Supplementary Material (ESI) for Soft Matter

Equimolar Mixtures of Lysophosphatidylcholine and *O*-Stearoylethanolamine Form Bilayers

Pradip K. Tarafdar and Musti J. Swamy*

School of Chemistry, University of Hyderabad, Hyderabad-500046, India

***Corresponding Author**

Tel: +91-40-2313-4807, Fax: +91-40-2301-2460, E-mail: mjssc@uohyd.ernet.in, mjswamy1@gmail.com, Website: http://202.41.85.161/~mjs/

SUPPLEMENTARY INFORMATION

This section contains the Experimental Section for this manuscript and related references.

Supplementary Material (ESI) for Soft Matter

EXPERIMENTAL SECTION

Materials. Fatty acids, *N, N'*-dicyclohexylcarbodiimide and 4-dimethylaminopyridine were purchased from Sigma-Aldrich (USA). ELPC was obtained from Avanti polar lipids. Oxalyl chloride and dioxane were obtained from Merck (Germany). Ethanolamine, BOC-anhydride, solvents and other chemicals were purchased locally. Milli-Q water was used in all experiments.

Synthesis of O-Stearoylethanolamine and N-Acylethanolamines. *O*-Stearoylethanolamine hydrochloride (OSEA) was synthesized as described earlier.[SR1] *N*-Myristoylethanolamine (NMEA), *N*-palmitoylethanolamine (NPEA) and NSEA were synthesized and characterized as reported in Ramakrishnan et al (1997).[SR2]

^{31}P-NMR Spectroscopy. Accurately weighted quantities of ELPC and OSEA (or NAE) were mixed to give an equimolar ratio and dissolved in dichloromethane/methanol (1:1, v/v). The solvent was removed under a stream of dry nitrogen gas and the resulting lipid film was vacuum desiccated for 5-6 hours and then hydrated with 150 mM NaCl containing 0.2 mM EDTA. The hydrated lipid film was subjected to five cycles of freeze thawing, using liquid nitrogen and hot water (ca. 65 °C) before the ^{31}P-NMR measurements. Proton decoupled ^{31}P-NMR spectra were recorded at 162 MHz on a Bruker Avance 400 FT-NMR spectrometer using the zgpg30 pulse program, using the following settings: decoupling power, 14db; $\pi/2$ pulse width, 9.5 μs (for ^{31}P); recycle delay, 1 s; number of scans, 2048 to 4096; spectral width, 400 ppm. The free induction decay for each spectrum was processed using 100 Hz of line broadening to improve s/n ratio. Temperature was regulated by a thermostatted air-flow system.

Atomic Force Microscopy. For AFM measurements, a drop of 0.2 mg/ml equimolar ELPC-OSEA solution in 150 mM NaCl was placed on a freshly cleaved mica sheet and dried immediately under nitrogen gas. The salt deposits were removed by washing with Milli-Q water and the samples were dried with nitrogen gas. All the images were recorded in air under ambient conditions in semi-contact mode with a scan rate of 0.8 Hz using a SOLVER PRO-M AFM instrument (NTMDT, Moscow). The force was kept at the lowest possible value by continuously adjusting the set-point and feed-back gain during imaging. Images were analyzed using NOVA software, supplied by NTMDT.

Differential Scanning Calorimetry. DSC studies were performed using a VP-DSC equipment from MicroCal LLC (Northampton, MA, USA). Accurately weighed lipid samples were dissolved in dry dichloromethane/methanol (1:1, v/v) and then the solvent was removed under a stream of dry nitrogen gas. The resulting lipid film was desiccated under vacuum for 5-6 hrs and hydrated with 150 mM NaCl. DSC measurements were carried out essentially as described in Kamlekar et al. (2006).[SR3]

Turbidimetric Studies. Samples for turbidimetric study were prepared as described in Kamlekar et al. (2006),[SR3] except that the lipid film was hydrated with 150 mM NaCl. Measurements were performed at various temperatures (25 to 60 °C) using a Cary 100 UV-Visible spectrophotometer equipped with a Peltier thermostat. Turbidity was measured as the optical density at 400 mm.

Fluorescence Spectroscopy. Fluorescence measurements were made on a SPEX FLUOROMAX-4 fluorescence spectrometer equipped with a Peltier temperature controller. Lipid films containing ca. 0.25 mol% pyrene, prepared essentially in the same manner as described above, were hydrated with 150 mM NaCl and the optical density of the sample at

335 nm was maintained below 0.1. Samples were excited at 335 nm and emission spectra were recorded between 350 and 550 nm using slit widths of 1.5 nm for both excitation and emission. Each spectrum was blank subtracted and corrected for lamp intensity variation during measurement.

References:

SR1) P. K. Tarafdar and M. J. Swamy, *Biochim. Biophys. Acta*, 2010, **1798**, 872-881.

SR2) M. Ramakrishnan, V. Sheeba, S. S. Komath and M. J. Swamy, *Biochim. Biophys. Acta*, 1997, **1329**, 302-310.

SR3) R. K. Kamlekar and M. J. Swamy, *Biosci. Rep*., 2006, **26**, 387-398.