Title: SOLID-STATE ²H NMR SHOWS EQUIVALENCE OF DEHYDRATION AND

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Supporting material

SOLID-STATE ^{2}H NMR SHOWS EQUIVALENCE OF DEHYDRATION AND OSMOTIC PRESSURES IN LIPID MEMBRANE DEFORMATION

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EXPERIMENTAL METHODS

Lipid sample preparation

Samples for NMR experiments were prepared in custom-designed glass ampoules having a narrow 2-mm × 150-mm constriction in the center, with an 8-mm spherical enclosure at the bottom and an 8-mm × 100-mm opening at the top. 1,2-diperdeuteriomyristoyl-*sn*-glycero-3-phosphocholine (DMPC-d_{54}) (Avanti Polar Lipids; Alabaster, AL) was added as a powder (10–30 mg) and the ampoule was immediately sealed with a stopper. Approximately 30–40 μL of hexane and 1–2 drops of absolute ethanol were added and the sample was vortexed until clear. The phospholipid solution was lyophilized (about 12–14 h) under vacuum (100 mTorr) to constant weight. Controlled hydration of the samples was achieved by addition of deuterium-depleted water (ISOTEC; Miamisburg, OH) using a 10-μL Hamilton syringe. The sample was reweighed, briefly centrifuged at 5000 rpm, and then flash frozen in liquid nitrogen with the phospholipid dispersion in the spherical bottom. The top region of the ampoule was flame sealed under vacuum (40 mTorr); thereafter the sample was homogenized by 3–5 freeze thaw cycles, followed by repeated centrifugation at 7000 rpm of the lipid dispersion back and forth through the constriction until homogeneous by visual inspection. With the sample at the bottom of the spherical enclosure (under vacuum), the ampoule was cut and flame sealed, keeping the bottom portion in a liquid nitrogen bath. Changes in hydration of the samples were checked by inspection of the ^{2}H NMR spectra which remained constant over the duration (months) of the experiments. The number of waters per lipid was calculated using their gravimetric proportion of weights. The samples were kept at –20 °C when not in use. The osmolyte method followed the same procedure, except that an appropriate quantity of poly(ethylene glycol) (PEG 1500) (Sigma-Aldrich; St. Louis, MO) were added to the lipid following the lyophilization step. No phospholipid hydrolysis was observed in any of the samples, as verified by thin-layer chromatography with $CHCl_3$/MeOH/H_2O (65:25:10) and charring with 40% H_2SO_4 in ethanol.

Osmotic pressure measurements

We carried out osmotic pressure measurements using a Vapro Osmometer 5520 (Wescor Inc., Logan, UT) for poly(ethylene glycol) (M_r 1500) (PEG 1500), which agreed very well with tabulated data (1). In the present analysis, we used both the reported values as well our

independent measure for specific concentrations of PEG 1500 corresponding to the NMR results, i.e., 0 MPa (0 wt. % PEG), 5.67 MPa (50 wt. % PEG), 12.16 MPa (70 wt. % PEG), and 22.6 MPa (87.6 wt. % PEG) at T = 30 °C.

Deuterium NMR spectroscopy

^{2}H NMR measurements were conducted using a Bruker AMX-300 spectrometer operating at 7.05 T (^{2}H frequency of 46.07 MHz) with a phase-cycled, quadrupolar echo sequence $(\pi/2)_x - \tau_2 - (\pi/2)_y - \tau_2 -$ acquire. A locally constructed solid-state probe with an 8-mm diameter coil and high-voltage capacitors (Polyflon; Norwal, CT) was used together with a narrow-band radio-frequency boost amplifier (Tempo 2006, Henry Radio; Los Angeles, CA) in series with the spectrometer output to enable 90° pulse durations of 3–4 μs. The transient NMR signals were shunted to the preamplifier (Miteq; Hauppauge, NY) using a Lowe-Tarr series crossed-diode circuit, and were acquired with a Bruker fast digitizer. Typical spectral acquisition parameters involved a pulse spacing (τ_2) of 42 μs, a dwell time of 0.5 μs, and the collection of 8192 data points. Recycle times were generally 1 s, and typically 38000 transients were collected and Fourier transformed beginning at the maximum of the quadrupolar (solid) echo (2). The radio-frequency coil was enclosed in a glass Dewar, and the temperature was controlled with a flow of compressed air by the Bruker AMX 300 variable temperature controller interfaced to a heater/sensor located directly in the probe. The temperature was monitored before and after each measurement with a thermistor inserted directly above the radio-frequency coil, and typically was found to vary by less than 0.5 °C during a given run. The temperatures are estimated accurate to within ±1 °C of the reported values.

