ROESY: A Technique for Establishing the Existence of Chemical Exchange in Paramagnetic Model Hemes with Short T_1 and T_2 Relaxation Times

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Two-dimensional nuclear Overhauser enhancement and chemical exchange spectroscopy in laboratory (NOESY)[4-7] and rotating (ROESY)[8-12] frames have become powerful methods for investigating molecular structures and dynamics.[4-14] Both methods provide information about cross relaxation among spatially proximal and chemically exchanging spins. Whereas NOESY or its phase-sensitive version[4,13,14] is not always suitable for differentiation of the two effects, it has been suggested that ROESY provides an attractive alternative to distinguish chemical exchange from cross-relaxation.[9-14] The fundamental advantage of ROESY over NOESY is that spin-locked NOEs are always positive; i.e., there is no region of $\omega\tau_c$ for which enhancements are small or zero, as in NOESY. Furthermore in the spin-locked experiment, cross peaks due to NOEs are inverted relative to diagonal peaks, whereas those due to chemical exchange are in phase with the diagonal.[9]

Only a few reports have appeared of use of 2-D methods for conformational and dynamic analysis of paramagnetic molecules[15-17] including heme proteins and heme model complexes.[18-22] The lack of studies is largely due to the fact that paramagnetic molecules exhibit drastically enhanced relaxation rates, which hamper the use of the 2-D methodology.[18-23] Nevertheless, we will show that ROESY provides an elegant method for unambiguously establishing the existence of chemical exchange in a paramagnetic complex. We have chosen the bis(*N*-methylimidazole) complex of (mono(*o*-dialkylamido)tetraphenylporphinato)iron(III) chloride (**1**), where the carboxamide substituent is derived from 3-aza-bicyclo[3.2.2]nonane,[23,24] to demonstrate both the power and limitations of the ROESY technique. NOESY and saturation transfer experiments performed at several temperature nicely complement the ROESY studies.

The pyrrole protons of **1** exhibit a unique resonance pattern, as reported earlier.[23,24] Eight individual signals are observed, which coalesce into two broad peaks[25] at the high-temperature limit of the solvent ($CDCl_3$). To further investigate the exchange process we have acquired ROESY spectra of **1** on a 500-MHz spectrometer at room temperature and at −20 °C.[26] The ROESY map shown in Figure 1a clearly reveals cross peaks among the pairs of resonances 1,4; 2,3; 5,8; and 6,7. They have the same phase as the diagonal and are therefore chemical exchange cross peaks. No direct NOE correlations, which would be inverted relative to the diagonal, are detectable. This is consistent with the prediction that the relaxation mechanism in this paramagnetic complex is mainly determined by dipolar interactions of the proton spins with the spin of the unpaired electron and not by dipole–dipole interactions between proton spins.[27] The dipolar interactions between protons and the unpaired electron thus effectively quench cross-relaxation in the rotating frame. Chemical exchange, resulting from hindered rotation of the axial ligand on the same side of the porphyrin plane as the carboxamide substituent, is still rapid on the NMR time scale at −20 °C and is the dominant contribution to the magnetization transfer.[28] To determine whether the observed cross-correlations might be overshadowed by homonuclear Hartmann–Hahn artifacts,[10,29-31] HOHAHA experiments were also performed, but no cross peaks were detectable under any experimental condition.

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(25) Each of these broad peaks is believed to consist of two unresolved resonances, as is observed for the dimethylcarboxamido derivative at high temperatures.[23]
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(28) Axial ligand exchange is not the dominant contribution to magnetization transfer at this temperature, since the calculated rate constants for exchange of the two *N*-methylimidazole lignads are 0.053 and 0.449 s^{-1}.[24] Hence the lifetimes of bound *N*-MeIm molecules are 19 and 2.2 s, respectively, long in comparison to mixing times used in the ROESY experiment.
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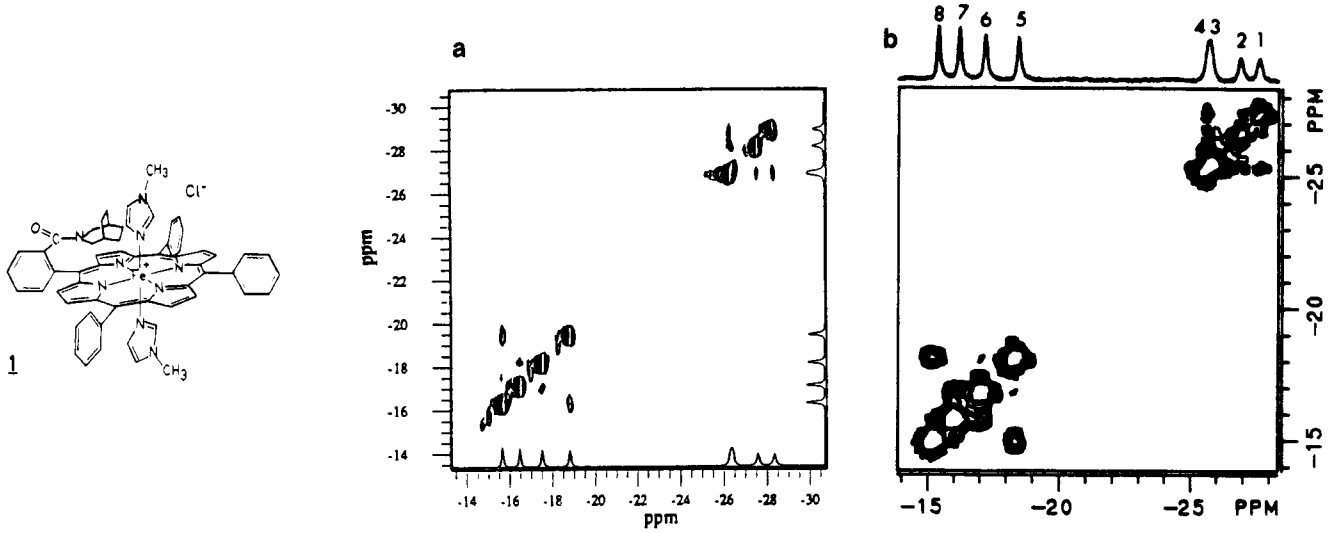

