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Generating long-lasting ^{1}H and ^{13}C hyperpolarization in small molecules with parahydrogen-induced polarization

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Recently, Levitt and co-workers demonstrated that conserving the population of long-lasting nuclear singlet states in weak magnetic fields can lead to a preservation of nuclear spin information over times substantially longer than governed by the (high-field) spin-lattice relaxation time T_1. Potential benefits of the prolonged spin information for magnetic resonance imaging and spectroscopy were pointed out, particularly when combined with the parahydrogen induced polarization (PHIP) methodology. In this contribution, we demonstrate that an increase of the effective relaxation time by a factor up to three is achieved experimentally, when molecules hyperpolarized by PHIP are kept in a weak magnetic field instead of the strong field of a typical NMR magnet. This increased lifetime of spin information makes the known PHIP phenomena more compatible with the time scales of biological processes and, thus, more attractive for future investigations. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2209235]

INTRODUCTION

Because of the low magnetic energy of nuclear spins compared to the thermal energy of the surrounding lattice, magnetic resonance (MR) is a method of low intrinsic sensitivity in which spin energy levels are only polarized to a few parts per million (e.g., 5 parts per million at 1.5 Tesla and 293 K). Even high magnetic field strengths and the use of sensitive nuclei increase the polarization by no more than an order of magnitude. For nuclei with low magnetic moments or low natural abundance (e.g., ^{13}C: 25% of the sensitivity of ^{1}H and only 1.1% natural abundance), an even lower polarization of spin energy levels is observed. In recent years, many approaches were presented to enhance the sensitivity of MR imaging (MRI) and spectroscopy (MRS) by applying different concepts of hyperpolarization, i.e., by achieving non-Boltzmann population among the spin energy levels.

Even though hyperpolarization through parahydrogen induced polarization (PHIP) was discovered almost 20 years ago,[1–3] its application has been rather limited to the investigation of hydrogenation reaction mechanisms.[4,5] Only recently, PHIP was applied to address biomedical questions, yielding promising results in fields like *in vivo* and *in vitro* MRI.[6,7] In contrast to other, more established hyperpolarization approaches such as noble-gas techniques (e.g., using hyperpolarized ^{3}He or ^{129}Xe),[8] PHIP allows for the selective generation of hyperpolarized target compounds. These compounds may either be used directly as MRI contrast agents or as precursor material for investigating biochemical processes with *in vivo* MRS. Especially the last approach is attractive but not possible with hyperpolarized noble gases because they are generally not metabolized.

A major challenge for using PHIP-generated ^{1}H hyperpolarization is the short time span during which the spin populations usually return to their Boltzmann distribution. While relaxation times of several hours are observed for hyperpolarized noble gases, PHIP-generated ^{1}H hyperpolarization typically decays within seconds or even less. These substantial differences in relaxation times are due to more effective relaxation mechanisms in hydrogenation products compared with noble gases. Even though procedures to administer PHIP-hyperpolarized substances may be very fast, one is most likely left with a very small fraction of the original hyperpolarization. Hence, effective means for preserving nuclear spin information over extended times are needed.

In a recent series of publications, Levitt and co-workers introduced a concept that allows for the storage of nuclear spin information over periods substantially longer than governed by the spin lattice relaxation time, T_1.[9–11] In systems

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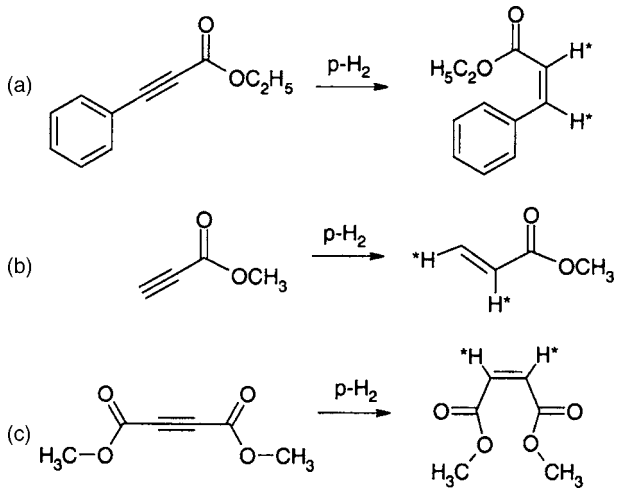

