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Study of Local Diffusion coefficients of the Hydration layer of Lipid Vesicle bilayers

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Abstract

Water molecules near the surface of vesicle bilayers exhibit slow dynamics with respect to that of pure bulk water as they belong to the hydration layer. We present a unique analysis tool for the selective detection of local water of the hydration layer on the surface of unilamellar vesicles and the determination of its diffusion coefficients. We utilized stable nitroxide radicals covalently attached to the hydrophilic head groups of DOPC lipid chains that incorporate along with other lipids into vesicles. Through the use of dynamic nuclear polarization (DNP) the ^{1}H NMR signal of local water interacting with the radical is amplified, and we present here an analysis of the local diffusion coefficients of this hydration layer.

Keywords

Dynamic Nuclear Polarization, Overhauser Effect, Hydration layer, Local Diffusion coefficients, Unilamellar Vesicles.

1. Introduction

Measurement of localized diffusion constants of the hydration layer of soft matter systems is difficult, because schemes for isolating just the surface dynamics of solvent are not common. Dynamic nuclear polarization can help in providing a contrast by selectively enhancing nuclear spins interacting with the electron spins of the free radical. The technique needs only small quantities of sample (5 µL) and small radical concentrations (< 500 µM). As translational diffusion dominates the dipolar interactions between the water protons and spin labels on lipid vesicles, we can model it to quantify the local diffusion coefficients. We have also shown this value to be sensitive to the chemical nature of the lipid head groups composing the vesicle.

2. Materials and Methods

Measured stock solutions of the lipids DOTAP and DOPG, and spin labels Tempo-PC (Avanti Polar Lipids), 16-DS and 5-DS (Sigma Aldrich) were dissolved in chloroform/ethanol and mixed. Lipid concentrations of 35 mM and spin label concentrations of 675 μM were maintained for all samples. Volumes of 5.0 μL of the vesicles were prepared and then transferred to 0.8 mm inner diameter capillaries for the experiments.

ESR spectra were acquired on a Bruker EMX X-band spectrometer at 295 K equipped with a rectangular TE_{102} cavity using a center field of 0.35 T and microwave frequency of 9.8 GHz. Typically, a modulation amplitude of 0.12 Gauss, a modulation frequency of 100 kHz and a field sweep range of 100 Gauss were used. DNP-enhanced NMR experiments were carried out as described previously [1]. A home-built U-shape NMR coil made of 0.013 inch diameter Teflon coated silver wire was introduced into the tuned and matched TE_{102} ESR cavity. The coil was tuned to 14.8 MHz and connected to the broadband channel of a Bruker Avance NMR spectrometer. During DNP operation, the unpaired electrons are irradiated with a power output between 100 mW and 1.5 W. It has been shown that for sample setups and volumes as employed here, minimal sample heating effects occur [1].

3. Theory

In a system of two interacting spins (here a water proton and an unpaired electron of a nitroxide radical) coupled together through dipolar or scalar interactions, non-equilibrium proton spin polarization can result from the saturation of electron spin's magnetic transitions with an oscillating magnetic field. If the cross-relaxation between electron and proton spins is mediated by molecular motion (translation or rotation), this DNP mechanism is known as the Overhauser effect, which is more effective in liquid samples and at lower magnetic fields (< 1 T, depending on the translational and rotational correlation times of the solvent and/or radicals, τ_{rot} and τ_{t}, respectively) [2]. In liquids, the DNP-induced NMR signal enhancement at extrapolated maximum power, $E_{\max}$, depends on the interaction between the electron and proton by:

$$\mathrm{E}_{\max} = 1 - \rho f s_{\max} \frac{|\gamma_s|}{\gamma_I}, \tag{1}$$

where ρ is the coupling factor describing the coupling between the electron and proton, f is the leakage factor describing the proton T_1 relaxation due to dipolar or scalar interactions with the electron spin over other proton relaxation mechanisms, $s_{\max}$ is the saturation factor describing the saturation of the electron spin Zeeman transition at maximum microwave power, γ_S is the magnetogyric ratio of the electron and γ_I is the magnetogyric ratio of the proton; thus $|\gamma_S|/\gamma_I = 658$. The negative enhancement for the Overhauser effect for protons originates from the dipolar nature of their motional coupling to the electrons (positive coupling factor), and manifests itself as NMR signal with negative amplitude compared to the equilibrium NMR signal.

