Physicochemical Properties of Phosphatidylcholine (PC) Monolayers with Different Alkyl Chains, at the Air/Water Interface

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Physicochemical properties of a series of PC monolayers with different alkyl chains (C24, C20, C16, and C8), at the air/water interface were investigated. The surface pressure is influenced mainly by the hydrophobicity of the PCs, which is confirmed by the curve shape and the on-set value of π-A isotherms at the air/water interface by increasing the number of alkyl chain. The on-set values of surface pressure were 125 Å^2/molecule for DOPC(C8), 87 Å^2/molecule for DPPC(C16), 75 Å^2/molecule for DAPC(C20), and 55 Å^2/molecule for DLPC(C24), respectively. The orientations of alkyl chains at the air/water interface are closely connected with the rigidity of the monolayers, and it was confirmed by the tendency of monolayer thickness in ellipsometry data. The temperature dependence of a series of PCs shows that the surface pressure decreases by increasing temperature, because the longer the alkyl chain length, the larger the hydrophobic interaction in surface pressure. The temperature effects and the conformational changes of unsaturated and saturated PCs were confirmed by the computer simulation study of the cis-trans transition with POPC and DPPC(C16). The cis-trans conformational energy difference of POPC is 62.06 kcal/mol and that of DPPC(C16) is 6.75 kcal/mol. Due to the high conformational energy barrier of POPC, phase transition of POPC is limited in comparison with DPPC(C16).

Key Words : Phosphatidylcholine, Langmuir-Blodgett monolayer, Hydrophobicity, Unsaturated phosphatidylcholine

Introduction

Phospholipids are major components of biological membranes and have gained current interest in many fields such as biochemistry, chemistry, and polymer science. Recently, molecular films of biomolecules have received increased attention because of their potential applications in biological sensors and other applications in materials science.

As a model system of biological membrane, Langmuir-Blodgett films have been investigated by many researchers for many years.[1-3] The characteristic properties of molecules at the air/water interface are generally characterized by π-A isotherms[4-9] which are crucial in obtaining a proper understanding of what is occurring at the molecular level. For example, Gibbs elasticity of a monolayer is determined from the slope of the π-A isotherms. It is also known that the shapes of the π-A isotherms depend on many variations including temperature,[10] impurities,[11] and speed of compression. By simply analyzing the π-A isotherms, the process of monolayer formation and the physical properties of the single molecule can be inferred from the shape and slope of the π-A isotherms.[12] The combined effects of hydrophobic and hydrophilic interactions are two most important non-covalent interactions dominated in the phospholipid bilayer structure in solution as well.[13]

The phospholipids were influenced by crystallization of fatty acids, so that they could be used as bioactive components *in vivo*.[14,15] Especially, PC (phosphatidylcholine) phospholipids have gained the interest because of its pharmaceutical usefulness to promote metabolism through the cell membrane. For example, PCs with short chain, C6-C8 atoms, are endowed with detergent-like properties. These compounds form micelle rather than bilayer when dispersed in water with a relatively high critical micelle concentration (CMC).[16] They have been applied successfully in the solubilization of biological membranes and the reconstitution of membrane proteins into simple, well-defined membrane systems (proteoliposomes).[17] For another example to facilitate breathing, it is required that surface tension at the air-alveolar interface in the mammalian lung be reduced to near zero from the normal value of 72 mN/m at the air/water interface.[18] *In vivo*, surface tension is regulated by pulmonary surfactant, a lipid-protein complex. Deficiency of this material causes respiratory distress syndrome (RDS) in premature infants,[19] and has also been implicated in the adult version of RDS, which is often trauma-related.[20] The gross composition of typical surfactant isolates is 95% lipid and 5% protein by weight, with the main lipid classes being the phosphocholines and phosphoglycerols. Pulmonary lung surfactant is approximately 50% 1,2-dipalmitoyl-sn-glycero-3-phosphocholine by weight. DPPC(C16) (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) is generally accepted as being the lipid responsible for generating a near-zero surface tension at the interface during compression.[21] The two saturated alkyl chains enable the lipid to form a tightly packed mono-

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layer which can generate low surface tension without collapsing. The unsaturated PC lipids might be important in the formation of a lipid reservoir, in the initial adsorption of lipids to the interface or in the regulation of surface tension during the respiratory cycle. Other functions in intracellular events, such as lamellar body assembly, transport or secretion, are also possible.[22] These biophysical approaches are also necessary for the rational design of exogenous surfactants for therapeutic purpose.

Although many studies with these lipids have been performed because of their various applications, there are lacks of studies for the saturated derivatives PC and the unsaturated PC to provide the influence of the hydrophobic forces of phosphatidylcholine on the self assembled structure.

