Dynamic nuclear polarization properties of nitroxyl radical in high viscous liquid using Overhauser-enhanced Magnetic Resonance Imaging (OMRI)

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ABSTRACT

The dynamic nuclear polarization (DNP) studies were carried out for ^{15}N labeled carbamoyl-PROXYL in pure water and pure water/glycerol mixtures of different viscosities (1.8 cP, 7 cP and 14 cP). The dependence of DNP parameters was demonstrated over a range of agent concentration, viscosities, RF power levels and ESR irradiation time. DNP spectra were also recorded for 2 mM concentration of ^{15}N labeled carbamoyl-PROXYL in pure water and pure water/glycerol mixtures of different viscosities. The DNP factors were measured as a function of ESR irradiation time, which increases linearly up to 2 mM agent concentration in pure water and pure water/glycerol mixtures of different viscosities. The DNP factor started declining in the higher concentration region ($\sim$3 mM), which is due to the ESR line width broadening. The water proton spin–lattice relaxation time was measured at very low Zeeman field (14.529 mT). The increased DNP factor (35%) was observed for solvent 2 (η = 1.8 cP) compared with solvent 1 (η = 1 cP). The increase in the DNP factor was brought about by the shortening of water proton spin–lattice relaxation time of solvent 2. The decreased DNP factors (30% and 53%) were observed for solvent 3 (η = 7 cP) and solvent 4 (η = 14 cP) compared with solvent 2, which is mainly due to the low value of coupling parameter in high viscous liquid samples. The longitudinal relaxivity, leakage factor and coupling parameter were estimated. The coupling parameter values reveal that the dipolar interaction as the major mechanism. The longitudinal relaxivity increases with the increasing viscosity of pure water/glycerol mixtures. The leakage factor showed an asymptotic increase with the increasing agent concentration. It is envisaged that the results reported here may provide guidelines for the design of new viscosity prone nitroxyl radicals, suited to the biological applications of DNP.

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1. Introduction

Nitroxyl radicals are less toxic and stable organic free radicals, which belong to the six-membered or five membered ring

structure. These radicals have been widely used as a spin probe for invivo/invitro electron spin resonance (ESR)/Overhauser-enhanced magnetic resonance (OMR) imaging techniques to analyze the interactions, reduction and oxidation status of free radicals and tissue oxygenation in living animals [1–9]. ESR imaging provides the spatial distribution of the nitroxyl radical where as anatomic information is not available. Overhauser-enhanced magnetic resonance imaging (OMRI) is a double resonance technique that uses the presence of paramagnetic agents to enhance the signal intensity from nuclear spins by means of a process known as dynamic nuclear polarization (DNP) or Overhauser effect [10,11]. In this phenomenon, the relatively stronger magnetic moment of the electron is used to enhance the polarization of nuclear spins, thereby enhancing their signal. The unique advantage of this technique is high spatial resolution of the image and short acquisition

time. The significant contrast-to-noise ratio obtained by this technique makes OMRI advantageous in obtaining physiological information. OMRI is a promising technique for imaging the distribution and dynamics of free radicals [12–15], which is also used to demonstrate the reduction and oxidation process of ^{14}N and ^{15}N labeled nitroxyl radicals simultaneously [16].

Recently, Utsumi et al. used the membrane-impermeable spin probe, carboxy PROXYL and visualized the whole body kinetic image of mice with better spatiotemporal resolution, which provided both anatomic and physiological information of organ function for preclinical and clinical studies of oxidative diseases [17]. The nitroxyl spin probe, 3-carbamoyl-PROXYL has a partition coefficient that is intermediate between carboxy-PROXYL and methoxycarbonyl-PROXYL, which is expected to have intermediate level membrane permeable activity, and thus may penetrate cerebrovascular cell membranes with low efficiency. The nitroxyl spin probe 3-carbamoyl-PROXYL is one of the best spin probe for measuring nitroxide dynamics and redox imaging, based on magnetic resonance imaging of organic contrast agents in mice [18]. The carbamoyl-PROXYL, a redox sensitive water soluble spin probe was used to observe the small disruptions in brain vascular permeability by using magnetic resonance imaging technique [19]. The in vivo ESR/NMR co-imaging of the nitroxyl spin probe 3-carbamoyl-PROXYL in living mice enabled in vivo organ specific mapping of free radical metabolism and redox stress and the alternations occur in the parthogenesis of disease [20]. The isotopic substitution of the nitrogen atom from the naturally abundant ^{14}N to ^{15}N provides enhanced detection sensitivity by decreasing the spectral multiplicity, the saturation of one ESR line in ^{15}N nitroxyl radical induces a 17% increase in the proton polarization [21]. Hence, the ^{15}N labeled carbamoyl-PROXYL nitroxyl radical is chosen as a spin probe for the present study.

The DNP studies have provoked considerable interest in the field of biomedical imaging. The dynamic nuclear polarization properties of nitroxyl radicals in biological fluids was reported [22]. The dependence of image intensity on proton mobility was demonstrated for samples of the nitroxyl radical in water/glycerol mixtures of different viscosities [23,24]. Recently, the ESR studies on nitroxyl radicals in high viscous liquids have been reported, which reveals that the nitroxyl radicals having narrowest linewidth is suitable for in vivo/in vitro studies using ESRI/OMRI techniques [25].

