Calculating order parameter profiles utilizing magnetically aligned phospholipid bilayers for H-2 solid-state NMR studies

ARTICLE *in* SOLID STATE NUCLEAR MAGNETIC RESONANCE · SEPTEMBER 2003

Impact Factor: 2.27 · DOI: 10.1016/S0926-2040(03)00052-3 · Source: PubMed

4 AUTHORS, INCLUDING:

Elvis K Tiburu
University of Ghana
20 PUBLICATIONS **351** CITATIONS
SEE PROFILE

Nisreen A Nusair
Walsh University
6 PUBLICATIONS **60** CITATIONS
SEE PROFILE

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Solid State Nucl. Magn. Reson. 24 (2003) 137–149
Solid State Nuclear Magnetic Resonance
http://www.elsevier.com/locate/ssnmr

Calculating order parameter profiles utilizing magnetically aligned phospholipid bilayers for ^{2}H solid-state NMR studies

Paresh C. Dave, Elvis K. Tiburu, Nisreen A. Nusair, and Gary A. Lorigan*

Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA

Received November 15, 2002

Abstract

Solid-state deuterium NMR spectroscopy was used to study the structural and dynamic properties of stearic acid-d_{35} in magnetically aligned phospholipid bilayers as a function of temperature. Magnetically aligned phospholipid bilayers or bicelles are model systems, which mimic biological membranes for magnetic resonance studies. Paramagnetic lanthanide ions (Yb^{3+}) were added to align the bicelles such that the bilayer normal is colinear with the direction of the static magnetic field. The corresponding order parameters of the stearic acid-d_{35} probe were calculated and compared with values obtained from unoriented samples in the literature. The addition of cholesterol to the bicelle system decreases the fluidity of the phospholipid bilayers and increases the ordering of the acyl chains of stearic acid-d_{35}. This study demonstrates the feasibility of utilizing magnetically aligned bicelles for calculating ^{2}H order parameter profiles for non-biological systems such as polymer-grafted membranes and Schiff's base complexes.

Keywords: Solid-state NMR; Bicelles; Magnetically aligned bilayers; Stearic acid; Order parameter; Cholesterol

1. Introduction

Magnetically aligned phospholipid bilayers or bicelles have recently emerged as a model membrane system useful in a variety of NMR studies [1–4]. Bicelles are

*Corresponding author. Fax: +1-513-529-5715.
E-mail address: lorigag@muohio.edu (G.A. Lorigan).

0926-2040/03/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0926-2040(03)00052-3

formed upon mixing long-chain phospholipids, such as 1,2 dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with short-chain phospholipids, such as 1,2 dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC). DHPC coats the rim of the bilayer and isolates the hydrophobic core from water. The size of the bicelle discs increases as the molar ratio $q = n_l/n_s$ between the long-chain (n_l) and short-chain (n_s) phospholipids increases [1,5]. A particularly useful characteristic of the bicelle discs is that they spontaneously align when placed in a strong magnetic field. The magnetic alignment of bicelles is due to the anisotropy of the overall magnetic susceptibility of the system [1]. The negative sign of the diamagnetic susceptibility tensor ($\Delta\chi < 0$) for phospholipid bilayers dictates that the bicelle discs align with their bilayer normal oriented perpendicular to the direction of the static magnetic field. The addition of paramagnetic lanthanide ions with large positive magnetic susceptibilities (Eu^{3+}, Er^{3+}, Tm^{3+}, and Yb^{3+}) can cause the bicelles to flip 90° such that the average bilayer normal is colinear with the direction of the static magnetic field [1]. The ions are thought to associate with the phospholipid headgroups of the bicelles, changing the overall magnetic susceptibility. The spontaneous alignment of bicelles offers significant advantages for performing solid-state NMR studies [6]. In our lab, new methods have been developed to study the structural and dynamic properties of membrane protein systems utilizing spin-labeled EPR spectroscopic techniques to complement solid-state NMR spectroscopic techniques [7–11]. Also, bicellar solutions may consequently be used as a macro-ordered matrix that permits residual dipolar coupling to be used in the NMR structure determination of proteins. The use of lyotropic liquid crystals has been previously proposed for structural biology work, but phospholipid bicelles have proved particularly valuable for this purpose because enzymes have been shown to maintain activity in this environment [12]. High-resolution NMR techniques are now routinely employed to study the structure of complex macromolecules in solution [13–16]. An alternative approach to structural studies of membrane macromolecules is the determination of the orientational and structural properties via solid-state NMR spectroscopy [16]. Aligned bicelles allow the acquisition of high-resolution solid-state NMR spectra of comparable quality to those obtained with samples that have been mechanically oriented on glass or polymer solids [17,18]. The use of bicelles to align membrane proteins is attractive for several reasons, but in particular because a wide variety of peptides and proteins can be easily reconstituted into bicelle discs while their biological activity is retained [1,12].