In this study, the physicochemical properties of PCs (C8, C16, C20 and C24) monolayers, at the air/water interface were studied by Langmuir-Blodgett technique. The conformational changes of unsaturated POPC and saturated DPPC (C16) by the increase of surface pressure at the air/water interface were observed by π-A isotherms and were confirmed by the computer simulation with CHARMM.

Materials and Experimental Methods

Materials. The phospholipids used in this study were 1,2-dioctanoyl-sn-glycerol-3-phosphocholine (DOPC(C8)), 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC(C16)), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC(C20)), 1,2-dilignoceroyl-sn-glycero-3-ph-osphocholine (DLPC(C24)), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), all obtained from Avanti Polar Lipids, Inc., Alabaster, AL. Their chemical structures are in Figure 1. They all had purities of 99% and were used as received. Spectroscopic grade chloroform (Aldrich) was used as the spreading solvent. Water was prepared using a Millipore Milli-Q system, which had a resistivity of $>18.2\ M\Omega^{-1}$.

Experimental Methods.

Langmuir-Blodgett technique: An automatically controlled Langmuir film balance KSV3000 (KSV Instruments Ltd., Finland) equipped with a platinum Wilhelmy plate, was used to obtain the surface pressure-area (π-A) isotherms of

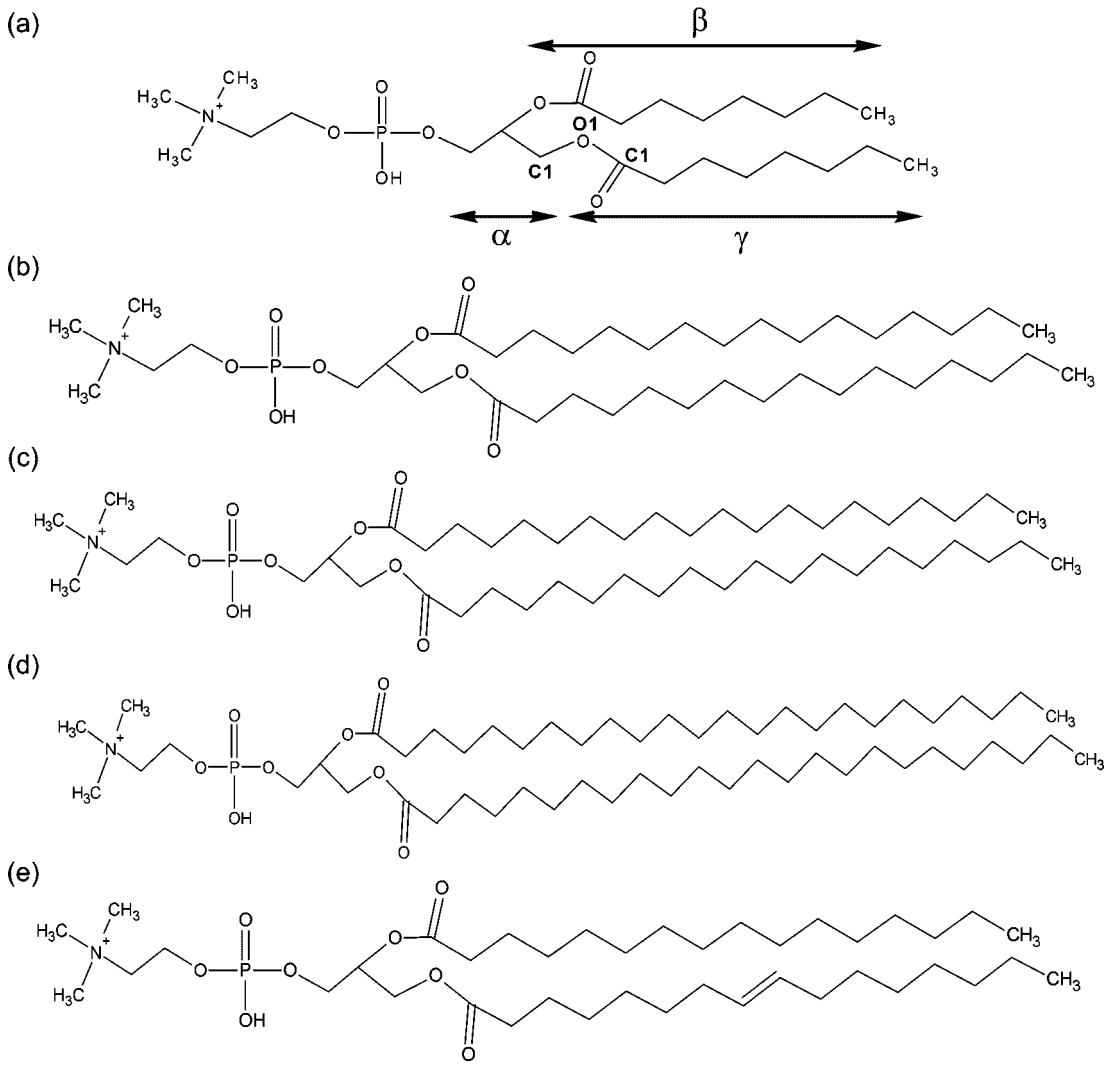


Figure 1. The chemical structure of (a) DOPC(C8), (b) DPPC(C16), (c) DAPC(C20), (d) DLPC(C24), (e) POPC.

