Relationships between Equilibrium Spreading Pressure and Phase Equilibria of Phospholipid Bilayers and Monolayers at the Air−Water Interface

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The intricate interplay between the bilayer and monolayer properties of phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) phospholipids, in relation to their polar headgroup properties, and the effects of chain permutations on those polar headgroup properties have been demonstrated for the first time with a set of time-independent bilayer−monolayer equilibria studies. Bilayer and monolayer phase behavior for PE is quite different than that observed for PC and PG. This difference is attributed to the characteristic biophysical PE polar headgroup property of favorable intermolecular hydrogen-bonding and electrostatic interactions in both the bilayer and monolayer states. This characteristic hydrogen-bonding ability of the PE polar headgroup is reflected in the condensed nature of PE monolayers and a decrease in equilibrium monolayer collapse pressure at temperatures below the monolayer critical temperature, T_c (whether above or below the monolayer triple point temperature, T_t). This interesting phenomena is compared to equilibrated PC and PG monolayers which collapse to form bilayers at 45 mN/m at temperatures both above and below monolayer T_c. Additionally, it has been demonstrated by measurements of the equilibrium spreading pressure, π_e, that at temperatures above the bilayer main gel-to-liquid-crystalline phase-transition temperature, T_m, all liquid-crystalline phospholipid bilayers spread to form monolayers with π_e around 45 mN/m, and spread liquid-expanded equilibrated monolayers collapse at 45 mN/m to form their respective thermodynamically stable liquid-crystalline bilayers. At temperatures below bilayer T_m, PC and PG gel bilayers exhibit a drop in bilayer π_e values $\leq$0.2 mN/m forming gaseous monolayers, whereas the value of π_c of spread monolayers remains around 45 mN/m. This suggests that spread equilibrated PC and PG monolayers collapse to a metastable liquid-crystalline bilayer structure at temperatures below bilayer T_m (where the thermodynamically stable bilayer liquid-crystalline phase does not exist) and with a surface pressure of 45 mN/m, a surface chemical property characteristically observed at temperatures above bilayer T_m (monolayer T_c). In contrast, PE gel bilayers, which exist at temperatures below bilayer T_m but above bilayer T_s (bilayer crystal-to-gel phase-transition temperature), exhibit gel bilayer spreading to form equilibrated monolayers with intermediate π_e values in the range of 30−40 mN/m; however, bilayer π_e and monolayer π_c values remain equal in value to one another. Contrastingly, at temperatures below bilayer T_s, PE crystalline bilayers exhibit bilayer π_e values $\leq$0.2 mN/m forming equilibrated gaseous monolayers, whereas spread monolayers collapse at a value of π_c remaining around 30 mN/m, indicative of metastable gel bilayer formation.

Introduction

The tendencies of water-insoluble amphiphilic molecules to spontaneously spread as a monomolecular layer from a bulk phase placed on an aqueous surface have been the subject of much research for decades.[1,2] Such studies have revealed the importance of the physical state of the bulk phase in determining the extent and rate of spreading, with an accompanying reduction in the surface free energy at the air−water interface, for example, crystal versus melt,[3−6] differences between polymorphic crystalline forms,[7] or in the case of phospholipids, gel versus liquid-crystalline fully hydrated bilayer states.[7−10]

The motivation for studying the spreading of phospholipids arises because of the desire to better understand their fundamental properties as spread monolayers[11−14] and to relate this to the biophysical properties of biological membranes[15] and the molecular interactions of therapeutic drugs with biological

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membranes.[16−19] The process of spreading and formation of phospholipid monolayers at the air−water interface also is of interest since it appears to mimic the physiological role of lung surfactant. Lung surfactant, a mixture of primarily phospholipids and smaller amounts of surface-active proteins, is essential in the breathing process by decreasing the work of breathing and regulating pulmonary immunity and provides other related physiological pulmonary function by spreading over the aqueous alveolar−air interface and reducing the surface tension to very low values.[20] Such studies also have been useful in providing information required to prepare artificial lung surfactant for the therapeutic treatment of various lung diseases[21] and the use of phospholipid colloidal dispersions as self-assemblies for drug delivery.[22−24]

One of the earliest studies of the spreading of synthetic phospholipids from a dry powder applied to the aqueous surface revealed that dipalmitoylphosphatidylcholine (DPPC) exhibited no measurable equilibrium spreading pressure below its main bilayer phase-transition temperature, T_m, of 41 °C but exhibited significant spreading above this temperature.[8] That spontaneous spreading of phosphatidylcholines to high-surface pressures occurs only above T_m was further shown by studying several shorter-chain and unsaturated derivatives.[7,8] However, studies with dilauroyl- and dimyristoyl-phosphatidylethanolamine (DLPE and DMPE, respectively), which have T_m values that are higher than those of their corresponding PC derivatives, indicated spreading of these compounds below their T_m values.[7] Interestingly, studies of the spreading of dipalmitoylphosphatidylglycerol (DPPG) from its bilayer, and its relationship to T_m, indicated an essentially identical value of T_m as that of DPPC and the same sharp increase in π_e just above its T_m.[25]

More recent phospholipid spreading studies have been focused on dimyristoylphosphatidylcholine (DMPC) because its main transition occurs at about 23 °C, making it convenient to carry out experiments below and above T_m. Gershfeld and Tajima[9,10] were able to show that below its bilayer T_m DMPC spreads to form a gaseous monolayer with a π_e only in the μN/m range, while above bilayer T_m the value of π_e rises sharply to a constant value of about 50 mN/m. Interestingly, evidence was presented to suggest that over a very limited range of temperature, just above bilayer T_m, an equilibrium exists between the bulk phase and both a monolayer and bilayer surface phase. Above this range of temperatures, again, a monolayer with significant surface pressure is formed. In more recent years, there has been considerable interest in measuring the DMPC π_e values produced by dispersing unilamellar and multilamellar vesicles into the aqueous phase and by comparing the rate and extent of surface pressure change with that obtained by placing the dry powder directly onto the surface.[26−28] Typically, the widely used and standard surface chemical method employed in these spreading studies involving dry powder, aqueous dispersions, and solutions is a Langmuir film balance with the Wilhelmy Plate technique. Other experimental approaches that might be used for studying spreading from solutions and dispersions have been recently developed.[29,30] The important role of the state of the bulk form of the phospholipid was confirmed, and differences in the rate of change were attributed to differences in the physical state of the bilayer, including its initial state of hydration. Recently, it was suggested that the kinetics of spreading may relate better to the phase properties of the monolayer.[31] It also has been suggested that under some conditions spread DMPC vesicles might exist at the surface as partial bilayer fragments and not as a continuous monolayer.[32,33]

In view of the many interesting questions raised in previous work associated with the phospholipid spreading and the limitation of the most recent studies to the use of DMPC, in this study it was deemed useful to carefully examine such behavior with a range of rationally selected phospholipids, differing in acyl chains and three different polar groups, namely, phosphatidylcholine (PC), sodium phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) as one-component bilayer and monolayer systems, under equilibrium (time-independent) conditions, at three temperatures of 25 °C, 37 °C, and 45 °C. In view of concerns as to whether phospholipids actually form monolayers when spread from the bulk phase, emphasis first was placed on measuring equilibrium, rather than kinetic, behavior and on comparing these values to the surface pressures at time-independent monolayer collapse from monolayer $\pi-A$ (surface pressure vs area per molecule) isotherms. Specifically, the objectives of this systematic and comprehensive study are (1) to examine and characterize, under equilibrium conditions, the surface phase behavior and spreading of phospholipid monolayers; (2) to examine and characterize equilibrium bilayer spreading and bilayer phase behavior; (3) to examine, characterize, and correlate the relationship between equilibrium bilayer π_e and monolayer π_c; and (4) to examine and characterize the equilibria between the bilayer and monolayer states, under equilibrium conditions, such that a useful predictive phospholipid bilayer−monolayer equilibria model at the air−water interface can be attained. The differences in spreading reported for the PC and

