

Communication

Long-lived nuclear spin states in the solution NMR of four-spin systems

Giuseppe Pileio^{a,b}, Maria Concistrè^b, Marina Carravetta^b, Malcolm H. Levitt^{b,*}

^a Department of Chemistry, University of Calabria, via P. Bucci, Italy

^b School of Chemistry, University of Southampton, Southampton SO17 1BJ, UK

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Abstract

The existence of long-lived nuclear spin states in four-spin systems is explored by solution-state NMR experiments. Long-lived states are proved to exist in three different natural product molecules, each containing either a $AA'BB'$ or a $AA'XX'$ proton spin system. The measured state lifetimes are between four and eight times the spin–lattice relaxation time constants.

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1. Introduction

In normal circumstances, the longitudinal relaxation time T_1 sets a rough upper limit on the lifetime of spin order in a nuclear spin system. However, we recently showed that *singlet states* with much longer lifetimes than T_1 may be established in systems containing isolated pairs of coupled spins-1/2, even in the absence of magnetic equivalence [1–4]. The singlet states exhibit long life times providing that singlet–triplet interconversion is suppressed. This is done either by transporting the sample into a low-field region [1,2], or by using resonant radiofrequency irradiation in high magnetic field [3,4]. Singlet lifetimes T_S of up to $18T_1$ were demonstrated [3]. The long lifetimes arise because the dominant relaxation mechanism, namely the intrapair dipole–dipole coupling, is completely ineffective for the singlet states of spin-1/2 pairs [2].

Long-lived nuclear spin states have a number of possible applications. They could be useful for studying slow-motional processes such as molecular conformational changes and spatial diffusion [4]. Long-lived states could find application in the transport of systems exhibiting nuclear spin

hyperpolarization, such as those prepared by techniques such as dynamic nuclear polarization (DNP) [5] or by chemical reactions of parahydrogen [6–12].

Many potential applications of long-lived nuclear spin states involve molecules that contain more than two coupled spins-1/2. However, it has not yet been clear whether the symmetry conditions which lead to long singlet lifetimes in spin-1/2 pairs also apply (in modified form) to larger spin systems. In this communication, we demonstrate the existence of long-lived states in four-spin systems of the type $AA'BB'$ or $AA'XX'$. We have measured long-lived state lifetimes of between four and eight times T_1 in solutions of three substances, namely 4-aminobenzoic acid (PABA), 4-hydroxybenzoic acid (PHBA) and citric acid (CA) (for molecular structures, see Fig. 1). In each case, the non-labile protons form an $AA'BB'$ or $AA'XX'$ spin system with shift and coupling parameters shown in Table 1.

The work described in this paper does not establish the *nature* of the long-lived spin states in four-spin systems. Since the nature of these states is still under investigation, we use the non-committal term *long-lived spin state* for systems of more than two spins. The characteristic singlet states of two-spin systems are special cases of multiple-spin long-lived states.

* Corresponding author. Fax: +44 23 8059 3781.

E-mail address: mhl@soton.ac.uk (M.H. Levitt).

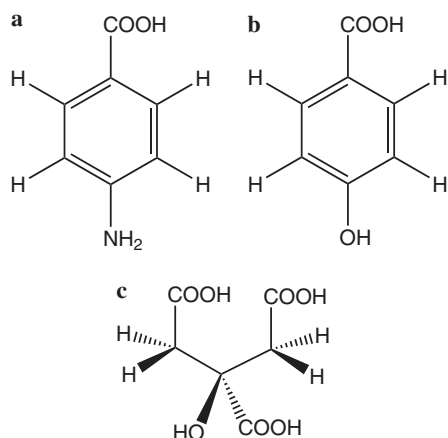


Fig. 1. Molecular structures of (a) 4-aminobenzoic acid, (b) 4-hydroxybenzoic acid and (c) citric acid.

The relaxation time constants of the long-lived states, investigated in this article, are denoted T_{LLS} . In the case of two-spin singlet states, this relaxation time is equal to the singlet lifetime T_S .

2. Samples

2.1. 4-Aminobenzoic acid (PABA)

4-Aminobenzoic acid, also known as *para*-aminobenzoic acid or PABA, is a non-protein amino acid widely distributed in nature, including the human metabolism [13]. Its molecular structure is shown in Fig. 1a. The PABA sample was prepared by dissolving 25 mg of PABA and 20 mg of sodium phosphate in 0.5 ml of D₂O, followed by degassing with three freeze–pump–thaw cycles. The four aromatic protons form a $AA'XX'$ spin system. The aromatic region of the proton NMR spectrum (see Fig. 2a) is identical to that expected for a single AX spin system with the parameters given in Table 1.