monolayers at the air/water interface. For all experiments, the trough was filled with purified water as the subphase, and the temperature was maintained by external water bath circulation. The air/water interface could be compressed and expanded symmetrically with two teflon barriers at a desired rate. The cleanliness of the trough and subphase was ensured before each experiment by aspirating the surface of subphase. When the surface pressure fluctuation was found to be less than 0.1 mN/m during the compression cycle, a sample containing monolayer-forming materials was spread on the subphase surface by using a microsyringe (Hamilton Co., USA). Ten minutes were allowed for solvent evaporation and monolayer equilibration before an experiment was started. After allowing for the solvent to evaporate, the monolayer at the air/water interface was continuously compressed at a rate of 7.5 cm^2/min to obtain the π-A isotherms. As a monolayer was compressed to be in a condensed phase, the isotherm generally exhibited a sharp collapse followed by an abnormal change of surface pressure upon further compression, which was referred to as the collapse point of the monolayer under the given experimental condition.

Ellipsometry: Auto EL nulling ellipsometer (Rudolph scientific) was used to measure the thickness of LB monolayer. The compensator is set at an azimuth angle of 70° to sample surface. The optical parameter Δ and Ψ were used for calculating apparent thickness of film material. Average values from 5 measurements on Si/SiO_2 served as reference, and the average value for film thickness obtains from 6 measurements on each sample.

Results and Discussion

π-A isotherms of PC monolayers with different length of alkyl chain. Figure 2 shows the π-A isotherms for the pure DOPC(C8), DPPC(C16), DAPC(C20), and DLPC (C24) at 25 °C. As the length of alkyl chain increase, the on-set values of surface pressure shift toward lower area per molecule (Am). The on-set values of surface pressure were 125 $Å^2$/molecule for DOPC(C8), 87 $Å^2$/molecule for DPPC (C16), 75 $Å^2$/molecule for DAPC(C20), and 55 $Å^2$/molecule for DLPC(C24), respectively. The π-A isotherms show that PCs with short alkyl chain have smooth increase of surface pressure, which could be explained as the monolayer fluidity of DOPC(C8), because they are dominated by the weak order of its relatively short hydrocarbon chains. In contrast, PCs with long alkyl chains have stiff increase of surface pressure. The monolayers of DAPC(C20) and DLPC(C24) are rather rigid than those of short alkyl chains because of condense order of its relatively long hydrocarbon chains. Orientation of alkyl chains at the air/water interface is the main factor for the rigidity of monolayers. Orientation of the alkyl chains of long PCs is rather horizontal with the respect to the surface normal, which represents that DAPC(C20) and DLPC(C24) are arranged vertically at the air/water surface of large Am, and it is confirmed by ellipsometry data as in Figure 3.

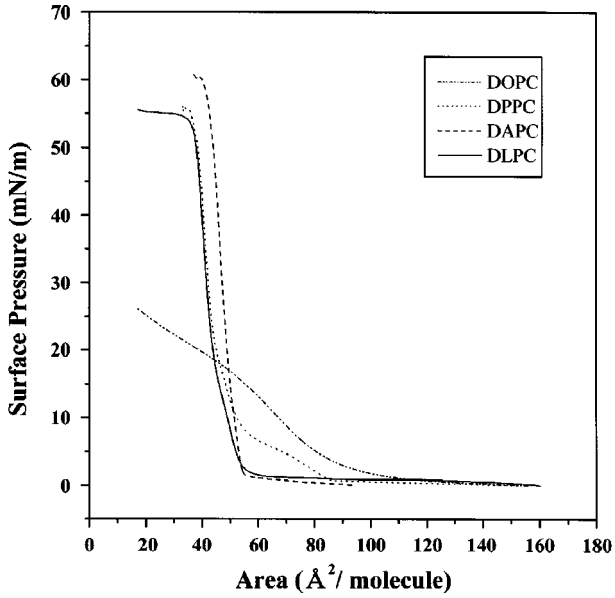


Figure 2. The π-A isotherms of PCs with different alkyl chain lengths, DOPC(C8), DPPC(C16), DAPC(C20), and DLPC(C24), at 25 °C.

