J. Am. Chem. Soc. 1981, 103, 6988-6989

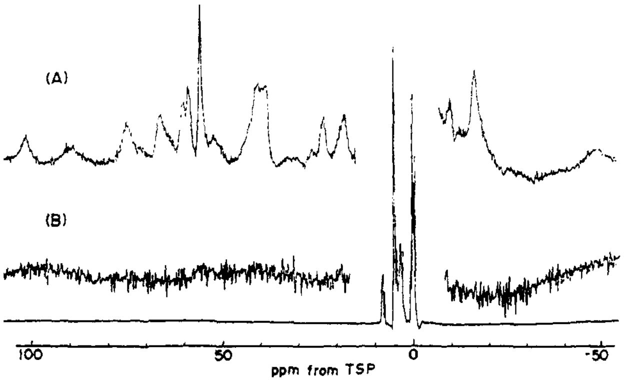

Figure 2. 220-MHz FT ^{1}H NMR spectra of (A) PYML-Fe(II) complex and (B) PYML-Fe(II)-CO complex at 20 °C and pD 7.6.

adduct complexes[14,17] (see Table II). The ESR spectra of PYML-Co(II) (Figure 1 and Table I) strongly support the presence of a nitrogen atom in the axial position. Similar to the BLM-Fe(II) complex, the PYML-Fe(II) complex showed large proton paramagnetic shifts which are due to the contact and pseudocontact effect of the central Fe(II) ion (see Figure 2). The magnitude of the proton chemical shifts suggests the presence of a high-spin Fe(II) ion ($S = 2$) for the PYML-Fe(II) complex. Upon CO binding to the PYML-Fe(II) complex, the pronounced proton paramagnetic shifts completely disappeared, indicating the presence of a diamagnetic Fe(II) ion ($S = 0$) in the CO adduct.[8e] The spin-trapping experiments[14] using *N-tert*-butyl-α-phenylnitron (BPN) or 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) at pH 6.9 clearly revealed that hydroxyl radicals are generated in the PYML-Fe(II)-O_2 complex system.[19] The ESR pattern and parameters were as follows: BPN spin adduct (triplet of doublet, $g = 2.0057$, and $a^N = 15.3$ G) and DMPO spin adduct (quartet, $g = 2.0058$, and $a^N = a_\beta{}^H = 15.2$ G). In contrast with the corresponding PYML-Fe(II)-O_2 complex system, the CO introduction strongly interfered with the O_2 activation by the PYML-Fe(II) complex. Carbon monoxide is in competition with dioxygen for interaction with the PYML-Fe(II) complex and is a typical O_2 antagonist, just as with the BLM-Fe(II) complex.

Of special significance is the fact that even such a simple oligopeptide like the synthetic model compound **8** is able to mimick the metal binding and dioxygen reduction by BLM ligand. In addition, the present results strongly support the proposed metal-binding site in which (1) the β-aminoalanine-pyrimidine-β-hydroxyhistidine region of the BLM molecule is substantially important for the Fe(II) and dioxygen interaction and (2) the gulose-mannose and methylvalerate moieties in BLM are not necessarily participating as direct ligands toward the Fe(II) binding.

On the basis of the results described here, we believe that there is good hope for the design of simple synthetic systems that will approach the mechanism of the reductive activation of molecular oxygen and explore the nature of chemical and biochemical oxidation with the PYML-Fe(II)-O_2 complex. Work is continuing in this effort.

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(17) Burger, R. M.; Peisach, J.; Blumberg, W. E.; Horwitz, S. B. *J. Biol. Chem.* **1979**, *254*, 10906.

(18) Pillai, R. P.; Lenkinski, R. E.; Sakai, T. T.; Geckle, J. M.; Krishna, N. R.; Glickson, J. D. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 341.