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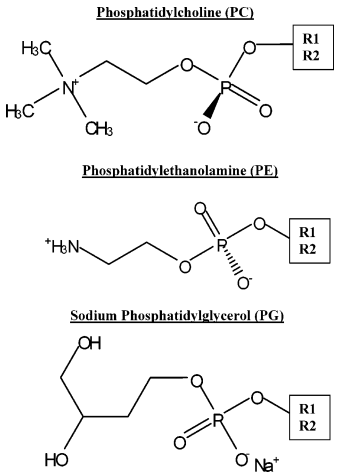
Table 1. The Diacyl Chain Lengths, Names, and Their Respective Abbreviations for the Various Diacyl and Mixed Fatty Acid Chain Configurations Used in These Studies

abbreviation	chain lengths (R1, R2)	1-acyl 2-acyl
DS	18:0−18:0	1,2-distearoyl
DP	16:0−16:0	1,2-dipalmitoyl
PS	16:0−18:0	1-palmitoyl-2-stearoyl
PO	16:0−18:1	1-palmitoyl-2-oleoyl
DM	14:0−14:0	1,2-dimyristoyl
DL	12:0−12:0	1,2-dilauryl
PL	16:0−12:0	1-palmitoyl-2-lauryl
DO	18:1−18:1	1,2-dioleoyl

Scheme 1. Chemical Structures of the Three Zwitterionic and Anionic (Sodium Salt) Phospholipid Polar Headgroups Used in These Studies



PE derivatives relative to their values of bilayer T_m[7], and the similarity between DPPC and DPPG,[25] as described above, were of particular interest, as was the suggested possible greater dependence of spreading from the bilayer on the monolayer rather than bilayer phase properties.[28]

Experimental Section

Materials. All of the synthetic phospholipids used in this study were obtained from Avanti Polar Lipids (Alabaster, AL) with a stated purity of >99%, and were used as received. Table 1 lists the various acyl chain configurations, and the molecular structures of the three polar headgroups used in these studies are depicted in Scheme 1 (ChemDraw Ultra 10.0, CambridgeSoft). All powdered samples were stored at −21 °C for no more than 3 months, which was shown to be a period where no changes in bilayer and monolayer properties occur, including chemical instability. Chloroform and methanol, used as the organic solvents for the various monolayer spreading experiments, were obtained from Sigma-Aldrich in 99.93% ACS HPLC grade (Sigma Aldrich Company, Inc., Milwaukee, WI). Tris, ultrapure hydroxy-methylaminomethane 99.9% (United States Biochemical Co.), and sodium chloride 99.999% (Sigma-Aldrich) were used as received. The water used in all experiments was house-distilled water passed through a five-cartridge Barnstead PCS water purification system with a resistivity of 18 MΩ-cm. This was then twice distilled from an alkaline permanganate solution and from dilute sulfuric acid and was collected in a glass container, and this highly purified water was used for all bilayer and monolayer biophysical and surface chemical experimental studies.

Methods. *Determination of the Main Bilayer Phase-Transition Temperature, T_m.* All phospholipid samples were prepared as 15% (w/w) aqueous dispersions in a pH 7.4 Tris buffer with 150 mM NaCl. The weights of the dry phospholipid samples in differential scanning calorimetry (DSC) pans were in the range of 1.00−3.00 mg, as determined with an Ohaus GA 200-D electronic balance (Ohaus Corp., Florharn Park, NJ). The pH 7.40 150 mM NaCl 10 mM Tris ultrapure buffer was added to the dry phospholipid samples in the aluminum differential scanning calorimetry (DSC) pans in sufficient amount to make the lipid concentration 15% on a weight−weight (w/w) basis. This composition was shown to represent a fully hydrated bilayer system for all of the phospholipids.[34,35] Samples of each dispersion were placed into 15-μL Seiko aluminum differential scanning calorimetry (DSC) pans (Seiko Instruments Inc., Horsham, PA) and were sealed hermetically with a Seiko hermetic sealer (Seiko I, Seiko Instruments Inc., Horsham, PA). Samples were then mixed with a vortexer at 50−70 °C (depending on the phospholipid) for 10−20 min to ensure good dispersion. The measurement of T_m, the gel-to-liquid-crystalline bilayer phase-transition temperature, was measured under dry nitrogen gas purge (Praxair, Inc., Danbury, CT) connected to a laboratory gas drying unit consisting of DrierRite (anhydrous $CaSO_4$) and Indicating Drierite (97% $CaSO_4$ + 3% $CoCl_2$) in a Seiko SS 220C differential scanning calorimeter (Seiko Instruments, Horsham, PA) fitted with a fully automated liquid nitrogen cooling accessory. DSC data were analyzed with a coupled Seiko 5200 data station that consisted of a Hewlett-Packard computer (Model 712/60) that used a UNIX platform. Samples of 1−10 mg were heated and cooled at a scanning rate of 1.0 °C/min (at a sampling interval of 0.5 s), previously shown to give values of T_m identical to those obtained at 0.5 °C/min. This, plus good agreement with previously reported values, assured that sufficient time to equilibrate had been allowed during a scan. For the DSC heating scans, fully hydrated samples were held at the higher program temperature (approximately 15−20 °C above the T_m of interest) for 30−45 min to ensure complete sample hydration and homogeneity. The lowest temperature used for most systems during cooling cycles for fully hydrated samples was −10 °C to avoid freezing of the water and consequent T_m elevation because of lipid dehydration. The DSC was calibrated for melting points and heats of fusion using pure indium (156.6 °C, 28.59 J/g) for high temperatures, gallium (29.8 °C, 80.17 J/g) for intermediate temperatures, cyclohexane (6.5 °C, 31.25 J/g) for low temperatures, and dodecane (−9.6 °C, 214.75 J/g) for very low temperatures. At least four melting and four cooling scans were carried out to ensure reproducibility. All reported transition temperatures were taken from heating endotherms. Preliminary studies, however, indicated good reproducibility when comparing cooling and heating results under these conditions. All reported T_m values were taken from heating endotherms, as determined from the intersection of the endothermic baseline and the tangent of the onset of the melting peak.

Measurement of Bilayer Equilibrium Spreading Pressure, π_e. In preliminary studies, samples of representative phospholipids were applied to the surface of the pH 7.4 Tris, 150 mM NaCl buffer solution, held at constant temperature in a jacketed glass beaker, by three methods: (1) deposition of dry powder directly onto the surface, (2) deposition of a fully hydrated 15% (w/w) aqueous dispersion of phospholipid multilamellar vesicles (MLVs), and (3) small unilamellar vesicles (SUVs) onto the surface and injection of these dispersions below the surface into the bulk solution. Colloidal dispersions were prepared in a manner similar to the method described above for MLVs, and then the MLV dispersions were sonicated for approximately 30 min until the colloidal dispersion became a visibly clear aqueous solution of SUVs. The surface tension of the solution before and after application of the phospholipid was measured by the Wilhelmy plate method and Langmuir film balance, using a sandblasted platinum plate (2.58 × 1.0 × 0.1 cm, 2.55 × 1.0 × 0.1 cm, or 2.56 × 1.0 × 0.1 cm) attached to a Cahn model 2000 Electrobalance (Cahn Co., ThermoCahn Cooperation, Madison, WI). Subphase and air temperatures were maintained at 25 °C, 37 °C, and 45 °C by a circulating water bath and temperature feedback system, respectively. All experiments were carried out in an aluminum box to prevent surface contamination and air drafts and to maintain a relative humidity of 80% or more with open evaporating dishes

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containing triply distilled water and wet filter paper. The surface tension of the pure aqueous buffer subphase, γ_o, was always recorded first and was 72.3 ± 0.4 mN/m, in good agreement with the expected value of 71.97 mN/m for pure water at 25 °C. The equilibrium surface tension, γ_{eq}, was determined when any change in γ of the monolayer was less than 0.2 mN/h.