Figure 3(a) shows the π-A isotherm and the monolayer thickness of DOPC(C8) depending on the area per molecules. We observed smooth increase of film thickness through the decreased surface area, in which the alkyl chains are arranged slowly and vertically to the plane of subphase surface. Monolayer thickness was 5 Å at the 160 $Å^2$/molecule and change to 9 Å at the 20 $Å^2$/molecule. In contrast, Figure 3(c) of DLPC(C24) shows stiff increase of monolayer thickness and surface pressure due to the large hydrophobicity of long alkyl chains. Figure 3(b) is the π-A isotherm and the monolayer thickness of DPPC(C16) which has two transitions: a small transition at around 80 $Å^2$/molecule and a stiff transition at around 50 $Å^2$/molecule. A small transition is generally known as the liquid condensed state transition in which the molecules are closed packed with the hydrocarbon chains in a more ordered state. Relatively long alkyl chains of DLPC(C24) generate the strong hydrophobic interactions between the hydrocarbons, but short chains of DOPC(C8) make relatively flexible and weak interaction between the chains. The PCs having 16 hydrocarbons have competition between the hydrophobic intermolecular interaction and the chain flexibility.

Temperature effects of PC monolayers with different alkyl chain lengths. Figure 4(a) and 4(b) show the temperature dependent π-A isotherms of DOPC(C8) and DPPC(C16), respectively. In Figure 4(a), by increasing temperature, the surface pressure decreases. At 60 $Å^2$/molecule, the surface pressure values have 17 mN/m for 10 °C, 13 mN/m for 20 °C, 9 mN/m for 30 °C 5 mN/m for 40 °C. It explains that π-A isotherms are dominated by the combined effect of hydrophobicity of alkyl chains and interaction between hydrophilic parts and aqueous subphase. The isotherm of DOPC(C8) is dominated by the interaction between the hydrophilic part and the aqueous surface

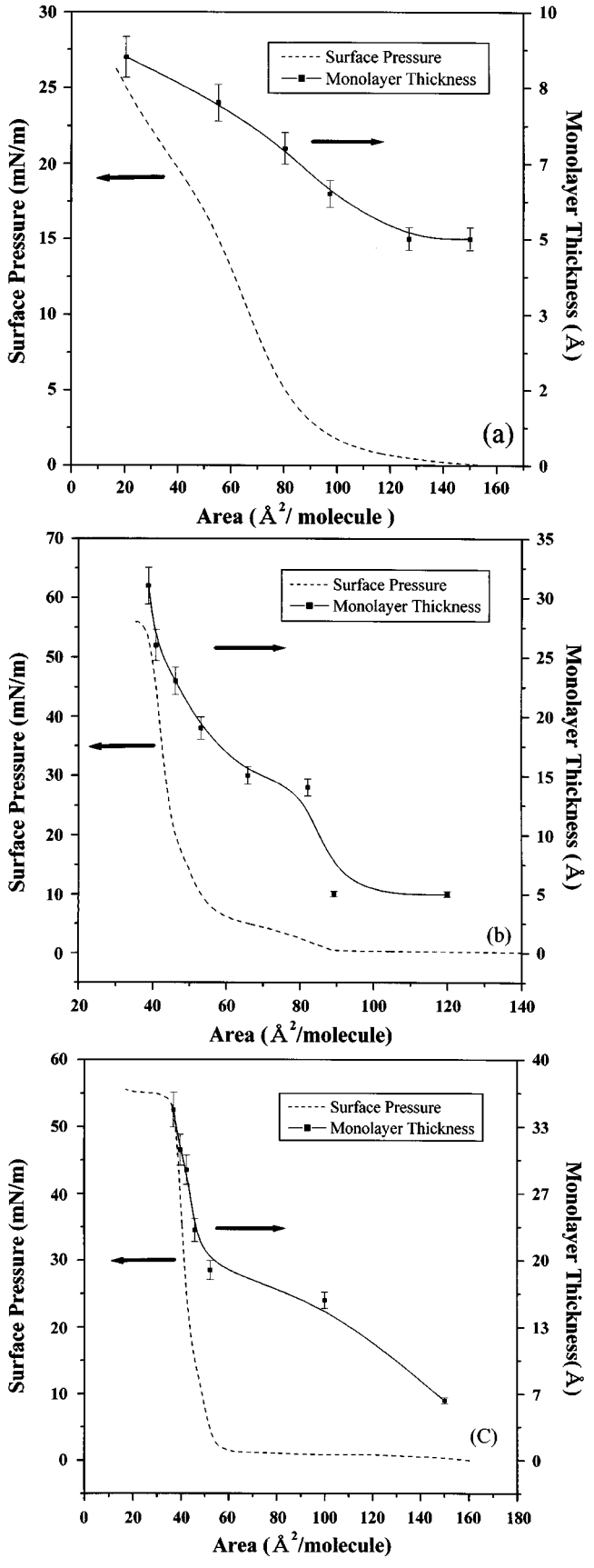


Figure 3. The π-A isotherms (—) and monolayer thickness vs. area per molecule (Am) curves (■) of (a) DOPC(C8), (b) DPPC(C16), and (c) DLPC(C24), at the air-water interface. The monolayer thickness was measured by ellipsometer.