Recently, there has been considerable interest in the development of biomembrane-mimetic materials that can provide an ordered matrix in which biomolecules such as proteins and peptides can be spatially organized [1,5,11,12]. For example, polymer-grafted membrane liquid crystalline gels, consisting of a quaternary mixture of a phospholipid, a lipopolymer comprising low-molecular-weight poly(ethylene) oxide (PEG) terminally grafted onto the phosphate head group of phospholipids, and a co-surfactant dispersed in water has been reported [19,20]. The magnetically induced alignment process has been studied by using ^2H solid-state NMR spectroscopy. Moreover, our group has incorporated a small amount of PEG polymer into the DMPC/DHPC bicelle discs for EPR and NMR experiments [8,21].

The addition of the PEG polymer has increased the stability of the bicelle discs and is used routinely for low field EPR studies. Interestingly, various liquid crystalline materials have been developed in recent years for a wide variety of different technological applications (for e.g. liquid crystalline displays) [22]. Liquid crystals consist of rod like shapes such as Schiff's base complexes, β-Diketonates, or disc like shapes such as phospholipid bicelles, porphyrins, and phthalocyanine complexes. They have orientational and positional dependence characteristics. These properties change with temperature and can be studied using ^{2}H order parameter profiles. Our present report is exploring the possibility for easily calculating ^{2}H order parameter profiles for different types of oriented systems. The oriented deuterium spectrum does not require spectral deconvolution or the so called de-paking, to resolve multiple splittings in the ^{2}H NMR spectra [23,24]. The quadrupolar splittings provide a direct measure of the orientational and dynamic ordering of C–D bonds (S_{CD}) embedded in anisotropic media.

Solid-state NMR experiments were carried out to evaluate quadrupolar splittings from magnetically aligned phospholipid bilayers. Our aim is to investigate the order parameter profiles obtained by inserting the isotopically labeled NMR probe, stearic acid-d_{35} into magnetically aligned phospholipid bilayers. Previous studies have determined that deuterated fatty acids make excellent, non-perturbing NMR spin probes [25,26]. We incorporated this ^{2}H-labeled fatty acid into a DMPC/DHPC bicelle system ($q = 3.5$) and examined the results via solid-state NMR spectroscopy. Also, cholesterol has been added to the DMPC/DHPC bicelle matrix to examine the effect cholesterol has on the physical properties of the phospholipid bilayers [27,28]. Several articles have discussed the interactions of cholesterol and lipid bilayers, while few reports mention the effect of cholesterol on bicelles [29–31]. We carried out temperature-dependent solid-state NMR experiments at two different cholesterol concentrations (0 and 10 mol% with respect to DMPC). ^{2}H NMR experiments were conducted in the presence of Yb^{3+}. In the present paper, we are focusing on three main points: (1) the calculation of order parameters using quadrupolar splittings obtained directly from magnetically aligned ^{2}H NMR spectra, (2) the effects of cholesterol and temperature on a DMPC/DHPC/ Yb^{3+}/fatty acid bicelle system and (3) the future application of this technique to non-biological systems.

2. Materials and method

2.1. Materials

DMPC, DHPC, and stearic acid-d_{35} were purchased from Avanti Polar Lipids (Alabaster, AL). All phospholipids were dissolved in chloroform and stored at -20°C prior to use. Ytterbium(III) chloride hexahydrate and HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-2-ethanesulfonic acid) were obtained from Sigma-Aldrich. Deuterium-depleted water was obtained from Isotec (Miamisburg, OH). Cholesterol powder was purchased from Alfa Aesar (Ward Hill, MA).

2.2. Sample preparation

DMPC/DHPC bicelle samples ($q = 3.5$) consisting of 25% (w/w) phospholipid to water, were made in 25 ml pear-shaped flasks. DMPC, DHPC and stearic acid-d_{35} dissolved in chloroform were mixed together in the flask at millimolar ratios of 0.1/0.035/0.017 [11]. 10 mol% cholesterol was added with respect to DMPC to some samples. The mixture in the flask was rotovaped down to remove the chloroform from the phospholipid mixture and the flask was placed under high vacuum overnight.

The following day, a 100 mM HEPES buffer of pH 7.0 was prepared using deuterium-depleted water and the appropriate amount was added to the flask. The flask was then vortexed briefly, sonicated with a FS30 (Fisher Scientific) bath sonicator with the heater turned off. Several freeze/thaw (77 K/room temperature) cycles were made until the dispersion was homogenous. The freeze/thaw cycles removed all of the air bubbles in the sample. An aqueous solution of ytterbium(III) chloride hexahydrate was prepared fresh with deuterium-depleted water (38.7 mg in 1 ml) and added (10 μl) to the bicelle sample. The sample was placed on ice for 1 h and then transferred to a 21 mm NMR flat bottom tube with a 5 mm OD via a Pasteur pipette.