(19) The ESR spectra were obtained by oxygen bubbling of 0.5 mM PYML-Fe(II) complex, 0.5 mM PYML-Fe(II) complex plus 5.0 mM $Na_2S_2O_4$, and 0.5 mM PYML-Fe(II)-CO complex in the presence of 0.08 M BPN. Conditions of ESR spectroscopy: microwave power, 10mW; modulation amplitude, 0.5 G; time constant, 0.03 s; scan time, 4 min. The radical spin concentration of the PYML-Fe(II) complex system was estimated to be approximately 20% of that of the BLM-Fe (II) complex system. Therefore, it is inferred that the sugar portion of BLM contributes to the more effectve dioxygen activation by the Fe(II) complex.

Use of Spy Nuclei for Relaxation Studies in Nuclear Magnetic Resonance

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It is well-known that the study of spin-lattice relaxation in multilevel spin systems, such as coupled $I = {}^1/_2$ nuclei or quadrupolar nuclei, provides valuable information on molecular motional processes.[2] We should like to demonstrate how the measurement of the relaxation rates can be greatly simplified by the introduction of an additional nonparticipating "spy" nucleus.

The time evolution of the energy level populations P_i is governed by the master equation[3]

$$\frac{d}{dt}\mathbf{P}(t) = \mathbf{W}[\mathbf{P}(t) - \mathbf{P}_{eq}] \qquad (1)$$

where the relaxation matrix $\mathbf{W}$ contains all transition probabilities W_{ij} between pairs of energy levels. In conventional relaxation measurements, one is confined to measuring population *differences* as functions of an interval τ between initial perturbation and observation. Even within the initial rate approximation,[4] a difference $P_i - P_j$ is invariably affected by the transition probabilities W_{ik} and W_{jk} for all values of k. Their separation requires a laborious analysis based on extensive sets of experiments obtained with carefully chosen selective perturbations.[4]

Consider an additional nucleus (such as carbon-13) which is introduced into the spin system under investigation (e.g., a set of coupled protons). Provided the "spy" nucleus interacts through scalar or dipolar coupling with all nuclei in the original set, the spectrum of the spy will consist of a multiplet where each transition T_i corresponds to one particular state i of the spins under investigation. The migration of population from state i to state j resulting from an initial perturbation can now be observed through the migration of intensity between the transitions T_i and T_j of the "spy" nucleus. Experiments of this kind can be carried out most efficiently by two-dimensional exchange spectroscopy[5,6] which has hitherto been applied to chemical exchange[7,8] and spin diffusion or transient Overhauser effects.[9-11]

The spy nucleus is subjected to a pair of 90° pulses, separated by an evolution period t_1, and followed by a mixing interval τ_m which terminates with a 90° observation pulse with subsequent data acquisition. If signal intensity migrates from spy transition T_i to T_j in the τ_m period, a peak appears in the 2-D spectrum at $\omega_1 = \omega_i$ and $\omega_2 = \omega_j$. Within the limits of the initial rate approximation (neglecting relaxation actively involving the spy nucleus), the amplitude of such a peak is simply proportional to the transition probability, $A_{ij} \propto W_{ij}\tau_m$. With an appropriate form of difference spectroscopy,[12] it is also possible to obtain diagonal

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(1) Physics Department, Huadong Normal University Shanghai, People's Republic of China.

(2) Vold, R. L.; Vold, R. R. *Prog.* NMR *Spectrosc.* **1978**, *12*, 79.

(3) Abragam, A. "The Principles of Nuclear Magnetism"; Clarendon Press: Oxford, England, 1961; Chapter VIII.

(4) Freeman, R.; Wittekoek, S.; Ernst, R. R. *J. Chem. Phys.* **1970**, *52*, 1529.

(5) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.

(6) Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259.

(7) Meier, B. H.; Ernst, R. R. *J. Am. Chem. Soc.* **1979**, *101*, 6441.

(8) Huang, Y.; Macura, S.; Ernst, R. R. *J. Am. Chem. Soc.* **1981**, *103*, 5327.

(9) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95.

(10) Kumar, Anil; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1.