because of relatively short alkyl chain. So, by increasing temperature, hydrogen bonding between head group and aqueous phase is broken, and the surface pressure through full range is reduced. By contraries, in Figure 4(b), the higher temperature, the larger surface pressure, at 50 Å^2/ molecule, and the surface pressure values have 0.2 mN/m for 10 °C, 7.5 mN/m for 20 °C, 25 mN/m for 30 °C, and 36.5 mN/m for 40 °C. It could explain that the isotherms of DPPC(C16) are controlled by the hydrophobicity of alkyl chains. So, by increasing temperature, the thermal motion of alkyl chains is increased, and the surface pressure is increased. By all accounts, with expansion of alkyl chain length, the predominance of hydrophobic interaction in surface pressure is getting bigger. The longer hydrocarbons in DAPC(C20) and DLPC(C24) make strong chain interactions, so they do

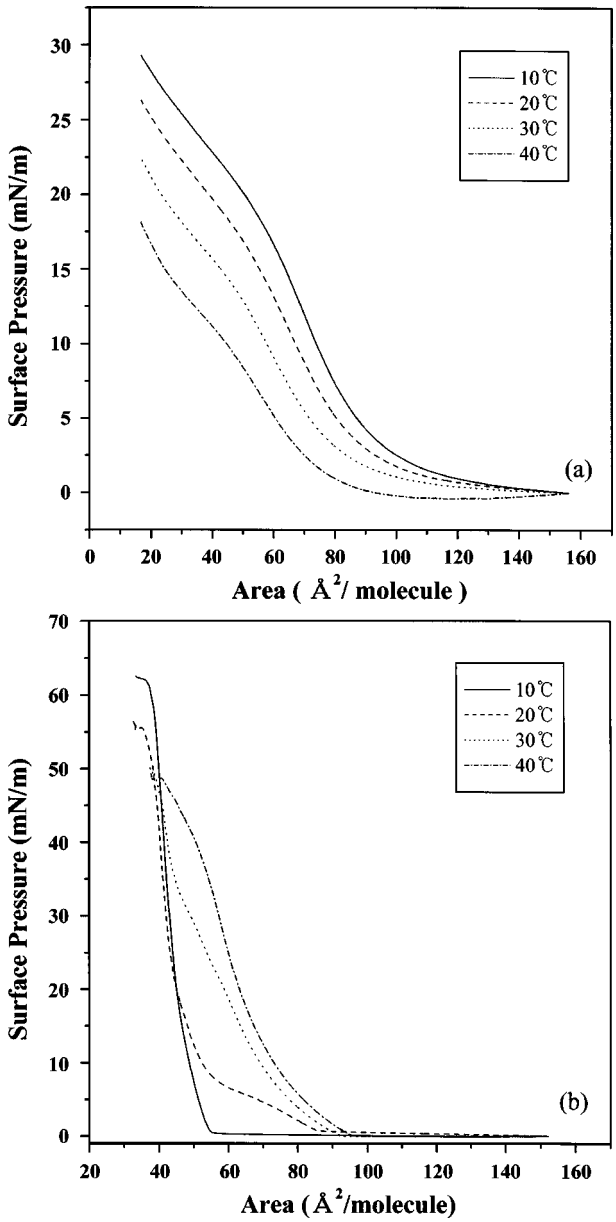
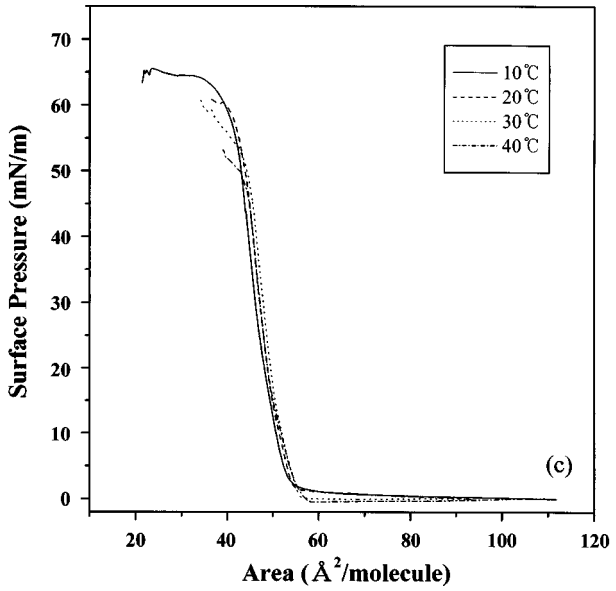
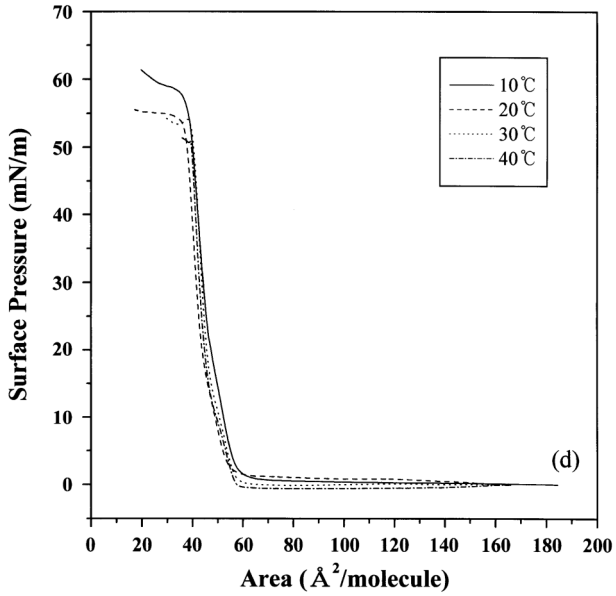