2.3. NMR spectroscopy

All solid-state NMR experiments were carried out on a modified Bruker AVANCE 7.05 T narrow bore 300/54 magnet configured to conduct high-power solid-state NMR studies. The solid-state NMR spectra were gathered with a static double-tuned 5 mm round-coil solid-state NMR probe purchased from Doty Scientific. ^{2}H NMR spectra were recorded at 46.07 MHz using a standard quadrupole-echo pulse sequence (3.0 μs 90° pulses, 45 μs innerpulse delay, 5.12 ms acquisition time, 0.4 s recycle delay, and a 150 kHz sweep width). Typically, 20 000 scans were accumulated. An exponential line broadening of 300 Hz was applied to the free induction decay before Fourier transformation. The data were gathered over the temperature range from 30°C to 60°C in 4°C or 5°C increments.

2.4. Order parameter calculations

Order parameters depend upon several averaging modes provided by intramolecular, intermolecular, and collective motions. The relative ease in deuterating molecules and using solid-state NMR spectroscopy has enabled C–D bond order parameter profiles (S_{CD}), to be easily calculated. This C–D bond profile has been proposed by Seelig and co-workers as a measure of the thickness and fluidity of the hydrophobic phospholipid bilayer [32–34]. S_{CD} describes local orientational or dynamic perturbations of the C–D bond vector from its standard state due to perturbations of DMPC phospholipid conformations or dynamics as a result of the addition of DHPC and cholesterol [35,36]. The segmental order parameters were calculated using the equations given by Prosser et al. [37]. In our study, the order

parameter defined as S^i_{CD} is analogous to S^i_{lp} as previously defined by Prosser and co-workers [37]

$$S^i_{CD} = \Delta^i_l/\Delta^i_p, \tag{1}$$

where S^i_{CD} is the order parameter for a deuteron attached to the ith carbon of the fatty acid, Δ^i_l is the observed quadrupolar splitting for a deuteron attached to ith carbon and Δ^i_p is the splitting that would be observed for a stationary deuteron in a C–D bond pointing along the direction of the external magnetic field. Order parameters were calculated assuming a quadrupole coupling constant of $e^2qQ/h = 168$ kHz [37]. In the case where the bicelle discs are aligned parallel to the static magnetic field (addition of Yb^{3+}), Eq. (1) reduces to

$$S^i_{CD} = \Delta^i_l/252, \tag{2}$$

when Δ^i_p is equal to 3/2 e^2qQ/h. The carbon numbering system for the deuterated NMR probe stearic acid-d_{35} is given in Fig. 1. Thus, the corresponding order parameters for the individual C–D methylene groups and the terminal methyl groups of the acyl chains were directly evaluated from the quadrupolar splittings of the ^2H NMR spectra. The ^2H peaks in the NMR spectra were assigned based upon the dynamic properties of the individual CD_2 and CD_3 groups. The quadrupolar splittings for the CD_3 methyl groups at the end of the fatty acid are the smallest and closest to 0 kHz. The next smallest splitting was assigned to the ^2H attached to C-16 and so forth along the fatty acid as shown in Fig. 1. The quadrupolar splittings for the deuterons in the plateau region were estimated by integration of the last broad peak according to the literature [38]. The order parameters calculated for the CD_3 quadrupolar splittings have been multiplied by three [25,27].

3. Results and discussion:

3.1. Magnetic alignment of DMPC/DHPC bicelles investigated by stearic acid-d_{35}

The bicelle system under investigation is composed of DMPC and DHPC phospholipids at a molar ratio of 3.5:1. Solid-state NMR studies have indicated that the addition of fatty acids in small concentrations to phospholipid bilayers has no marked effects on the bilayers, this is in contrast to the dramatic effects of other systems such as cholesterol and long chain alcohols [25,28]. Our goal is to calculate

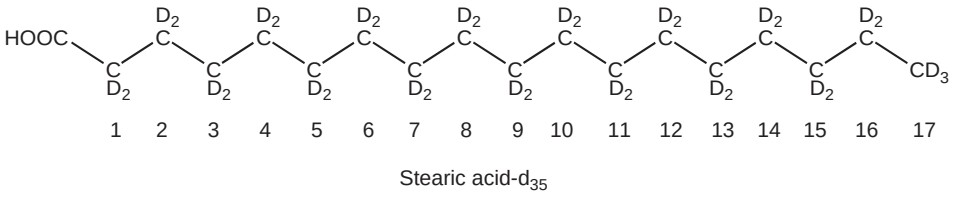

Fig. 1. Structure and numbering of deuterium labeled stearic acid-d_{35} used for this ^2H NMR study.

order parameters utilizing stearic acid-d_{35} as a NMR probe incorporated into a DMPC/DHPC bicelle system. We have carried out ^{2}H NMR experiments on the stearic acid-d_{35} incorporated into bicelles in the presence of Yb^{3+}. Stearic acid incorporates into bicelle discs such that the acyl chains of the DMPC phospholipids and the stearic acid are collinear with respect to each other. Thus, the molecular axis of stearic acid-d_{35} is parallel to the bilayer normal axis of the bicelle discs and the static magnetic field. Fig. 2 represents the ^{2}H-NMR spectra for stearic acid-d_{35} incorporated into a DMPC/DHPC/ Yb^{3+} bilayer with 0 mol% cholesterol as a function of temperature. The spectra are all characteristic of well-aligned phospholipid bilayers. As the temperature increases, the resolution of the ^{2}H peaks increases because the Yb^{3+}-doped bicelle discs are in the magnetically aligned liquid crystalline smectic phase [37]. The quadrupolar splittings are increased by a factor of

