–4),[40] it appears that the field gradient created at the center of the cavity of **1**-4H$^+$ is about 19 times greater than that at the center of the cavity of **1**. For the cryptates **2** and **3** where a tetrahedral arrangement of $^+$N–H$\cdots$Cl$^-$ is also formed, the χ value increases slightly as the symmetry of the ligand is reduced.

For the cryptated chloride in **4**-6H$^+$, the environment is an octahedral arrangement of the $^+$N–H bonds and a trigonal bipyramid of three oxygens and two nitrogen atoms.[19] The χ value increases to 4.4 MHz. Finally for **5**-2H$^+$ where the bonding pattern, by analogy with the X-ray structure of an other katapinate,[5] is reduced to two $^+$N–H$\cdots$Cl bonds, a χ value of 7.2 MHz is reached. From this series of χ values it appears that the QCC of cryptated chloride is controlled by the symmetry of the environment and that small differences in the $^+$N–H$\cdots$Cl$^-$ bond length suffice to create appreciable field gradients. The classical electrostatic model of quadrupolar interaction[41] predicts a $1/r^5$ relation between the values of the field gradient and the distance between the nucleus and the fluctuating dipole, that is a doubling of χ when two dipole–nucleus distances differ by about 15%. This model predicts quite nicely the behavior of aquo halide ions[42] where the distances are equal to or greater than the sum of van der Waals radii, but it fails here where some of the $^+$N–H$\cdots$Cl$^-$ distances as in [Cl$^-$ $\subset$ **1**-4H$^+$][18] and [Cl$^-$ $\subset$ **4**-6H$^+$][19] are smaller than the sum of the ionic radii of Cl$^-$ (1.81 Å)[43] and $^+$NH$_4$ (1.48 Å).[43]

A theory of chemical shifts of spherical ions predicts a linear relation between δ and χ values.[44] Such a relation has been found experimentally for ^{23}Na NMR on sodium cryptates[45] and also for other sodium complexes.[46] No correlation is found here between the deshielding of ^{35}Cl and the increasing value of χ. A possible explanation for this discrepancy might be that ^{23}Na parameters are much less sensitive than ^{35}Cl parameters to the modification of the coordination sphere.

Conclusion

Cryptate complexes allow physicochemical studies to be performed on substrates bound in a well-defined, "frozen" coordination shell. Various earlier studies have taken advantage of this feature for cationic substrates. The present results give insight into the properties of bound chloride anions and emphasize the contributions which the well-defined anion cryptates may bring to the study of the general phenomenon of anion coordination to receptor molecules of either chemical or biological nature.

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Registry No. **1**-4H$^+$Cl$^-$, 61136-83-2; **2**-4H$^+$Cl$^-$, 61136-87-6; **3**-4H$^+$Cl$^-$, 87585-87-3; **4**-6H$^+$Cl, 87585-88-4; **5**-2H$^+$Cl$^-$, 61136-91-2.

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Origin of Asymmetry in Nitrogen Hyperfine Interactions in Metmyoglobin

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Abstract: The aim of this study was to investigate possible sources that could explain the experimentally observed differences in the electron–nuclear hyperfine interactions from diagonally opposite nitrogens of heme in metmyoglobin. The Self-Consistent-Charge-Extended Hückel (SCCEH) technique was used to investigate the perturbation on heme nitrogen electron spin densities from two possible sources: First, the direct electronic orbital influence of the side chains attached to pyrrole rings was found to provide a difference in ^{14}N hyperfine couplings substantially smaller than that found by experiment. This conclusion is unchanged even when one changes the orientation of the vinyl group or the charge on the propionic acid side chains. Second, the effect on nitrogen hyperfine couplings from small distortions in the position of diagonally opposite nitrogens was investigated. The relative hyperfine couplings of diagonally opposite nitrogens were found to be strongly influenced by differences in pyrrole Fe–N bond distances. A difference of 0.018 Å between Fe–N bond distances corresponding to diagonally opposite nitrogens was sufficient to explain the difference in hyperfine couplings. Such a distortion in geometry appears to involve only a small energy of the order of 100 cal/mol and could arise out of small differences in heme–protein interactions associated with diagonally opposite pyrrole rings. Thus, while one cannot rule out other possible causes, a difference in Fe–N bond distances does appear to be a plausible source for the origin of the observed difference in ^{14}N hyperfine interactions in metmyoglobin.

