DNP and EPR properties of a biocompatible macromolecule for EPRI and in vivo MRI

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Introduction

The application of ^{13}C (or other low γ nuclei) NMR spectroscopy and imaging for clinical diagnosis has been constrained by the extremely long imaging and spectroscopy acquisition times that are required to obtain high SNR under physiological conditions (low natural abundance of ^{13}C, low concentration of ^{13}C-compounds, physiological temperature etc.). However, this obstacle could be overcome by in vitro hyperpolarization of a ^{13}C containing molecule with long spin lattice relaxation time via dynamic nuclear polarization (DNP) or parahydrogen induced polarization (PHIP) and subsequent injection into the animal or patient of investigation [1, 2, 3]. One major issue in this concept is the toxicity of the dissolved radicals. There are mainly two approaches to overcome this obstacle. The first is the subsequent separation of polarizing agent and the solution to be injected [4]. The second is the use of non-toxic polarizing agents without separation of the radicals. Here we present spin-labeled biocompatible heparin macromolecules investigated by EPR which show high ^{1}H DNP enhancements [5].

Material and Methods

For EPR irradiation and NMR/EPR detection a Bruker (Karlsruhe, Germany) probehead originally designed for electron nuclear double resonance (ENDOR) was used. For the DNP/EPR experiments the field strength of the electromagnet was adjusted to 0.35 T, corresponding to electron and ^{1}H Larmor frequencies of 9.7 GHz and 14.7 MHz, respectively. Continuous-wave (CW) EPR spectra were recorded at room temperature whereas the echo-detected and double electron-electron resonance (DEER) measurements were performed at a temperature of 50 K. For NMR detection a low field spectrometer (Kea, Magritek, Wellington, New Zealand) was employed (1-30 MHz). Novel heparin-nitroxide derivatives with four different labeling degrees and positions were used for the DNP experiments. 1. H-NR 1: Nitroxide conjugated with heparin via carboxyl group without linker (20 % disaccharides modified by Tempo). 2. H-NR 2: Nitroxide conjugated with heparin via carboxyl group without linker (72 % disaccharides modified by Tempo). 3. H-NR 3: Nitroxide conjugated with heparin via carboxyl group with a linker (45 % disaccharides modified by Tempo). 4. H-NR 4: Nitroxide conjugated with heparin via amino group without linker (65 % disaccharides modified by Tempo). The spin-labeled macromolecules have an average molecular mass of M_w=18,000. Each of the heparin-nitroxides was dissolved in deionized water to give a radical concentration ranging from 1.5 mmol to 10 mmol.

Results

For the characterization of the heparin-nitroxides we analyzed the measurements of CW and echo-detected (ED) EPR spectra as well as DEER spectra. As an example Figure 2 shows the two CW EPR spectra of two spin-labeled heparin molecules which differ only in their degree of labeling. In CW spectra, strong interactions between the electron spins manifest themselves in a broadening of the lines. In Figure 2 the heparin-nitroxide with the higher radical-labeling (H-NR 2) shows broader lines than the one with the lower labeling degree (H-NR 1). The complementary methods ED EPR and DEER confirm the stronger dipolar coupling between the electron spins for higher labeled heparins. DNP measurements for the four investigated spin-labeled heparins yielded ^{1}H DNP enhancement factors of -74 (@ 12 W mw power, 6 mmol), –84 (@ 12 W mw power, 10 mmol), –68 (@ 12 W mw power, 6 mmol) and –91 (@ 12 W mw power, 6 mmol) for H-NR 1 (20 % labeling degree), H-NR 2 (72 % labeling degree), H-NR 3 (45 % labeling degree) and H-NR 4 (65 % labeling degree), respectively. In order to evaluate the maximum DNP enhancements, which can be obtained with the different spin-labeled macromolecules at our experimental conditions, we measured the DNP enhancement factors for each heparin-nitroxide at different microwave powers. By plotting the reciprocal Overhauser enhancement 1/(1-enhancement factor) against the reciprocal EPR power level 1/P the maximum achievable DNP enhancement factors can be extrapolated [2, 6]. Our data show the expected linear relation and the maximum enhancement factors obtained by this method are: -86 for H-NR 1, -94 for H-NR 2, -73 for H-NR 3 and -102 for H-NR 4. For the free radical Tempol we found an enhancement of -100 and a maximum enhancement of -101.

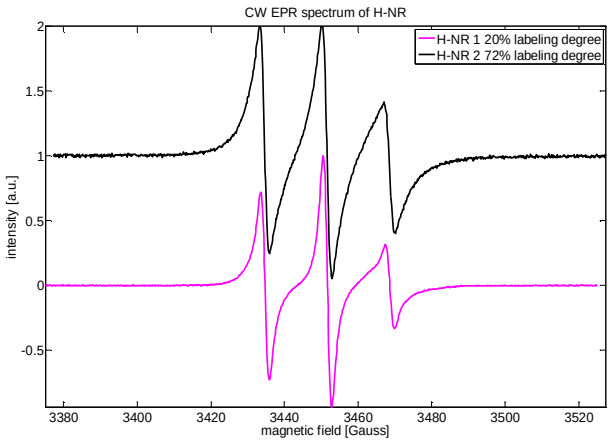

Fig. 1:
General structure of the heparin-nitroxide labeled via the carboxyl groups.

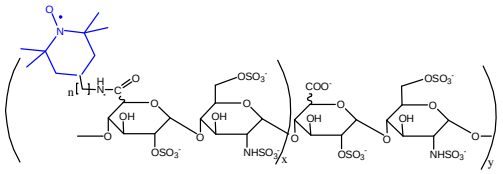

Fig. 2:
CW EPR spectra at room temperature of heparin-nitroxides dissolved in water with a radical concentration of 6 mmol.
Magenta: Heparin-nitroxide H-NR 1 (20 % Tempo radical-bearing disaccharides, Tempo bound covalently to carboxyl group)
Black: Heparin-nitroxide H-NR 2 (72 % Tempo radical-bearing disaccharides, Tempo bound covalently to carboxyl group)

Discussion

Our results demonstrate a versatile and biocompatible spin-labeled macromolecule that can be used for EPRI and *in vivo* MRI [5]. One striking result concerning the very broad lines of the heparin-nitroxides in the EPR spectra is the fact that the achievable enhancements are comparable to free Tempol. Usually, for free radicals broad lines correspond to low achievable enhancement factors. This effect might be due to the broad range of intramolecular and intermolecular radical-radical distances and hence of dipolar couplings between electron spins that can be expected because of the statistical nature of the labeling and the manifold of conformations that a polysaccharide in solution may adopt [7]. In this respect, the spin-labeled heparin can be seen as a "broad-band" polarizing agent that might also be suitable for an efficient hyperpolarization of ^{13}C containing molecules.

References

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