$E_{\max}$ can be experimentally determined for each sample by measuring E while varying the applied microwave power, and then extrapolating to infinite power. The maximum saturation value, $s_{\max}$, approaches 1 for nitroxide spin labels across a wide spectrum of rotational freedom ($\tau_{\mathrm{rot}} \sim 7\times10^{-10}$ s - 5×10^{-7} s), including typical rotational diffusion in micelle, vesicle or protein aggregate systems [3]. Also, f can be experimentally determined by measuring the solvent ^{1}H T_1 time with and without dissolved radical. So, it is straightforward to translate the DNP enhancement at infinite power ($E_{\max}$) for a wide range of conditions, into the coupling factor, ρ. The coupling factor for ^{1}H NMR enhancement of water through dissolved nitroxide spin labels at 0.35 T was determined to be around 0.22, which is expected to decrease in more viscous, and increase in less viscous, local environments [3].

We use the pure translational model for Overhauser-driven DNP enhancement of water protons by dissolved stable nitroxide radicals as described by Hausser and Stehlik [2]. The correlation time of the motion between the protons of water and the unpaired electron of the radicals can be approximated by a single translational correlation time, τ_t, given by [2]

$$\tau_t = \frac{d^2}{D_I + D_S} \qquad (2).$$

This is valid if the relaxation times (T_{1e}, T_{2e}, and T_e, the Heisenberg exchange relaxation time) of the electron spin are long compared to τ_t, which is the case for nitroxide radicals in fluid water under ambient condition. The value of D_I can be obtained by assuming a distance of closest approach of 4.5 Å (same as in the case of free in solution) and ignoring D_s which is expected to be at least an order of magnitude smaller.

4. Results and Discussion

The results are summarized in table 1. The rotational correlation times of the spin labeled molecules (here surfactants) were obtained by fitting the ESR data with the simulation and fitting program package (EasySpin, ETH). The modeling program can be used to estimate axial rotational rates of the spin labeled molecular segments. The differing values of the parallel and perpendicular values of the correlations times indicate the anisotropy in the system. The hyperfine coupling constant, a_N, that measures the hyperfine splitting of the unpaired electron due to the nitroxide's ^{14}N nuclear spin were obtained from the cw ESR spectra, and the observed values of around ~16 Gauss are representative of a hydrated environment. For the diffusing protons, approximately 90 % of the relaxation is caused by motions within the first 10Å from the nitroxide center [4]. The diffusion coefficients obtained for water in the hydration layer ($0.9\text{-}1.3\times10^{-9}$ m^2/s) differ by a factor of 2 or more from the bulk value (2.3×10^{-9} m^2/s). The diffusion coefficients also have been found to depend on the chemical nature of the head group as water molecules near DOTAP vesicles (headgroup carries positive charge due to absence of phosphate groups) have higher diffusion compared to DOPG vesicles (headgroup is zwitterionic).

Lipids	DOPG+ DOPC	DOTAP
$R_\perp$	$3.0\times10^7 s^{-1}$	$3.4\times10^7 s^{-1}$
$R_\parallel$	$95\times10^7 s^{-1}$	$125\times10^7 s^{-1}$
a_N	16.1 Gauss	16.2 Gauss
ρ	0.075	0.12
D	$0.87\times10^{-9} m^2/s$	$1.31\times10^{-9} m^2/s$

Table 1: The rotational correlation times $R_\parallel$ and $R_\perp$, and the hyperfine coupling constant a_N have been obtained by fitting the ESR spectra. The Diffusion coefficients have been obtained by measuring the ρ value from the DNP enhancements, and then employing the spectral density function appropriate for pure translational correlations between the coupled spins.

We conclude that DNP is a powerful technique to measure local diffusion coefficients of the hydration layer water of soft matter systems especially since these cannot be determined using conventional techniques like PGSE-NMR. This is because of the lack of chemical shift resolution and small signal amplitude of this water. As the sample volume needed for the experiment is only a few microliters and the experiments are carried out under physiological conditions, the technique will prove to be very useful for biologically relevant samples.

References

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