Recent ENDOR experiments[1] on aquometmyoglobin single crystals have shown that the hyperfine couplings of diagonally opposite pyrrole nitrogens are significantly different from each other. Earlier theoretical investigations[2-5] of high-spin heme systems have provided overall explanations of both the absolute values and trends in experimentally observed hyperfine couplings[6-8] at ^{57}Fe, ^{14}N, and ^{1}H nuclei among different heme derivatives, such as between different halogen-substituted five-liganded heme

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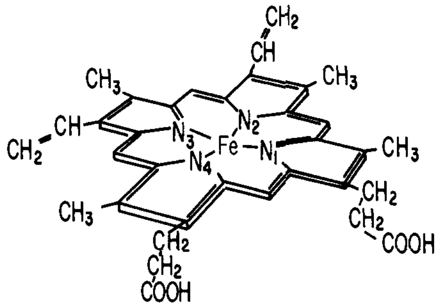

Figure 1. Protoporphyrin IX unit of metmyoglobin showing side chains of the porphyrin ring. The fifth and sixth ligands of iron (not shown) were taken to be imidazole and water as in ref 5.

systems and between these systems and aquomet– and fluoromet–myoglobin systems. In these earlier investigations,[2–5] the porphyrin base had all side chains replaced for simplicity by protons and was assumed to have tetragonal symmetry.

In the present work, we use the earlier theoretical methods to investigate finer details of the electronic structures of heme derivatives in order to explain the ENDOR observations.[1] We have examined the electronic effects of two possible sources that could contribute to the departure, as measured at the heme nitrogens, from tetragonal symmetry. First, we study the direct electronic influence at pyrrole nitrogens from asymmetrically arranged outer side chains, and second, we study the effects of the geometric inequivalences between the nitrogen atoms of the pyrroles.

Experimental Findings

The experimentally measured difference between the contact hyperfine couplings for diagonally opposite heme nitrogen pairs [(N_1,N_3) or (N_2,N_4) in Figure 1] was 0.34 MHz out of an average ^{14}N isotropic Fermi contact coupling of 7.95 MHz; this is a difference of 4.4%. A similar percentage difference for ^{15}N was also observed when the heme was 90% enriched with ^{15}N (see Figures 3–8 of ref 1). The complete hyperfine tensor was determined from the single-crystal ENDOR work. This determination showed that it was primarily the isotropic Fermi contact term a_F that accounts for the majority of the overall hyperfine interaction and which differs between heme nitrogens. The nitrogens on the two pyrrole rings with vinyl side chains (N_2 and N_3) had smaller hyperfine couplings than those on the rings with propionic acid side chains (N_1 and N_4).

Procedures, Results, and Discussion

In earlier work on metmyoglobin,[2,4,5] the protoporphyrin of the heme unit was approximated to be tetragonal and the side chains were all replaced by hydrogens, the fifth and sixth groups being taken as water and imidazole. In this model system, the porphyrin nitrogens in the molecule were almost completely equivalent, the influence of the fifth and sixth ligands on the symmetry of the nitrogens being negligible. The SCCEH procedure[9] employed in these investigations[2,4] gave a fairly good explanation of the porphyrin ^{14}N hyperfine constants and their ratio with respect to the imidazole $^{14}N_\epsilon$. In the present work, we are interested in the small observed differences[1] in hyperfine constants between members of diagonal pairs (N_1,N_3) and (N_2,N_4). We have studied the effects of two possible sources for these differences in a perturbative sense by analyzing the changes caused by these sources in the coefficients $C_{\mu i}$ in the LCAO molecular wave functions

$$\psi_\mu = \sum_i C_{\mu i}\chi_i \qquad (1)$$

corresponding to the atomic orbitals χ_i representing porphyrin N-atom orbitals.