Final π_e values, time-independent equilibrium values, were determined when the surface tension change was less than 0.2mN/m per hour, the lower limit of surface pressure detection. The equilibrium surface pressure, π_e, represents the difference between the time-independent surface tension values obtained before (γ_o) and after (γ_{eq}) adding the phospholipid. These preliminary studies showed that each technique gave the same final value of π_e, but as also observed in earlier studies,[26−28] there were differences in the rate of attaining equilibrium; applying the dry powder onto the surface of the aqueous subphase produced the fastest rate of attaining equilibrium. Consequently, this latter technique was used throughout the rest of the study. All values were independent of the amount of excess phospholipid added and the total surface area.

Measurement of Monolayer Surface Pressure (π) vs Area/Molecule (A_m) Isotherms. Surface pressure−area ($\pi-A$) isotherms were measured with equipment and techniques previously described in detail.[25,36] Briefly, using a Hamilton microliter syringe (Hamilton, Reno, NV), a known volume of phospholipid organic solution of known concentration was carefully deposited onto the aqueous buffer subphase which was contained in a Teflon trough designed to maintain a constant temperature (25 °C, 37 °C, and 45 °C) by circulating thermostatted water through a glass coil at the bottom of the trough for surface pressure−area ($\pi-A$) monolayer experiments. In addition to controlling the aqueous buffer temperature, to avoid a temperature gradient at the air−water interface, the air temperature was maintained at 25 °C, 37 °C, and 45 °C by a temperature feedback system. The surface tension before and after application of the phospholipid solution deposition and monolayer equilibration was measured by the Wilhelmy plate method and Langmuir film balance, using a sandblasted platinum plate ($2.58 \times 1.0 \times 0.1$ cm, $2.55 \times 1.0 \times 0.1$ cm, or $2.56 \times 1.0 \times 0.1$ cm) attached to a Cahn model 2000 Electrobalance (Cahn Co., ThermoCahn Cooperation, Madison, WI). All experiments were carried out in an aluminum box enclosure to prevent surface contamination and air drafts and to maintain a relative humidity of 80% or more through the use of open evaporating dishes containing triply distilled water and wet filter paper, thereby preventing surface evaporation during the course of any experiment.

The area per molecule (total area divided by the number of molecules on the surface), A_m, was varied by three methods using a Langmuir surface film microbalance and the Wilhelmy Plate technique: (1) stepwise addition of molecules to a surface of constant area with each addition made after attaining an equilibrium (time-independent) surface pressure with the previous addition; (2) compression: stepwise reduction of surface area (after equilibrium is reached) with a movable barrier with a fixed amount of deposited material on the surface; and (3) "single- shot" addition of an amount of phospholipid organic solution required to reach a given area per molecule spread onto the clean surface, attainment of equilibrium, followed by complete cleaning of the surface, Langmuir trough, and sandblasted platinum plate before the next phospholipid film was spread from a volatile organic solution onto the clean surface. Good agreement among the three methods was confirmed with both expanded and condensed regions of selected monolayers. To further ensure that no possible overcompression (nonequilibrium) occurred, each isotherm in its entirety was also created and verified by method 3 (single shot). Immediately prior to each experiment, the surface was cleaned via in-house water-powered aspiration using a Pasteur pipet to remove any residual surface contaminants. Depending on the monolayer state and the phospholipid, 1−24 h after the volatile organic solvent completely evaporated was required to attain equilibrium. These π_e (and π_c) values were stable and invariant for extended time periods on the order of days for a variety of phospholipids varying in polar headgroup and chains. For all surface chemical studies, A_c is determined to be the point on the time-independent isotherm at which the monolayer just reaches the equilibrated collapse surface pressure, π_c. All monolayer experiments were carried out under equilibrium (time-independent) conditions as determined by no more than a change of 1 mg per hour in meniscus mass which is equal to a change of 0.2 mN/m per hour. The surface tension of the pure aqueous buffer subphase, γ_o, was always recorded first and was 72.3 ± 0.4 mN/m, in good agreement with the expected value of 71.97 mN/m for pure water at 25 °C. The equilibrium surface tension, γ_{eq}, was determined when any change in γ of the monolayer was less than 0.2 mN/h.

Fluorescence microscopic measurements were carried out with techniques and equipment previously described in detail[25,36,37] to confirm the presence of either a single monolayer phase or two phases in any region of coexistence. Briefly, surface FM setup consisted of a commercially available microscope (Micromaster, Model E, Fisher Scientific, Pittsburgh, PA) that was connected to a high sensitivity Olympus DEI-750D CCD video camera system (Olympus America, Inc., Melville, NY) that was interfaced to a Mitsubishi HS-U795 SVHS video recorder (Mitsubishi Digital Electronics America, Inc., Irvine, CA) connected to a Sony Trinitron monitor. The interface could be observed directly by eye via the monitor, while images were recorded onto SVHS videotape (Mitsubishi HS-U65) and also were digitally captured by a Gateway Windows 98 computer via Optronics DEI-750 Acquire software. The incoming excitation light from a tungsten-halogen lamp (Fiber-Lite, Dolan-Jenner Industries, Inc.) was made to pass through a filter (Zeiss, Inc.) which only passes light with $\lambda = 480 \pm 20$ nm and then was reflected down to the aqueous surface via a dichroic mirror (Zeiss, Inc.). The fluorescence from the surface is collected by the objective lens (40× magnification) and is passed through the dichroic mirror and an additional barrier filter (Zeiss, Inc.) which both only pass light with $\lambda > 500$ nm. Surface pressure in the fluorescent microscope assembly was determined using a Wilhelmy sandblasted platinum plate (3.56 cm^2) with a Nima pressure transducer (Nima, Inc., Coventry, England). The Wilhelmy Plate apparatus and the Teflon trough (85.37 cm^2) were mounted on a vertical translator, which allowed for both coarse and fine focusing. A vertically mobile (but horizontally immobile) circular Teflon barrier was present at the bottom of the trough to capture and confine a small area of the surface-flowing elevation to the interface region, hence minimizing flow effects. The subphase temperature, air temperature, and relative humidity were controlled as described above. The fluorescent probe used was NBD-PC, 1-palmitoyl-2-amino]dodecanoyl-sn-glycerol-3-phosphocholine. It was used at a level of 1% in the spread monolayer, a level previously shown not to affect the properties of the monolayer.[36,37]

Results

In Table 2, bilayer T_m values (±0.1 °C) are presented for three groups of phospholipids differing in polar group, PC, PG, and PE and for five groups of acyl chains along with their corresponding bilayer-to-monolayer equilibrium spreading pressures, π_e, at 25°, 37° and 45 °C. In addition, π_e values are given in the footnote to Table 2 for DMPC and DMPG at 5° and 19 °C and for DMPE and POPE at 5 °C, all temperatures below their bilayer T_m. The value of the bilayer main transition temperature usually reported for disaturated PE derivatives represents a crystal-to-gel-to-liquid-crystalline bilayer transition region where the gel phase is metastable and no clearly delineated crystal-to-gel bilayer phase transition, T_s, and gel-to-liquid-crystalline bilayer phase transition, T_m, are observed in the

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Table 2. Comparison of Fully Hydrated Phospholipid Bilayer Gel-to-Liquid Crystalline Main Phase-Transition Temperature, T_m, and Bilayer Equilibrium Spreading Pressure, π_e, at 25 °C, 37 °C, and 45 °C along with the Thermodynamically Stable Bilayer Phase Present at Each Temperature

