Hyperpolarization of hyperbranched polymers for molecular imaging

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Introduction

Magnetic Resonance Imaging (MRI) has revolutionised medical diagnosis, however, the relatively low sensitivity of MRI and lack of contrast between diseased and healthy tissue can be a drawback. Numerous methodologies have been employed to enhance contrast in MRI. None-the-less, limitations still exist in both the sensitivity and effectiveness of these agents. In order to overcome the issues of sensitivity, different "hyperpolarisation" techniques have been proposed that create non-equilibrium spin populations compared to the thermal distribution. For the hyperpolarization of molecules the two most important methods are dynamic nuclear polarization (DNP) and parahydrogen-induced polarization (PHIP). Dynamic Nuclear Polarisation (DNP) have been reported to show signal enhancements of up to 10^4 by transferring polarisation from unpaired electrons to the nuclear spins of interest [1]. PHIP produces hyperpolarization by chemical means: it transfers the nuclear spin alignment of para-H_2 (where the two nuclear spins are in an antiparallel configuration) to a molecule of interest, either by a hydrogenation reaction [2] or by reversible chemical exchange [3]. The use of PHIP and DNP to enhance the sensitivity of polymeric imaging agents has not been reported, thus in this contribution we demonstrate ^{1}H-PHIP and ^{19}F-DNP enhancement of biocompatible hyperbranched polymers which have been used for molecular imaging employing ^{19}F MRI [4].

Material and Methods

The hyperbranched polymers were synthesised using reversible addition fragmentation chain-transfer (RAFT) polymerization. As for PHIP unsaturated moieties in the molecule are necessary, the molecular structure of the hyperbranched polymer was varied such that a defined number of alkyne-terminated chains were contained in each molecule. The structures of the polymers hyperpolarized via DNP and PHIP are depicted as insets in figure 1 and 2, respectively. For the Overhauser DNP experiments we used an electro magnet (adjusted to 0.345 T) and an ENDOR probehead, both manufactured by Bruker (Karlsruhe Germany). TEMPOL radicals (20mM) were added to the ^{19}F containing hyperbranched polymers dissolved in water as a source of unpaired electron spins. The ^{19}F-DNP experiments were performed at room temperature with cw irradiation (4W) at the larmor frequency of the electrons. PHIP experiments were performed in d_6-acetone due to the high solubility of the hydrogenation catalyst [1,4-bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate in this solvent. In a typical experiment, the polymer and catalyst is dissolved in d_6-acetone under an inert atmosphere followed by addition of para-hydrogen (enriched to 95%) at a pressure of 3.5 atm. The NMR tube is heated to 60°C, shaken at low magnetic field, immediately inserted into a 300MHz NMR spectrometer and the resulting ^{1}H spectrum is recorded.

Results

The highest observed ^{19}F DNP enhancement was -37 which equals an acquisition time saving of 1400 for ^{19}F NMR measurements. Indeed, the DNP-enhanced spectrum (single shot) showed a slightly better signal-to-noise ratio than the reference spectrum acquired with 1000 scans (Fig.1). Interestingly, the fluorine atoms are located at the inner parts of the hyperbranched molecule which hinders the direct radical-^{19}F contact. This steric hindrance significantly lowers the DNP coupling factor which explains the reduced enhancement as compared to water protons (-110) under these conditions. The result of the PHIP experiment is shown in Fig.2. The alkene protons at 6.02 and 5.22 ppm were assigned to the strongly polarised peaks (signal enhancement of 1500) exhibiting the typical signature of absorption and emission signals for ALTADENA PHIP enhancement. It should be noted that the integrated intensity of the alkene chain-ends is much greater than the peak at 4.16 ppm which arose from the thermally polarised PEGMA repeating monomer unit. Surprisingly, a large signal enhancement is also observed for cyclooctene (the positive and negative peaks at 5.61 and 1.46 ppm, respectively) which arises through hydrogenation of the cyclooctadiene ligand of the PHIP catalyst.

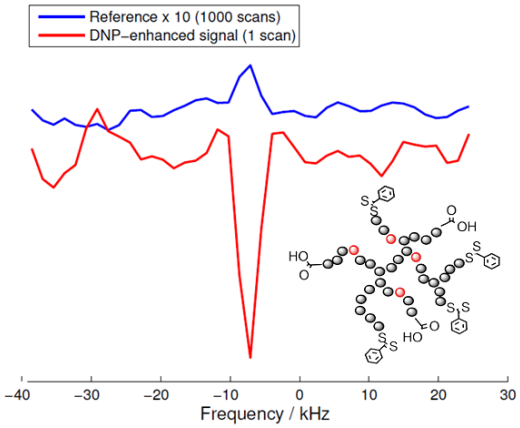

Fig. 1: Comparison of the reference spectrum (1000 scans) and the DNP-enhanced single-shot ^{19}F signal (1 scan) acquired on the same sample. The position of the ^{19}F containing and hyperpolarized moiety is indicated in red in the molecular structure of the used hyperbranched polymer.

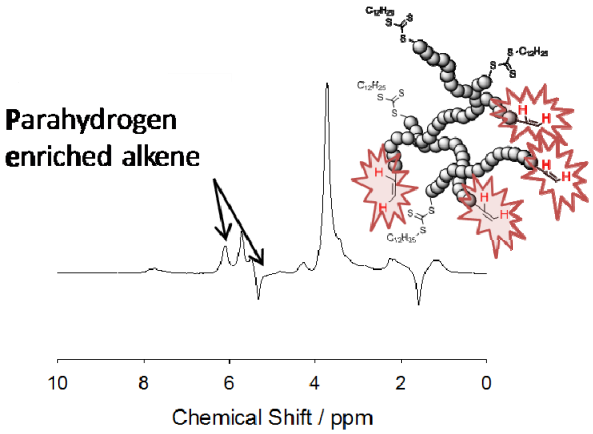

Fig. 2: ^{1}H NMR spectrum of a hyperbranched polymer following PHIP polarisation. Note the intensity of the hyperpolarized alkene end-groups is comparable to the intensity of the thermally polarized protons in the whole polymer. The chemical structure of the hyperpolarized polymer is shown and the hyperpolarized protons are highlighted.

Discussion

The DNP enhancement of a dissolved ^{19}F containing molecule presented here can be seen as a proof of principle for the applicability of Overhauser DNP for direct hyperpolarization of ^{19}F in large polymer molecules. This approach can enhance the sensitivity in polymeric ^{19}F MRI and can be envisaged to allow for the monitoring of dynamic processes happening on a short time scale. Moreover, we demonstrated dramatic ^{1}H signal enhancements in a hyperbranched polymer upon PHIP hyperpolarization despite the short T_1 relaxation time of the polymer and the level of steric hindrance such a system have towards a reactive catalyst species [5]. These results open up new possibilities for designing dual-modal MR imaging agents that combine highly sensitive PHIP or DNP enhancements for measuring relatively fast biological processes with an additional imaging mechanism that is likely much less sensitive (normal ^{19}F MRI), but which would provide contrast over a longer time-scale.

References

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