The first source that we analyzed was the influence of the groups attached to the pyrrole rings in the protoporphyrin unit. From Figure 1, it can be seen that all four pyrrole rings have methyl groups attached. Therefore these groups cannot contribute to the observed differences in the ^{14}N hyperfine interactions. It is the electronic perturbations of the vinyl (side chains of pyrroles containing N_2 and N_3) vs. propionic acid (side chains of pyrroles containing N_1 and N_3) that appear to be important in this respect.

Table I. Percentage Difference in Pyrrole $^{14}N_2$ and $^{14}N_4$ Hyperfine Interaction Constants due to Side Chains on the Porphyrin Ring

	percentage difference in *f* between $^{14}N_4$ and $^{14}N_2$			
	vinyl off plane		vinyl on plane	
side chain	neutral propionic acid group	charged propionic acid group	neutral propionic acid group	charged propionic acid group
vinyl (pyrrole 2)	0.29	0.29	0.70	0.70
propionic acid (pyrrole 4)	0.88	1.31	0.88	1.31
propionic acid (pyrrole 1)	−0.45	−0.65	−0.45	−0.65
vinyl (pyrrole 3)	0.00	0.00	−1.30	−1.30
total	0.72	0.95	−0.17	0.06

The conformations of the vinyl and propionic acid groups are available from experimental structural information. This information indicated that the second carbon atoms of the vinyl groups (Figure 1) lie 1.15 Å above the porphyrin plane, so that they are not coplanar with the porphyrin. A change in conformation of the vinyl group toward coplanarity could provide stronger π bonding with the pyrrole groups. To study this effect, we have also considered a situation in which the vinyl groups are coplanar with porphyrin. Further, the hydrogen atom in the COOH group of propionic acid could be dissociated in an aqueous medium. To analyze the influence of this effect on the $^{14}N_2$ and $^{14}N_4$ hyperfine interactions, we have investigated the cases of both COOH or COO^- occurring in the propionic acid groups. Thus, there are four possibilities that we have studied which arise from the coplanar or noncoplanar orientations of vinyl and the presence of charged or neutral propionic acid groups. The results for the four cases are listed in Table I.

The change in the hyperfine constant for a particular pyrrole nitrogen nucleus due to the influence of the side chains is given as

$$\delta A_N = A_N - A_N{}^0 = \frac{8\pi}{6S}\gamma_e\gamma_N\hbar^2\sum_\mu\{|\psi_\mu(N)|^2 - |\psi_\mu{}^0(N)|^2\} \qquad (2)$$

where μ extends over the five unpaired spin states, γ_e and γ_N referring to the electronic and nuclear gyromagnetic ratios, respectively, with S ($= {}^5/_2$) referring to the electronic spin of the molecule. The superscript zero refers to the unperturbed systems in the absence of side chains.

The contribution to the density at a nitrogen nucleus due to an unpaired molecular orbital arises from the atomic orbitals χ_{2s} of the corresponding nitrogen atom. Consequently one can write

$$\delta A_N = \frac{8\pi}{6S}\gamma_e\gamma_N\hbar^2\sum_\mu\{|C_{\mu,2s}|^2 - |C_{\mu,2s}{}^0|^2\}\chi_{2s}{}^2(N) \qquad (3)$$

and

$$f = \frac{\sum_\mu\{|C_{\mu,2s}|^2 - |C_{\mu,2s}{}^0|^2\}}{\sum_\mu |C_{\mu,2s}{}^0|^2} \qquad (4)$$

Since a similar percentage difference in the hyperfine interaction was observed for the diagonally opposite pairs N_1 and N_3 and N_2 and N_4, we shall consider only the pair (N_2,N_4) in the rest of the paper.