		25 °C		37 °C		45 °C	
T_m (°C)	molecular weight (g/mol)	π_e (mN/m)	bilayer phase	π_e (mN/m)	bilayer phase	π_e (mN/m)	bilayer phase
			Distearoyl (DS)				
DSPC 55.0	790.15	≤0.2	crystal	≤0.2	gel	≤0.2	ripple
DSPG 54.0	801.07	≤0.2	crystal	≤0.2	gel	≤0.2	ripple
DSPE 74.0	748.08	≤0.2	crystal	≤0.2	crystal	≤0.2	crystal
			Dipalmitoyl (DP)				
DPPC 41.2	734.05	≤0.2	gel	≤0.2	ripple	45.4	liquid crystal
DPPG 40.3	744.96	≤0.2	gel	≤0.2	ripple	45.4	liquid crystal
DPPE 63.4	691.97	≤0.2	crystal	30.6	gel	38.2	gel
			Dimyristoyl (DM)				
DMPC[a] 23.0	677.95	48.5	liquid crystal	47.5	liquid crystal	48.5	liquid crystal
DMPG[a] 22.8	688.86	45.3	liquid crystal	46.9	liquid crystal	46.7	liquid crystal
DMPE[b] 50.0	635.86	31.6	gel	38.5	gel	38.5	gel
			Dilauroyl (DL)				
DLPC −2.0	621.89	49.6	liquid crystal	47.1	liquid crystal	47.3	liquid crystal
DLPG −3.0	632.75	49.5	liquid crystal	47.5	liquid crystal	47.5	liquid crystal
DLPE 30.0	579.76	37.8	gel	47.5	liquid crystal	49.5	liquid crystal
			Palmitoyloleoyl (PO)				
POPC −3.2	760.10	44.3	liquid crystal	44.7	liquid crystal	44.2	liquid crystal
POPG −3.4	771.00	46.1	liquid crystal	46.3	liquid crystal	46.2	liquid crystal
POPE[c] 23.7	718.01	46.0	liquid crystal	46.4	liquid crystal	46.6	liquid crystal

[a] At 5 °C and 19 °C, below the T_m of DMPC and DMPG (gel phase) π_e < 0.2 mN/m. [b] At 5 °C, below the T_m of DMPE (gel phase) $\pi_e \sim 5$ mN/m. [c] At 5 °C, below the T_m of POPE (gel phase) $\pi_e \sim 20$ mN/m.

lyotropic bilayer phase diagrams for PE.[35,38−42] From these reported lyotropic phase diagrams for DPPE bilayers, it is clear that at 25 °C, fully hydrated DPPE bilayers exist in the crystalline phase, whereas at 37 °C and 45 °C, fully hydrated DPPE bilayers exist in the metastable gel phase. However, to be consistent with convention, this major transition temperature for the fully hydrated material is referred to as T_m for PE bilayers. Contrastingly, reported lyotropic phase diagram for DPPC[35,43] bilayers shows a clear demarcation for the crystal-to-gel phase, T_s, and gel-to-ripple phase, T_p, and ripple-liquid-crystalline phase, T_m, bilayer phase-transition temperatures such that T_s is below 20 °C for DPPC bilayer colloidal dispersions containing 20% water and higher. Hence, for the fully hydrated DPPC bilayers, T_s is below 25 °C. Wherever a ripple phase occurs, for example, as in the case of DPPC and DPPG bilayers, the T_m value reported is actually that of the main transition from the ripple phase to the liquid-crystalline phase. The values of T_m and π_e agree very well for those phospholipids for which values have been reported previously. Specifically, all of the T_m values reported in this study have been reported by others and are in good agreement, whereas only π_e values for a few select phospholipids have been reported, including DPPC, DPPG, DMPC, DLPC, POPC, and POPG at select temperatures.[7−10,25] In all cases, spreading on an aqueous surface from the dry powder to form a thin film having a time-independent surface pressure value was almost instantaneous in the length of time to occur and reach its invariant and time-independent, equilibrium value. As expected, the T_m values for all compounds decrease as the acyl chain length is decreased and unsaturation is introduced. For each acyl chain, the PC and PG derivatives exhibit about the same bilayer T_m values and essentially identical π_e values at each temperature. Also, for PC and PG bilayers at temperatures just below T_m, the π_e value is ≤0.2 mN/m; however, at temperatures above T_m, π_e is much more significant at 45−50 mN/m.

For the various PE derivatives, somewhat different behavior can be observed in Table 2. First, the bilayer T_m values for each group of acyl chains are about 20−30 °C greater than those observed for the corresponding PC and PG derivatives. Second, from the results at 25 °C for DPPE and DSPE, it appears that the value of π_e was always ≤0.2 mN/m when the temperature was significantly below bilayer T_m and only reached its maximum value, which was in the range of 45−50 mN/m, when bilayer T_m was exceeded, for example, see DLPE at 37 °C and POPE at 25 °C. However, for intermediate temperatures that are below bilayer T_m, spreading occurs to a form film having a π_e value in the range of 30.6−38.5 mN/m, which is less than the maximal π_e value (45−50 mN/m) which occurs at temperatures above bilayer T_m for PC, PG, and PE phospholipid systems. Note also in the footnote to Table 2 that at 5 °C, DMPE and POPE exhibit bilayer equilibrium spreading to form films at π_e values around 5 mN/m and 20 mN/m, respectively. Thus, PE derivatives, which generally have higher bilayer T_m values than their corresponding PC and PG derivatives, spread to form films at intermediate values of equilibrium spreading pressure at higher temperatures that are still below bilayer T_m, as observed, for example, with DMPE at 45 °C and DLPE at 25 °C.

To further address the factors that influence the behavior described above, in particular, the possible relationship of monolayer phase behavior to bilayer spreading, additional studies were carried out measuring monolayer $\pi-A$ isotherms at 25°,

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37°, and 45 °C for DPPC,[25] DPPG,[25] and DPPE, and only at 25 °C for POPC, POPG, and POPE, 25 °C being much higher than the bilayer T_m of all three monounsaturated phospholipids. As indicated above, the surface pressure values in each of these isotherms, including those at collapse, were time-independent for the various methods used to change the area per molecule (A_m). This is in contrast to many studies carried out with rapid compression rates, where the resulting isotherms depend on the rate of compression.[44] In Figures 1 and 2, the monolayer $\pi-A$ isotherms are given for the dipalmitoyl and palmitoyloleoyl derivatives, respectively. As reported in previous studies,[25,36] and shown in Figure 1a and 1b, at temperatures below the T_m of the corresponding bilayer (see Table 3), DPPC and DPPG monolayers exhibit regions of coexistence between the liquid-expanded (LE) and liquid-condensed (LC) surface phases, whereas at 45 °C (above bilayer T_m), these one-component monolayers remain in the LE state until the monolayer-to-bilayer collapse surface pressure, π_c, is reached. The existence or absence of a coexistence region (the invariant two-phase region of constant π over a finite range of A_m in the equilibrium $\pi-A$ isotherm) between the LE and LC surface phases was confirmed by FM measurements over the entire monolayer $\pi-A$ isotherm, as shown previously[25,36,37] (data not shown). This supports the earlier suggestion that the monolayer critical temperature,[14] T_c (the temperature at which a monolayer LE−LC coexistence region no longer exists and the monolayer is in the LE state all along the isotherm up to π_c), is equal in value to the bilayer T_m.[45,46] In comparing DPPC and DPPG monolayers, the $\pi-A$ isotherms are very similar at all A_m at 45 °C but are significantly different at 25 °C and 37 °C all along the isotherm until both undergo their surface LE−LC surface phase transition to the LC state. In Figure 1c, it can be seen that DPPE also exhibits regions of coexistence between LE and LC surface phases at 37° and 45 °C but only with a gaseous (G) to LC surface phase transition at 25 °C, with no tendency to form an LE surface phase. In other other words, at 25 °C, the DPPE monolayer is below its monolayer triple point temperature, T_t, and its G surface phase is in equilibrium with its LC surface phase. The lack of a completely LE isotherm over all A_m up to A_c at any temperature is consistent with the fact that the bilayer T_m of DPPE, and therefore, its monolayer T_c, is greater than 45 °C. In Figure 2, as expected from the monolayer results with the saturated phospholipids, it can be seen that the three palmitoyloleoyl derivatives, with bilayer T_m values and hence monolayer T_c, all below 25 °C, are LE at 25 °C over all A_m up to A_c. Fluorescence microscopy of the entire isotherm revealed the lack of phase domains and surface-phase homogeneity which are characteristic of the LE phase.

