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Probing membrane topology by high-resolution ^{1}H–^{13}C heteronuclear dipolar solid-state NMR spectroscopy

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Abstract

Membrane topology changes introduced by the association of biologically pertinent molecules with membranes were analyzed utilizing the ^{1}H–^{13}C heteronuclear dipolar solid-state NMR spectroscopy technique (SAMMY) on magnetically aligned phospholipid bilayers (bicelles). The phospholipids ^{1}H–^{13}C dipolar coupling profiles lipid motions at the headgroup, glycerol backbone, and the acyl chain region. The transmembrane segment of phospholamban, the antimicrobial peptide $(KIGAKI)_3$ and cholesterol were incorporated into the bicelles, respectively. The lipids ^{1}H–^{13}C dipolar coupling profiles exhibit different shifts in the dipolar coupling contour positions upon the addition of these molecules, demonstrating a variety of interaction mechanisms exist between the biological molecules and the membranes. The membrane topology changes revealed by the SAMMY pulse sequence provide a complete screening method for analyzing how these biologically active molecules interact with the membrane.

Keywords: SAMMY; Phospholipid bilayers (bicelles); Biologically pertinent molecules; ^{1}H–^{13}C dipolar coupling; Membrane topology

1. Introduction

Functioning cell membranes contain a large variety of biological additives. The interaction between biological pertinent molecules and lipids plays a key role in modulating the physical characteristics of membranes, and directly determines the activity of cell membranes [1,2]. The most common method for probing these interactions utilizing solid-state NMR techniques has been a combination of both ^{2}H and ^{31}P NMR spectroscopy. In this communication, a recently reported high-resolution heteronuclear dipolar solid-state NMR spectroscopy technique (SAMMY) [3] is applied to magnetically aligned phospholipid bilayers (bicelles) [4] to detect and characterize natural abundant ^{13}C lipid motion perturbations induced by the association of biological molecules with the membrane bilayer.

The dipole–dipole interaction between two nuclei is related to the internuclear distance, the orientation of the dipolar interaction tensor with respect to the magnetic field, and the motion of the molecule. For phospholipid molecules in a hydrated bilayer sample, the heteronuclear dipolar coupling interaction between ^{13}C and the covalently bonded ^{1}H reflects the ordering and the motional rates of the individual segments of the lipid molecules [5]. The addition of biologically pertinent molecules to membrane bilayers modulate the ordering and motions of the phospholipids [5]. Therefore, the ^{1}H–^{13}C dipolar coupling of phospholipid bilayers reflects the interaction between the lipids and the molecules, and elucidates pertinent membrane-protein topology information.

To obtain a high-resolution solid-state NMR spectrum of a randomly dispersed bilayer sample, magic-angle spinning of the sample at relatively high speeds is needed which will simultaneously attenuate the anisotropic dipolar interactions. These dipolar interactions can be ascertained utilizing dipolar recoupling pulse schemes, such as FSLG-CP [6,7], R-PDLF [8], DIPSHIFT [9] at a specific sample

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spinning speed. Alternatively, the PISEMA or SAMMY solid-state NMR pulse technique on an oriented membrane sample provides a direct way to obtain a high-resolution two-dimensional ^{1}H–^{13}C heteronuclear dipolar couplings [10]. The SAMMY pulse sequence was chosen because it has a better signal-to-noise ratio, more reliable scale factors for all resonances and is much easier to setup, when compared to the PISEMA sequence [3]. The bicelle alignment method was used because the membrane system can be easily aligned in a solution NMR spectrometer above the gel to liquid crystalline phase transition temperature. The aligned bicelles make high-resolution NMR spectra possible without spinning the sample at the magic angle [11]. Furthermore, the fully hydrated bicelles provide an environment close to physiologically relevant conditions [4]. The experiments were carried out on a standard 500 MHz narrow bore using a soft SAMMY pulse sequence that does not require a high power amplifier, wide bore magnet, and magic-angle spinning capability. Thus, this method is potentially widely applicable to a wide range of users.

