^-	34		

[a] Estimated by using a dimension for H^- anion from crystal anion–cation distances and comparing them with those of alkali halides. It leads to a value of $\Delta H_f^\circ_{298}[H^-(aq)]$ = 34 kcal/mol. [b] Estimated in this paper.

Table III. Electron Affinities of Some Oxy Radicals

ref	radical	EA, eV
3	O_2	0.44
3	O	1.46
3	OH	1.83
3, 10	CH_3O	1.8 ± 0.1
3, 10	*n*-PrO	1.9 ± 0.1
a	HO_2	1.85 ± 0.12
10	O_3	2.89
a	RO_2	1.85 ± 0.12

[a] Estimated here. R is taken as an alkyl group.

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Hemoprotein Models: NMR of Imidazole Chelated Protohemin Cyanide Complexes

Sir:

^1H NMR studies of low-spin ferric ($S = 1/2$) cyanide complexes of hemoproteins have shown the heme methyl resonances to be differentially hyperfine shifted so as to spread them from 8 to 28 ppm downfield of their diamagnetic positions.[1-7] By contrast, the biscyanide complex of protohemin

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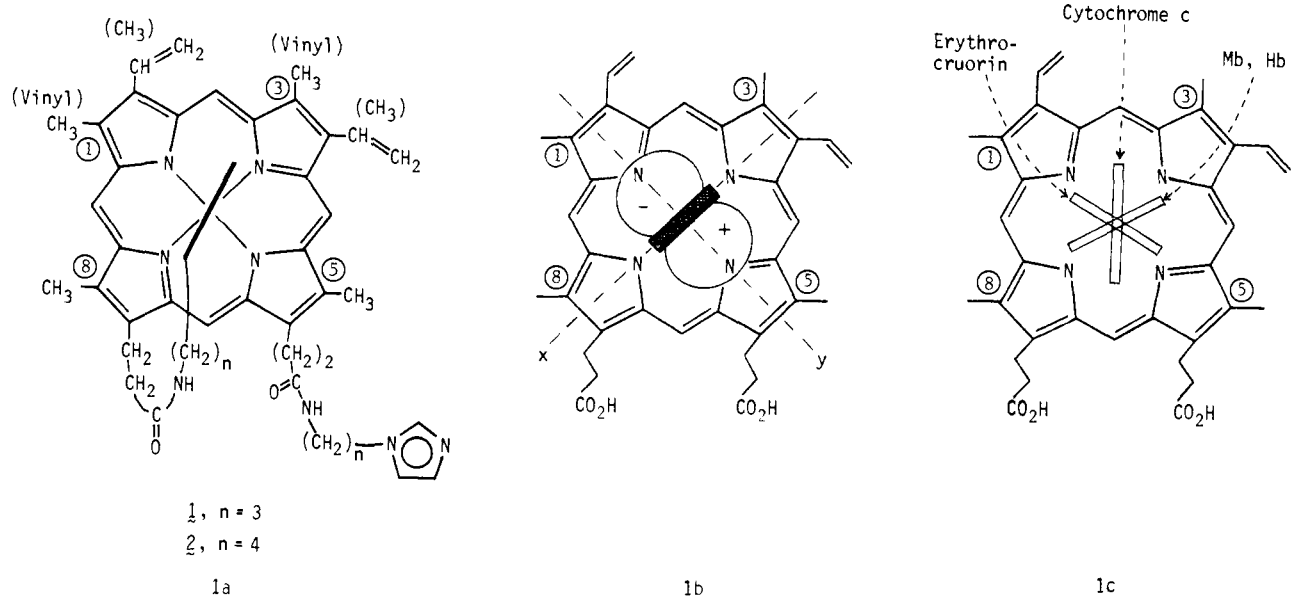

1, n = 3
2, n = 4

1a 1b 1c

Figure 1. (a) Dichelated protohemin monocyanides. One of the four isomers in this diastereomeric mixture is shown. (b) Interactions of the imidazole π orbitals and the heme orbitals postulated for selective shifting of the 1- and 5-methyl resonances in the protohemin cyanide of myoglobin and of dichelated protohemin cyanide ($\mathbf{1}^{+}CN^{-}$). (c) Orientations of the imidazole plane in some hemoproteins as described in ref 19-22.