Figure 1. (a) ROESY spectrum of **1** in $CDCl_3$ at −20 °C, recorded at 500 MHz. Cross peaks are observed between resonances 1,4; 2,3; 5,8; and 6,7, which have the same phase as the diagonal. (b) NOESY spectrum of **1** in $CDCl_3$ at −15 °C, recorded at 300 MHz. In addition to the same sets of cross peaks observed in the ROESY spectrum shown in Figure 1a, additional cross peaks are observed between peaks 5,6 and 7,8.

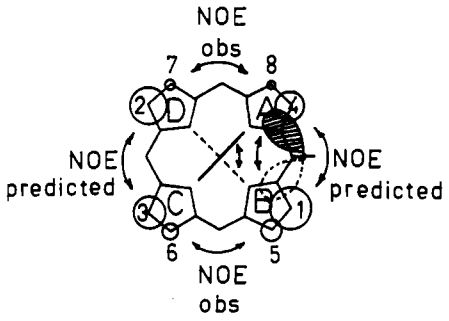

Figure 2. Proposed model for the assignment of pyrrole proton peaks of **1**, which is consistent with both chemical exchange and NOE cross peaks observed in the NOESY spectrum shown in Figure 1b. In this revised model (peaks 6 and 7 reversed from the original[24]), NOE cross peaks arise from cross-relaxations that occur between protons in pyrrole rings A and D and rings B and C. As proposed previously,[24] chemical exchange occurs as both the carboxamide and axial ligand switch from being positioned over pyrrole ring A to being over B.

In spite of the short T_1 relaxation times of the complex (3–5 ms at −25 °C),[32,33] NOESY spectra were successfully obtained at +20, 0, −15, −20, and −25 °C, with optimized mixing times of 30, 25, 20, 15 and 10 ms, respectively.[34] The NOESY map at −15 °C, shown in Figure 1b, reveals the same cross-correlations as does the ROESY spectrum (Figure 1a). Additional cross peaks are also observed between resonances 5,6 and 7,8. These cross peaks must be due to NOE cross-relaxations between protons that are in close proximity, since they become relatively more pronounced at lower temperatures, where chemical exchange is suppressed. Unfortunately, NOE correlations are not observed among other resonances, due to the significantly shorter T_1s of signals 1–4, which may render cross peak intensities undetectable, as discussed previously by La Mar[18] and Bertini.[20–22] Strong paramagnetically-induced relaxation precludes observation of any cross peaks below −25 °C.

To determine whether the correlations observed among the resonances 5,6 and 7,8 are due to NOE cross-relaxations between protons within the same pyrrole ring, we acquired COSY spectra of **1**. However, no couplings were observed, due to the short T_2 relaxation times of the complex[15,18,19] (3.0–4.4 ms at 25 °C[35]) and/or the fact that the protons that give rise to the 5,6 and 7,8 cross-correlations in the NOESY spectrum are in *different* pyrrole rings, facing each other on either side of phenyl rings, as suggested in Figure 2. This model for the electron densities at the pyrrole-H positions is slightly revised from that proposed earlier,[24] in which the assignments of resonances 6 and 7 are reversed. On the basis of the model shown in Figure 2, COSY cross-correlations are expected among the pairs of resonances 1,5; 2,7; 3,6; and 4,8, while NOE cross peaks are expected between resonances 5,6; 7,8; 1,4; and 2,3. Although we have been unable to observe any COSY cross-correlations, the observation of NOE cross peaks between resonances 5,6 and 7,8 are consistent with the revised model shown in Figure 2.[36]

Saturation transfer experiments, performed from +25 to −30 °C, are consistent with the ROESY and NOESY results. At the lowest temperatures, irradiation of resonances that are related through chemical exchange has no effect on the intensity of exchange related resonances, whereas when the temperature is raised, irradiation results in remarkable reduction of signal intensity, as shown in Figure 3 (supplementary material).

These studies show that the ROESY experiment can be used to verify the existence of chemical exchange in paramagnetic complexes such as model hemes. Furthermore, for the investigation of chemical exchange, the ROESY experiment provides an attractive alternative to NOESY and saturation transfer experiments carried out at several temperatures. These experiments give the same information, but are more time-consuming, since they must be performed at several temperatures, and experimental parameters must be optimized at each temperature.

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Supplementary Material Available: Figure 3, one-dimensional saturation transfer spectra of the pyrrole protons of **1** at 0 and −15 °C (1 page). Ordering information is given on any current masthead page.

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