31P Dynamic Nuclear Polarization Applied to Phosphonates for MRS/MRI Applications.

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Introduction: In the recent years, Dynamic Nuclear Polarization (DNP) has emerged as a very promising technique for enhancing the sensitivity of the magnetic resonance spectroscopy and imaging (MRSI). A number of nuclei, namely ^{13}C, ^{15}N, ^{29}Si, ^{89}Y, and ^{129}Xe, have been successfully polarized and a few of them have been employed in in-vivo studies for functional imaging and metabolism. Although ^{31}P dynamic nuclear hyperpolarization has been applied to deoxynucleotides, triphosphates and oligonucleotides for NMR studies of biomolecules such as DNA, RNA, proteins, for structural identification purposes, this study presents the first application of ^{31}P DNP applied to phosphonates for functional imaging and spectroscopy[1]. The ^{31}P isotope is 100% naturally abundant and is present ubiquitously in the bodies. Hyperpolarized ^{31}P nuclei in phosphonates have applications in functional imaging studies in vitro and in vivo. In this work, we specifically develop Dimethyl methyl phosphonate (DMMP) as a hyperpolarized phosphonate, which may be a reporter of intracellular volume[2] and may also be used as a freely diffusible tracer for perfusion studies. Moreover, one other benefit of hyperpolarized DMMP is its use with other phosphonates e.g., 3-aminopropylphosphonic acid (3-APP), an intracellular pH indicator, for the simultaneous characterization of blood perfusion, cell volume and extracellular pH. This technique is potentially beneficial for the hyperpolarized imaging of different biomarkers using a single nucleus.

Methods: The polarization buildup and signal enhancements were optimized for two different radicals, a nitroxyl radical Tempo and a trityl radical OX063. For the polarization of ^{31}P, neat solvent DMMP was mixed with glassing agent (50/50 v/v D_2O/glycerol mixture) in the volume ratio of 1:1 and doped with 15mM of OX063 and 42mM of TEMPO as the polarizing agents. The samples were polarized at 3.35T and 1.28K in the Oxford Hypersense Polarizer. The maximum polarization build-up time of 3 hours and the microwave irradiation frequencies of 94.080 GHz for OX063 and 94.100 GHz for TEMPO were optimized using a solid state probe tunable to the Larmor frequency ^{31}P frequency at 3.35T (hyperpolarizer conditions). For the in-vivo experiment, 100 µL of the DMMP sample prepared with glassing agent and doped with15 mM OX063 were polarized and injected in a male nude mouse weighing approximately 27 g following the dissolution with 1.5 mL of D_2O/EDTA buffer. The mouse was anesthetized using 2% isoflurane and restrained in a mouse cradle. Respiration and temperature were monitored using the SAII system (Small Animal Instruments, Inc.) and temperature control achieved by heated air (37±1 °C). A total of 250 µL of the hyperpolarized solution (containing 7.8 µL of DMMP) was injected into the animal via a tail vein catheter, which also contained 100 µL of saline solution to compensate for the dead volume. MRI was acquired with a 7-T horizontal magnet ASR 310 (Agilent Technologies, Inc.) equipped with nested 205/120/HDS gradient insert and a bore size of 310 mm. Using a 33 mm double resonance ^{1}H,^{31}P RF coil (Doty Scientific, Inc., Columbia, SC) gradient echo multi-slice (GEMS) images (TR/TE = 4/2.02 ms, matrix size = 32×32, FOV = 8 cm (axial) × 4 cm (transverse)) of hyperpolarized DMMP were acquired with a slice thickness of 30 mm and a flip angle of 20 degrees. GEMS sequences were alternated with single pulse spectrum acquisitions (SPULS) (flip angle=15 degrees, TR=50 ms) for the observation of temporal delivery of hyperpolarized DMMP (data not shown). The hyperpolarized DMMP image was registered with the ^{1}H coronal section GEMS anatomical images (TR/TE = 90 ms/4.11 ms, 20 degrees flip angle, FOV = 8 cm (axial) × 4 cm (transverse), 1.50 mm slice thickness and data matrix= 128×128). For the polarization calculations, hyperpolarized and thermal equilibrium data were acquired with 15 and 90 degrees flip angles respectively and evaluated using in-house scripts in MATALB (Mathworks, Inc).

Results and Discussions: Hyperpolarizing DMMP with TEMPO and OX063 resulted in the polarization buildup of 0.72% (≈ 767 fold signal enhancement) and 2.15% (≈2300 fold signal enhancement) respectively. The hyperpolarized DMMP in-vivo image (registered with the ^{1}H anatomical image) shown in the figure 1 was obtained about 4-5 seconds after the injection. It is interesting to note that the NMR tube filled with DMMP (used as a reference) is not visible in the hyperpolarized images as they were acquired with a single average; whereas the tube is clearly visible in gems ^{31}P images that were acquired with 128 averages. This exemplifies the potential advantages of hyperpolarization where a neat solvent with nearly 100% ^{31}P abundance does not show up in an image with a single scan at these low spatial resolutions.

These results demonstrate the first ever application of ^{31}P DNP in phosphonates and indicate the potential application of hyperpolarized DMMP in the functional imaging and spectroscopic studies. In figure 2, the Hyperpolarized DMMP can be seen in the blood stream and probably diffusing into the heart .Our future plans include the improvement of pulse sequences and the experimental protocols to obtain localized spectra of hyperpolarized DMMP and to track it's diffusion in-vivo.

Furthermore, we are also interested in the investigation of other phosphonates that can be used for hyperpolarized MRI/MRS applications.

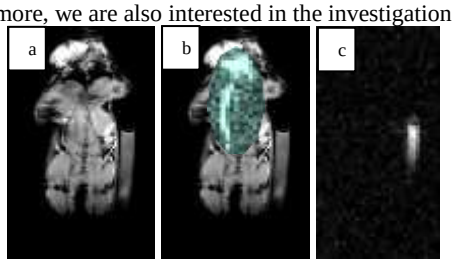

Figure 1. Hyperpolarized DMMP in-vivo image. **(a)** The anatomical image used for the registration. **(b)** hyperpolarized DMMP image registered with the 1H anatomical image. **(c)** Gems image of the reference NMR tube with 100% neat solvent DMMP acquired with 256 averages

References:

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