**Paramagnetic relaxation of nuclear singlet states**

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Paramagnetic relaxation of nuclear singlet states

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Nuclear singlet states often display lifetimes that are much longer than conventional nuclear spin relaxation times. Here we investigate the effect of dissolved paramagnetic species on the singlet relaxation of proton pairs in solution. We find a linear correlation between the singlet relaxation rate constant $T_S^{-1}$ and the longitudinal relaxation rate constant $T_1^{-1}$. The slope of the correlation depends on the nature of the paramagnetic relaxation agent, but typically, singlet states are between two to three times less sensitive to paramagnetic relaxation than conventional nuclear magnetization. These observations may be interpreted using a model of partially-correlated local fields acting on the nuclear sites. We explore the effect on singlet relaxation of adding a metal-ion-chelating agent to the solution. We also investigate the effect of ascorbate, which reacts with dissolved oxygen.

Nuclear singlet states are exchange-antisymmetric states of spin-$1/2$ pairs that often display unusually long relaxation time constants. The paradigm of a nuclear singlet state is provided by the form of dihydrogen known as para-hydrogen, which is often so long-lived that the para-hydrogen nuclear singlet state and the ortho-hydrogen nuclear triplet states may be regarded as separate physical and chemical species, i.e. allotropes of hydrogen gas. The exceptional stability of para-hydrogen allows one to generate metastable nuclear spin states of hydrogen gas that are far from thermal equilibrium: reactions of such species with suitable substrates generate enormously enhanced nuclear magnetic resonance (NMR) signals.

It is possible to construct nuclear singlet states in ordinary chemical compounds containing pairs of spins-$1/2$, and these may also display unusually-long lifetimes. The NMR observation of nuclear singlet states in polyatomic molecules relies on a finite chemical shift difference, or other symmetry-breaking interaction, between the participating spins. This allows thermal magnetic triplet order to be converted into non-magnetic singlet order, and returned to triplet order at a later time for observation of the NMR signal. The singlet-to-triplet transitions may be suppressed temporarily by transporting the sample into a region of low magnetic field, or by applying a resonant spin-locking field. Singlet lifetimes as long as 25 minutes have been observed for $^{15}\text{N}$-labeled nitrous oxide in a deuterated solvent.

The conversion between para- and ortho- isomers of $\text{H}_2$ is catalyzed by the near approach of molecules to a paramagnetic surface. Instantaneous differences in the local magnetic field are experienced by the hydrogen nuclei, causing singlet-to-triplet transitions. Paramagnet-induced ortho-para conversion has been studied both experimentally and theoretically for the case of free $\text{H}_2$ in solution, and also for $\text{H}_2$ encapsulated in fullerene cages.

Nuclear singlet-triplet conversion in polyatomic molecules is also induced by paramagnetic species. The question arises as to whether this conversion is faster or slower than the conventional spin-lattice relaxation of longitudinal magnetization. This question is particularly relevant to the possible in vivo applications of nuclear singlet states. It has been postulated that the long lifetimes of nuclear singlet states may be useful for storing and transporting hyperpolarized nuclear spin order, as generated by dynamic nuclear polarization (DNP) or by para-hydrogen-induced polarization (PHIP). In many cases these applications require the introduction of hyperpolarized agents into the circulatory system of a living animal or human being, where the close proximity of paramagnetic agents such as blood haemoglobin causes strong relaxation. For example, the longitudinal $(T_1)$ relaxation of hyperpolarized $^{129}\text{Xe}$ is more than 12 times faster for a solution in blood than for a saline solution. Can hyperpolarized nuclear singlet states be expected to persist for a longer time than ordinary magnetization, in contact with blood?

To characterize the influence of paramagnetic agents we studied the pair of inequivalent glycine $\alpha$-protons of the dipeptide alanlyglycine (AG) (fig. 1). Singlet relaxation of this proton pair has been studied previously. In the absence of paramagnetic agents, the singlet decay constant $T_S$ is almost 40 times longer than the conventional magnetization relaxation time $T_1$, mainly because the dominant dipole-dipole relaxation mechanism is highly effective for $T_1$, (due to the close proximity of the two protons), but completely ineffective for $T_S$ (due to a symmetry-induced selection rule).