Figure 4. The π-A isotherms of (a) DOPC(C8), (b) DPPC(C16), (c) DAPC(C20), (d) DLPC(C24), and (e) POPC with various temperatures at the air/water interface.





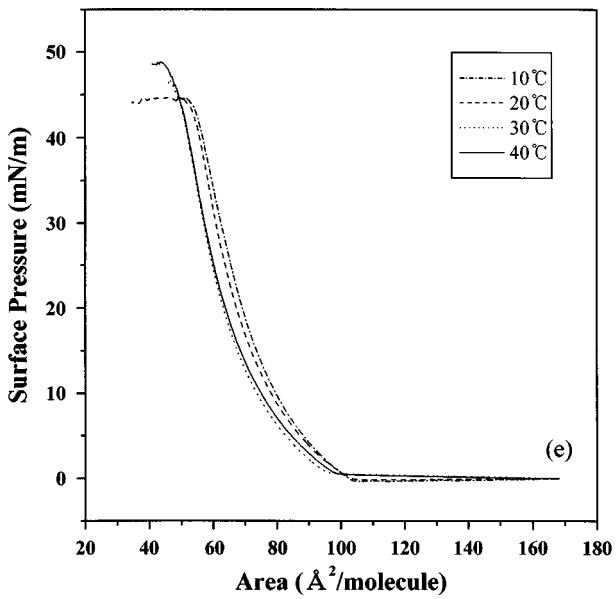
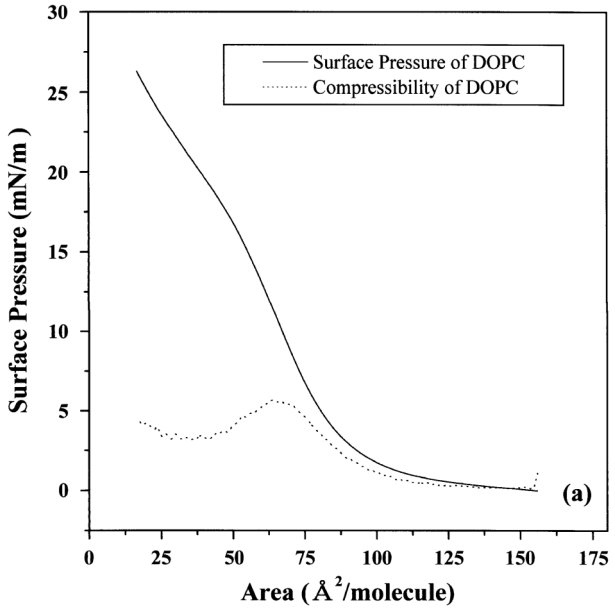


Figure 4. Continued.

not change dramatically their π-A isotherms by temperature.

Comparing the π-A isotherms of PC series with computer simulation. The DPPC(C16) and the DOPC(C8) have the same head group, and the alkyl chain length of DPPC(C16) is twice as large as that of DOPC(C8). There are at least two distinguishable liquid phases at the air-water interface. The first was called liquid expanded having rather high compressibility and the second was called liquid condensed phase of a low compressibility as discussed in reference 23. Their π-A isotherms of surface pressure and compressibility in Figure 5(a) show that DOPC(C8) has smooth curve with one phase transition at 66.44 Å^2/molecule, but as shown Figure 5(b), DPPC(C16) has three phase transition at 80.49 Å^2/molecule, 48.29 Å^2/molecule, 40.58 Å^2/molecule. These experiments reveal that DPPC(C16) has liquid expanded-liquid condensed coexistence region by compression.



