Long-Lived Spin States

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Long-Lived ^{1}H Nuclear Spin Singlet in Dimethyl Maleate Revealed by Addition of Thiols**

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Abstract: *Nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) have become important techniques in many research areas. One major limitation is the relatively low sensitivity of these methods, which recently has been addressed by hyperpolarization. However, once hyperpolarization is imparted on a molecule, the magnetization typically decays within relatively short times. Singlet states are well isolated from the environment, such that they acquire long lifetimes. We describe herein a model reaction for read-out of a hyperpolarized long-lived state in dimethyl maleate using thiol conjugate addition. This type of reaction could lend itself to monitoring oxidative stress or hypoxia by sensitive detection of thiols. Similar reactions could be used in biosensors or assays that exploit molecular switching. Singlet lifetimes of about 4.7 min for ^{1}H spins in [D_4]MeOH are seen in this system.*

Hyperpolarization has become a very active research area in NMR spectroscopy and MRI because of the prospect of sensitivity enhancements[1] on the order of 10^2–10^5. One long-term goal is the establishment of contrast agents that can, after sensitivity enhancement, be injected in the body and later measured independently of the weaker background signals.[2] Other areas of application may be the monitoring of chemical reactors,[3] or the use of biosensors, such as Xe-biosensors.[4] One sticking point in many of these proposals is the lifetime (on the order of T_1 within 20 s) of the magnetization states that are imparted on the molecules. The short lifetimes can be circumvented by the use of long-lived states, which can be based on nuclear singlet states (or other relaxation-minimized subspaces). For ^{1}H spins in solution a record was achieved of a 4 min half-life,[5] while longer times are technically possible for low-gamma nuclei generally due to weaker relaxation mechanisms.[6] While this manuscript was in preparation, desymmetrization (conversion of an A_2 spin system into an AB or AX spin system by a chemical reaction) of hyperpolarized ethylene gas by electrophilic addition with an arenesulfenyl chloride was reported, demonstrating much longer singlet life times for singlet states in ethylene in the gas phase.[7] Switching in and out of such long-lived states, in particular singlet or near-singlet states, has been performed with field-cycling[5,8] and by pulse sequences.[6b,9] It has also been shown by desymmetrization of ^{13}C-enriched diacetyl, and a ^{13}C half-life of over 1 min was reported.[7,10] The very sensitive response to changes in solvent proved the principle of symmetry-switching, opening the door to the possibility that a probe could be developed that would respond to a molecular analyte.

Here we describe a desymmetrization reaction suitable for unlocking hyperpolarized singlet states in a reaction inspired by the enzyme maleate isomerase, whose mechanism of action involves addition of a thiol to maleate.[11] ^{1}H lifetimes of up to 4.7 min in [D_4]MeOH were achieved (a 24-fold enhancement over T_1). We also discuss the relaxation mechanisms that limit the lifetimes of these states.

The symmetry switch strategy is shown in Figure 1 a where hydrogenation of dimethyl acetylene dicarboxylate (DMAD; **1**) with parahydrogen yields hyperpolarized dimethyl maleate (DMM; **2**). Due to magnetic equivalence, the nuclear singlet state of **2** is preserved and remains invisible to NMR

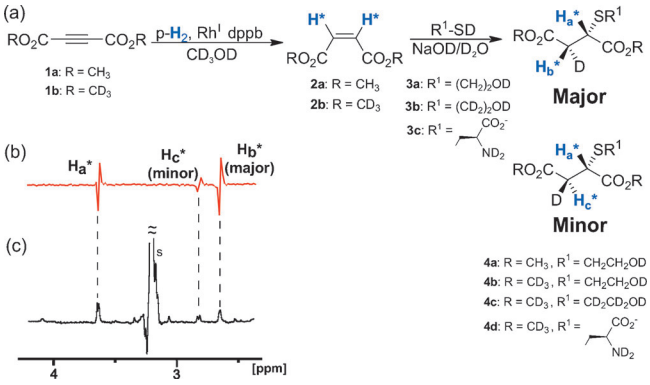

Figure 1. Revelation of long-lived nuclear spin singlet in compound **2** by desymmetrization: a) Reaction scheme. b) ^{1}H OPSY spectrum of hyperpolarized **4c**. c) Thermal spectrum of **4c** after the hyperpolarization decayed. "S" denotes solvent peak. The relative stereochemistry of the protons in the major (70%) and minor (30%) products was derived from the splitting pattern of the peaks and the coupling constants. The peak corresponding to H_c* is a doublet with a large coupling constant ($^3J = 10$ Hz), indicating a dihedral angle between H_c* and H_a* of about 180°. H_b* is broadened because the dihedral angle between H_b* and H_a* is about 60°, and thus, the fine splitting pattern due to a small coupling constant was not resolved.