(11) Bösch, C.; Kumar, Anil; Baumann, R.; Ernst, R. R.; Wüthrich, K., *J. Magn. Reson.* **1981**, *42*, 159.

(12) Bodenhausen, G.; Huang, Y.; Ernst, R. R., to be published.

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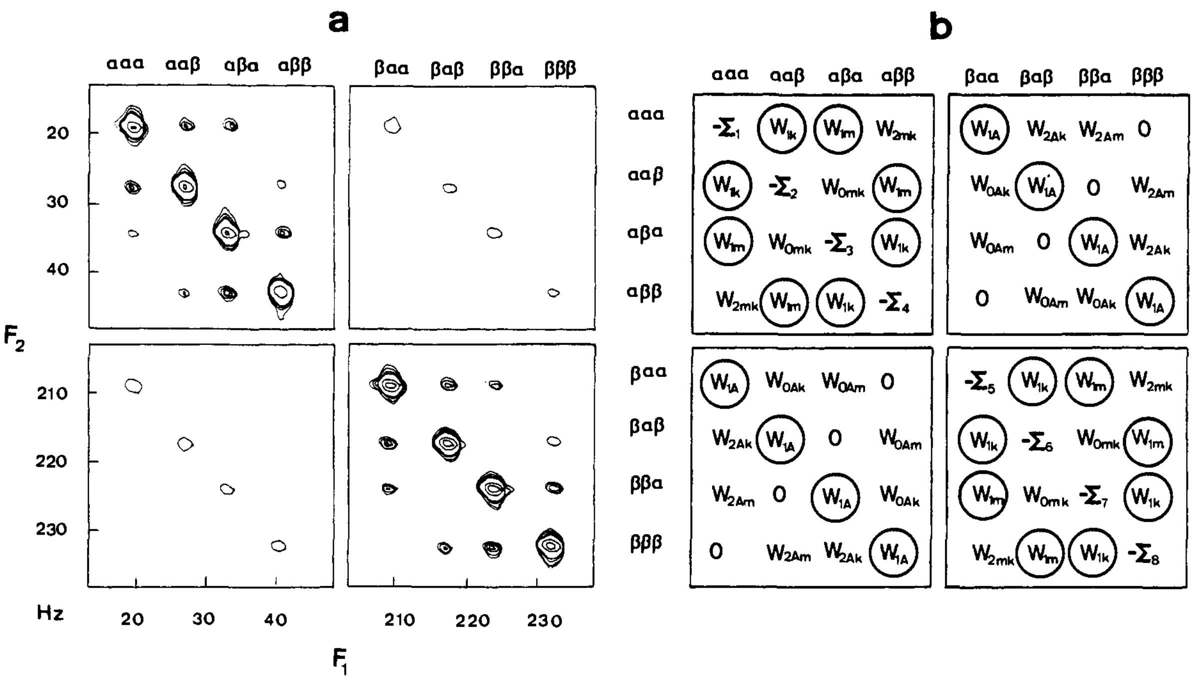
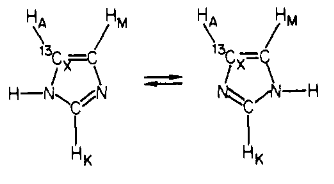


Figure 1. (a) Absolute value contour plot of 2-D relaxation map of the C_X resonance in imidazole (I) obtained with a 3.2 M solution in Me_2SO with 5×10^{-5} M $Gd(fod)_3$ to enhance external random field proton relaxation. The spectrum was obtained at 75 MHz with a Bruker CXP 300 spectrometer with 512 t_1 values in increments of 2 ms with a mixing time $\tau_m = 2.5$ s. Along the main diagonal, the eight peaks of the proton-coupled carbon-13 multiplet appear ($J_{AX} = 189$, $J_{MX} = 13$, $J_{KX} = 8$ Hz). The off-diagonal peaks have amplitudes proportional to the relaxation rates W_{ij} between proton states. (b) The theoretical relaxation matrix W contains 24 single-quantum, 12 zero-quantum, and 12 double-quantum off-diagonal elements. In the presence of $Gd(fod)_3$, the single quantum pathways (in circles) dominate, leading to the distribution of peak amplitudes evident from the experimental spectrum.

peaks with intensities A_{ij} proportional to W_{ij}.