Table I. Hyperfine Shifts of Heme Methyl Resonances in $S = 1/2$ Model Complexes and Hemoproteins[a]

	3[b]	**2**[b]	**1**[b]	Mb^{+}CN[c]	$Hb^{4+}(N_3^-)_4$[d] α	β	cytochrome[e] C^{+}CN^{-}	chironomus Hb^{+}CN^{-}[f]
	13.3	13.7	18.0	23.2 (5)	24.1	23.8	19.3	25.2 (x)
	12.9	13.0	16.5	14.5 (1)	19.0	18.8	17.5	25.1 (y)
	9.2	11.9	15.6	9.7 (8)	8.9	10.9	12.4	18.3 (x)
	8.7	11.4	11.2	<6 (3)	*g*	*g*	7.8	16.0 (y)
		10.7	7.2					*g*
		8.9	4.4					
		8.6	3.9					
		7.7	0.9					
spread	4.6	6.0	17.1[h]	>17			11.5	

[a] Abbreviations: Mb, myoglobin; Hb, hemoglobin. + refers to ferric heme. Hyperfine shifts are obtained by subtracting 3.6 ppm from the observed resonances as in ref 8. [b] In Me_2SO-d_6. The chemical shifts determined here did not show marked changes with changes in concentration. [c] Numbers in parentheses are ring methyl assignments and apply only to Mb.[13] Other resonances are listed in decreasing order of chemical shift and no correlation across this table is implied. [d] Reference 1. [e] Reference 7. [f] x and y denote major and minor isomer pair.[3] [g] Other resonances are obscured by globin. [h] The relevant spread of one of the two diasteriomers of **1** could be as little as 14.1 ppm (18.0-3.9). The conclusions would not be changed.

has methyl resonances occurring in the narrow range of 13-18 ppm.[8] Two interpretations have been offered for this increased asymmetry in the proteins. Morishima,[1] Shulman,[6] and Horrocks[9] and their co-workers suggested an interaction of the hemin with the proximal imidazole, and La Mar et al.[8] have suggested additional effects of the closest globin contacts.

Our studies of the imidazole-cyanide complexes of dichelated protohemins **1** and **2** (Figure 1), with fixed imidazole geometry, reveal a spread of hyperfine shifts which closely resembles the proteins. This is in contrast to the symmetrical spectra of the dicyanohemin or 1-methylimidazole-cyanide-protohemin (*N,N*-dimethyl)diamide (**3**). We conclude that this asymmetry is caused by the restricted rotation of the imidazole in **1**, which models the fixed heme-imidazole orientation of the proteins.

The chelated protohemins were synthesized (as the chloride) and studied in Me_2SO-d_6, as previously described.[10] The monocyanide complexes were generated by titration of the hemin with a 1 M solution of KCN in D_2O. Addition of >1 equiv of cyanide led to formation of the biscyanide complex, displacing imidazole. For this reason the monocyanide spectra shown are contaminated with small amounts of the biscyanide complex.

The methyl and vinyl resonances of the dicyanide complexes corresponding to compounds **1**, **2**, and **3** are identical, showing that these three compounds have identical electronic environments in the absence of the internal imidazole binding.

The spectra of **1**, **2**, and **3** are shown in Figure 2. Even more striking than the spread in the resonances is the finding of 8-methyl and 4-vinyl group resonances for the chelated hemes. Closing one of the two imidazole arms above or below the heme plane produces an enantiomeric pair (as shown in Figure 1a). Similar closures of the other arm produce the diasteriomers (with methyls and vinyls interchanged as shown in parentheses in Figure 1a). The enantiomers are NMR equivalent and the diasteriomers differ only with regard to the rotation angle of the imidazole plane with respect to the unsymmetrical placement of the vinyl groups, documented elsewhere.[10-12] The imidazole is also relatively fixed, but unstrained, in **1**, and much looser in **2**.[12a] As a result, **1** resembles Mb^{+}CN^{-} in its NMR behavior (Figure 2), while **2** appears to be somewhere between **1** and the freely rotating complex **3**. Some hyperfine shifts obtained for the model compounds are compared with hemoproteins in Table I.

Previous studies of model $S = 1/2$ complexes have shown the hyperfine shifts to primarily reflect spin density in the filled