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detection.[5] To access this long-lived nuclear singlet state, we used a facile base-catalyzed thiol addition reaction with **2**. In the product, **4**, the two hyperpolarized hydrogen atoms are magnetically inequivalent, and so, readily convert to the triplet state, which is observed by an only parahydrogen spectroscopy (OPSY) pulse sequence.[12] The OPSY spectrum in Figure 1 b shows the new anti-phase peaks characteristic of parahydrogen induced polarization (PHIP). The addition of thiols to **2** is known to be diastereoselective[13] leading to the appearance of hyperpolarized protons H_b* (major) and H_c* (minor) and resulting in additional line splitting.

We adapted the hydrogenation reaction of **1** to proceed in deuterated methanol (rather than in acetone),[5] since base-catalyzed nucleophilic addition of aliphatic thiols to **2** is sluggish in acetone. The choice of a protic solvent is also a better intermediate step towards an eventual goal of performing water-based reactions. When **1** was hydrogenated with parahydrogen at earth field and then transported to the observation field (Figure 2, path A), the hyperpolarized signals due to nuclear triplet states decayed within 2 min (Figure S1 in the Supporting Information). The hydrogenation reaction was found to be completed within 30 s (Figure S2).

For singlet state read-out, we implemented the thiol addition reaction. This reaction could potentially be further developed into an enzyme-responsive system. Mercaptoethanol (**3a**) and its deuterated analog (**3b**) were used for base-catalyzed thiol addition experiments.[14] The reaction between **2a** and **3a** was facile and the half-life was estimated to be 12 s from following the consumption rate of **2a** by UV/Vis spectroscopy. In order to inject the thiol solution into the NMR sample tube in the spectrometer, we inserted a segment of a long tubing through a septum cap into the NMR sample solution. The tubing was connected to a syringe containing the thiol solution.[15] A typical experiment proceeded in the following manner (Figure 2): After hydrogenation of **1** with parahydrogen at earth field, we transported the NMR sample tube, along with the tubing, to the observation field and applied a spoiler pulse. After several minutes (T_w), the thiol solution was injected simultaneously with the initiation of the OPSY pulse sequence.

To ensure that hydrogenation of **1** did not re-initiate upon thiol addition (the average yield of **2** was 80% with 20% of unreacted **1**), we injected the thiol solution prior to hydrogenation with parahydrogen. No hyperpolarized peaks or formation of **2** were observed because **3a** added readily to **1**, making it unavailable for hydrogenation (Figure S3).

We plotted the ^{1}H OPSY signal and enhancement factor (EF) of individual hyperpolarized protons as a function of T_w (Figure 3). In consecutive OPSY experiments, the persistent hyperpolarized signals detected for at least a minute arise from the ongoing thiol reaction. In **4a**, T_1 of H_c is 12 s while for **4c**, T_1 values of H_a, H_b and H_c are 21 s (average of two diastereomers), 14 s and 18 s respectively. One can deduce that the thiol reaction is complete (Figure S4 c) after one minute. Hydrolysis of compounds **2a** and **4a** was not observed (Figure S4 c). For **4a** and **4b**, data is only available for H_c, which is isolated in the thermal spectrum. As a next step, we implemented the reaction with a biologically relevant thiol (cysteine) as the chemical trigger (Figure 4).

