Models of the Cytochromes *b.* 10. Application of ^{1}H COSY To Delineate the Spin Density Distribution at the Pyrrole β Positions of Unsymmetrically Substituted Low-Spin Iron(III) Tetraphenylporphyrins

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Received May 4, 1992

The correlation of the pattern of spin delocalization with the molecular orbital coefficients of the wave functions of each carbon and nitrogen atom within the porphyrin core is a very important consideration in understanding the π-bonding effects and the mechanism of spin delocalization in model hemes.[1] For some time we have suggested that the ^{1}H NMR hyperfine shifts for the pyrrole-H resonances in a variety of low-spin (tetraphenylporphinato)iron(III) complexes may be used to describe the electron density distribution in the porphyrin π orbitals.[2–5] We have shown previously that the unpaired electron of low-spin Fe(III) is delocalized into the 3e(π) porphyrin orbitals by Por → Fe π donation to the hole in the metal d_{xz},d_{yz} set, as shown in Figure 1A,B.[1,2] The sizes of the circles denote the electron density at porphyrin ring positions and are proportional to the wave function coefficients c_i^2 at each carbon and nitrogen atom;[6] the filled/unfilled pattern of the circles denotes the nodal properties of these π orbitals, which match those of the d_π orbitals, d_{xz} and d_{yz}.

We will show in this communication that the cross-correlations obtained from homonuclear correlation spectroscopy (COSY) experiments substantiate the theoretical predictions,[1,2] based on molecular orbital calculations,[6] of the pattern of spin delocalization in the 3e(π) porphyrin orbitals of low-spin Fe(III) complexes of unsymmetrically substituted tetraphenylporphyrins. Mono-ortho-substituted derivatives of $[\text{TPPFe(NMeIm)}_2]^+$ have been investigated by two-dimensional NMR spectroscopy[7] in an attempt to delineate the inductive or conjugative effect of the phenyl ring bearing the ortho substituent on the pattern of spin delocalization in model hemes. The COSY-45 spectra of two representative examples, $[(o\text{-OEt})_1\text{TPPFe(NMeIm)}_2]\text{Cl}$[5] (**1**) and $[(o\text{-Cl})_1(p\text{-OCH}_3)_3\text{TPPFe(NMeIm)}_2]\text{Cl}$[8,10] (**2**) reveal cross-correlations that are consistent with those expected for the electron density distribution in these orbitals,[1,2,6] as modified by the unique substituent[2–5] (Figure 1C–F), as will be discussed in detail below.