In Table 3, the values of bilayer T_m, bilayer-to-monolayer π_e, and monolayer-to-bilayer π_c for the various phospholipids are given. As described in the Experimental Section, the values of π_c were independent of the manner in which A_m was changed, remaining constant over a considerable time period (hours and days, as described in the Methods section). If the monolayer and bilayer upon collapse attain an equilibrium state, the values of

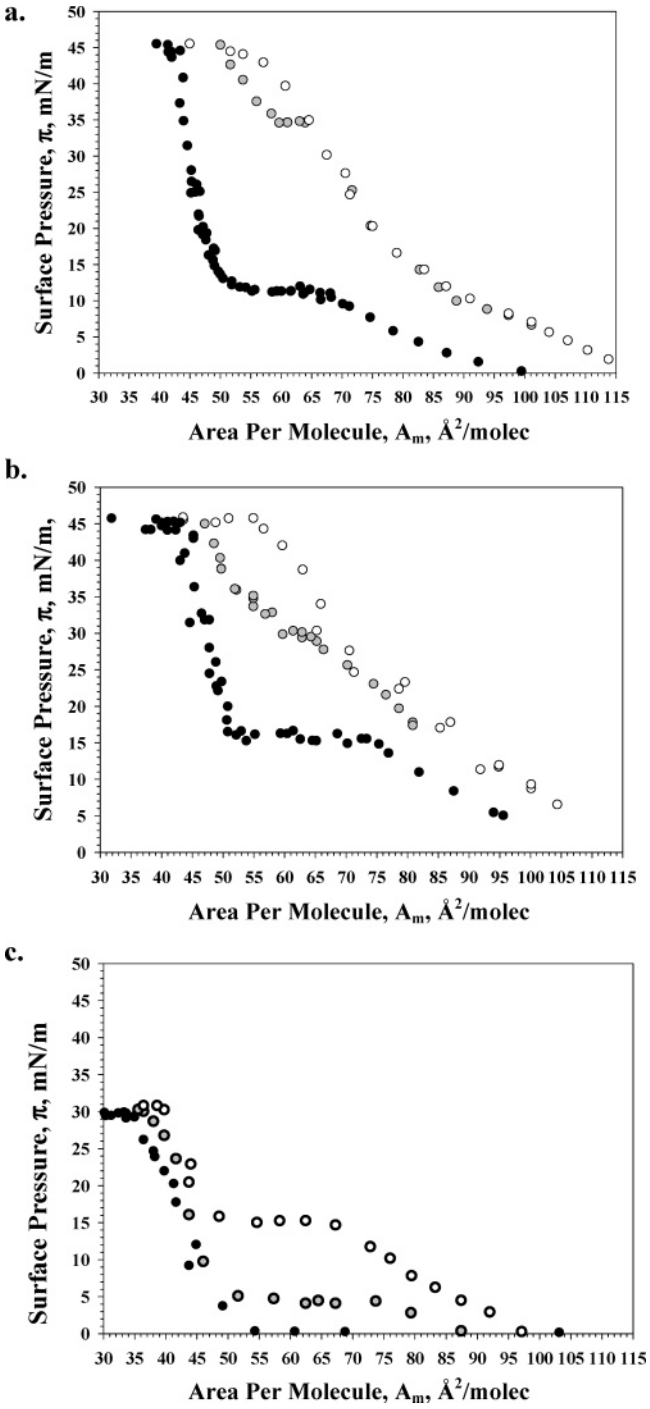

Figure 1. Surface pressure (π) vs area per molecule (A_m) isotherms for dipalmitoyl (DP) phospholipid monolayers with varying polar headgroups at the air−water interface spread on pH 7.40 Tris buffer with 150 mM NaCl at 25 °C (●), 37 °C (gray circles), and 45 °C (○) for phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE): (a) DPPC; (b) DPPG; and (c) DPPE.

π_e and π_c should be equal.[2,47] If the values are different, but π_c is relatively independent of time, it can be assumed that the monolayer at monolayer-to-bilayer collapse was overcompressed to a metastable state and that a metastable "equilibrium" exists between these states of the bilayer and monolayer. From Table 3, it also can be seen that, on the basis of these criteria, all of the collapsed phospholipid monolayers in coexistance with their

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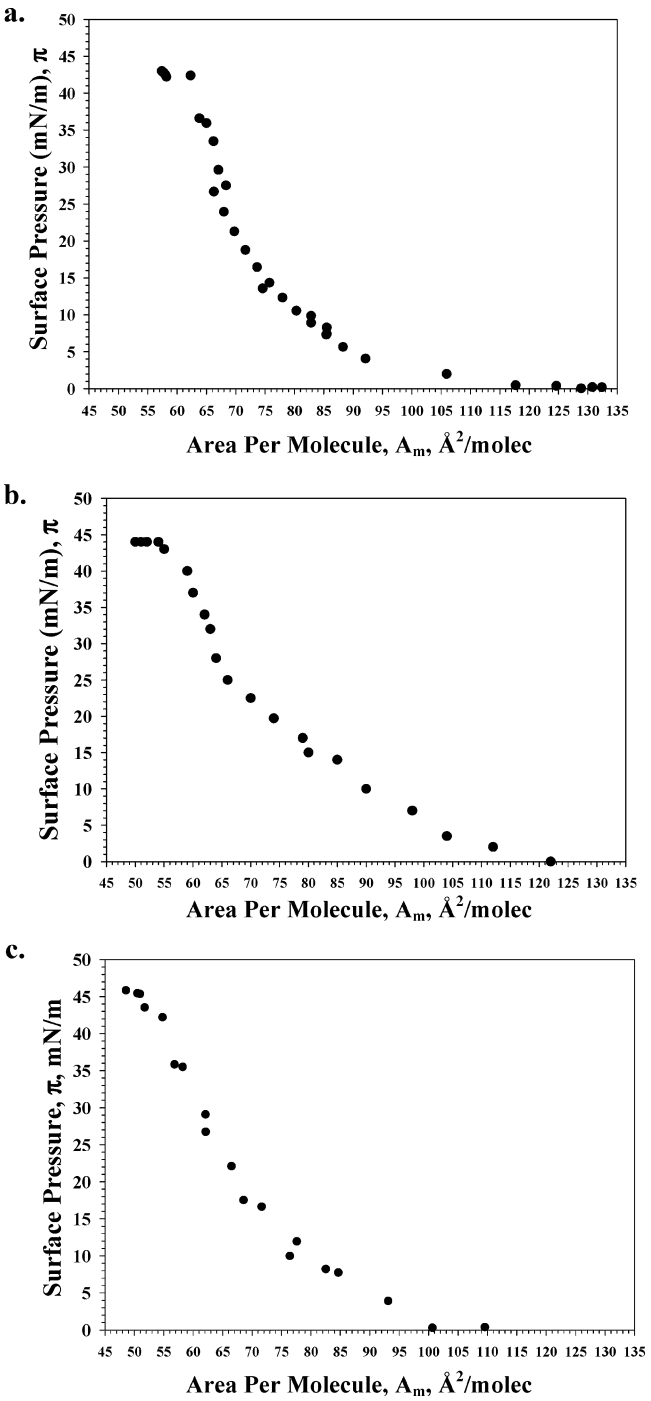


Figure 2. Surface pressure (π) vs area per molecule (A_m) isotherms for palmitoyloleoyl (PO) phospholipid monolayers with varying polar headgroups at the air−water interface spread on pH 7.40 Tris buffer with 150 mM NaCl at 25 °C (●) for phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE): (a) POPC; (b) POPG; and (c) POPE.