SCHEME 1. Hydrogenation of (A) phenyl propiolic acid ethylester to phenyl acrylic acid ethylester, (B) propiolic acid methylester to acrylic acid methylester, and (C) acetylene dicarboxylic acid dimethylester to maleic acid dimethylester. The hydrogen atoms in the product molecules, which originate from parahydrogen, are marked with asterisks.

with pairs of protons, long-lasting singlet states exist at low magnetic fields which, because of their symmetry, are not affected by intramolecular dipole–dipole relaxation. Hence, the relaxation of spin information stored in highly symmetric singlet states will be less effective, and the low-field singlet relaxation time constant, T_{LFS}, may be up to seven times longer than T_1 at high field. Already in their first publication,[9] Levitt and co-workers pointed out potential benefits for NMR imaging and spectroscopy, when the extended relaxation times would become available using PHIP. In the following, we show experimentally that, at low fields, relaxation times are indeed substantially prolonged for compounds hyperpolarized with PHIP.

MATERIALS AND METHODS

To investigate the effects of weak magnetic field on PHIP relaxation, we distinguish between three different methods of sample handling, which we termed according to a notation of Bowers:[12]

(i) "ALTADENA waiting": The PHIP-generating hydrogenation is conducted in a weak magnetic field (i.e., the earth's magnetic field of 50 μT), and the sample is kept therein for an evolution time that allows for nuclear spin relaxation. Thereafter, the sample is quickly transferred into the high magnetic field of an NMR spectrometer and a spectrum is recorded without delay.

(ii) "ALTADENA direct": As before, the PHIP-generating hydrogenation is conducted in a weak magnetic field and, immediately afterwards, the sample is transferred into the high magnetic field of an NMR spectrometer. After an evolution time that allows for nuclear spin relaxation, an NMR spectrum is recorded.

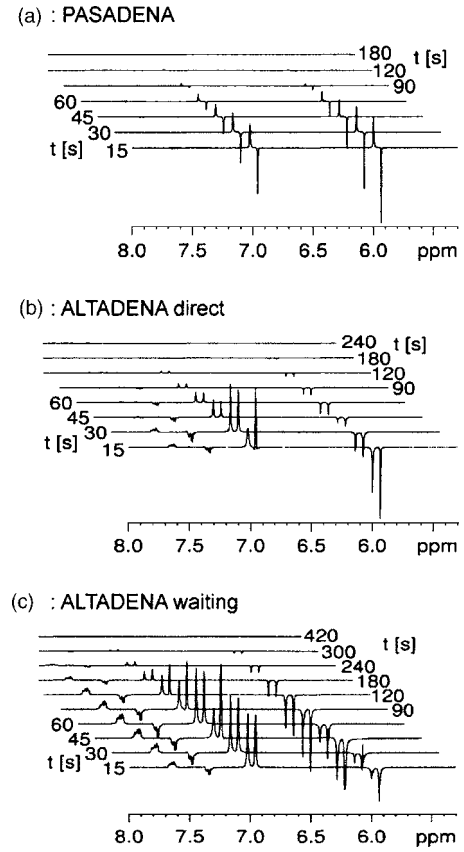

FIG. 1. ^{1}H-PHIP NMR spectra recorded for the hydrogenation of phenylpropiolic acid ethylester to phenylacrylic acid ethylester utilizing the different procedures (A) "ALTADENA waiting", (B) "ALTADENA direct", and (C) "PASADENA".

(iii) "PASADENA": The PHIP-generating hydrogenation is conducted in the spectrometer's high magnetic field (4.7 T) and the sample is kept therein for both the evolution time and the acquisition of the NMR data.

Even though "PASADENA" should be sufficient to reflect the relaxation effects at high field strength, the experimental procedure (ii) termed "ALTADENA direct" was added to account for differences in the population of spin energy levels between ALTADENA and PASADENA and to monitor their influence on relaxation times. All experiments were performed with a Bruker Avance DRX 200 spectrometer operating at 4.7 T (200 MHz proton resonance frequency).