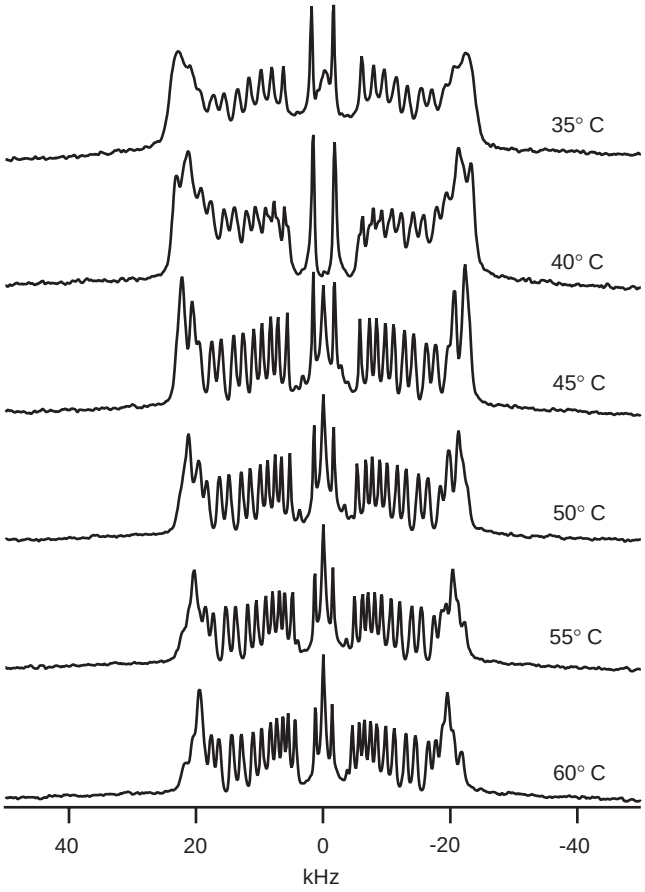

Fig. 2. ^{2}H NMR spectra of stearic acid-d_{35} embedded inside magnetically aligned DMPC/DHPC phospholipid bilayers in the presence of Yb^{3+} investigated as function of temperature. No cholesterol was added to the sample.

2 when compared to the splittings observed in the case where the bilayer normal is aligned perpendicular to the static magnetic field (data not shown). The increase is due to the parallel orientation of the phospholipid bilayers with respect to the magnetic field. In Fig. 2, the sharp quadrupolar splittings closest to 0 kHz represent the terminal CD_3 groups, while the others represent the methylene (CD_2) groups along the acyl chain. In the case of stearic acid-d_{35}, seventeen different peaks should be resolved in the ^2H NMR spectra (sixteen from CD_2 and one from CD_3).

Fig. 3 illustrates the ^2H quadrupole echo NMR spectra of DMPC/DHPC bicelles containing chain deuterated stearic acid-d_{35} and 10 mol% cholesterol with respect to DMPC. The spectra (Figs. 2 and 3) clearly indicate that by increasing the temperature the resolution of the ^2H acyl chain peaks increases. The addition of cholesterol also has been shown to increase the phase transition temperature of the bicelle discs [28]. Also, the deuterium quadrupolar splittings increase with the

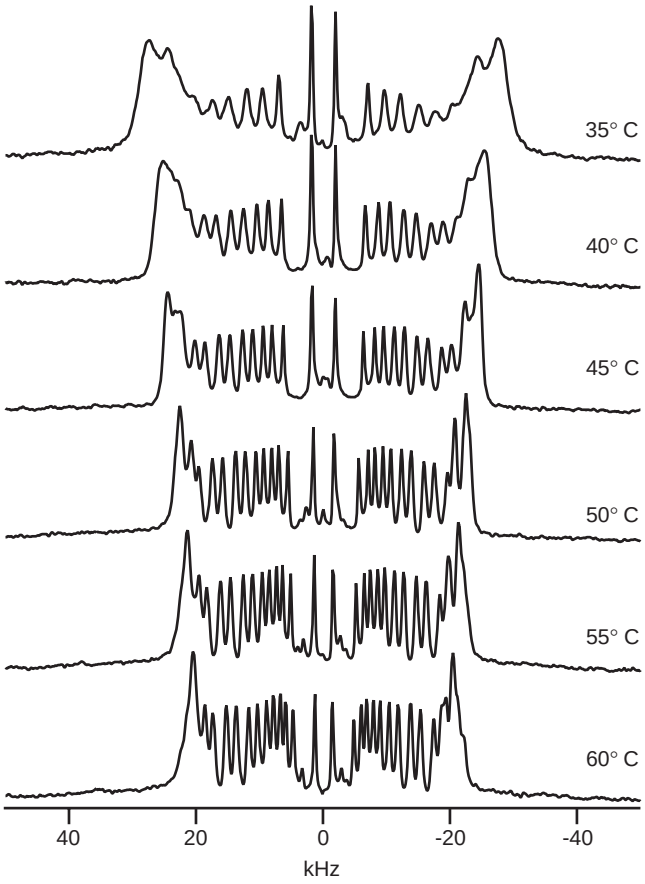

Fig. 3. ^2H NMR spectra of stearic acid-d_{35} embedded inside magnetically aligned DMPC/DHPC phospholipid bilayers in the presence of Yb^{3+} and 10 mol% cholesterol with respect to DMPC investigated as a function of temperature.

addition of cholesterol into the bicelle discs. This result suggests that in the presence of cholesterol, the fluidity of the bicelle discs decreases and the acyl chains are more ordered in the liquid crystalline phase.