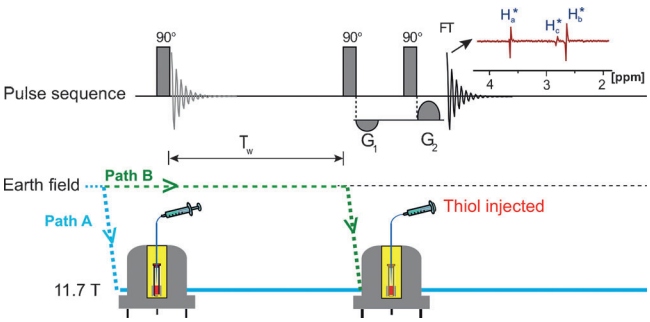

Figure 2. Pulse sequence and the magnetic field path experienced by the sample. At earth field, **1** was hydrogenated with parahydrogen to yield **2**. The NMR sample tube, along with the syringe containing the thiol solution, was inserted into the magnet (11.7 T) following path A (blue). Subsequently, a 90° pulse was used to spoil the signal resulting from transportation across different magnetic fields. After waiting for time T_w, the thiol solution was injected into the NMR tube to desymmetrize **2**, yielding **4** and the hyperpolarized signals for H_a*, H_b* and H_c* observed using the OPSY sequence. The OPSY spectrum is displayed beside its free induction decay (FID). In another set of experiments, the hydrogenated sample was left at earth field for various waiting times T_w, then transported to observation field for thiol addition (path B, green).

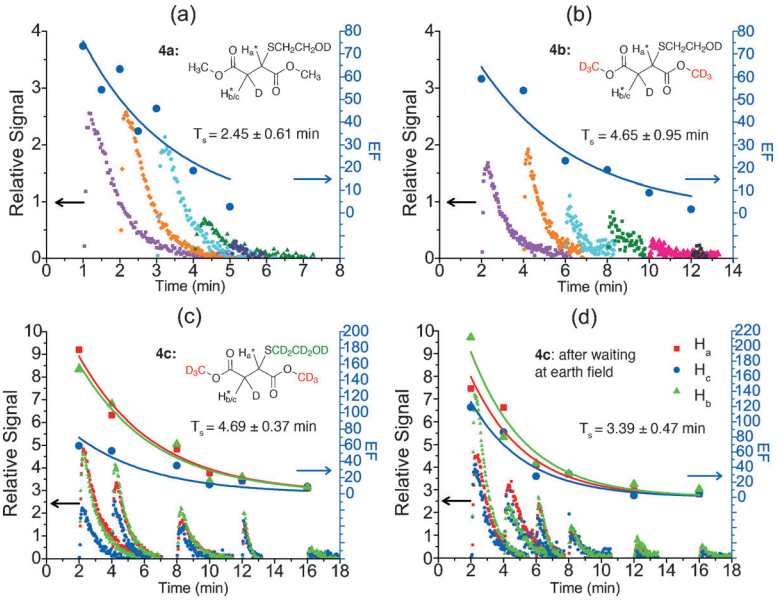

Figure 3. Decay of long-lived singlet state unveiled by chemical switching: ^{1}H OPSY signal (normalized to the thermal peak, shown on the left *y*-axis) and enhancement factor (right *y*-axis) of hyperpolarized ^{1}H decays as a function of T_w at 11.7 T (a, b, c) or at earth field (d). EF (bigger symbols) of H_a (■), H_c (●) and H_b (▲) are fitted to an exponential decay to determine T_s. a) H_c of **4a**: T_s of **2a** at 11.7 T is 2.45 ± 0.61 min; b) H_c of **4b**: T_s of **2b** at 11.7 T is 4.65 ± 0.95 min; c) H_a, H_b and H_c of **4c**: T_s of **2b** at 11.7 T is 4.69 ± 0.37 min; d) H_a, H_b and H_c of **4c**: T_s of **2b** at earth field is 3.39 ± 0.47 min.