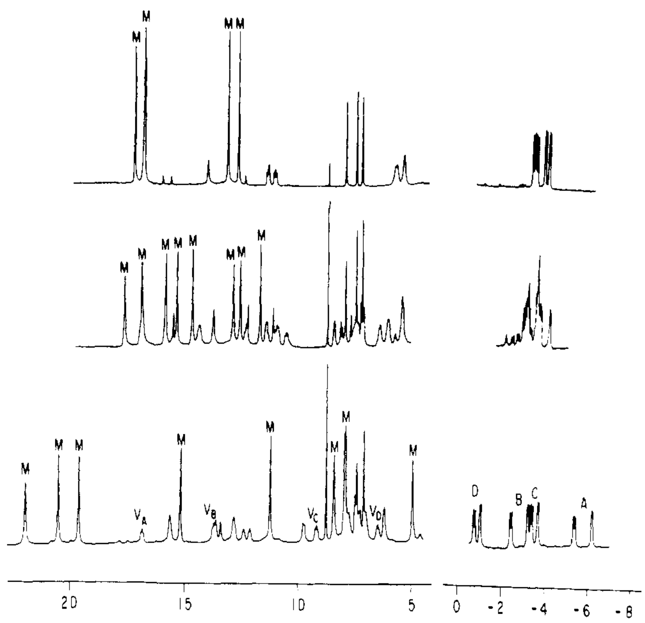

Figure 2. NMR spectra of cyanide complexes of the hemins **1** (lower trace), **2** (middle trace), and the complex with freely rotating *N*-methylimidazole **3** (upper trace). Methyl group resonances are identified as M and vinyl group resonances are identified as V_A, V_B, V_C, and V_D (methines), and A, B, C, and D (methylenes).

π orbitals of the pyrrole rings.[14] A hyperconjugative spin-polarization mechanism has been proposed,[15] consistent with the observation that heme methyl protons and vinyl methines are shifted downfield, while vinyl methylenes are shifted upfield. The vinyl methines and methylenes were paired by decoupling experiments on **1** and are shown as vinyls A, B, C, and D in Figure 2 (a full assignment of the NMR spectra of **1** and **2** will appear elsewhere[12b]). It is found that maximum upfield shifts correlate with maximum downfield shifts, and vice versa. This shows the asymmetry arises from a redistribution of spin density among the pyrroles, and not an anisotropy in the dipolar shift.[16]

A number of workers have proposed that the NMR shifts and **g**-tensor anisotropy[17] of Mb arise from an imidazole-heme interaction.[1,6,9,18] The filled pπ orbitals of the imidazole are of proper symmetry to interact with the iron d orbitals, which, in $S = 1/2$ ferric complexes, have a configuration equivalent to $d_{xy}{}^2(d_{xz},d_{yz})^3$. These d orbitals interact with the appropriate porphyrin π orbitals. In simple models such as **3**, which have freely rotating ligands, the highest of these pπ molecular orbitals would share the electron spin density equally.[18]

Now consider a complex which has the imidazole fixed in relation to the heme (Figure 1b). The imidazole π density will maximally interact with an appropriate heme molecular orbital, raising it in energy and lifting degeneracy.[18] The result is that the paired spin will reside in the lower orbital, and the unpaired spin density will be orthogonal to the imidazole plane. Through overlap with this orbital, we should find that pyrrole substituents along the y axis have the greatest hyperfine shifts.

We can test this hypothesis in the hemoproteins, using published structure determinations. Figure 1c shows the orientation of the proximal histidine in liganded Mb,[19] liganded Hb,[20] cytochrome *c*,[21] and chironomous hemoglobin,[22] and Table I lists the pertinent NMR data.

The heme methyl resonances of Mb^+CN^- have been assigned by reconstitution with selectively deuterated hemes.[13] The assignments in Table I show that methyls 1 and 5 are shifted further with respect to 3 and 8, in complete accord with our interpretation. Since the orientation in Hb is similar, the magnitude of the hyperfine shifts is also similar. The differences between the α- and β-chain resonances reflect the small differences in their orientation with respect to the heme.[20] The more symmetrical orientation in cytochrome *c* cyanide results in a more symmetrical pattern of hyperfine shifts.

Cytochrome b_5 displays a mixture of resonances.[4] This has been ascribed to varying imidazole-heme orientations caused by isomerization of the peptide backbone, and is reminiscent of the isomerism found in **1**.

A similar effect is seen for the two methyl resonances of chironomus hemoglobin.[3] We believe this is also explained by two different imidazole orientations. Our results are consistent with assignment of these resonances[23] to methyls 3 and 8 rather than 1 and 5, owing to reversal of the heme-imidazole orientation from that of Mb.

In conclusion, the orientation of the unpaired spin density in $S = 1/2$ hemin derivatives is strongly influenced by the orientation of the proximal histidine, and the NMR behavior of hemoproteins and models can be used as a diagnostic indicator of subtle changes in the heme-imidazole geometry. The chelated heme models mimic not only the electronic spectra and the dioxygen and carbon monoxide kinetic behavior[10,11,24] of Hb, and the activity of horseradish peroxidase,[25] but also the effects of imidazole-heme rotation found in hemoproteins. Further studies of the effect of this parameter on the chemistry and NMR behavior of hemoproteins is in progress.

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