An isotropic component is observed at 45°C and above in the absence of cholesterol (Fig. 2), while the isotropic peak is absent in the presence of 10 mol% cholesterol (Fig. 3). On the NMR timescale, small molecules undergo fast isotropic tumbling due to the low viscosity of the medium and can give rise to an isotropic peak. It is difficult to pinpoint the exact origin of the isotropic component in the NMR spectra. However, the slight presence of mixed micelles or small discoidal objects could cause this. A similar small isotropic component is observed at 35°C as well.

3.2. Calculating order parameters for stearic acid-d_{35} inserted into magnetically aligned phospholipid bilayers

Fig. 4 depicts the orientational order parameter profiles for the fatty acid chain derived from the spectra in Figs. 2 and 3 for (A) 0 mol% and (B) 10 mol% cholesterol with respect to DMPC as a function of temperature. S_{CD} was calculated using Eq. (2) as a function of the carbon number i. The numbering scheme for stearic acid-d_{35} is shown in Fig. 1. S_{CD} reflects the internal dynamic properties of the stearic acid incorporated to the bicelle discs. The magnitude of the order parameters indicates that the bicelle discs are in the liquid crystalline phase ($S_{CD} \sim 0.2$–0.3). Fig. 4 reveals that the order parameters for the C–D bond decrease in the acyl chains from the top of the fatty acid to the CD_3 end. As generally observed for the acyl chains of the phospholipid bilayers, two regions can be distinguished: a plateau region from C_1 to C_7 with a maximal value of $S_{CD} = 0.23$ and a region in which S_{CD} gradually decreases from a value of approximately 0.17 to a final value of 0.03 for the terminal C_{17} methyl group. The values for S_{CD} for stearic acid-d_{35} in a DMPC/DHPC bicelle system closely resemble previous studies of stearic acid-d_{35} incorporated into unoriented monounsaturated and polyunsaturated phosphocholine bilayer systems [38]. The data indicates that there is more disorder and motion in the center and at the ends of the acyl chains when compared to the head group regions. Also, the order parameter profiles indicate that by increasing the temperature, the value of S_{CD} decreases and the mobility of the acyl chains increases. The effect temperature has on the order parameters of stearic acid-d_{35} agrees well with the previous report for stearic acid-d_{35} incorporated into egg lecithin [26].

X-ray neutron diffraction, and ^2H NMR spectroscopic studies indicate that cholesterol embeds into phospholipid bilayers in such a way that its polar hydroxyl group is located in the aqueous phase and the hydrophobic steroid ring is oriented parallel to and buried in the hydrocarbon chains of the phospholipids [22]. Thus, the acyl chains of stearic acid-d_{35} are parallel to the normal of the long axis of cholesterol. The effect of cholesterol in the bilayer is well reported in the literature [28]. Based on the S_{CD} profiles for 0 and 10 mol% cholesterol with respect to DMPC, acyl chain mobility of stearic acid-d_{35} decreases with the addition of