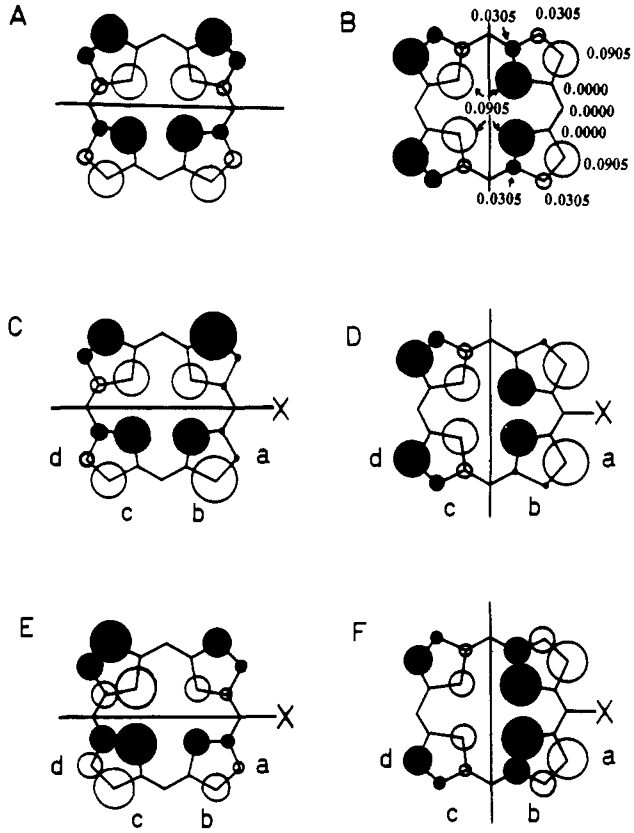

Figure 1. Symmetry properties of and electron density distribution in the filled 3e(π) porphyrin orbitals[6] that have proper symmetry for overlap with the d_{xz} and d_{yz} orbitals of low-spin Fe(III): (A,B) Linear combinations of the 3e(π) orbitals that are appropriate for meso-substituted porphyrins;[2] (C,D) exaggerated modifications of these orbitals due to the presence of one uniquely substituted phenyl ring, X, if the effect of the substituent is felt only as far as the adjacent pyrrole positions a and b; (E,F) exaggerated modifications of these orbitals if the effect of the unique substituent is felt over all four pyrrole positions a–d. Since we expect the proton attached to the carbon with largest spin density to have the largest hyperfine shift, the coupling pattern predicted by these simple modifications is a,b, and c,d, or pyrrole-H peaks 1,4 and 2,3 in the case of C and D or peaks 1,3 and 2,4 in the case of E and F.[20,21]

As can be seen in Figure 2A,C, four resonances are observed for the eight pyrrole protons of **1** and **2**, due to unsymmetrical substitution.[12] On the average, the phenyl rings are expected to be perpendicular to the macrocycle,[14] thus producing four symmetry-related pyrrole positions, labeled a–d in Figure 1C–F, where the electronic effect of the unique substituent has been

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(7) Mono-ortho-substituted complexes were chosen because they typically have four resolved pyrrole-H resonances,[4,5] while unsymmetrically-substituted derivatives of $[\text{TPPFe(NMeIm)}_2]^+$ having only meta or para substituents typically have only three pyrrole-H resonances, one of which is twice the intensity of the other two.[2–4]

(8) The mono-ortho-substituted tetraphenylporphyrin free bases were synthesized and purified as described previously.[5,9] Iron was inserted, and the bis(*N*-methylimidazole) complexes were formed as described previously.[3] Detailed characterization of the unsymmetrically ortho-substituted tetraphenylporphyrin free bases from which the Fe(III) porphyrins of this study were prepared and investigation of other physical properties and chemical reactions of their Fe(III) complexes are, or will be, described elsewhere.[5,10,11]

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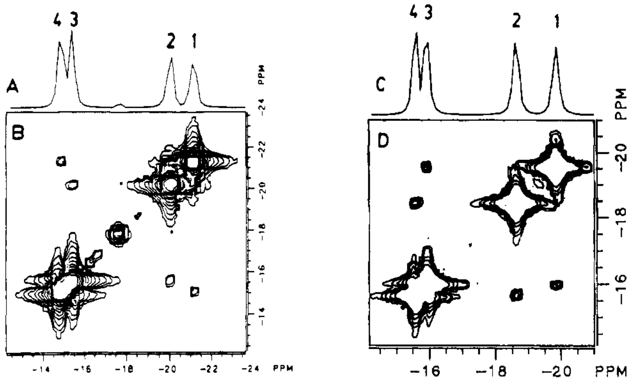

Figure 2. (a) One-dimensional NMR spectrum of the pyrrole-H region of $[(o\text{-OEt})_1\text{TPPFe(NMeIm)}_2]\text{Cl}$[5] (**1**) in CD_2Cl_2 recorded at 300 MHz at room temperature. The small peak at −17.5 ppm is due to the presence of a small amount of $[\text{TPPFe(NMeIm)}_2]^+$ in the sample. (B) COSY-45 spectrum of the pyrrole-H region of **1** recorded at room temperature, showing the 1,4 and 2,3 coupling pattern. (C) One-dimensional NMR spectrum of the pyrrole-H region of $[(o\text{-Cl})_1(p\text{-OCH}_3)_3\text{TPPFe(NMeIm)}_2]\text{Cl}$[8,10] (**2**) in CD_2Cl_2 recorded at room temperature. (D) COSY-45 spectrum of the pyrrole-H region of **2** recorded at room temperature, showing the 1,3 and 2,4 coupling pattern.