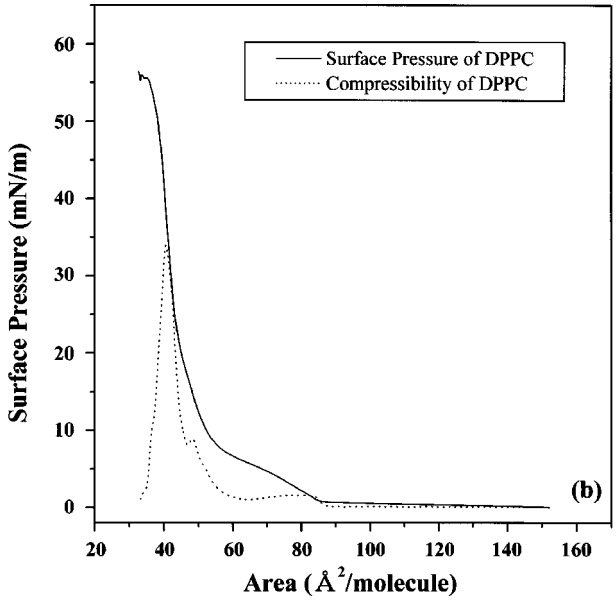


Figure 5. The π-A isotherms (—) and compressibility (---) of (a) DOPC(C8) and (b) DPPC(C16) at 20 °C.

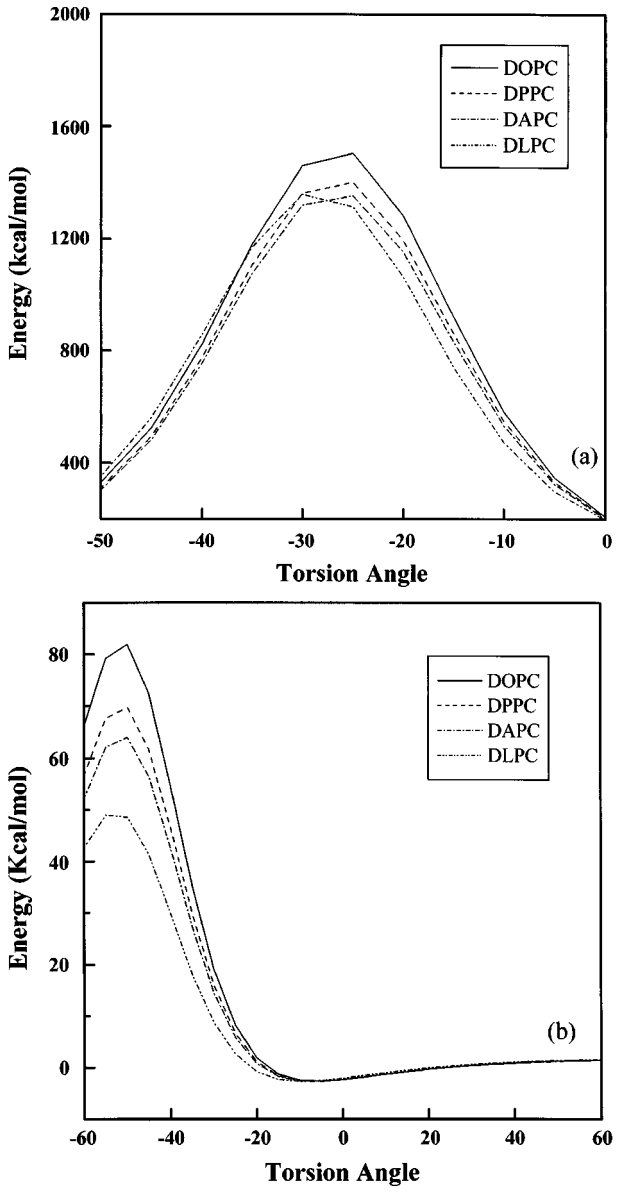


Figure 6. (a) Conformational energy curves of DOPC(C8), DPPC(C16), DAPC(C20), and DLPC(C24), (b) Interaction energy curve between two (β, γ) chains of DOPC(C8), DPPC(C16), DAPC(C20), and DLPC(C24) by CHARMM lipid force field.

The increased order as a smaller surface can result from changes in the internal conformation of the lipid by the chain dihedral distributions or by the overall molecular orientation.[24] To separate these two effects, the potential energies for rotation about the αC1-γO1-γC1-γC2 (as in Figure 1) dihedral angles of DOPC(C8), DPPC(C16), DAPC(C20), and DLPC(C24) molecules were calculated along 10 degree rotation. The β chain was anchored and fixed on αC1 carbon and the γ chain was rotated clockwise through 180° to -180°. The lipid force field of all-hydrogen atomic model with CHARMM was used. In the case of DOPC(C8), the region that has above 200 kcal/mol conformational energy difference is from -55° to -0° and maximum conformational energy difference is 1498.09 kcal/mol. For DPPC(C16), however, the region that has above 200 kcal/mol conformational energy difference is from -50° to -5° and maximum conformational energy difference is 1391.36 kcal/mol. Therefore the shorter alkyl chains length, the broader high conformational energy region as shown in Figure 6(a). These calculations tell us that flexibility of molecule itself have increased tendency with alkyl chain length and consist with result of Figure 5. In Figure 5(a), we have seen the smooth phase transition of DOPC(C8) and the stiff phase transition of DPPC(C16) at Figure 5(b). The high peaks of compressibility curve of DPPC(C16), in Figure 5(b), coincide with narrow conformation energy region in Figure 6(a), and it could explain that if the molecule has wide relatively low conformational energy region, molecule has a lot chance to phase transition and then compressibility value is large. And in comparison with DPPC(C16), low curve of compressibility of DOPC(C8), in Figure 5(a), coincides with wide relatively high conformational energy region.