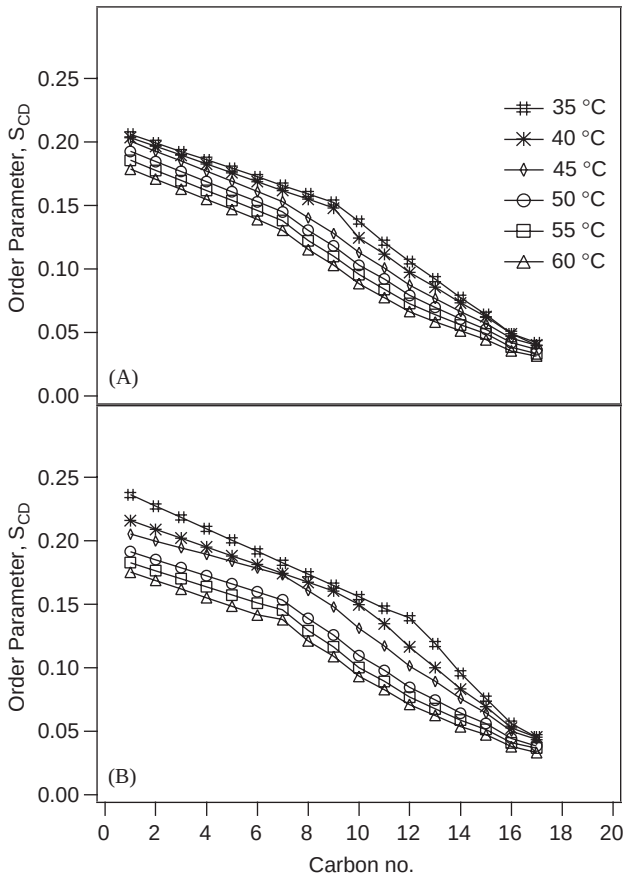

Fig. 4. Temperature-dependent S_{CD} order parameter profiles for the acyl chains of stearic acid-d_{35} incorporated into magnetically aligned DMPC/DHPC phospholipid bilayers in the presence of Yb^{3+}. (A) In the absence of cholesterol corresponding to the ^2H solid-state NMR spectra in Fig. 2 and (B) in the presence of 10 mol% cholesterol with respect to DMPC corresponding to the spectra in Fig. 3.

10 mol% cholesterol to the bilayer. Similar trends were also observed with 15 and 20 mol% cholesterol (data not presented). It is difficult to incorporate greater than 25 mol% cholesterol into the bicelle discs. However, it is interesting to note that the cholesterol concentration varies for different types of membrane systems. For example, plasma membranes contain 45–50 mol% cholesterol, while endoplasmic reticulum membranes contain 10–12 mol% cholesterol [39]. The bicelle membrane system discussed in the present paper mimics the natural membrane system, which consists of a lower mol% of cholesterol. Distal deuterons have a higher mobility when compared to the deuterons close to the head group as reflected in the order parameter profiles in Fig. 4. It should be noted that the temperature variation has no significant effect on the order parameter profiles of the distal deuterons. Figs. 4(A) and (B) suggest that the order parameters for the acyl chains decrease slowly from

35°C to 45°C in the absence of cholesterol when compared to the 10 mol% cholesterol sample. For simplicity, Fig. 5(A) shows the ^{2}H NMR spectra of stearic acid-d_{35} in the absence and in the presence of 10 mol% cholesterol with respect to DMPC at 40°C. The spectra clearly indicate that the quadrupolar splittings for the C–D bonds increase upon addition of cholesterol to the phospholipid bilayers. Fig. 5(B) displays the corresponding order parameter profiles for the ^{2}H NMR spectra in Fig. 5(A). The data indicates that the acyl chains of the deuterated fatty

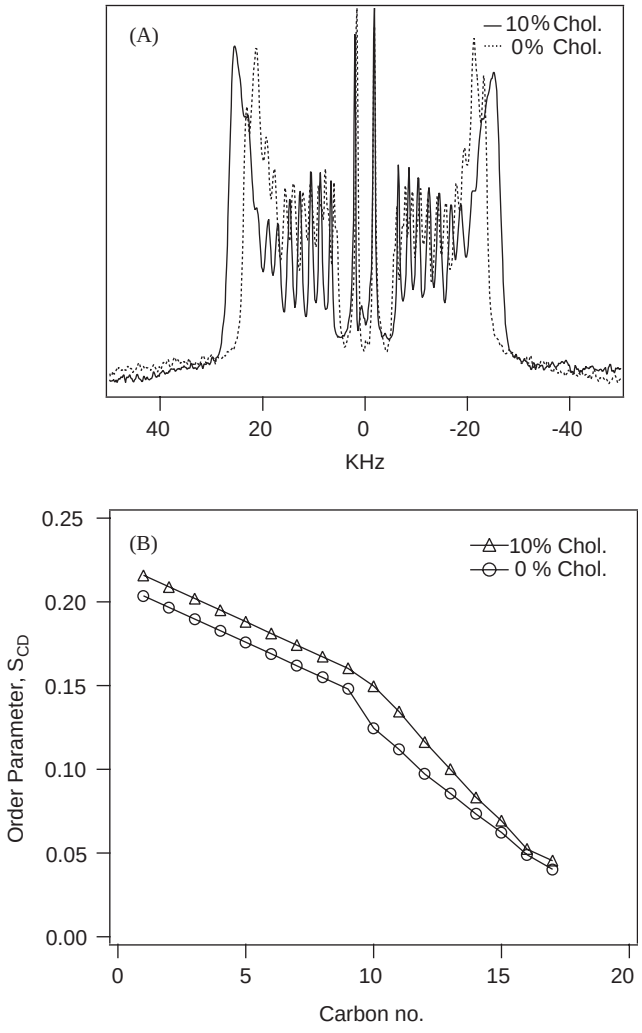

Fig. 5. (A) ^{2}H NMR spectra of stearic acid-d_{35} incorporated into magnetically aligned DMPC/DHPC phospholipid bilayers at 40°C. The boldface line spectrum contains 10 mol% cholesterol with respect to DMPC and the dotted line spectrum contains no cholestrol. (B) Chain-order parameter profiles corresponding to the stearic acid-d_{35} spectra represented in (A).

acid are well ordered in the presence of cholesterol in the liquid crystalline phase at 40°C. The order parameter profiles of the last three carbons of the fatty acid with and without cholesterol are nearly identical. Thus, cholesterol does not significantly alter the dynamic properties of the CD_2 and CD_3 groups near the end of the acyl chain of the fatty acid. This is not surprising because cholesterol is located near the top of the phospholipid head groups and the cholesterol rings are in close proximity to positions 2–10 of the acyl chains of the DMPC phospholipids.

