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.[1] 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.[2,3]

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,[4,5] or by applying a resonant spin-locking field.[6,7] Singlet lifetimes as long as 25 minutes have been observed for ^{15}N-labeled nitrous oxide in a deuterated solvent.[8]

The conversion between *para*- and *ortho*- isomers of H_2 is catalyzed by the near approach of molecules to a paramagnetic surface.[9] 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 H_2 in solution, and also for H_2 encapsulated in fullerene cages.[1,10–14]

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)[15] or by *para*-hydrogen-induced polarization (PHIP).[3] 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}Xe is more than 12 times faster for a solution in blood than for a saline solution.[16] 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 α-protons of the dipeptide alanylglycine (AG) (fig. 1). Singlet relaxation of this proton pair has been studied previously.[17–20] 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[5,21]).

We measured the NMR lifetimes for AG in solutions (40 mM in 500 μL D_2O, degassed) containing a dissolved tran-

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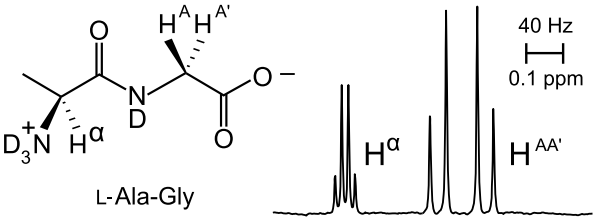

Fig. 1 ^{1}H NMR spectrum of the AG dipeptide (9.4 T, D_2O)

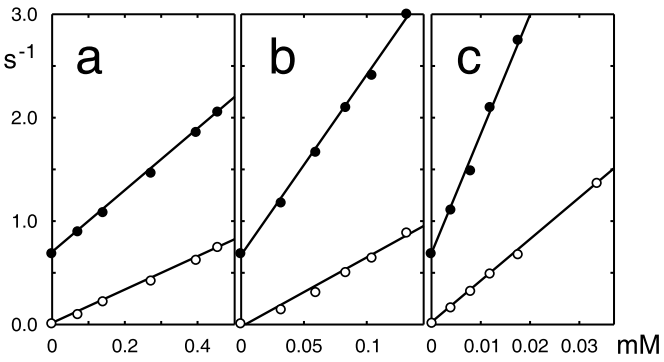

Fig. 2 Longitudinal ($\bullet\, T_1^{-1}$) and singlet ($\circ\, T_S^{-1}$) relaxation rate constants in D_2O of the glycine protons as a function of metal ion concentration for (a) $Cu^{II}Cl_2$; (b) $Mn^{II}Cl_2$; (c) $Gd^{III}Cl_3$. The time constants at zero concentration are $T_S(0) = 32$ s and $T_1(0) = 1.4$ s

sition metal or lanthanide salt. Solutions were prepared to known concentrations by adding aliquots from a D_2O stock solution of the paramagnetic salt. For each concentration, the singlet lifetime of the glycine protons was determined via the method of Sarkar *et al.* (see ref.[22] and SI of ref.[23]), at a field of 9.4 T. During the relaxation periods the singlet states were locked by applying a WALTZ-16 modulated irradiation of amplitude 2.5 kHz. The spin-lattice relaxation time constants T_1 were measured by inversion-recovery, the signal fitting to exponential recovery curves.

Fig. 2 shows how the rate constants T_1^{-1} and T_S^{-1} were found to increase linearly with concentration obeying a law $T_i([X])^{-1} = k_i[X] + T_i(0)^{-1}$, here $[X]$ being the concentration of the paramagnet, and $T_i(0)$ the time constant at zero concentration, caused predominantly by the magnetic dipole coupling between the glycyl protons (apparent in $T_S(0) \gg T_1(0)$). The coefficients k_1 and k_S, or *relaxivities*,[24] for the longitudinal and singlet relaxation, were fitted from the slopes

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

X	k_1 ($mM^{-1}s^{-1}$)	k_S ($mM^{-1}s^{-1}$)	k_S/k_1
$Cu^{2+}_{(aq)}$	3.0 ± 0.1	1.6 ± 0.08	0.51 ± 0.04
$Mn^{2+}_{(aq)}$	17.4 ± 1.0	6.7 ± 0.3	0.38 ± 0.02
$Gd^{3+}_{(aq)}$	115 ± 5	40.1 ± 2	0.35 ± 0.02

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 H_2,[10] and 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 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 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 μL D_2O), and the relaxation constants measured. Further additions of O_2 were made, repeating the relaxation measurements until no change was observed in T_i. 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 $k_S = O(1)$ $mM^{-1}s^{-1}$ on assuming the *approximate* saturation of 40 mg/L (1.2 mM) O_2 in D_2O 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 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:

$$T_S^{-1} = 2\gamma_H^2(B_1^2 + B_2^2 - 2CB_1B_2)(j_0 + 2j_1)/3 \quad (1)$$

$$T_1^{-1} = \gamma_H^2(B_1^2 + B_2^2)j_1 \quad (2)$$

where the sizes of T_S^{-1} and T_1^{-1} depend on the root-mean-square (rms) magnitudes $B_i = \langle \boldsymbol{B}_i \cdot \boldsymbol{B}_i \rangle^{1/2}$ of the random fields at the nuclear sites, and the extent $C = \langle \boldsymbol{B}_1 \cdot \boldsymbol{B}_2 \rangle / B_1B_2$ that they are correlated with one other. Here γ_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

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 treament 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 ± 2 s on adding 1 mM ethylenediamine tetraacetate (EDTA). A similar effect occurs with Cu^{II}. 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 ($O_2^{\bullet -}$), hydroperoxide ($HOO^{\bullet}$) 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]

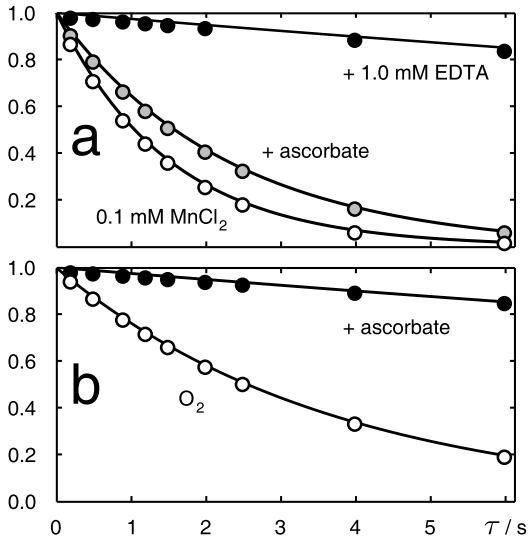

Fig. 3 Quenching of paramagnetic relaxation agents. Curves show the nuclear singlet decay $\propto \exp(-\tau/T_S)$ at 9.4 T for solutions of 40 mM AG plus (a) 0.1 mM $MnCl_2$ only ($\circ$, $T_S = 1.5 \pm 0.1$ s), with sodium ascorbate ($\bullet$, $T_S = 2.3 \pm 0.1$ s), with 1 mM EDTA ($\bullet$, $T_S = 37 \pm 2$ s); (b) dissolved oxygen ($\circ$, $T_S = 3.9 \pm 0.1$ s), then following addition of ascorbate[18,20] ($\bullet$, $T_S = 40 \pm 3$ s)

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