For three different reagents 2.5 ml of a master solution were prepared, each containing 1 ml of substrate in acetone-d_6 and 15 mg (c=8.3 mM) of the hydrogenation catalyst precursor [Rh(dppb)COD] BF_4 (M=724,36 g/mol, Sigma-Aldrich), where dppb and COD denote the ligands 1,4-bis(diphenylphosphino)butane and 1,5-cyclooctadiene, respectively. According to this formulation, the following concentrations and substrate-to-catalyst ratios (SCR) were achieved for the different reagents: (a) phenyl propiolic acid ethylester: c=4.04 M, SCR=487; (b) propiolic acid methylester: c=7.5 M, SCR=893; (c) acetylene dicarboxylic acid dimethylester: c=5.4 M, SCR=643. From the master solu-

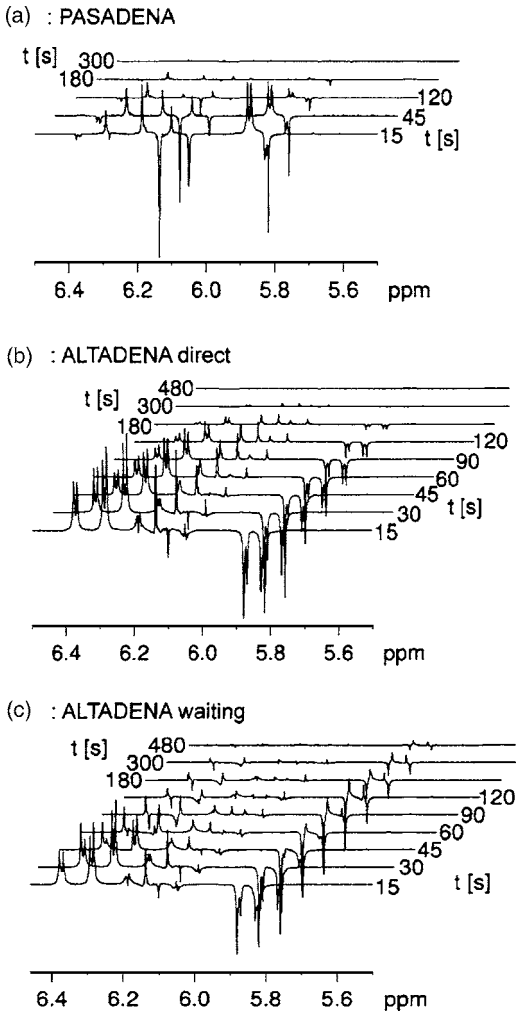

FIG. 2. ^{1}H-PHIP NMR spectra recorded for the hydrogenation of propiolic acid methylester to acrylic acid methylester utilizing the different procedures (A) "ALTADENA waiting", (B) "ALTADENA direct", and (C) "PASADENA.