The contributions to the percentage differences Δf in f between the $^{14}N_2$ and $^{14}N_4$ hyperfine constants from the effects of the various side chains and the total effect are listed in Table I. The total differences Δf for $^{14}N_2$ and $^{14}N_4$ listed in the last row of Table

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I have been obtained by combining the individual contributions from the two vinyl and two propionic acid groups attached to the pyrroles. To examine if the effects of the individual groups were mutually additive, we have studied for one of the four cases in Table I, represented by column 1, the net value of Δf when all four groups were attached. This value was found to be only 3% larger than the result in Table I obtained by summing the individual contributions. The closeness of the two results demonstrates that the effects of the various side chains are indeed additive, a feature that can be understood by noting from Table I that the individual contributions are themselves rather small, so that the influence of one group on the contribution from another is not expected to be significant. The second common feature of the results in Table I was that the major contribution to Δf in the summation over μ in eq 4 occurred through the d_{xz}- and d_{yz}-like orbitals. A likely explanation of this feature is that the d_{xz}- and d_{yz}-like orbitals are π-like and are therefore the only ones that can communicate long-range effects of the type associated with the influence of the side chains, attached to pyrrole rings, on the porphyrin nitrogens.

The net Δf for all four possibilities listed in Table I are seen to be too small to explain the observed difference in the hyperfine interaction constants for $^{14}N_2$ and $^{14}N_4$. Before discussing other plausible sources for the explanation of the experimental result,[1] we would like to comment on the trends in Δf among the four cases considered in Table I. Thus, the differences between the results in either the first and second rows or the third and fourth rows are indicative of the difference in the effects of neutral and charged propionic acid groups. The overall results in the two cases are very similar, with the contributions for the charged propionic acid group being somewhat larger. This is to be expected since the negatively charged group is a source of stronger perturbation than the neutral one. Similarly, the differences between the first and third cases or the second and fourth cases are indicative of the influence of coplanarity of the vinyl group with the porphyrin ring. These differences are seen to be somewhat stronger than that between neutral and charged propionic acid groups, leading to stronger cancellation between the contributions of individual groups and thus smaller Δf for the coplanar vinyl conformation. We would like to point out that the total energy in the case of the coplanar conformation was found to be 1.86 eV larger than that for the noncoplanar situation found from X-ray structural measurements.[10,11]

Thus, the influence of the side chains of the porphyrin ring on the spin densities on the nitrogen atoms cannot explain the observed difference in the hyperfine interaction constants of $^{14}N_2$ and $^{14}N_4$. One has therefore to investigate other sources for explaining this observed difference. One possible source that we have explored is a difference in the relative geometry of nitrogen atoms 2 and 4 (and of 1 and 3) with respect to the iron. In this connection, in recent work on nickel tetramethylporphyrin, X-ray work[12] has indicated a small but significant difference (about 1 per cent) among the Ni-N distances with Ni-N_1 and Ni-N_3 equal but different from Ni-N_2 and Ni-N_4 which are also equal. These differences have been ascribed as originating from intermolecular interactions between neighboring molecules. One might expect that differences in geometry involving similar orders of magnitude could be present in metmyoglobin from the influence of intermolecular interactions between the protein chain and the protoporphyrin IX unit. In the rest of this work we shall investigate what types of changes in the relative geometry of Fe and the four nitrogen atoms of porphyrin can explain the observed difference in $^{14}N_2$ and $^{14}N_4$ hyperfine constants and if such changes are plausible or not.

In examining the possible influence of changes in geometry on the $^{14}N_2$ and $^{14}N_4$ hyperfine interactions, we note that the spin densities in the ligand nitrogen atoms arise from the nitrogen 2s orbital component in the unpaired spin molecular orbitals in eq 1 which in the absence of mixing between the iron d orbitals and ligand orbitals would have consisted purely of the former. In a molecular orbital procedure, this mixing arises from the off-diagonal matrix elements $\mathcal{H}_{ij}$ of the electronic Hamiltonian between iron 3d and nitrogen orbitals. In the SCCEH procedure, the off-diagonal matrix elements $\mathcal{H}_{ij}$ are proportional to the overlap integral S_{ij} between the corresponding atomic orbital and the neighboring atoms, and