2. Results and discussion

Fig. 1 exhibits the two-dimensional ^{1}H–^{13}C dipolar coupling/^{13}C chemical shift spectrum of aligned DMPC/DHPC bicelles with a DMPC to DHPC molar ratio of 3.5 at 37 °C utilizing the SAMMY pulse sequence. The Hartmann–Hahn contact time was 1 ms. The ^{1}H 90° pulse was 10.1 μs and the recycle delay was 3 s. The one-dimensional ^{13}C chemical shift spectrum of the same bicelle sample (shown at the top) was obtained separately using a standard cross-polarization sequence. The assignment of the ^{13}C chemical shifts of phospholipids is based on previous studies utilizing magic-angle sample spinning (MAS)-NMR spectroscopy for a randomly dispersed bilayer sample [6,12] and Sanders assignment on bicelles [13]. The ^{13}C chemical shift values for the lipid acyl chain region are consistent with those obtained for the bilayers. However, at the lipids headgroup and glycerol regions the bicelles exhibit a slight shift in peak positions when compared to the bilayers. The C_{β}, GC_1 and GC_3 peaks become crowded together with much lower intensity for glycerol groups. It is difficult to distinguish between the GC_1 and GC_3 peaks. The spectrum of the aligned sample exhibits excellent linewidths with comparable spectral resolution as that of the randomly oriented sample. For bicelles, the bilayer normal of phospholipids are aligned with its long molecular axis perpendicular to the static magnetic field [11,14].

In the one-dimensional ^{13}C spectrum, the ^{13}C chemical shifts originating from individual carbons of less abundant DHPC molecules may overlap with those from DMPC and affect the spectral resolution. In this research, no significant DHPC contribution was observed in the ^{13}C chemical shift dimension. However, in the two-dimensional SAMMY spectrum, some ^{1}H–^{13}C dipolar coupling contours with small splitting values were observed, which is probably caused by DHPC molecules with relatively faster motion