exaggerated. If the phenyl substituent is electron-donating, the modified π orbital shown in Figure 1C,E should be slightly lower in energy than the orbital shown in Figure 1D,F, while if the substituent is electron-withdrawing, the π orbital shown in Figure 1D,F should be slightly lower in energy than that shown in Figure 1C,E. Thus, one of these two $3e(\pi)$ orbitals will be favored for spin delocalization over the other (or more precisely, *a larger fraction of one than the other* will be favored for spin delocalization). On the basis of these considerations, scalar couplings are expected among the pairs of protons H_a,H_b and H_c,H_d or between resonances labeled 1,4 and 2,3 (Figure 1C,D) or 1,3 and 2,4 (Figure 1E,F), depending upon the distance over which the inductive and/or conjugative effects of the unique phenyl substituent operate. The COSY-45 maps[15,16] of complexes **1** and **2**, shown in Figure 2B,D, indeed illustrate these two coupling patterns. In spite of the short T_2 relaxation times (8–9 ms),[17-19] cross-correlations are observed between resonances 1 and 4 and resonances 2 and 3 for **1** (Figure 2B). In contrast, complex **2** shows cross peaks among the pairs of resonances 1,3 and 2,4 (Figure 2D). Cross-peak intensities for these complexes are significantly diminished when a COSY-90 experiment is performed instead of a COSY-45.

The correlations observed (Figure 2B,D) clearly demonstrate that *the spin densities at the pyrrole-H positions are large and small within each pyrrole ring*, consistent with the expected electron density distribution in the $3e(\pi)$ orbitals depicted in either part A or B of Figure 1. They show, furthermore, that this π electron density distribution is differently modified by the electronic properties of the unique substituent in each case. If the substituent effect is felt only over the immediately adjacent pyrrole rings (that is, if C_a simply "borrows" (or "donates") π electron density from (to) C_b, leaving the π electron density in the other pyrrole ring unperturbed), then the modification of the $3e(\pi)$ orbitals is expected to be that shown in Figure 1C,D. This pattern gives rise to the 1,4 and 2,3 cross-correlations shown by compound **1** in Figure 2B. Other complexes that also have this coupling pattern include $[(o\text{-OCH}_2\text{Ph})_1\text{TPPFe(NMeIm)}_2]^+$ [5] and $[(o\text{-COOH})_1\text{TPPFe(NMeIm)}_2]^+$.[5,8,11] This pattern suggests that the effect of the phenyl ring bearing the substituent on the π system of the porphyrin ring is inductive in nature, since it extends only as far as pyrrole position b. In contrast, if the electronic effect of the substituent is distributed over both sets of pyrrole rings, i.e., positions a–d (that is, if both C_a and C_b "borrow" (or "donate") π electron density from (to) C_c and C_d), then the modification of the $3e(\pi)$ orbitals is expected to be that shown in Figure 1E,F. This pattern gives rise to the 1,3 and 2,4 cross-correlations observed in the COSY-45 map of by compound **2** (Figure 2D) and suggests that the phenyl ring bearing the ortho substituent may be in resonance with the porphyrin ring at least some fraction of the time. Other complexes that show this coupling pattern include $[(2,6\text{-Cl}_2)_1(p\text{-OCH}_3)_3\text{TPPFe(NMeIm)}_2]^+$.[8,10] However, while the exact amount of spin density present at the pyrrole-H positions c and d differs for **1** and **2**, the similarity in the chemical shifts of the two resonances 3 and 4 dictates that the amount of spin delocalization to these two positions is both relatively small and similar in the two cases. This indicates that the perturbation of the $3e(\pi)$ orbitals shown in figure 1A,B, beyond position a, by the ortho substituent is relatively small in both cases. We are currently investigating additional unsymmetrically substituted $[\text{TPPFe(NMeIm)}_2]^+$ complexes by COSY and NOESY (nuclear Overhauser and exchange spectroscopy) methods in order to understand more fully how spin density is distributed in these important model complexes and to be able to predict whether the substituent in the ortho position of one phenyl ring is electron-donating or electron-withdrawing.[20] Preliminary NOESY studies at low temperatures[21] show additional cross peaks that may lead to an absolute assignment of the four pyrrole proton resonances.