Table II. Percentage Changes in ^{14}N Hyperfine Constant due to Changes in Fe-N Bond Length

change in Fe-N bond length, Å	percentage change in ^{14}N hyperfine constant
+0.025	−5.06
−0.025	7.20

$$\mathcal{H}_{ij} = \frac{1}{2}K(\mathcal{H}_{ii} + \mathcal{H}_{jj})S_{ij} \tag{5}$$

with $\mathcal{H}_{ii}$ and $\mathcal{H}_{jj}$ the diagonal elements for the atomic orbitals χ_i and χ_j and K an empirical number commonly chosen as 1.89 for heme systems[9]. The diagonal elements $\mathcal{H}_{ii}$ and $\mathcal{H}_{jj}$ are obtained from the ionization energies of the neutral atom and charged ion in question by using linear interpolation between the two on the basis of the charge on the atom in the molecule. This charge in turn is related to the atomic orbital coefficients $C_{\mu i}$ in eq 1, through the Mulliken approximation.[9]

Thus, in the SCCEH procedure the changes in the matrix elements $\mathcal{H}_{ij}$ are proportional to changes in the S_{ij} for small variations in geometry of the molecule. The overlap integral between orbitals on two atoms can change because of bond distance change and/or changes in the angular orientations[13] of the orbitals with respect to the direction of the bond between the atoms. In our tests of possible changes in Fermi contact hyperfine effects due to changes in geometry, we have put most emphasis on the overlap of iron 3d orbitals and nitrogen 2s orbitals. Such overlap changes could occur through several alternative changes in geometry, notably, (a) a stretching of the Fe-N bond, (b) a puckering movement of the pyrrole N perpendicular to the heme plane, or (c) a displacement of the pyrrole N atom perpendicular to the Fe-N bond. The first type of change was found to be the mose efficient in producing overlap change between iron 3d orbitals and the nitrogen 2s orbital. The unperturbed 3d-2s overlap was −0.0577 in value, and for the three types of geometric change, the respective changes in overlap were 0.108/Å, 0.007/Å, and 0.008/Å. We were interested in the fractional change in nitrogen 2s population which would yield the observed fractional change[1] in Fermi contact interaction. If δS_{ij} is the change in overlap, one could use $2\delta S_{ij}/S_{ij}$ as an approximate measure of the fractional change in 2s spin population and hence of the fractional difference in N_2 and N_4 hyperfine constants. With this approximation, one requires large (~0.3 Å) differential vertical motions of opposite nitrogens for causes b and c to produce the 4.4% difference in Fermi contact couplings. Such a significant distortion of the porphyrin ring from planarity would probably have been seen in X-ray studies.[11]

The overlap of 3d and 2s orbitals varied most strongly with Fe-N bond length, so detailed calculations of the electronic wave function were performed for bond lengths differing from the normal (2.04 Å) Fe-N pyrrole bond length by small distortions, $\Delta = \pm 0.012$ Å. The resultant fractional changes in hyperfine constants for the slightly elongated or contracted Fe-N bonds are presented in Table II. We have fitted the fractional changes to a function $f = \alpha\Delta + \beta\Delta^2$ and obtained a value of $\Delta = 0.018$ Å that would explain the 4.4% fractional difference between hyperfine couplings of N_2 and N_4, the 0.018 Å corresponding to the difference in bond lengths between Fe-N_2 and Fe-N_4. This difference could al-

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ternatively be considered as a +0.009 Å extension of the $\mathrm{Fe{-}N_2}$ bond and a −0.009 Å contraction of the $\mathrm{Fe{-}N_4}$ bond away from a mean equilibrium distance. A change of bond length of this order is beyond the resolving power of protein X-ray techniques and perhaps of the EXAFS method.[14]

From the sum of the energies of the occupied molecular orbitals for different extensions of Fe–N bond lengths one can make an approximate estimate of the distortion energy needed to cause the small ±0.009 Å Fe–N bond length change. This energy turns out to be ~100 cal/mol. Whatever the source of the distortion energy may be, it appears that such small distortions are less than kT at room temperature, so that near room temperature the porphyrin system might sample a number of such thermally accessible, slightly distorted conformations.[15] In the ENDOR measurements[1] that we have tried to interpret in this work, the temperature involved was ~2 K, so that the porphyrin system can be frozen into a particular configuration with different Fe–N bond distances, leading to the observed difference in $^{14}N_2$ and $^{14}N_4$ hyperfine constants. Thus, while our investigations do not rule out other possible sources for this observed difference, they do suggest that a difference in $\mathrm{Fe{-}N_2}$ and $\mathrm{Fe{-}N_4}$ bond lengths at low temperatures is a reasonable source.