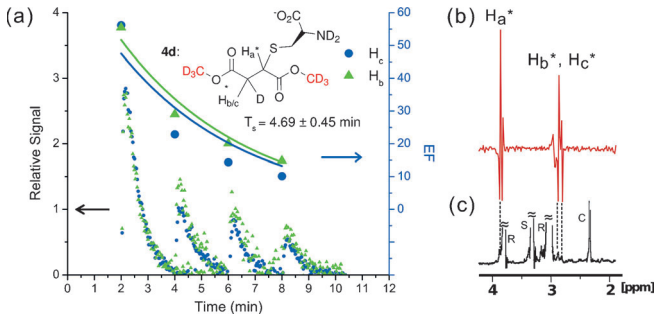

Figure 4. Decay of the long-lived singlet state unveiled by adding cysteine: a) ^{1}H OPSY signal (normalized to the thermal peak, shown on the left *y*-axis) and EF (right *y*-axis) of hyperpolarized ^{1}H decays as a function T_w at 11.7 T. Data for H_a is unavailable because the peak is unresolved in the thermal spectrum. EF of H_c (●) and H_b (▲) were fitted to an exponential decay to determine the T_s of **2b** at 11.7 T to be 4.69 ± 0.45 min. b) ^{1}H OPSY spectrum of hyperpolarized **4d**. c) Thermal spectrum of **4d** after the hyperpolarization decayed. "S" denotes solvent peak, "R" denotes unreacted cysteine peaks, "C" denotes catalyst peaks.

The lifetime of the singlet state, T_s, of **2a** at 11.7 T was found to be 2.45 ± 0.61 min (Figure 3a) in both the thiol addition and the field cycling[5] (Figure S5) procedures. We expected a somewhat shorter lifetime in [D_4]MeOH as compared to [D_6]acetone as used in Ref. [5] (4 min at 7 T). In solvents that contain hydrogen bonds (even deuterated ones) such as [D_4]MeOH, singlet lifetimes have been reported to be much shorter as compared to aprotic solvents.[16]

Deuterated DMM (**2b**) has a longer T_s, 4.65 ± 0.95 min (Figure 3b), because of the reduced number of significant coupling partners.[17] It was verified that field cycling did not work for **2b** (Figure S6b), due to magnetic equivalence in this compound. Technically, long-range coupling to deuterium introduces a tiny degree of magnetic inequivalence in the system. However, due to the weakness of such coupling constants, field-cycling would produce extremely weak singlet-to-triplet conversion, and/or would require a long residence time at the resonance field in order for an appreciable amount to be transferred, something that is not achievable or desired in practice.

It is interesting to note that the T_s of **2a** is 2.45 ± 0.61 min while T_s of **2b** is 4.65 ± 0.95 min even though the T_1 values of the vinylene protons in **2a** and **2b** are both about 21 s. This trend showed that deuteration had a minimal effect on T_1 while singlet relaxation was strongly affected. Intrapair dipole–dipole (DD) coupling makes a strong contribution to T_1 relaxation,[18] but is largely ineffective for singlet relaxation. Other contributions to relaxation, such as out-of-pair DD coupling, can then form the residual relaxation mechanisms that limit T_s. With deuteration of the methyl group, the out-of-pair DD coupling is reduced so that the T_s is longer. Chemical shift anisotropy (CSA) and spin-rotation relaxation could be additional lifetime limiting factors.[6b]

Since products from a common singlet precursor are shown in Figure 3b,c and 4a, the singlet decay constants should be the same, which is indeed observed. The T_s of **2b** at earth field is 3.39 ± 0.47 min (Figure 3d), shorter than that at 11.7 T. This trend indicates that the residual relaxation mechanisms are in a relatively fast motion regime.

Signal-revealing chemical reactions are desirable for their ability to detect certain molecular events. Many different molecular switches have been reported, including those that respond to electromagnetic field, oxidation potential, chemical reaction, and others.[19] Diverse properties have been explored as read-out for molecular switches, especially optical, magnetic, and electronic effects.[20] Environment-sensitive MRI probes have attracted significant interest in order to take advantage of the deep-tissue access afforded by MRI to image the expression of molecular phenomena associated with normal or pathogenic physiology or metabolism.[20c,21] A few examples of MRI contrast agents that demonstrate differential relaxivity in the presence of an environmental change have been reported,[21,22] but no example has been reported of a probe that shows a turn-on spectroscopic response to a biologically relevant stimulus.

In conclusion, nuclear singlet order created by PHIP was stored in a chemically symmetrical molecule at high field with half-lives of up to 4.7 min. Hyperpolarization was unveiled by a chemical reaction with thiols. Thiols represent a particularly useful analyte, opening prospects for responsive and biosensor systems with environmentally induced NMR spectroscopic signal turn-on mechanisms.

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