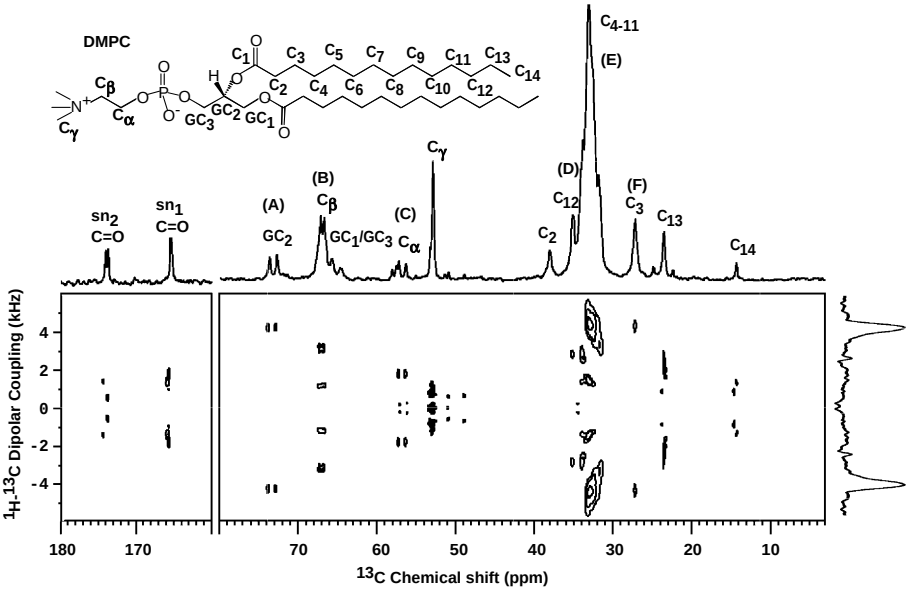

Fig. 1. Two-dimensional ^{1}H–^{13}C dipolar coupling/^{13}C Chemical shift spectrum of a DMPC/DHPC bicelle sample (1:3 w/w phospholipids in 100 mM Hepes buffer) utilizing the SAMMY pulse sequence on a Bruker 500 MHz narrow bore NMR spectrometer at 37 °C. The Hartmann–Hahn contact time was 1 ms. The ^{1}H 90° pulse was 10.1 μs and the recycle delay was 3 s. The spectra were collected with 200 increments in the $t1$ dimension and 1024 data points in the $t2$ dimension. Scans (512) were accumulated for each $t1$ increment. The ^{1}H–^{13}C dipolar coupling value is the apparent dipolar coupling value without considering the scaling factor. The one-dimensional ^{13}C chemical shift spectrum was obtained using a standard cross-polarization sequence with proton decoupling. The carbon position labeling is defined in the inset with the DMPC structure. The resonance peaks corresponding to the carbonyl carbons of phospholipids (*sn1* and *sn2* acyl chains) are shown in the left panel. The slice on the dipolar dimension represents the ^{1}H–^{13}C dipolar coupling taken from GC_2 group. Labels (A–F) were used to indicate the spectral regions exhibited in Fig. 2.

than DMPC molecules [14]. It is possible in the future to use fully deuterated DHPC in preparing the bicelle samples to diminish the DHPC ^{13}C signals obtained by ^{1}H–^{13}C cross-polarization and reduce the number of ^{1}H–^{13}C dipolar coupling contours near the center of the SAMMY spectrum.

A number of doublets were observed in the ^{13}C chemical shift dimension in the two-dimensional SAMMY spectrum. These splittings were absent for the randomly dispersed bilayer sample obtained under MAS conditions. This observation is consistent with previous reports by Sanders [13]. They explained that the splittings are introduced by the dipolar coupling of the isotopically dilute carbons to the headgroup ^{31}P, which is averaged out by the sample spinning at the magic angle for randomly oriented samples. The doublet at around 73, 67, and 57 ppm exhibits the same apparent ^{1}H–^{13}C dipolar coupling values. These results confirm that each doublet originates from the same ^{13}C nucleus. Conversely, another doublet at around 174 ppm, corresponding to the DMPC carbonyl carbon for the *sn2* chain, exhibits a different phenomenon. Two different dipolar coupling values between ^{13}C and indirect coupled ^{1}H were revealed for the two peaks. Sanders implied that the splitting is also caused by ^{31}P–^{13}C dipolar coupling interactions [13]. However, the two-dimensional ^{1}H–^{13}C dipolar coupling/^{13}C chemical shift spectrum suggests that this splitting may be caused by a slight difference in the chemical shift instead of by coupling to a third nucleus (e.g., ^{31}P). One possible reason for the slight difference in the chemical shift is that there are two populations of DMPC carbonyl carbons with slightly different molecular conformation or motions, which is consistent with the ^{13}C–^{1}H dipolar coupling difference.

To probe membrane perturbations induced by biological molecules, two-dimensional ^{1}H–^{13}C dipolar coupling/^{13}C chemical shift spectra were also obtained for DMPC/DHPC bicelle samples after incorporating the transmembrane segment of phospholamban (TM-PLB), the antimicrobial peptide $(KIGAKI)_3$ and cholesterol (Fig. 2). Phospholamban is a transmembrane peptide that regulates cardiac contractility and spans the lipid bilayer [15,16]. $(KIGAKI)_3$ is a positively charged peptide designed to have high antimicrobial activity and is known to strongly interact with the membrane surface [1,17]. The peptide concentrations used were approximately 0.3 mol% with respect to DMPC. For bicelles incorporated with $(KIGAKI)_3$, a reduction of the bicelle alignment temperature was observed by ^{31}P NMR spectroscopy (spectra not shown). The SAMMY experiment was carried out at 34 °C, at which the $(KIGAKI)_3$ bicelles are optimally aligned. Cholesterol is a main component of eukaryotic cell membranes, and is implicated in several diseases, such as heart disease and stroke [2]. At very high concentrations (>10 mol%), cholesterol can alter the bicelle alignment temperature [18]. Five mole percent cholesterol (respect to DMPC) was incorporated into the bicelles to maintain the bicelle alignment at 37 °C.