Consider, for example, the three coupled protons of imidazole (I). The proton-coupled carbon-13 spectrum of C_X reveals eight lines belonging to a weakly coupled AMKX system (the N–H proton is not observable because of rapid chemical exchange). The eight carbon-13 lines, which appear along the diagonal of the 2-D "relaxation map" shown in Figure 1a, correspond to the eight possible proton states (ααα, ααβ,. . .etc.)



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There is a one-to-one correspondence between the 2-D map (which may contain a maximum of 8 × 8 peaks) and the W matrix, each element W_{ij} being represented by a peak intensity A_{ij}. The structure of the W matrix is shown in Figure 1b. W_{1A}, W_{1M}, and W_{1K} represent single-spin relaxation of the protons A, M, or K. Elements of the type W_{0AM} or W_{2AM} involve concerted flips of two spins A and M, either in the form of zero-quantum ($\alpha\beta \rightleftarrows \beta\alpha$) or double-quantum transitions ($\alpha\alpha \rightleftarrows \beta\beta$).

In order to test the correspondence between the 2-D map and the W matrix, the proton relaxation was enhanced by 5×10^{-5} M $Gd(fod)_3$. As a result, external random field relaxation dominates. The experimental spectrum clearly confirms that only single quantum processes W_{1A}, W_{1M}, and W_{1K} can be observed. Thus only 24 out of a maximum of 48 off-diagonal peaks appear.

Finally, we have to consider the (undesired) influence of the spy nucleus on the relaxation of the proton spins under investigation. Dipolar interaction between the spy X and the nuclei A, M, and K opens additional relaxation pathways which perturb the apparent relaxation rates of the protons. In general, only single quantum transition probabilities (e.g., W_{1A}, W_{1K} and W_{1X}) are perturbed while zero and double quantum transition probabilities such as W_{0AM} or W_{2AM} remain unaffected. In the present system, the perturbation is restricted to the A nucleus because of the weakness of the dipolar KX and MX interactions. The apparent transition probability W_{1A}^{app}, determined from the experimental peak amplitudes, is then

$$W_{1A}^{app} = W_{1A} + W_{1A}^{AX} - \tfrac{1}{2}(W_{0AX} + W_{2AX}) \qquad (2)$$

where W_{1A} is the transition probability in the *absence* of the spy, consisting of three contributions $W_{1A} = W_{1A}^{external} + W_{1A}^{AM} + W_{1A}^{AK}$. The three terms W_{1A}^{AX}, W_{0AX}, and W_{2AX} are single, zero, and double quantum transition probabilities induced by the dipolar AX interaction. These terms invariably lead to a reduction of the apparent transition probability (for extreme narrowing, we find $W_{1A}^{X} - {}^{1}/_{2}(W_{0AX} + W_{2AX}) = -{}^{4}/_{3}W_{1A}^{X}$). Close inspection of the experimental 2D spectrum indeed confirms that the peaks associated with the transition probability W_{1A} are weaker then expected.

Thus a single 2-D experiment provides a great deal of insight into a moderately complex multilevel spin system. An analysis with conventional means would represent a formidable task. The method is also readily applicable to relaxation of quadrupolar spins such as deuterium or nitrogen-14 which can be monitored indirectly via ^1H or ^{13}C resonance, provided a resolved coupling exists to relay the information. Applications to the indirect observation of electron spin relaxation in paramagnetic molecules are also conceivable.

We should mention that another experiment for the indirect measurement of relaxaiton rates has been proposed by Morris.[13] In contrast to the present scheme, it involves a transfer of magnetization between the two nuclear species and does not allow the direct separation of individual transition probabilities.

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(13) Morris, G. A. *J. Magn. Reson.* **1981**, *41*, 185.