three-dimensional phospholipid self-assembled bulk phase (namely, the bilayer state), except DPPC, DPPG, and DPPE at 25 °C and DPPC and DPPG at 37 °C, are in a state of equilibrium, strongly supporting the conclusion that, in such systems, coherent LE or LC monolayers, and not fragments of bilayer, are formed upon spreading from the three-dimensional bulk phase. For gel-phase DPPC, gel-phase DPPG, and crystalline-phase DPPE bilayers at 25 °C and for DPPC and DPPG ripple-phase bilayers at 37 °C, bilayer spreading occurs to form a gaseous monolayer with $\pi_e \leq 0.2$ mN/m, while time-independent compression of these monolayers, spread from organic solvent, leads to equilibrium monolayer collapse (to a three-dimensional self-assembled bulk phase) with π_c of about 30 mN/m for DPPE LC monolayers and 45 mN/m for DPPC and DPPG LC monolayers. Although it was not possible to measure $\pi-A$ isotherms for POPC and POPG and POPE at temperatures below their T_m values, the lack of any surface LC−LE coexistence region in the $\pi-A$ monolayer isotherms at 25 °C, shown in Figure 2, and the equality of π_e and π_c are consistent with this general interpretation. An interesting observation for all of the disaturated phospholipids, shown in Table 3, is that whether monolayer collapse to a three-dimensional self-assembled bulk phase (i.e., a bilayer) occurred from the LE collapsed monolayer state or from collapse of the overcompressed LC monolayer state, the time-independent values of π_c for any particular phospholipid appear to be the same. However, the characteristic value of A_m of a given phospholipid at equilibrium monolayer collapse, A_c, as a function of temperature is significantly different, as shown in Table 4, for DPPC, DPPG, and DPPE LC monolayers. The A_c values at 25 °C for POPC, POPG, and POPE collapsed LE monolayers are 57.8 A^2/molecule, 54.8 A^2/molecule, and 50.8 A^2/molecule, respectively. The A_c values of DPPC, DPPG, and DPPE collapsed LC monolayers at 25 °C are all close to those estimated using molecular modeling software, Chemsite Pro Version 3.01 (Pyramid Learning, Hiram, OH), indicating closest packing because of almost complete removal of water from between the molecules in the monolayer by the time collapse of the metastable monolayer occurs. The A_c values for DPPC and DPPG LE monolayers at 45 °C increase significantly, as expected for a collapsed LE monolayer at a higher state of hydration. The DPPE A_c value for an equilibrium-collapsed LC monolayer at 45 °C increases relative to that at 25 °C, but the increase is not as significant as with DPPC and DPPG. That these effects of temperature are not highly discontinuous at a critical temperature like bilayer T_m is seen by the more gradual change in the monolayer A_c as the temperature is increased from 25 °C to 37 °C, still below the bilayer T_m for DPPC, DPPG, and DPPE.

Discussion

From the results of this study, several generalizations have been made that relate to questions posed earlier concerning factors affecting the bilayer-to-monolayer spreading of phospholipids at the air−water interface. For DPPC, DPPG, and the three groups of monounsaturated phospholipid bilayers containing the PC, PG, and PE headgroups, at temperatures above T_m of a fully hydrated bilayer and with sufficient time to reach equilibrium, spreading produces a monolayer that has an equilibrium spreading pressure, π_e, around 45 mN/m. In all cases, this value of π_e is equivalent to the equilibrium collapse surface pressure, π_c, of the corresponding monolayer spread from a volatile solvent at temperatures above its monolayer critical temperature, T_c. The equivalence of π_e and π_c was shown to be true for the spreading of aqueous liposomal dispersions and dry powders, and therefore, under such conditions, any tendency for "incomplete spreading" of phospholipids, as liposomal dispersions above bilayer T_m that might leave fragments of bilayers at the surface as reported under certain conditions,[32,33] must be due to kinetic effects and not to any intrinsic equilibrium properties of the bilayer or monolayer. For PC and PG phospholipid bilayer systems, shown in Figure 3a and 3b, respectively, this transition appears to be very sharp as the temperature passes through T_m. In contrast, the spreading of the PE phospholipid bilayer derivatives as a function of temperature, as shown in Figure 3c, exhibits two discontinuities, one discontinuity as the temperature passes through bilayer T_m (as observed with PC and PG) and a second discontinuity at temperatures well below bilayer T_m. For PC, PG, and PE, it

Table 3. Comparison of Fully Hydrated Phospholipid Bilayer Gel-to-Liquid Crystalline Main Phase-Transition Temperature, Bilayer Equilibrium Spreading Pressure, π_e, and Monolayer Equilibrium Collapse Pressure, π_c, at 25 °C, 37 °C, and 45 °C along with the Monolayer Phase Present at Equilibrium Collapse

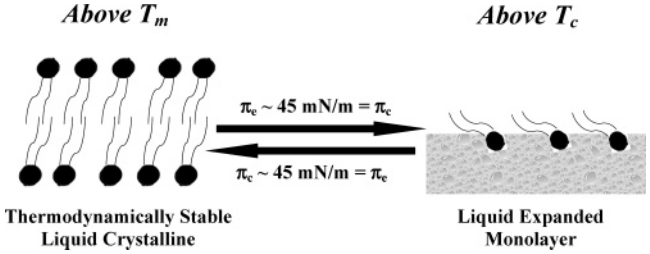
	molecular	25 °C		monolayer	37 °C		monolayer	45°C		monolayer
T_m (°C)	weight (g/mol)	π_e (mN/m)	π_c (mN/m)	phase at collapse	π_e (mN/m)	π_c (mN/m)	phase at collapse	π_e (mN/m)	π_c(mN/m)	phase at collapse
					Dipalmitoyl (DP)					
DPPC 41.2	734.05	≤0.2	45.4	liquid condensed	≤0.2	45.3	liquid condensed	45.4	45.3	liquid expanded
DPPG 40.3	744.96	≤0.2	45.4	liquid condensed	≤0.2	45.3	liquid condensed	45.4	45.3	liquid expanded
DPPE 63.4	691.97	≤0.2	30.2	liquid condensed	30.6	30.3	liquid condensed	34.9	31.0	liquid condensed
					Palmitoyloleoyl (PO)					
POPC −3.2	760.10	44.3	44.6	liquid expanded	44.7	44.3	liquid expanded	44.2	44.2	liquid expanded
POPG −3.4	771.00	46.1	44.6	liquid expanded	46.3	44.3	liquid expanded	46.2	44.3	liquid expanded
POPE 23.7	718.01	46.0	46.6	liquid expanded	46.4	46.4	liquid expanded	46.6	46.2	liquid expanded

Table 4. Comparison of the Area per Molecule at Equilibrium Collapse, A_c, at the Temperatures of 25 °C, 27 °C, and 45 °C or Disaturated Phospholipid Monolayers at the Air−Water Interface

	A_c (Å^2/molecule)[a]		
phospholipid monolayer	25 °C	37 °C	45 °C
DPPC	42.0	50.2	53.2
DPPG	44.1	48.0	55.1
DPPE	35.2	37.1	40.2

[a] A_c (25 °C) ± 0.3; A_c (25 °C) ± 0.5; A_c (25 °C) ± 0.8.

Scheme 2. Illustration Depicting Bilayer−Monolayer Equilibria at Temperatures above Bilayer T_m and Hence above Monolayer T_c for Phospholipids Systems



appears that monolayer T_c value is essentially the same as bilayer T_m value. These observations, therefore, indicate that for all three headgroups above bilayer T_m, the fluidity and energetics associated with the liquid-crystalline bilayer phase are quite similar to those of their corresponding LE monolayers, as depicted in Scheme 2. However, it must be recognized that the LE monolayer at time-independent equilibrium collapse and the fully hydrated liquid-crystalline bilayer, at equilibrium with each other, are not identical with respect to the value of A_m, for example, 53.2 A^2/molecule versus 62.9 A^2/molecule for DPPC[48] as an LE monolayer and a liquid-crystalline bilayer, respectively. Assuming that the effects of charge and size on molecular packing would be very similar for any one molecule in the bilayer and monolayer states, it would appear that a spread monolayer brought to equilibrium collapse to a three-dimensional bulk phase is less hydrated than a corresponding fully hydrated bilayer in the liquid-crystalline phase. This is likely due, in part, to the fact that interactions occurring normally between the chain-end methyl groups of the acyl chains of each layer of the bilayer do not occur in the monolayer. These terminal methyl groups are perpendicular to the interface and contribute to molecular dipole densities of the phospholipid monolayers making up the bilayer.[49] This also supports previous suggestions that a phospholipid molecule spread at a monolayer surface pressure below collapse, for example, in the region of 30 mN/m,[50] can be thought to be more structurally representative of a fully hydrated bilayer than that at the collapse pressure.