Acknowledgment. Financial support from the National Institutes of Health (Grant DK 31038 (F.A.W.)) is gratefully acknowledged. The Chemistry and Biochemistry Department at San Francisco State University gratefully acknowledges grants from the NIH (RR 02684) and the NSF (DMB-8516065) for the purchase of the NMR spectrometer.

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(12) Despite the existence of four resonances for **1** and **2**, the average chemical shift is in both cases very similar to that of the pyrrole-H resonance of the small amount of $[\text{TPPFe(NMeIm)}_2]^+$ present in **1** (−17.8 ppm (**1**), −17.5 ppm (**2**), −17.7 ppm ($[\text{TPPFe(NMeIm)}_2]^+$)), suggesting a fairly constant average amount of spin delocalization in all three cases, as observed previously for a variety of unsymmetrically substituted $[\text{TPPFe(NMeIm)}_2]^+$ derivatives.[4] This fairly constant average amount of spin delocalization is differently distributed, depending upon the symmetry and nature of the porphyrin substituents.[4,13]

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(14) Meyer, E. F.; Cullen, D. L. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. III, pp 513–529 and references therein.

(15) The magnitude COSY-45 spectra[16] were acquired on a 300-MHz NMR spectrometer at room temperature using a mixing pulse of 45°, 128 t_1 increments, and 256 real data points in t_2, with 64 transients per t_1 increment. The number of t_2 data points and the spectral bandwidth were chosen to yield an acquisition time of 14.85 ms, approximately 2 × (longest T_2). When the acquisition time in t_2 was chosen to be larger than $2 \times T_2$, cross-peak intensity diminished significantly. Prior to Fourier transformation, the 2-D matrix in each dimension was multiplied with an unshifted sine-bell-squared window function and zero-filled once in t_1 to give a 256 × 256 word square matrix.

(16) (a) Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; D. Reidel Publishing Co.: Dordrecht, Holland, 1984. (b) Bax, A.; Freeman, R.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 164.

(17) Short T_2^* relaxation times, such as those of this system (8–9 ms),[18] can lead to significant cancellation of the antiphase components, thus preventing detection of cross-peak intensity.[19]

(18) Determined from line widths at half-height, Δ: $T_2^* = (\pi\Delta)^{-1}$.

(19) Keating, K. A.; de Ropp, J. S.; La Mar, G. N.; Balch, A. L.; Shiau, F.-Y. *Inorg. Chem.* **1991**, *30*, 3258–3263.

(20) Results obtained from other techniques (formation constants for the *N*-methylimidazole complexes, derived slopes and intercepts of Curie plots) yield conflicting conclusions as to the electron-withdrawing/-donating properties of phenyl ortho substituents.[10]

(21) Preliminary NOESY investigations of **1** and **2** reveal cross peaks among the pairs of resonances 1,3 and 2,4 or 1,4 and 2,3, respectively. This is not surprising, since the scalar coupled protons a,b and c,d, are also close in space and thus should show NOEs.[22] For **2**, additional cross peaks are observed at low temperatures between resonances 1 and 2, consistent with the electron density distribution depicted in Figure 1E, and with the electronic effect of the substituent being distributed over both sets of pyrrole rings. For **1**, additional NOESY cross peaks between resonances 3 and 4 support the electron density distribution shown in Figure 1D. To rule out the possibility that these additional cross peaks may be due to chemical exchange with the high-spin five-coordinate intermediate, for which all pyrrole-H signals overlap, we are currently performing detailed NOESY and ROESY investigations of these and other complexes as a function of temperature.

(22) Bovey, F. A.; Jelinski, L.; Mirau, P. A. In *Nuclear Magnetic Resonance Spectroscopy*; Bovey, F. A., Ed.; Academic Press: New York, 1988.