Measurement of the Singlet-State Lifetime of N_2O in Rat Blood: Its Potential As An MRI Tracer

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INTRODUCTION: The utility of hyperpolarized MRI tracers is often limited by their longitudinal T_1 relaxation times which are usually quite short on the time scale of *in vivo* circulation, uptake, and metabolism. These rates are typically determined by intramolecular dipolar interactions. As has been previously shown [1], singlet-like (antisymmetric) spin states of pairs of isotopically-identical nuclei are immune to intra-pair dipolar relaxation, while other intra- and inter-molecular relaxation mechanisms can be greatly reduced. For example, the time constant for singlet-to-triplet conversion (T_S) of nitrous oxide ($^{15}N_2O$) dissolved in D_2O was measured to be 26 minutes. In this study, we report measurements of doubly-enriched $^{15}N_2O$ conversion times T_S in a variety of solvents including fresh whole blood in order to shed light on the mechanism of singlet-triplet conversion and to determine if the singlet state lifetimes *in vivo* can be sufficiently long to warrant the development of N_2O or an analogous agent as an MRI tracer. We note that although the singlet state has no magnetic moment, conversion into a triplet state by adiabatic transport to an imaging-strength magnetic field results in distinct, anti-phase NMR lines and makes it a suitable agent for high-sensitivity MRI.

MATERIALS AND METHODS: Doubly ^{15}N-labeled N_2O gas (98%+ ^{15}N fraction, Cambridge Isotope Laboratories, Inc.) was dissolved in various solvents in 5-mm NMR tubes (0.38 mm and 0.77 mm wall thickness, Wilmad Glass). With the exception of blood, solutions were deoxygenated under N_2 prior to introduction of N_2O. Additionally, 100 μM EDTA was added to aqueous solutions (H_2O and D_2O) as a metal-ion chelating agent. N_2O was introduced by cryopumping from a calibrated volume into a section of the NMR tube which was in contact with liquid N_2. The tube was then flame-sealed, allowed to warm up to room temperature, and inverted several times, after which the dissolved N_2O was in equilibrium with a gas pressure of 7–25 bar. For the experiments involving blood, heparinized rat blood (drawn from the tail vein of Sprague-Dawley rats) was used. After loading the blood into an NMR tube, a small section of blood (~6 mm tall) was separated from the bulk of the blood by a few centimeters of an air bubble to serve as a cold plug. This section was frozen by placing liquid N_2 in contact with the test-tube wall to prevent cryopumping of oxygen from the rest of the blood (~100 mm tall), which was held at room temperature. NMR experiments were performed on a Varian 500 MHz vertical bore system. After the sample was fully thermally relaxed to the 11.75 T field, the N_2O singlet state was populated using a two-step process. First, a selective long (3.3 ms) and weak square π pulse centered on the NMR frequency of one of the ^{15}N spectral doublets was applied. The pulse length was chosen to have a spectral node at the frequency of the other ^{15}N doublet and to leave that nucleus unaffected. Subsequent adiabatic transport of the NMR tube from the bore of the 11.75-T magnet to the center of a low-field μ-metal shielding system resulted in non-equilibrium population of the m=0 singlet state. After a set time interval the sample was reintroduced into the bore of the 11.75-T magnet. A hard, non-selective π/2 pulse was applied to both nuclei to read out their polarization. The procedure was repeated after a wait of 5 times the longer of the two individual nuclear T_1's to allow for thermal re-polarization of the sample. The data was analyzed by fitting to two Lorentzian doublets of unequal amplitude. The model included a correction for the re-polarization of the nitrogen nuclei as they were reintroduced into the magnet. Singlet state (anti-phase) amplitude was quantified as the difference between the doublet amplitudes, after the correction was applied. Preceding and following the singlet-state lifetime measurement, a standard inversion-recovery experiment was performed at 11.75 T yielding the T_1 times of the two ^{15}N sites, which is also the time-constant for interconversion among the triplet sub-states. We note that the blood darkened visibly during the 4-6 hour duration of the experiments despite the sealed tube, although there was no apparent change in either T_1 or T_S.

RESULTS AND DISCUSSION: Sample selectively inverted spectra after re-insertion into the magnet are shown in Fig. 1a, along with the time-course of polarization decay in inversion-recovery measurements (Fig. 1b) and anti-phase amplitude decay in singlet experiments (Fig. 1c). Decay curves are fit to a bi-exponential representing the more rapid triplet interconversion and the slower singlet decay. Table 1 summarizes these results. Of particular note is the similarity between time-constants in deuterated and natural water. This strongly suggests that intermolecular dipole interactions do not contribute significantly to singlet-triplet interconversion in aqueous solution. With the exception of the measurements in blood, the ratio of T_S/T_1 is quite constant, and is consistent with the assumption that the differing spin-rotation couplings of the two nuclei are the dominant contributor to both singlet and T_1 relaxation [1]. The measurements in blood do not fit in this pattern, however.