In previous studies, the nearly identical bilayer properties (T_m and π_e) and monolayer properties (the LC phase at temperatures below T_c, the LE phase at temperatures above T_c, and π_c) of PC and PG derivatives with the same acyl chains, and in contact with neutral aqueous solutions, have been reported and discussed.[25,32] Despite very different charge, hydration, and molecular size for the PC and PG headgroups, the acyl chains appear to be the major determinant of overall spreading from the bilayer. From these studies, it is clear that the properties of PE phospholipids are significantly different from those of PC and PG phospholipids, at constant chain length, and are more distinctly influenced by the nature of the PE headgroup fully hydrated at neutral pH. It follows, therefore, that the very different temperature dependence of spreading for DPPE, as opposed to DPPC and DPPG, particularly the ability to spread in a limited range of temperature below T_m must arise because of some of these critical differences.

For example, X-ray diffraction analysis of the acyl chain structure of DPPC and DPPG[51,52] reveals a tilted orientation in the fully hydrated bilayer as opposed to the more perpendicular orientation of DPPE. This perpendicular orientation of PE would appear to be due to closer packing (see Table 4) in the all-trans chain configuration strengthened by strong lateral hydrogen bonding, a property that PE phospholipid bilayer and monolayer systems possess. Interestingly, when DPPG is protonated to give the un-ionized form, with a greater tendency for lateral hydrogen bonding, this configuration becomes more perpendicular like that of DPPE, and the T_m correspondingly increases to a value more like that of DPPE.

The DPPE time-independent monolayer isotherm at 25 °C does not show an LE−LC transition but rather a direct G−LC surface-phase transition; the LE−LC surface-phase transition becomes clearly apparent at 37 °C and 45 °C. However, in contrast to DPPC and DPPG bilayer spreading at 37° and 45 °C, which are above their nearly identical bilayer T_m values, where the π_e value is typically around 45 mN/m, DPPE at 37° and 45 °C, which are temperatures below its fully hydrated bilayer T_m and monolayer T_c, spreads to an intermediate value of π_e of about 30−35 mN/m which is approximately equal to the monolayer equilibrium collapse pressures at these two temperatures. This

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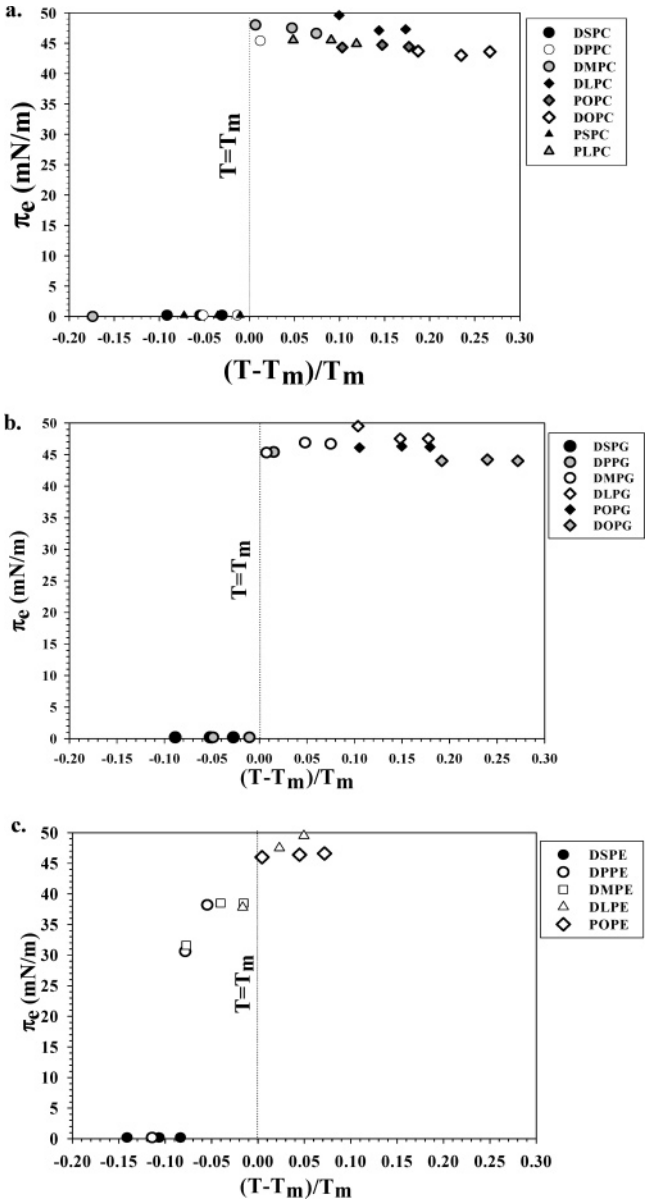

Figure 3. Bilayer spreading dependency on the experimental temperature, *T*, and the fully hydrated bilayer gel-to-liquid crystalline main phase-transition temperature, T_m, for distearoyl (DS), dipalmitoyl (DP), dimyristoyl (DM), dilauroyl (DL), and palmitoyleoyl (PO), dioleoyl (DO), and mixed acyl chain derivatives of phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamines (PE) phospholipids: (a) PC; (b) PG; and (c) PE.

represents a state of equilibrium between the DPPE bilayer and a liquid-condensed monolayer, as shown in Tables 2 and 3, with the occurrence of essentially the same intermediate values (in the range of 30−40 mN/m) of π_e and π_c for DPPE at 37° and 45 °C. Indeed, this tendency for PE to spread at particular temperatures below its bilayer T_m to monolayer films with values of π_e between 30 and 40 mN/m is also observed for DMPE (see Table 2). It would appear that a possible explanation for such behavior rests with an understanding of the unique bilayer phase behavior of DPPE relative to the other derivatives. Because of the very strong lateral intermolecular hydrogen bonding[53−59] that occurs between the phosphate and amine groups in DPPE and also in unsaturated acyl chain derivatives of PE, the acyl chains are more perpendicularly positioned which allows for maximal hydrophobic and van der Waals interactions and a T_m value that is much higher than those of DPPC and DPPG and for the PC and PG phospholipid systems, in general. This ability of PE phospholipids to pack more tightly in the bilayer and monolayer states than PC and PG phospholipids is also reflected in the smaller area per molecule observed in the fully hydrated PE bilayer,[53] higher bilayer phase-transition temperatures at various levels of water, low water uptake upon exposure to relatively high hydration levels, and gel-phase metastability with favorable reversion to crystallinity primarily because of intermolecular hydrogen bonding between adjacent PE headgroups in the bilayer leaflet (monolayer) and between facing PE moieties at the bilayer−bilayer interface of facing bilayers in a multilamellar dispersion,[35,38,40−42,58−60] the highly condensed state of its spread monolayers, with lower values of π_c compared to PC and PG monolayers (see Figures 1 and 2 for DP and PO chain derivatives, respectively), and the smaller area per molecule at equilibrium monolayer collapse (see Table 4). Lateral hydrogen bonding between polar headgroups in other nonphospholipid surfactants and its ordering restructuring effect on the hydrophobic chains of the surfactant molecules have been observed at the water−oil interface.[61] The characteristic PE polar headgroup properties of hydrogen-bonding and electrostatic interactions between adjacent PE headgroups in PE monolayers appear to have a similar ordering and restructuring of the hydrophobic acyl chains such that surface pressure at equilibrium collapse is restricted between ∼30−40 mN/m (as opposed to 45 mN/m, as observed with PC and PG monolayers) at the air−water interface. This characteristic biophysical property of PE systems has been reported[59] to be attributed to favorable hydrogen-bonding and electrostatic interactions between the PO_4^- and N^+H_3 moieties occurring between adjacent PE molecules in the monolayer and additionally at the bilayer−bilayer interface of facing PE bilayers leading to its inherent bilayer gel-phase metastability and thermodynamically favorable reversion to the bilayer crystalline phase.