2.2. 4-Hydroxybenzoic acid (PHBA)

4-Hydroxybenzoic acid, also known as *para*-hydroxybenzoic acid or PHBA, is also an human metabolite [13]. The sample was prepared by dissolving 30 mg of PHBA and 25 mg of sodium phosphate in 0.75 ml of D₂O, followed by degassing with three freeze–pump–thaw cycles. The NMR behaviour of PHBA is very similar to PABA. The four aromatic protons form a $AA'XX'$ spin system and generate a proton NMR spectrum which is identical

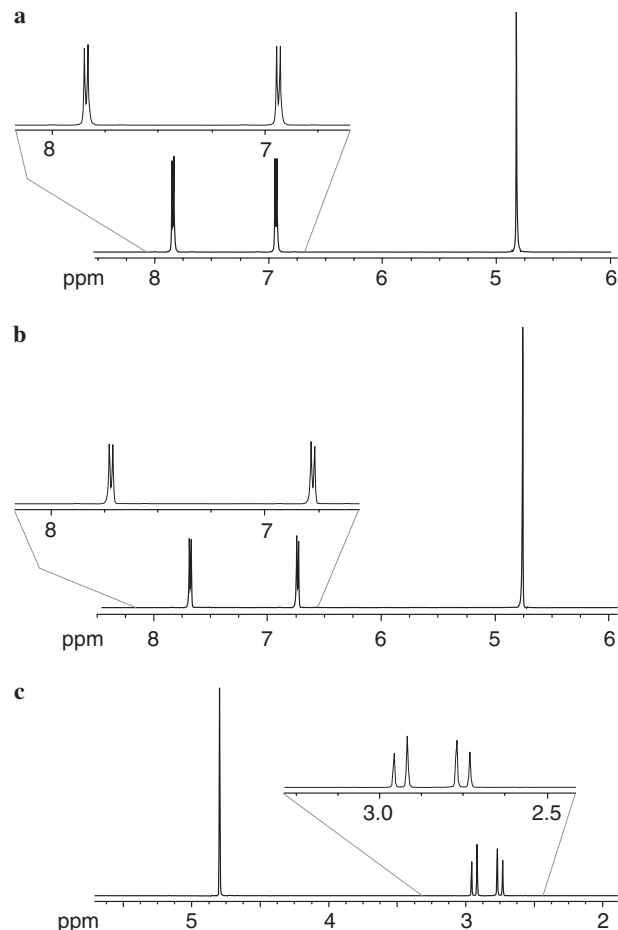


Fig. 2. Proton NMR spectra of solutions of (a) 4-aminobenzoic acid, (b) 4-hydroxybenzoic acid and (c) citric acid. The solvent is D₂O in all cases.

to that expected for a single AX spin system with the parameters given in Table 1 (see Fig. 2b).

2.3. Citric acid (CA)

Citric acid has the molecular structure shown in Fig. 1c. It is a central player in the Krebs cycle [14] and is a familiar constituent of fruits, vegetables, and soft drinks. The molecular structure in Fig. 1a indicates a plane of symmetry through the central carbon atom. Each of the two CH₂ groups forms a diastereotopic proton pair. If the labile-OH protons are ignored, the spin system classification is therefore $AA'BB'$.

The sample was prepared by dissolving 25 mg of CA in 0.5 ml of D₂O. Dissolved oxygen was removed by three

Table 1
Spin system and experimental parameters, and estimated relaxation time constants, for the three samples

Samples	Spin system			Experimental parameters					Relaxation times	
	δ_j (ppm)	δ_k (ppm)	J (Hz)	B^0 (T)	τ_1 (ms)	τ_2 (ms)	τ_3 (ms)	τ_5 (ms)	T_1 (s)	T_{LLS} (s)
PABA	7.72	6.81	8.5	11.744	29.27	30.34	0.53	0.53	5.15 ± 0.03	20.0 ± 0.8
PHBA	7.78	6.89	8.5	11.744	29.27	30.39	0.56	0.56	4.71 ± 0.02	21.7 ± 0.5
CA	2.94	2.76	15.7	9.395	11.43	5.25	3.64	6.23	0.63 ± 0.01	4.81 ± 0.07

freeze–pump–thaw cycles. The proton NMR spectrum exhibits a single narrow peak from the labile protons which exchange rapidly with the solvent and four narrow peaks from the CH₂ groups (see Fig. 2c). An analysis of the four-line pattern provides the chemical shifts and *J*-coupling given in Table 1, assuming that each CH₂ group behaves as an independent *AB* spin system. There is no spectral evidence of *J*-couplings between protons on different CH₂ groups.