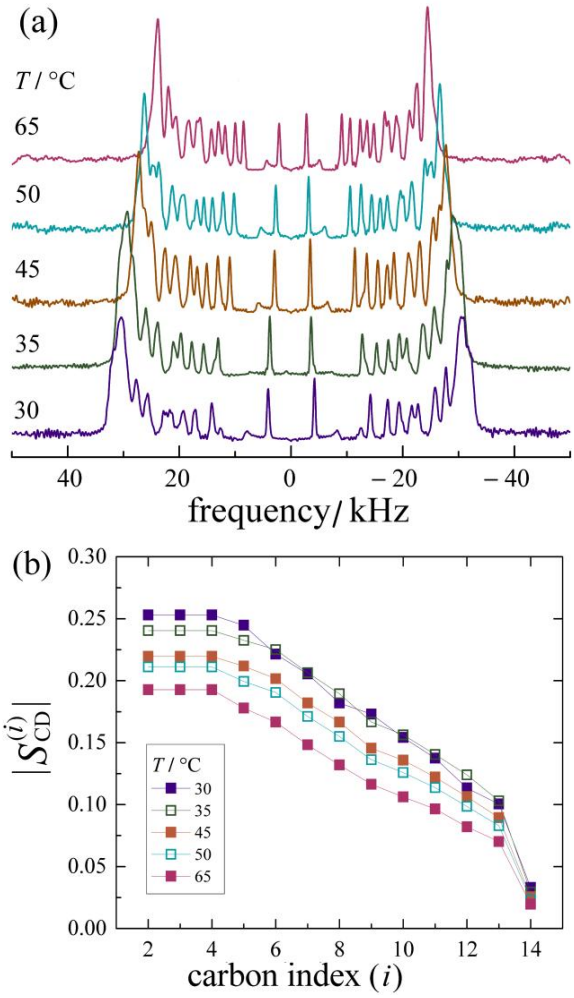

FIGURE S1. (a) ^{2}H NMR spectra and (b) C–^{2}H bond order parameters as a function of acyl chain segment index (i) for DMPC-d_{54} containing 20 wt. % H_2O at different absolute temperatures. Similar measurements were carried out for all the samples.

Reduction and analysis of ^{2}H NMR spectral data

The ^{2}H NMR spectral powder patterns were numerically deconvoluted (de-Paked) to yield sub-spectra corresponding to the θ = 0° bilayer orientation (i.e. bilayer normal parallel to the main external magnetic field) (3). Spectral assignments for DMPC-d_{54} were made by integration of de-Paked resonances and assumed the quadrupolar splittings decreased along the chain. The

order parameters, $S_{CD,}$ which describe the motional averaging of the various C–^{2}H labeled segments with respect to the bilayer normal (4), were evaluated from the well-resolved peaks in de-Paked ^{2}H NMR spectra, using the relation

$$|\Delta\nu_Q^{(i)}| = \tfrac{3}{2}\ \chi_Q\ |S_{\mathrm{CD}}^{(i)}|\ |P_2\,(\cos\theta)| \qquad (1)$$

Here the segmental order parameter is given by

$$S_{\mathrm{CD}}^{(i)}\ = \tfrac{1}{2}\ \langle 3\cos^2\beta_i - 1\rangle \qquad (2)$$

where β_i is the instantaneous angle between the C–^{2}H bond and the director axis (membrane normal). An order parameter of $-1/2$ corresponds to all-*trans* phospholipids rotating about the bilayer normal and a value of zero indicates an isotropic liquid, with typical values for liquid-crystalline bilayers falling between. In the above equation, $\Delta\nu_Q^{(i)}$ is the experimental *i*th quadrupolar splitting, $\chi_Q \equiv e^2qQ/h = 167$ kHz is the static quadrupolar coupling constant, and $P_2(\cos\theta) = (½)(3\cos^2\theta - 1)$ where θ is the angle between the membrane director and the static magnetic field. For powder-type ^{2}H NMR spectra of random multilamellar lipid dispersions, the sharp peaks correspond to θ = 90° leading to $P_2(\cos\theta) = -1/2$, whereas for the de-Paked ^{2}H NMR spectra θ = 0° yielding $P_2(\cos\theta) = 1$ with a two-fold increase in peak-to-peak splittings and a reversal of sign (4). The segmental order parameters $S_{\mathrm{CD}}^{(i)}$ are related to bilayer structural parameters including the mean area per lipid $\langle A\rangle$ and the volumetric bilayer thickness D_C in terms of a mean-torque model as described previously (5).

TABLE S1 Results for DMPC-d_{54} bilayer under osmotic stress due to different hydration levels and osmolyte concentrations at T = 30 °C

Method	T / °C	wt. %	Π / MPa*	n_w†	$\lvert S_{\mathrm{CD}}^{\mathrm{plat}}\rvert$
PEG 1500	30	0	0.00	41	0.2114
		50	5.67	10	0.2720
		70	12.16	5	0.3009
		87	22.60	1.5	0.3358
Gravimetric	30	50	0.00	41	0.2114
		40	0.02	26	0.2149
		30	0.05	18	0.2169
		20	2.76	10	0.2530
		10	8.42	4.88	0.2859
		6.8	9.67	2.98	0.2911
		3.1	23.12	1.34	0.3370

*Measured from vapor pressure osmometry for PEG 1500 samples and calculated for gravimetric samples.

†Measured for gravimetric samples and calculated for PEG 1500 samples.

Influence of temperature

For all the samples studied in the present investigation, de-Paked ^{2}H NMR spectra and corresponding segmental order parameter profiles were measured as a function of temperature (30, 35, 45, 50, and 65 °C). The samples were kept at the desired temperature for more than 30 min for temperature stabilization before recording the NMR spectrum. Representative data for DMPC-d_{54} containing 20 wt. % H_2O at all temperatures are presented in Fig. S1. Inspection of the peak positions of each spectrum demonstrates that the quadrupolar splittings and hence order parameters decrease noticeably with lower temperature. The quadrupolar splitting of the plateau peak region changes from 32.89 kHz (at 30 °C) to 24.29 kHz (at 65 °C) and the corresponding plateau order parameter changes from 0.253 (at 30 °C) to 0.193 (at 65 °C). The decrease in quadrupolar splittings and hence order parameters is due to greater mobility for the acyl chain segments and arises from an increase in their thermal energy. The order parameters, osmotic pressures, and number of waters per lipid corresponding to both gravimetric and osmolyte samples at T = 30 °C are summarized in the Table S1.

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