We measured the NMR lifetimes for AG in solutions (40 mM in 500 $\mu$L D$_2$O, degassed) containing a dissolved tran-
Longitudinal (\( T_{1} \)) and singlet (\( T_{2}^{-1} \)) relaxation rate constants in \( \text{D}_2\text{O} \) of the glycine protons as a function of metal ion concentration for (a) \( \text{Cu}^{2+}\text{Cl}_2 \); (b) \( \text{Mn}^{2+}\text{Cl}_2 \); (c) \( \text{Gd}^{3+}\text{Cl}_3 \). The time constants at zero concentration are \( T_{S}(0) = 32 \text{ s} \) and \( T_{1}(0) = 1.4 \text{ s} \) of \( T_{1}^{-1} \) and \( T_{S}^{-1} \) monitored over the range 0 < [X] < 0.4 mM. These are summarized in table 1.

The \( T_{1} \) relaxivity, \( k_{1} \), occupies a wide range of values, increasing approximately proportional to the square magnetic moment of the metal ion species as is consistent with the relaxation mechanism involving modulation of the proton-paramagnet hyperfine coupling.\(^{11,25} \) We find that singlet relaxation broadly follows this trend too, but the constants \( k_{S} \) are smaller, typically by a factor of 2 to 3. This means nuclear singlet order is less sensitive to paramagnet-induced relaxation than ordinary Zeeman magnetization.

The paramagnetic relaxivities \( k_{S} \), in table 1, are more than 10\( ^{5} \) times faster than the analogous parameters for ortho-para conversion in \( \text{H}_2 \),\(^{10} \) and \( \text{H}_2 \) trapped inside fullerenes.\(^{12,14} \) This probably results from a combination of factors, namely the much slower rotational correlation time of AG compared to \( \text{H}_2 \), and the partial coordination of the paramagnetic ions to the carboxyl groups of the peptide. In addition, the case of ortho-para conversion in \( \text{H}_2 \) involves a very large singlet-triplet energy splitting, which is mainly due to Pauli-principle entanglement of the nuclear spin states and the rotational angular momentum states.\(^{1} \)

Oxygen gas was bubbled through an initially degassed solution of AG (40 mM in 500 \( \mu \text{L} \), \( \text{D}_2\text{O} \)), and the relaxation constants measured. Further additions of \( \text{O}_2 \) were made, repeating the relaxation measurements until no change was observed in \( T_{1} \). We did not measure the levels of dissolved oxygen, hence values of \( k_{S} \) and \( k_{1} \) were not fitted. The lowest value of \( T_{S} \) recorded, however, was 1.4 seconds, which gives an estimate of the relaxivity \( k_{S} = O(1) \text{ mM}^{-1}\text{s}^{-1} \) on assuming the approximate saturation of 40 mg/L (1.2 mM) \( \text{O}_2 \) in \( \text{D}_2\text{O} \) at 293 K.\(^{26} \) The concentration-independent term \( k_{S}/k_{1} = 0.55 \pm 0.03 \) could be obtained from the slope of \( T_{S}^{-1} \) vs. \( T_{1}^{-1} \). Hence, the singlet state is about two-times less sensitive to relaxation caused by dissolved \( \text{O}_2 \) than ordinary magnetization.

The ratio \( k_{S}/k_{1} \) can be interpreted roughly by assuming the two protons each experience randomly fluctuating magnetic fields generated by the paramagnetic center.\(^{5,25} \) Using Redfield’s formalism we obtain formulae for the rate constants:

\[
T_{S}^{-1} = 2 \gamma_{H}^{2}(B_{1}^{2} + B_{2}^{2} - 2CB_{1}B_{2})(j_{0} + 2j_{1})/3 \quad (1)
\]

\[
T_{1}^{-1} = \gamma_{H}^{2}(B_{1}^{2} + B_{2}^{2})j_{1} \quad (2)
\]