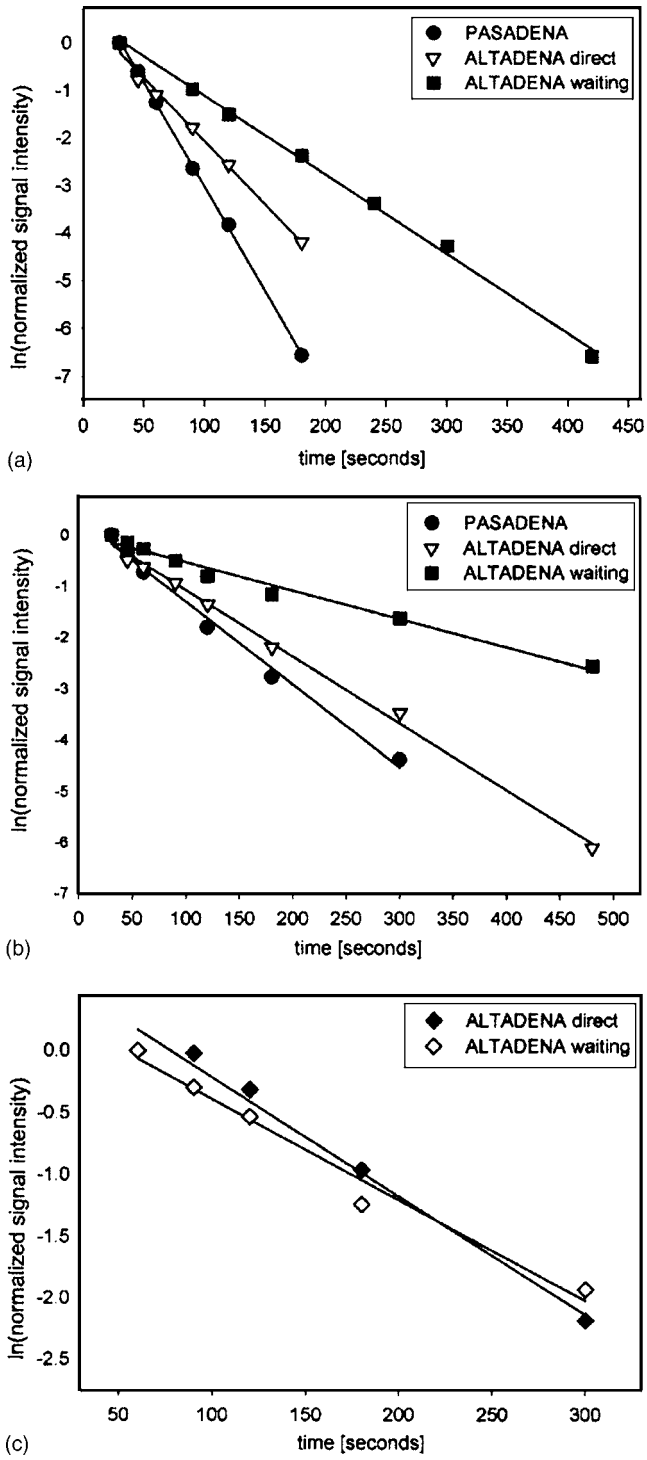

FIG. 3. Semi-logarithmic plots of signal intensities obtained from ^{1}H-PHIP NMR spectra as a function of evolution time. The normalized intensity data were extracted from NMR spectra of the hydrogenation products (A) phenylacrylic acid ethylester, (B) acrylic acid methylester, and (C) maleic acid dimethylester.

tion, 800 μl each were distributed to three NMR tubes used for the three procedures listed above. For the PHIP-generating hydrogenation, parahydrogen was enriched according to a previously described method[13] and always bubbled through the solution at ambient pressure and temperature. Thus, comparable reaction conditions for all samples were established. After 10 seconds of hydrogenation, samples were left either inside or outside the magnetic field allowing the evolution time to pass before a ^{1}H-NMR spectrum was recorded. Because of the high SCRs and the small amounts of hydrogen in solution, only a very small fraction of substrate was consumed during a single experiment. Accordingly, the same sample could be reused for all experiments in a series of varying evolution times. For the relaxation time analysis, the averaged signal intensities of the transferred ^{1}H nuclei were used.

RESULTS

In Scheme 1 the three hydrogenation reactions which we used in this study are shown. In particular, phenyl propiolic ester (reaction A) and propiolic acid ester (reaction B) were used side by side as reagents to elucidate relaxation effects that might originate from additional coupling nuclei while acetylene dicarboxylic acid dimethylester (reaction C) was used for generating strong ^{13}C hyperpolarization.[14] In three series of experiments, each sample was treated according to the three different procedures "ALTADENA waiting" (i), "ALTADENA direct" (ii), and "PASADENA" (iii) described in the Materials and Methods section. Figures 1 and 2 show a series of ^{1}H-PHIP NMR spectra recorded from the prod-

TABLE I. Relaxation times measured for three different reagents each processed with "ALTADENA waiting", "ALTADENA direct", and "PASADENA". Values are provided with their best-fit standard deviation.

Relaxation time [s]	PASADENA	ALTADENA direct	ALTADENA waiting
Phenylacrylic acid ethylester	22.9±0.2	37.5±1.6	60.1±1.2
Acrylic acid methylester	62.0±3.0	77.0±1.9	180.7±9.9
Maleic acid dimethylester	n/a	104.0±7.0	122.5±10.0

ucts of reactions (A) and (B) as a function of evolution time. It is noted that significantly different signal patterns are obtained from the "PASADENA" hydrogenations as compared to the "ALTADENA" hydrogenations. This phenomenon is well-known[11] but its discussion was considered not relevant for our relaxation-time analysis. Because it is also known that the transfer of hyperpolarization from ^{1}H to ^{13}C is relatively ineffective at high field strength,[15] the "PASADENA" experiment was not conducted for the hydrogenation of acetylene dicarboxylic acid dimethylester forming maleic acid dimethylester.