The two-dimensional strip plots in Fig. 2 reveal the ^{13}C chemical shift values between 75 and 55 ppm that represents the lipid glycerol and headgroup region, while the ^{13}C chemical shift range between 36 to 25 ppm represents the lipid acyl chain region. The control peaks prepared with DMPC/DHPC only are shown in black. The individual lipid group dipolar coupling value is in the same range as that obtained on DMPC bilayers using a power-demanding separated local-field experiment (CT-DIP-SHIFT) [5]. The ^{1}H–^{13}C dipolar splittings decrease in both the lipid headgroup/glycerol region and the acyl chain region when TM-PLB (red) was incorporated into the bicelles, indicating that both the DMPC headgroup and the acyl chains experience a disordering effect. These results are consistent with previous ^{2}H and ^{31}P NMR experimental results on TM-PLB [15]. A more pronounced reduction in the ^{1}H–^{13}C dipolar coupling was observed when $(KIGAKI)_3$ (blue) was added to the lipid bilayers. Close examination also reveals that the lipid headgroups and glycerol region exhibit displacements in the chemical shift. These results indicate that the surface peptide $(KIGAKI)_3$ disturbs the membrane surface and alters the lipid motions. However, the decreased dipolar couplings in Figs. 2D–F suggest that it also perturbs the lipids acyl chain carbons. ^{2}H NMR order parameter experiments carried out on randomly dispersed POPC bilayers exhibited a decrease in the quadrupolar splitting values of each deuteron along the POPC lipids acyl chain upon incorporation of the antimicrobial $(KIGAKI)_3$ peptide [19]. The ^{1}H–^{13}C SAMMY results presented here are consistent with these S_{CD} parameters. Different from TM-PLB and $(KIGAKI)_3$, only a slight increase in the ^{1}H–^{13}C dipolar coupling value in the middle (carbons 4–11) of the DMPC acyl chain region with a definite deshielding in the chemical shift (Fig. 2E) was observed upon addition of cholesterol (green) at 5 mol% to the bicelles. Meanwhile, the dipolar interaction in the glycerol, and headgroup regions of the lipids were nearly undisturbed. The cholesterol molecule has a rigid planar ring structure intercalated in the membrane and parallel to the lipid acyl chains. The addition of cholesterol can diminish the *trans–gauche* isomerization of the lipid acyl chains and increase order within the lipid acyl chains at higher concentrations [18]. Similar deshielding effects in the lipids acyl chain region were also observed in lipid bilayers upon incorporation of cholesterol due to higher molecular ordering [12]. Unlike the large headgroup of DMPC, cholesterol has only a small OH group positioned in the vicinity of the fatty ester groups of the phospholipids [20]. This explains the reduced perturbation observed in the lipid glycerol and headgroup regions upon incorporation of cholesterol. In general, the changes in ^{1}H–^{13}C dipolar coupling values reflect a perturbation effect of these biological pertinent molecules upon association with the bilayer membranes. However, the characteristic of the perturbation may be a motional change or a conformational change or both.