Conclusions

The analysis carried out here has shown that the side chains of the protoporphyrin have a rather small influence on the unpaired electron densities at the pyrrole nitrogen atoms. The smallness of this effect on the pyrrole nitrogen atom leads us to expect even smaller influence of these side chains on the hyperfine properties[2,6] at the Fe nucleus and at the nuclei on the fifth and sixth ligands. This conclusion thus provides a justification for replacing the side chains in the porphyrin ring by hydrogen atoms when overall hyperfine properties of the inner nuclei of heme are under investigation as was done in earlier work.[2-4]

In view of the inability of the perturbations due to the side chains of the pyrrole ring to provide an explanation for the observed difference in $^{14}N_2$ and $^{14}N_4$ hyperfine constants, one has to look into other sources for this difference. One such possibility that has been explored here is a difference in the relative geometry of the nitrogens N_2 and N_4 (N_1 and N_3) with respect to the Fe. It is shown that a difference between diagonally opposite Fe-N bond lengths of only 0.018 Å can explain the observed[1] hyperfine differences in metmyoglobin. This difference in Fe–N bond lengths could perhaps originate from a difference in the interactions of the various pyrrole rings with protein groups. Alternatively, this difference in $\mathrm{Fe{-}N_1}$ and $\mathrm{Fe{-}N_3}$ ($\mathrm{Fe{-}N_2}$ and $\mathrm{Fe{-}N_4}$) bond distances could be an intrinsic property of the protoporphyrin IX unit. The results of X-ray measurements on five-ligand heme systems[16] without the protein chain are inconclusive in this respect. ENDOR measurements on single-crystal hemin and other halogen derivatives would be useful in throwing light on this question.

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Alterdentate Ligands: Determination of the Energy Barrier for Intramolecular Metal Ion Exchange in Complexes of Ninhydrin and Alloxan Radical Ions with Zn^{2+}, Mg^{2+}, Cd^{2+}, and Y^{3+}

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Abstract: The anionic radical ligands derived from ninhydrin (I) and from alloxan (II) form complexes with the closed-shell metal ions Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Y^{3+}, and La^{3+}. The radicals are generated in the cavity of an ESR spectrometer by an electrochemical method in dimethylformamide solutions of the corresponding metal perchlorates. Coordination of a metal ion in one of the chelating sites A or A′ (III and IV) reduces the symmetry of the paramagnetic species from C_{2v} in the free ligand to C_s in the complex. The splitting pattern due to the ligand protons (and ^{14}N nuclei in the alloxan radical complexes) is therefore, in general, different in the complexes as compared with the free ligand. Some of the metal complexes (Ca^{2+}, La^{3+}) show nevertheless an apparent C_{2v} symmetry of the ligands. This is interpreted in terms of a rapid intramolecular exchange from site A to A′. In other cases this intramolecular exchange gives rise to selective line broadening in the ESR spectra. The exchange rate can be determined by using a two-site jump model. The observed exchange rate constants are in the range $k = 5 \times 10^4\ s^{-1}$ to $k = 2 \times 10^7\ s^{-1}$. Activation parameters are determined from the temperature dependence of the ESR spectra. These kinetic parameters for the exchange process suggest an associative mechanism for the ninhydrin radical and a dissociative mechanism for the alloxan radical. The series of metal ions with increasing intramolecular exchange rate is the same as that for water exchange in hydrated ions. INDO calculations and a molecular mechanics model indicate an out-of-plane motion of the metal ion on its pathway from site A to A′.

Alterdentate ligands offer, by definition, at least two equivalent sites for the binding of a metal ion.[3] Two ligands of this class are the radical anions of ninhydrin (I) and alloxan (II).[4]

Both ligands have in their uncoordinated forms C_{2v} symmetry, rendering the two pairs of protons (H^1/H^4 and H^2/H^3) in ninhydrin and the two NH groups in alloxan equivalent. This symmetry is mirrored in the ESR spectra of the free-radical anions,[5]

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