For each model substrate, Fig. 3 shows the average magnitude of all hyperpolarized signal intensities normalized by the intensity of the experiment with the shortest evolution time. The data are plotted as a function of evolution time, where different symbols reflect the three different reaction procedures. To easily identify changes in relaxation time, a logarithmic scale was chosen for the signal intensity data. In Table I, relaxation times are summarized for the three model substrates as obtained from the signal intensities of the three different procedures. For the reaction products phenylacrylic acid ethylester [reaction(A)] and acrylic acid methylester [reaction(B)], the relaxation times increase from 22.9 s (±0.2 s) to 37.5 s (±1.6 s) and from 62.0 s (±3.0 s) to 77.0 s (±1.9 s), respectively, when the procedure is changed from "PASADENA" to "ALTADENA direct". These increases (64% and 24%, respectively) are attributed to the different spin populations that are achieved when the PHIP-generating hydrogenation is conducted in a weak and in a strong magnetic field.[12] However, if the sample is stored outside the magnetic field ("ALTADENA waiting"), the relaxation times increase further to 60.1 s (±1.2 s) for phenylacrylic acid ethylester (increase of 162% compared to "PASADENA") and to 180.7 s(±9.9 s) for acrylic acid (increase of 191% compared to "PASADENA"). Accordingly, an increase by a factor close to three was measured when both the hydrogenation reaction and the evolution time was moved from the high magnetic field of the NMR spectrometer to a low field. Although a substantial relaxation-time prolongation was observed for both model substrates, its amount is rather different (162% versus 191%). Most likely, this effect is explained by the molecular structure, the number of coupling nuclear spins, and the symmetry of the substrates. However, because acrylic acid methyl ester exhibits the largest relaxation-time enhancement among our examples, the number of *J*-coupled spins or their coupling strength may not be viewed as the only factors in quenching singlet- state spin hyperpolarization.

For the ^{13}C hyperpolarization investigated with maleic acid dimethylester, the ^{13}C signal intensity of the reaction product's carbonyl carbon atom was used for the analysis. For both procedures, "ALTADENA direct" and "ALTADENA waiting", we found long relaxation times (104.0 s±7.0 s and 122.5 s±10.0 s, respectively). While longer spin-lattice relaxation times are generally expected for ^{13}C, only rather small differences were observed between high-field ("ALTADENA direct") and low field storage ("ALTADENA waiting"). This finding is largely in agreement with Levitt's theory of prolonged relaxation, because—in contrast to pairs of ^{1}H nuclei—^{13}C is not strongly coupled to another hyperpolarized nucleus, so that an altered magnetic field does not necessarily lead to a sufficiently significant change in the nuclear spin symmetry.[11] Therefore, we assume that the observed differences in ^{13}C relaxation time are the result of other factors, such as a difference in the efficiency of the polarization transfer from ^{1}H to ^{13}C.

CONCLUSIONS

Hyperpolarized molecules generated with PHIP experience substantially longer relaxation times for ^{1}H nuclei when stored in low magnetic fields rather than in the high magnetic fields of NMR spectrometers. Depending on the system under investigation, an increase in relaxation time by a factor up to three was measured in our study. Remarkably, the prolongation of relaxation times was achieved in multifaceted spin systems, where the former parahydrogen protons are *J*-coupled with different strengths to several other protons in the products. So far, enhanced lifetimes were only reported for two-spin systems untainted with coupling partners. For contrast agents or metabolic markers, this prolongation makes the application of hyperpolarized materials more attractive and the relaxation time scale more compatible with biological processes. Furthermore, our results indicate that tuning the substrates' structures may help to further increase the relaxation times of PHIP-generated hyperpolarization. In agreement with the underlying theory, no significant increase was measured for ^{13}C relaxation times. However, long intrinsic relaxation times still make ^{13}C a suitable nucleus for lasting hyperpolarization.

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