In this work, OMRI has been used to produce NMR images reflecting changes in the viscosity of aqueous free radical solutions, with a view to studying molecular dynamics in biological samples such as blood, plasma, serum, plasma membrane and possibly in vivo. The ^{15}N nitroxyl radical provides enhanced detection sensitivity by decreasing the spectral multiplicity. Hence the ^{15}N labeled carbamoyl-PROXYL is chosen for this study. The biological fluids viscosity is in the range of 1.8–14 cP. In order to understand and probe the viscosity effect on the DNP properties of ^{15}N labeled carbamoyl-PROXYL in various solvents with different viscosities. Here, the dynamic nuclear polarization studies of ^{15}N labeled carbamoyl-PROXYL in pure water and water/glycerol mixtures with different viscosities is reported.

2. Theory

Theoretical principles of OMRI are well documented [26–28]. Nevertheless, a brief outline of the principles relevant to the experiments reported herein is presented in this section. The enhancement, E of the NMR signal of the ^{1}H nuclei ($I = 1/2$) of water molecules with couplings to an unpaired electron spin $S = 1/2$ of a dissolved free radical, is given by

$$E = \frac{\langle I_z \rangle}{I_0} = 1 - \rho f s \frac{|\gamma_e|}{\gamma_N} \qquad (1)$$

Here $\langle I_z \rangle$ denotes the expectation value of the dynamic nuclear polarization, I_0 is its thermal equilibrium value, ρ is the coupling parameter, f is the leakage factor, s denotes the saturation parameter, and γ_e and γ_N are, respectively the electron and nuclear gyro magnetic ratios.

The leakage factor f in Eq. (1) that accounts for the loss of polarization, which is sensitive to the motion and it also depends upon the concentration of the nitroxyl agents, as given by,

$$f = 1 - \frac{T_1}{T_{10}} = \frac{kCT_{10}}{1 + kCT_{10}}, \qquad (2)$$

Here T_1 denotes the water proton spin–lattice relaxation time of the nitroxyl agent solution. Intrinsic nuclear relaxation rate of water proton in the absence of nitroxyl agent is denoted by $1/T_{10}$. The concentration of the nitroxyl agent is given by C, and k denotes the relaxivity constant. As the concentration of the agents is increased the leakage factor approaches to unity, because with increasing C, $kC \gg 1/T_{10}$

The saturation factor (s) is critical to the sensitivity of OMRI, which is given by the degree of saturation of the electron spin,

$$s = \frac{(S_0 - \langle S_z \rangle)}{S_0}, \qquad (3)$$

where S_0 is the equilibrium polarization of the electron spins and $\langle S_z \rangle$ is the polarization upon irradiation of the ESR resonance. The complete saturation occurs when the spin populations of the energy levels are equal and in this limit s approaches its maximum value of unity. For on resonance irradiation at the center frequency of one of the hyperfine components of the nitroxyl agent, by an oscillating magnetic field of amplitude B_1, the saturation factor is given by,

$$s = \frac{\gamma_e^2 B_1^2 T_{1e} T_{2e}}{1 + \gamma_e^2 B_1^2 T_{1e} T_{2e}} \qquad (4)$$

Here T_{1e} and T_{2e} are the electron spin–lattice relaxation time and spin–spin relaxation time, respectively. For complete saturation of one of the ESR transitions, and the condition that $1/T_{10} \ll 1/T_1$, the enhancement factor is given by,

$$1 - E = -\frac{(\gamma_e/\gamma_N)\rho}{(2I + 1)} \qquad (5)$$

where I is the relevant nuclear spin quantum number, (1 for ^{14}N and ½ for ^{15}N). The Overhauser enhancement reaches maximum values of 110 and 165, respectively for ^{14}N and ^{15}N nitroxyl agents for pure dipolar, and 220 and 330 for scalar interactions. Experimentally, these maximum values are not realized due to many factors. In nitroxyl agents, there is additional hyperfine interaction between the hydrogen nuclei and the unpaired electron. Hence the three (for ^{14}N) or two (for ^{15}N) ESR lines are inhomogeneously broadened due to the presence of the unresolved hydrogen hyperfine. An inhomogeneously broadened ESR line with very closely spaced hyperfine lines has Voigt line shape function, the convolution of a Lorentzian line shape with a Gaussian intensity profile [29]. Hence Eq. (4) will no longer hold good, and irradiation of one of the nitrogen hyperfine lines will result only in partial saturation. Nevertheless, the enhancement factor, achieved by irradiating a single ESR line of the nitrogen hyperfine line of the nitroxyl agent can be approximately given [23,30] by combining Eqs. (1), (2) and (5),

$$\frac{1}{1 - E} = \frac{1}{658}\left(1 + \frac{1}{kCT_{10}}\right)(2I + 1)\left(1 + \frac{1}{\alpha P}\right)\frac{1}{\rho} \qquad (6)$$

Here P is the applied ESR power level, which is proportional to B_1^2, and α is a constant related to the conversion efficiency of the coil and the relaxation times of the electron spins. Therefore, a plot of