Long-lived zero-quantum coherences have been noted previously in the NMR spectroscopy of citric acid [15].

3. NMR experiments

3.1. Spectrometers

Two different NMR spectrometers were used: a Varian Infinity + NMR spectrometer working at a field of 9.395 T (400 MHz for protons) and equipped with a 5 mm HX liquid probe, and a Bruker Avance 500 NMR spectrometer working at a field 11.744 T (500 MHz for protons) and equipped with a 5 mm *z*-gradient probe. The magnetic fields used for the three compounds are specified in Table 1. All experiments were performed on room-temperature samples.

3.2. Estimation of T_1

Conventional inversion–recovery pulse sequences were used to estimate the longitudinal relaxation time constants T_1 for the *AA'**BB'* or *AA'**XX'* protons. The relaxation curves for the three compounds are shown in Fig. 3. In each case, the recovery of longitudinal magnetization is exponential. The estimated relaxation time constants are summarized in Table 1. The T_1 values for the aromatic protons of PABA and PHBA are both around 5 s, while the T_1 for the CH₂ protons in citric acid is almost ten times shorter, presumably because of the strong dipole–dipole interaction between the CH₂ protons, as well as a relatively long rotational correlation time caused by hydrogen bonding and/or strong electrostatic interactions with the solvent.

3.3. Estimation of T_{LLS}

The pulse sequence shown in Fig. 4 allows the estimation of singlet relaxation time constants T_S for inequivalent spin pairs in high magnetic field [3]. The first three rf pulses and three intervals τ_1 , τ_2 and τ_3 convert the initial Zeeman polarization into a state of opposite singlet and triplet populations [3,4]. In the case of an isolated *AX* spin system, the rf reference frequency is set to the mean of the chemical shift frequencies of the two nuclei, and the optimal values for the three intervals are given by $\tau_1 = 1/(4J)$, $\tau_2 = 1/(4J) + \pi/(\Delta\omega^0)$, and $\tau_3 = \pi/(\Delta\omega^0)$, where $\Delta\omega^0$ is the difference in angular Larmor frequencies between the two spin sites [3]. A continuous, resonant rf field is applied for the interval τ_4 . This rf field suppresses the chemical shift differ-

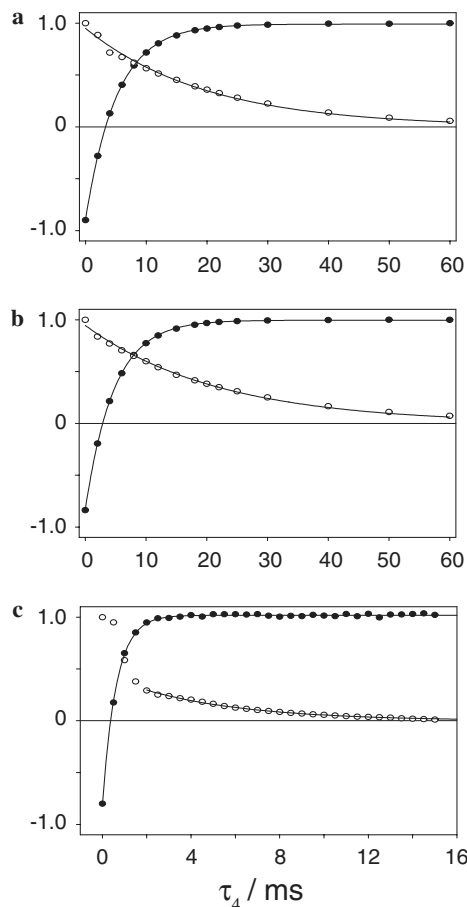


Fig. 3. Inversion–recovery relaxation curves (solid symbols) and singlet relaxation curves (open symbols), for the solutions of (a) 4-aminobenzoic acid, (b) 4-hydroxybenzoic acid and (c) citric acid.

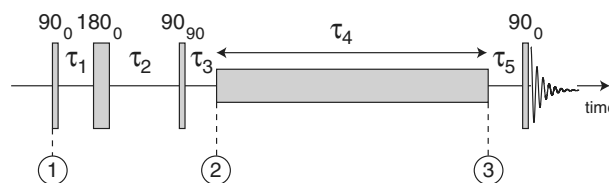


Fig. 4. RF pulse sequence for measuring the decay of long-lived nuclear spin states in high magnetic field.

ence between the two sites and isolates the singlet and triplet manifolds. During this interval, the three triplet populations equilibrate rapidly, while the singlet population decays slowly, with the time constant T_S . The singlet population at the end of τ_4 is monitored by apply an additional pulse after the interval τ_5 , which takes the optimal value $\tau_5 = \pi/(2\Delta\omega^0)$ in the case of an isolated *AX* spin system. Spurious signals are eliminated by subtracting a spectrum obtained using an identical sequence but with a phase change of π for the first rf pulse. Fourier transformation of the accumulated NMR signal produces a spectrum with a characteristic antiphase signal pattern [3]. Repetition of the experiment for several different values of the interval τ_4 allows estimation of the singlet decay time constant T_S .