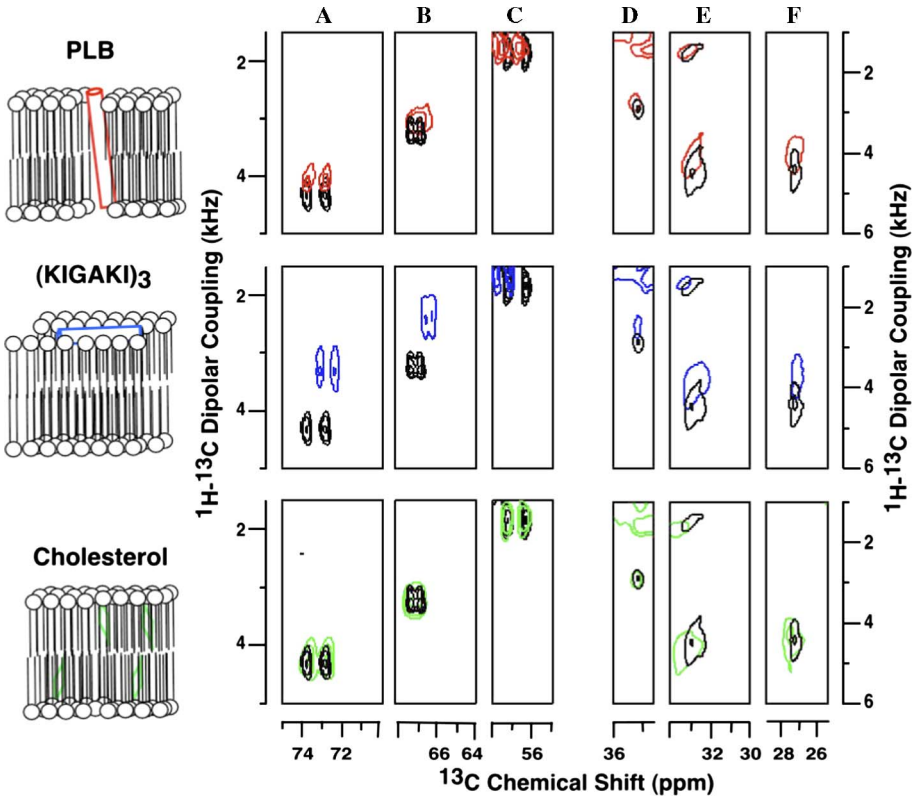

Fig. 2. ^{1}H–^{13}C dipolar coupling profiles comparing control DMPC/DHPC bicelles (black) and bicelles containing the transmembrane segment of PLB (red), antimicrobial peptide $(KIGAKI)_3$ (blue) and cholesterol molecules (green) utilizing a soft SAMMY pulse sequence. The left three bilayer models show the proposed modes of interaction between the lipid bilayer and the added biological molecules. Contours from the lipid glycerol sites (A), headgroup (B and C), and acyl chain region (D–F) are displayed. The bicelle dipolar coupling profile with $(KIGAKI)_3$ was obtained at 34 °C. All the other spectra were obtained at 37 °C.

3. Conclusion

In conclusion, the two-dimensional SAMMY pulse sequence (^{1}H–^{13}C dipolar coupling/^{13}C chemical shift) provides detail information on membrane topology and an easy method to probe the structural perturbations induced upon the addition of biologically relevant molecules. The motional and ordering properties of the headgroup and the acyl chain regions of phospholipid bilayers are usually examined with a combination of ^{31}P and ^{2}H solid-state NMR spectroscopy and different deuterated lipids. ^{2}H NMR spectra usually exhibit poor resolution for acyl chain segments with very rigid molecular motions (top of the acyl chain, carbons 1–6) [11]. The 2D ^{1}H–^{13}C dipolar coupling/^{13}C chemical shift experiment simultaneously reveal high-resolution spectra of the ^{13}C nuclei present in the phospholipid headgroups, glycerol sites, and the acyl chains. Currently, we are working towards complete assignment of the bicelle ^{1}H–^{13}C dipolar coupling/^{13}C chemical shift profile. Here biological pertinent molecules were incorporated at low concentrations into the bicelle samples with comparable large effect on the values of ^{1}H–^{13}C dipolar coupling. The alignment of bicelle samples was verified by ^{31}P NMR spectroscopy (observed sharp ^{31}P peaks at $\sigma_{\perp}$ position). The observed change in the ^{1}H–^{13}C dipolar coupling values is not a result of the decrease in the orientational order of the bicelles. Instead, a combination effect is observed in which changes of the phospholipids conformation and motion is responsible for the deviation of the ^{1}H–^{13}C dipolar coupling values.

The SAMMY spectra were obtained utilizing a standard solution 500 MHz NMR spectrometer and naturally abundant ^{13}C phospholipids. Thus, this membrane topology method is widely applicable because it does not require isotopic labeling, magic angle spinning, or an expensive wide-bore solid-state NMR spectrometer. This research also extends the application of the bicelles from an ideal membrane system for structure determination of membrane proteins to a complete membrane topology method good for characterizing the interaction between membrane lipids and biological molecules.

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