Another critical observation, particularly important for PE phospholipids, is how the bilayer crystal-to-gel phase-transition temperature, T_s, relates to the monolayer triple point temperature, T_t, where a direct gaseous (G)−LC surface-phase transition of a phospholipid monolayer occurs (analogous to a direct gaseous-to-solid-phase transition in three-dimensional systems). In DPPE at 25 °C, negligible spreading occurs from the bilayer, and the monolayer shows a direct G−LC surface-phase transition, since 25 °C is below monolayer T_t and, consequently, bilayer T_s. However, at 37 °C and 45 °C, which are above monolayer T_t and

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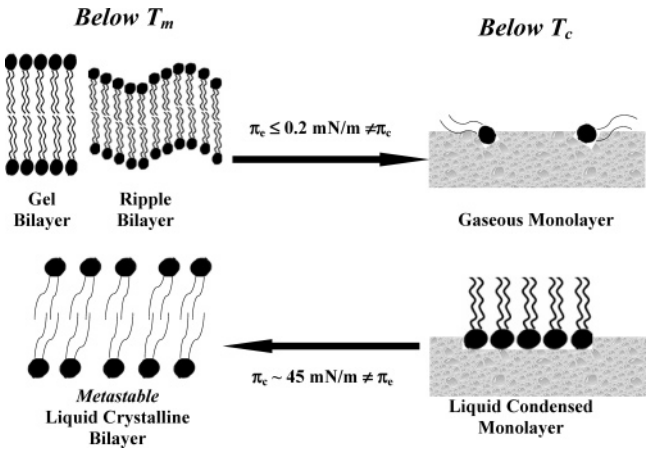
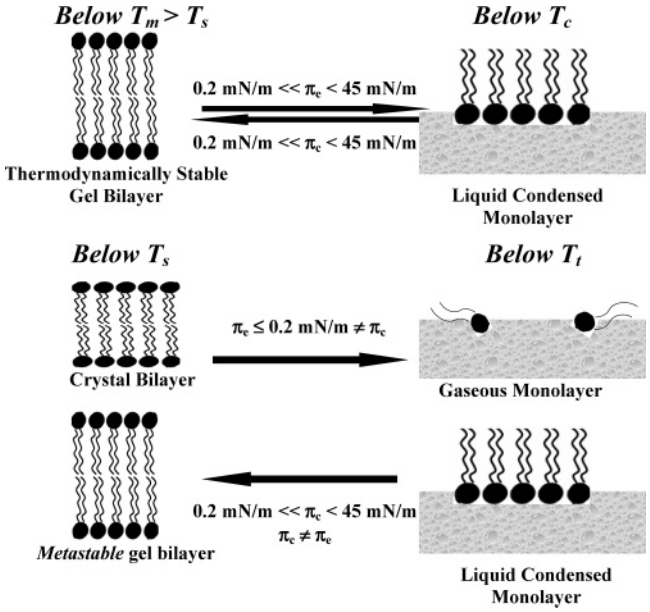
Scheme 3. Illustration Depicting Bilayer−Monolayer Equilibria at Temperatures below Bilayer T_m and Hence below Monolayer T_c for PC and PG Phospholipids Systems



Scheme 4. Illustration Depicting Bilayer−Monolayer Equilibria at Temperatures above Bilayer T_s (but below Bilayer T_m) and below Monolayer T_c and Bilayer−Monolayer Equilibria at Temperatures below Bilayer T_s and below Monolayer T_t for PE Phospholipids



consequently bilayer T_s but are below monolayer T_c and hence bilayer T_m, the monolayer exhibits a clearly distinct LE/LC surface-phase transition that collapses around 30 mN/m, which is essentially identical to π_e at these temperatures.

At temperatures below T_m, illustrated in Scheme 4, the crystal-to-gel bilayer phase-transition temperature, T_s, becomes an important parameter in the context of PE spreading tendencies below T_m. Specifically, unlike spreading of PC and PG below T_m (Scheme 3), PE gel-phase bilayers exhibit significant spreading tendencies to a liquid-condensed monolayer with an intermediate equilibrium spreading pressure value between 30 and 40 mN/m, where π_e and π_c values are approximately equal. This is considered an intermediate π_e value because the maximal value for PE phospholipids of 45−50 mN/m has not been reached. Since the PE polar headgroup is recognized to possess strong intermolecular hydrogen-bonding and electrostatic interactions (reflected in high T_m values and highly condensed monolayers) between the PO_4^- and N^+H_3 moieties, PE bilayers form fully hydrated crystals[38−40] that negligibly spread ($\pi_e \leq 0.2$ mN/m) to a gaseous monolayer at 25 °C. These intermolecular hydrogen-bonding interactions that give rise to tight packing result in a liquid-condensed monolayer collapsing at π_c less than 45 mN/m (unlike PC and PG) forming a metastable lamellar gel phase (unlike PC and PG collapsed monolayers which form a metastable liquid-crystalline phase as depicted in Scheme 3) at temperatures below the triple point of the monolayer, T_t, as is the case at 25 °C. This metastable lamellar gel phase coming out of the compressed monolayer is kinetically stable but thermodynamically unstable at temperatures below bilayer T_s.

Conclusions

The intricate interplay between the bilayer and monolayers properties of PC, PG, and PE phospholipids, in relation to their polar headgroup properties, and the effects of chain permutations on those polar headgroup properties have been demonstrated for the first time with a set of time-independent bilayer−monolayer equilibria studies. Gel-phase metastability and relaxation to the more molecularly ordered crystalline phase have been shown to play a critical role in bilayer and monolayer phase behavior for PE that is quite different than that observed for PC and PG. This is attributed to favorable PE polar headgroup−headgroup hydrogen-bonding and electrostatic interactions in both the bilayer and monolayer states. These characteristic properties of the PE polar headgroup are reflected in the condensed nature of PE monolayers and a decrease in equilibrium collapse pressure at temperatures below T_c (whether above or below T_t) compared to PC and PG monolayers which collapse to 45 mN/m at temperatures both above and below T_c.

Additionally, it has been demonstrated by measurements of the equilibrium spreading pressure, π_e, that above T_m, all liquid-crystalline phospholipid bilayers spread to form and coexist at equilibrium with LE monolayers at 45 mN/m; similarily, LE monolayers at equilibrium collapse to form and coexist with their respective liquid-crystalline bilayer at the same surface collapse pressure. At temperatures below bilayer T_m, PC and PG gel bilayers exhibit a drop in bilayer π_e values $\leq$0.2 mN/m forming gaseous monolayers, whereas the value of π_c of spread monolayers remains around 45 mN/m. This suggests that spread equilibrated PC and PG monolayers collapse to a metastable liquid-crystalline bilayer structure at temperatures below bilayer T_m and surface pressure of 45 mN/m (a surface chemical property characteristically observed at temperatures above T_m). In contrast, PE gel bilayers, which exist at temperatures below bilayer T_m but above bilayer T_s, exhibit gel bilayer spreading to form equilibrated monolayers with intermediate π_e values in the range of 30−40 mN/m; however, bilayer π_e and monolayer π_c values remain equal in value to one another.

Contrastingly, at temperatures below bilayer T_s, PE crystalline bilayers exhibit bilayer π_e values $\leq$0.2 mN/m forming equilibrated gaseous monolayers, whereas spread monolayers collapse at a value of π_c remaining around 30 mN/m, indicative of metastable gel bilayer formation. At temperatures below bilayer T_m and monolayer T_c, the characteristic biophysical property of PE headgroups to hydrogen bond with one another along with favorable electrostatic interactions is reflected in bilayer gel-phase metastability with thermodynamically favorable relaxation to its thermodynamically stable bilayer crystalline state and in a highly condensed spread monolayer which collapses under equilibrium conditions to its metastable gel bilayer state. This characteristic biophysical property which affects both the bilayer and the monolayer phase behavior of PE systems can be attributed to favorable hydrogen-bonding and electrostatic interactions between the PO_4^- and N^+H_3 moieties occurring between adjacent PE molecules in the monolayer and additionally at the bilayer−bilayer interface of facing bilayers.

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