In the present study, we demonstrated that the DMPC/DHPC bicelle discs align parallel to the magnetic field in the presence of Yb^{3+}. Quadrupolar splittings for individual deuterons were easily measured from the well-resolved ^2H NMR spectra of stearic acid-d_{35} incorporated into magnetically aligned bilayers. Our results agree with previous studies that have utilized a more complicated de-Paking method for measuring the quadrupolar splittings of stearic acid-d_{35} inserted into unoriented phospholipid bilayers. The S_{CD} order parameter profiles for stearic acid-d_{35} suggest that the mobility of the acyl chains increases with increasing temperature. Interestingly, the addition of cholesterol to the bicelle system decreases the fluidity of the membrane, except near the end of the acyl chains. This study indicates that non-perturbing NMR probes can be utilized for studying the structural and dynamic properties of membrane systems. Also, liquid crystalline molecules in a mesophase can be aligned in the presence of external magnetic field. Molecules in a crystalline lattice possess both orientational and positional order. Our present investigation suggests that the properties of non-biological liquid crystalline materials can be more easily studied by using solid-state ^2H NMR spectroscopy through the direct measurement of quadrupolar splittings of oriented systems.

Acknowledgments

This work was supported by an American Heart Association Scientist Development Grant (0130396N) and a National Institutes of Health Grant (GM60259-01).

References

[1] R.S. Prosser, I.V. Shiyanovskaya, Lanthanide ion assisted magnetic alignment of model membranes and macromolecules, Concept Magnetic Res. 13 (2001) 19–31.
[2] R. Fu, T.A. Cross, Solid-state nuclear magnetic resonance investigation of protein and polypeptide structure, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 235–268.
[3] B. Bechinger, M. Zasloff, S.J. Opella, Structure and orientation of the antibiotic peptide magainin in membranes, Protein Sci. 2 (1993) 2077–2084.
[4] S.O. Smith, Magic angle spinning NMR methods for internuclear distance measurement, Curr. Opin. Struct. Biol. 3 (1993) 755–759.
[5] C.R. Sanders, J.H. Prestegard, Magnetically orientable phospholipid bilayers containing small amounts of a bile salt analogue, CHAPSO, Biophys. J. 58 (1990) 447–460.
[6] J.W. Emsley, J.C. Lindon, NMR Spectroscopy Using Liquid Crystal Solvents, Pergamon Press, Oxford, 1975.