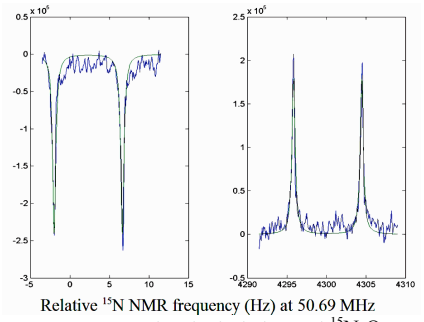

Figure 1 (a) Sample selectively inverted $^{15}N_2O$ spectra acquired in H_2O solution after a selective inversion of one doublet and 5 min. wait at zero field. The m=0 triplet component has decayed but the singlet component

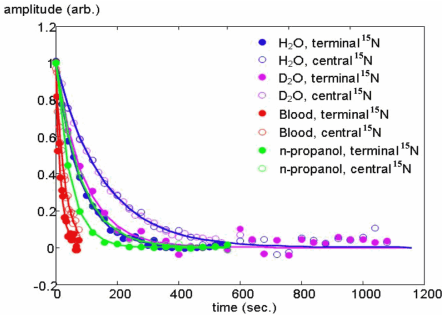

Figure 1 (b) T_1 relaxation measurements of dissolved $^{15}N_2O$ at 11.75 tesla fitted to T_1 curves for the two (terminal and central) ^{15}N sites in $^{15}N\equiv{}^{15}N{=}O$.

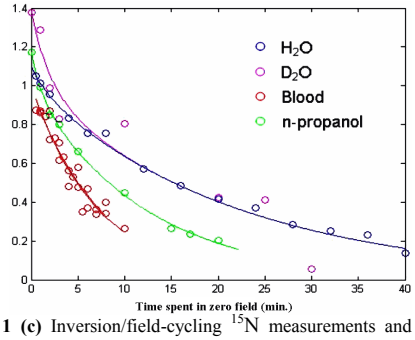

Figure 1 (c) Inversion/field-cycling ^{15}N measurements and T_S fitted curves. The values shown are proportional to the difference between the areas under the two doublets, corrected for individual repolarization during insertion using the measured T_1 values.

	J (Hz)	T_{1t}, T_{1c} (min.)	T_S (min.)	T_S / $\overline{T_1}$
n-propanol	8.69	0.81, 1.39	12.0±3.6	11.7
H_2O	8.21	1.34, 2.57	22.8±0.9	12.9
D_2O	8.2±0.1	1.55, 2.56	22.0±7.5	11.4
blood	8.2±0.1	0.28, 0.49	7.1±0.5	20.3

Table 1: Measured N-N scalar coupling (J), longitudinal relaxation times ($T_{1t,c}$ = terminal, central N), singlet-triplet interconversion time (T_S), and the ratio of T_S to the naively-assumed value (in the absence of correlation) of $\overline{T_1} = 2 / (1/T_{1t} + 1/T_{1c})$ for N_2O in four solvents.

The singlet lifetime of N_2O in blood exceeds the average nuclear T_1 by a factor of ~21, indicating that another mechanism, likely paramagnetic centers in hemoglobin or another blood component, dominate relaxation. To the extent that the local fields of these paramagnetic components spread over a long range and influence both ^{15}N nuclei equally, singlet-triplet conversion is suppressed. Regardless of mechanism, the unusually long T_S relative to T_1 provides an opportunity for longer duration of potential *in vivo* imaging experiments in which the agent is allowed to distribute in the body at low field and is imaged after re-introduction into the MRI scanner, in a manner analogous to the experiments described here. Although N_2O or another agent would be subject to *in vivo* T_1 relaxation once in the imaging field, intracellular or lipid-dissolved agent may exhibit a longer T_1 compared to one measured here in blood.

CONCLUSION: The singlet spin-state lifetime of doubly enriched $^{15}N_2O$ in blood is long enough to warrant its further investigation as a potential MRI tracer. The lifetimes of this state in various solvents have been measured and are consistent with relaxation dominated by spin-rotation coupling in water and alcohol, and by strong paramagnetic sites in blood. These residual relaxation mechanisms may be further reduced in agents in which the spin-rotation couplings of the nuclear pair are similar, and the close approach to paramagnetic blood components is minimized.

REFERENCES: [1] G. Pileio, M. Carravetta, E. Hughes, and M.H. Levitt, J. Am. Chem. Soc. 130, 12582–12583 (2008) DOI: 10.1021/ja803601d