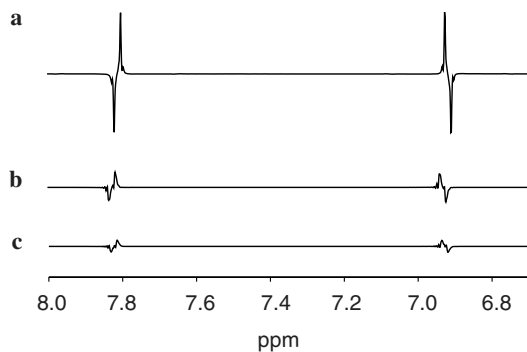


Fig. 5. Experimental ^1H NMR spectra of PHBA in D_2O solution at 300 K and 9.4 T using the pulse sequence in Fig. 4. The relaxation interval τ_4 is given by (a) 1 ms, (b) 20 s and (c) 40 s.

We performed the experiments on the four-spin systems using the same relationships between pulse sequence delays and the spin system parameters as apply for singlet generation and detection in two-spin systems.

The protons in PABA and PHBA form weakly coupled $AA'XX'$ spin systems with the spin system parameters in Table 1. The intervals τ_1 , τ_2 , τ_3 and τ_5 were set according to the formulae for AX systems given above and specified in Table 1. In both cases, an unmodulated rf field, with an amplitude corresponding to a nutation frequency of 3.5 kHz, was used during the “locking” interval τ_4 . Fig. 5 shows representative NMR spectra obtained for PHBA using several different values of the locking interval. The characteristic antiphase line-shapes decay as a function of τ_4 , maintaining the same relative peak amplitudes. The decays of a selected peak integral are shown in Fig. 3a and b. In both cases the decay is exponential, to a good approximation. The estimated decay time constants T_{LLS} the long-lived states are given in Table 1. The decay time constants T_{LLS} are around four times longer than the corresponding longitudinal relaxation time constants T_1 .

Since the protons in CA are strongly coupled, the pulse sequence intervals must be adjusted in order to obtain a good yield of the long-lived state. In practice, the delays were optimized numerically by calculating the spin dynamics of a strongly coupled AB spin system, and adjusting the intervals to obtain the highest singlet yield. The optimized pulse sequence intervals are specified in Table 1. An unmodulated rf field, with an amplitude corresponding to a nutation frequency of 3.5 kHz, was used during the “locking” interval τ_4 .

The decay of the long-lived state in citric acid, shown in Fig. 3c, has a different appearance to that in PABA and PHBA. The initial trajectory decays relatively rapidly, and is confused by spectral phase distortions (not shown). Nevertheless, a clean, long-lived signal component emerges for locking times of more than around 2.5 s. This component decays exponentially with a time constant of 4.8 s, which is almost eight times the longitudinal relaxation time T_1 .

The confused behaviour of the initial part of the trajectory is probably due to the strong coupling in citric acid,

which makes it difficult to achieve a clean interconversion of magnetization and long-lived spin order.

4. Discussion

These results prove that long-lived states do exist in systems of more than two coupled spins-1/2. The state lifetimes observed for the four-systems studied here clearly exceed T_1 by several factors, even if the lifetimes are not as spectacularly long as for some of the two-spin singlet states.

What are the long-lived four-spin states?

To a first approximation, the $AA'BB'$ or $AA'XX'$ spin systems may be regarded as independent pairs of AB or AX systems. The interpair J -couplings are very weak in the systems studied here, and are not evident in the NMR spectrum. It is therefore plausible that the long-lived states are simply singlet states localized on one of the spin pairs. We call this the “localized singlet hypothesis.”