[7] T.B. Cardon, E.K. Tiburu, A. Padmanabhan, K.P. Howard, G.A. Lorigan, Magnetically aligned phospholipid bilayers at the parallel and perpendicular orientations for X-band spin-label EPR studies, J. Am. Chem. Soc. 123 (2001) 2913–2914.
[8] S.M. Garber, G.A. Lorigan, K.P. Howard, Magnetically oriented phospholipid bilayers for spin label EPR studies, J. Am. Chem. Soc. 121 (1999) 3240–3241.
[9] M.L. Mangels, T.B. Cardon, A.C. Harper, K.P. Howard, G.A. Lorigan, Spectroscopic characterization of spin-labeled magnetically oriented phospholipid bilayers by EPR spectroscopy, J. Am. Chem. Soc. 122 (2000) 7052–7058.
[10] M.L. Mangels, A.C. Harper, A.I. Smirnov, K.P. Howard, G.A. Lorigan, Investigating magnetically aligned phospholipid bilayers with EPR spectroscopy at 94 GHz, J. Magn. Reson. 151 (2001) 253–259.
[11] E.K. Tiburu, D.M. Moton, G.A. Lorigan, Development of magnetically aligned phospholipid bilayers in mixtures of palmitoylstearolyphosphotidylcholine and dihexanoylphosphatidylcholine by solid state NMR spectroscopy, Biochim. Biophys. Acta 1512 (2001) 206–214.
[12] C.R. Sanders, G.C. Landis, Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies, Biochemistry 43 (1995) 4030–4040.
[13] R.R. Vold, R.S. Prosser, A.J. Deese, Isotropic solutions of phospholipid bicelles: a new membrane mimetic for high-resolution NMR studies of polypeptides, J. Biomol. NMR 9 (1997) 329–335.
[14] M. Ottiger, A. Bax, Bicelle-based liquid crystals for NMR-measurement of dipolar couplings at acidic and basic pH values, J. Biomol. NMR 13 (1999) 187–191.
[15] S. Cavagnero, H.J. Dyson, P.E. Wright, Improved low pH bicelle system for orienting macromolecules over a wide temperature range, J. Biomol. NMR 13 (1999) 387–391.
[16] N. Tjandra, A.D. Bax, Direct measurement of distances and angels in biomolecules bu NMR in a dilute liquid crystalline medium, Science 278 (1997) 1111–1114.
[17] B. Bechinger, S.J. Opella, Flat-coil probe for NMR spectroscopy of oriented membrane samples, J. Magn. Reson. 95 (1991) 585–588.
[18] B. Bechinger, Biophysical investigations of membrane perturbations by polypeptides using solid-state NMR spectroscopy, Mol. Mem. Biol. 17 (2000) 135–142.
[19] M.A. Firestone, P. Thiyagarajan, D.M. Tiede, Structure and optical properties of a thermoresponsive polymer-grafted, lipid-based complex fluid, Langmuir 14 (1998) 4688–4698.
[20] M.A. Firestone, D.M. Tiede, S. Seifer, Magnetic field-induced ordering of a polymer-grafted biomembrane-mimetic hydrogel, J. Phys. Chem. B 104 (2000) 2433–2438.
[21] V. King, M. Parker, K.P. Howard, Pegylation of magnetically oriented lipid bilayers, J. Magn. Reson. 142 (2000) 177–182.
[22] P.A. Luchette, T.N. Vetman, R.S. Prosser, R.E.W. Hancock, M. Nieh, C.J. Glinka, S. Kruger, J. Katsaras, Morphology of fast-tumbling bicelles: a small angle neutron scattering and NMR study, Biochim. Biophys. Acta 1513 (2001) 83–94.
[23] M. Bloom, J.H. Davis, A.L. Mackay, Direct determination of the oriented sample NMR spectrum for systems with local axial symmetry, Chem. Phys. Lett. 80 (1981) 198–201.
[24] E. Sternin, M. Bloom, A.L. Mackay, De-Pake-ing of NMR spectra, J. Magn. Reson. 55 (1983) 274–282.
[25] G.W. Stockson, C.F. Polnaszek, A.P. Tulloch, F. Hasan, I.C.P. Hasan, Molecular motion and order in single bilayer vesicles and multilamellar dispersions of egg lecithin and lecithin–cholesterol mixtures. A deuterium nuclear magnetic resonance study of specifically labeld lipids, Biochemistry 15 (1976) 954–966.
[26] I.C.P. Smith, G.W. Stockson, A.P. Tulloch, C.F. Polnaszek, K.G. Johnson, Deuterium NMR and spin label ESR as probes of membrane organization, J. Colloid Interface Sci. 58 (1977) 439–451.
[27] E.J. Dufourc, E.J. Parish, S. Chitrakorn, I.C.P. Smith, Structural and dynamical details of cholesterol–lipid interaction as revealed by deuterium NMR, Biochemistry 23 (1984) 6062–6071.
[28] H. Ohvo-Rekila, B. Ramstedt, P. Leppimaki, J.P. Slotte, Cholesterol interactions with phospholipids in membranes, Progr. Lipid Res. 41 (2002) 66–97.
[29] A.M. Smondyrev, M.L. Berkowitz, Structure of dipalmitoylphosphatidylcholine/cholesterol bilayer at low and high cholesterol concentrations: molecular dynamics simulation, Biophys. J. 77 (1999) 2075–2089.

[30] A. Leonard, C. Escrive, M. Laguerre, E. Pebay-Peyroula, W. Neri, T. Pott, J. Katsaras, E.J. Dufourc, Location of cholesterol in DMPC membranes. A comparative study by neutron diffraction and molecular mechanics simulations, Langmuir 17 (2001) 2019–2030.
[31] A. Kessel, N. Ben-tal, S. May, Interactions of cholesterol with lipid bilayers: the preferred configuration and fluctuations, Biophys. J. 81 (2001) 643–658.
[32] C.R. Sanders, J.P. Schwonek, Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid state NMR, Biochemistry 31 (1992) 8898–8905.
[33] A. Seelig, J. Seelig, The dynamic structure of fatty acyl chains in a phosphatidylcholine bilayer measured by deuterium magnetic resonance, Biochemistry 13 (1974) 4839–4845.
[34] J. Seelig, W. Niederberger, Deuterium-labeled lipids as structural probes in liquid crystalline bilayers. A deuterium magnetic resonance, J. Am. Chem. Soc. 96 (1974) 2069–2072.
[35] J.P. Douliez, A. Leonard, E.J. Dufourc, Restatement of order parameter in biomembranes: calculation of C–C bond order parameters from C–D quadrupolar splittings, Biophys. J. 68 (1995) 1727–1739.
[36] D. Otten, M.F. Brown, K. Beyer, Softening of membrane bilayers by detergents elucidated by deuterium NMR spectroscopy, J. Phys. Chem. B 104 (2000) 12119–12129.
[37] R.S. Prosser, J.S. Hwang, R.R. Vold, Magnetically aligned phospholipid bilayers with positive ordering: a new model membrane system, Biophys. J. 74 (1998) 2405–2418.
[38] D. Huster, K. Arnold, K. Garwrisch, Influence of docosahexaenoic acid and cholesterol on lateral lipid organization, Biochemistry 37 (1998) 17299–17308.
[39] P.L. Yeagle, Cholesterol and the cell membrane, Biochem. Biophys. Acta 822 (1985) 267–287.