where the sizes of \( T_{S}^{-1} \) and \( T_{1}^{-1} \) depend on the root-mean-square (rms) magnitudes \( B_{i} = \langle B_{i} \cdot B_{i} \rangle^{1/2} \) of the random fields at the nuclear sites, and the extent \( C = \langle B_{1} \cdot B_{2} \rangle/B_{1}B_{2} \) that they are correlated with one other. Here \( \gamma_{H} \) is the proton gyromagnetic ratio and \( j_{m} = j_{1}(m\gamma_{H}B_{0}) \) signifies the non-normalized rank-1 spectral densities of the random field fluctuation.

Under the condition of extreme narrowing (\( j_{m} = j_{1}(0) \)) and equal rms field amplitudes \( B_{1} = B_{2} \), the ratio \( k_{S}/k_{1} \)

---

**Table 1** Relaxivity of AG at 293 K at a field of 9.4 T (400 MHz)

| \( X \) | \( k_{1} \) (mM\(^{-1}\text{s}^{-1}) | \( k_{S} \) (mM\(^{-1}\text{s}^{-1}) | \( k_{S}/k_{1} \)
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<td>Cu(^{2+}\text{aq} )</td>
<td>3.0 ± 0.1</td>
<td>1.6 ± 0.08</td>
<td>0.51 ± 0.04</td>
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<tr>
<td>Mn(^{2+}\text{aq} )</td>
<td>17.4 ± 1.0</td>
<td>6.7 ± 0.3</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Gd(^{3+}\text{aq} )</td>
<td>115 ± 5</td>
<td>40.1 ± 2</td>
<td>0.35 ± 0.02</td>
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depends only upon the field correlation, $C$. If the fields are completely uncorrelated ($C = 0$), $k_S = 2k_1$. Towards $C = 1$ at the opposite extreme, $k_S/k_1$ tends to zero: note at this point the Hamiltonian becomes symmetric under permutation, disallowing singlet-triplet transitions. The findings $k_S \ll k_1$ thus confer a strong correlation in the induced fields; values of $C$ are c. 0.8 to 0.9, under the assumption of extreme narrowing.

Wokaun and Ernst$^{27}$ obtained similar correlation values for the random-field model by comparing zero-, single- and double-quantum linewidths of a proton pair. They remarked that a lower $C$ is indicative of a smaller mean-approach distance of the paramagnet to the nuclear spins.$^{27}$ Elaborations on such theory, and proper treatment of the spectral densities, may allow quantitation of the proton-paramagnet distances, if desired.$^{24}$

Paramagnet-induced relaxation may be reduced or eliminated either by chemical transformation of the relaxing agent to a diamagnetic form$^{18}$ or, in the case of metal ions, by addition of suitable chelating agents. As shown in fig. 3a, the lifetime $T_S = 1.5 \pm 0.1$ s, for AG in the presence of 0.1 mM MnCl$_2$ improves to $37 \pm 2$ s on adding 1 mM ethylenediamine tetracetate (EDTA). A similar effect occurs with Cu$^{1+}$. These observations support a relaxation mechanism involving transient formation of complexes between the ions and AG. Paramagnetic ions remain physically present in solution, but the chelate formation with EDTA outcompetes their association with AG, increasing the observed relaxation times.

Fig. 3 also shows that the addition of ascorbate$^{18}$ markedly reduces the relaxation effects of dissolved oxygen. It is well known that ascorbate reduces superoxide ($\text{O}_2^-$), hydroperoxide (HOO$^-$) and dioxygen radicals in solution.$^{18,20}$

In summary, we have observed and quantified the relation $T_S \gg T_1$ for aqueous solutions of a dipeptide containing dissolved paramagnetic species. The singlet lifetime is sensitive to very-low levels of paramagnetic metal ions in solution, but this effect can be removed by addition of suitable complexing agents. The relative insensitivity of singlet states to paramagnetic relaxation agents, caused by local field correlations at the sites of the coupled spins, is favourable for experiments involving hyperpolarized nuclear singlet states.$^{17}$

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**References**