Consider a $AA'XX'$ spin system. The population operator corresponding to a localized singlet is given by

$$P(S_{AX}) = |S_{AX}\rangle\langle S_{AX}|, \quad (1)$$

where the localized singlet ket for spins A and X is

$$|S_{AX}\rangle = \frac{1}{\sqrt{2}}\{|\alpha_A\rangle \otimes |\beta_X\rangle - |\beta_A\rangle \otimes |\alpha_X\rangle\}. \quad (2)$$

In the presence of the resonant rf field, the singlet population $P(S_{AX})$ evolves under the relaxation superoperator $\hat{\Gamma}$ as well as the superoperator $\hat{\mathcal{H}}_J$ for commutation with the J -coupling Hamiltonian:

$$\mathcal{H}_J = \sum_{j < k} 2\pi J_{jk} \mathbf{I}_j \cdot \mathbf{I}_k. \quad (3)$$

The localized singlet population $P(S_{AX})$ is immune to relaxation under the intrapair DD coupling, which is the dominant relaxation mechanism:

$$\hat{\Gamma}_{AX}^{\text{DD}} P(S_{AX}) = 0. \quad (4)$$

However, the localized singlet population commutes with the J -coupling Hamiltonian only if all interpair J -couplings are identical:

$$\hat{\mathcal{H}}_J P(S_{AX}) = 0 \quad \text{iff} \quad J_{AA'} = J_{AX'} = J_{A'X} = J_{XX'}, \quad (5)$$

where “iff” means “if and only if.”

This is a very strong condition. For example, in the case of 4-hydroxybenzoic acid, Eq. (5) requires that the four-bond J -coupling between the A and A' protons should be identical to the five-bond J -coupling between the A and X' protons.

There is at present no reason to assume that the condition in Eq. (5) may be waived if the interpair J -couplings are unresolved in the spectrum. We must therefore assume that a localized singlet state is interconverted with short-lived states by long-range J -couplings, even if such couplings are too small to observe directly. The lifetime of a localized singlet state is expected to be limited by the differences in interpair J -couplings.

We used Gaussian 03 software to estimate the unresolved interpair couplings in 4-hydroxybenzoic acid. Density functional calculations were performed using a B3LYP functional and 6-31G* basis set, after geometry optimization. The estimated J -couplings for 4-hydroxybenzoic acid are

$$\begin{aligned} J_{AA'} &= 1.0 \text{ Hz} \\ J_{AX'} &= -0.1 \text{ Hz} \\ J_{AX} &= 8.2 \text{ Hz} \\ J_{XX'} &= 1.6 \text{ Hz} \\ J_{A'X} &= -0.1 \text{ Hz} \\ J_{A'X'} &= 7.9 \text{ Hz}. \end{aligned} \quad (6)$$

The J -couplings are therefore expected to deviate from the condition in Eq. (5) by at least 0.5 Hz. Although this deviation is less than the experimental linewidth, it is an order of magnitude more than the inverse of the observed lifetime T_{LLS} . A localized singlet state should therefore be interconverted into a short-lived state by the unresolved interpair J -couplings, on a timescale of around 2 s. The observed lifetime of around 20 s is about an order of magnitude longer. We conclude that either (i) the localized singlet hypothesis is invalid, which still leaves open the identity of the observed long-lived state, or (ii) there is a currently unknown mechanism which stabilizes the localized singlet state, despite the presence of long-range J -couplings.

If verified, the existence of a stabilizing mechanism for localized singlet states in multiple-spin systems would greatly increase the usefulness of these states.

Citric acid is a different matter. The observed value of T_{LLS} is only around 5 s, and the inter-pair couplings are expected to be much smaller than in the benzoic acid derivatives. In this case, it is likely that the long-lived states are equivalent to localized singlet states within one of the two spin pairs.

Incidentally, the condition in Eq. (5) is *not* the same as that leading to magnetic equivalence of the A and A' spins, and of the X and X' spins. Magnetic equivalence is defined by the different condition

$$J_{AX} = J_{AX'} = J_{A'X} = J_{A'X'} \quad (7)$$

If Eq. (7) is satisfied, the spin system is classified as A_2X_2 or A_2B_2 . This condition is not relevant to the systems studied here.

Although the nature of the long-lived four-spin states still requires elucidation, the demonstrated existence of long-lived spin states in multiple-spin systems is encouraging for potential applications of these states in studying slow motion, infrequent chemical exchange, and in hyperpolarized NMR. Work is in progress to establish the nature of the long-lived states, and to understand their decay mechanisms.

Note added in proof

G. Bodenhausen has pointed out that suppression of chemical shifts by the resonant rf field causes all of the spin

systems described here to conform to the classification A_4 , i.e. full magnetic equivalence is imposed by the resonant rf field. Under such conditions, the evolution of the total spin angular momenta is independent of all J -couplings. However, it does not necessarily follow that the localized singlet states are also immune to the J -couplings under conditions of imposed magnetic equivalence.

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