We also calculated the hydrocarbon interaction energy between the β chain and the γ chain during the rotation. The interaction energy function is partitioned as

$$E_I = \sum_{i,j>1} \left(\frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right) + \sum_{i,j>1} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right)$$

Where first term is electrostatic energy; $q_i q_j$ is atomic charges of each atom, ε_0 is dielectric constant, r_{ij} is interatomic distance and second term is van der Walls energy; A_{ij} and B_{ij} are derived from the atomic polarizabilities and the effective number of outer shell electrons, respectively.[25]

In Figure 6(b), the interaction energy of DOPC(C8) has from -2.60 kcal/mol to 81.84 kcal/mol and that of DPPC (C16) has from -2.62 kcal/mol to 69.71 kcal/mol, where the maximum interaction energy region is -5°. These results support that the longer alkyl chain length, the more increased intermolecular interaction. So the formed aggregates of the molecule are rigid because of the hydrophobic interaction between chains. From the above results, Lipid molecules have lower conformational energy in liquid-expanded region, but in liquid-condensed region, lipid molecules have high conformational energy and stabilize molecule chain interaction. Because the longer alkyl chains have high flexible and good interaction of chains than those of shorter alkyl chain, and the molecules having longer alkyl chain have the tendency of stiff phase transition.

Comparing the π-A isotherms of saturated PC and unsaturated PC. Figure 4(e) shows the π-A isotherms of POPC, one of unsaturated phosphatidylcholine phospholipids. DPPC(C16) and POPC have the same hydrocarbon numbers, but POPC has cis-double bond in one of alkyl chains on γ chain. The phase transition between the liquid expanded (LE) and liquid condensed (LC) states of DPPC(C16) is prominent in the π-A isotherm, but there is no phase transition (plateau region) in that of POPC during compression in Figure 4(e). DPPC(C16) has internal dihedral transition but that of POPC is limited because one cis-double bond disturbs the motion of another alkyl chain. To reveal the relationship of π-A isotherms between POPC and

Table 1. The *cis-trans* conformational energy differences of DPPC and POPC. The unit is kcal/mol

	cis energy	trans energy	ΔE
DPPC	11.800	5.054	6.746
POPC	65.242	3.184	62.058

DPPC(C16), we calculated cis-trans conformational energy of the molecules. The cis-trans conformational energy difference of POPC has 62.058 kcal/mol whereas that of DPPC(C16) has 6.746 kal/mol. In general, molecules had double bond have high conformational energy barrier. The π-A isotherm of POPC doesn't show special phase transition because the conformational energy barrier of POPC is higher than that of DPPC(C16), so conformational change is limited in comparison with DPPC(C16). The effect of temperature on POPC is negligible because one alkyl chain with double bond interrupts motion of another alkyl chain and the thermal motion of molecules could be restricted, as in Figure 4(e). These results are summarized on Table 1.

Conclusion

At the air/water interface, the surface pressure is influenced by two interfaces: hydrophobic region of molecules/air interface and hydrophilic parts of molecules/aqueous subphase interface. By increasing the length of alkyl chain in PCs, the surface pressure is influenced mainly by hydrophobicity, that is confirmed by curve shape and on-set value of π-A isotherms, tendency of monolayer thickness, and computer simulations. The orientations of alkyl chains at the air/water interface are closely connected with the rigidity of the monolayers. The orientation of the PCs (C20, C24) is rather horizontal with respect to the surface normal and this explains that the PCs (C20, C24) are arranged vertically at the air/water surface of large Am, and it is confirmed by ellipsometry data. Also, the temperature dependence of a series of PCs shows that, by increasing temperature, the surface pressure decreases because the longer the alkyl chain length, the larger the hydrophobic interaction in surface pressure.

Comparing the π-A isotherms of saturated PC and of unsaturated PC, the transition between the liquid expanded (LE) and liquid condensed (LC) states of DPPC(C16) is prominent in the π-A isotherm, but there is no transition (plateau region) in that of POPC during compression. The alkyl chain of POPC having one *cis* double bond which disturbs the motion of another alkyl chain, so that conformational change is limited. This is confirmed by showing the energy difference of cis-trans transition of POPC and DPPC(C16) by computer simulation.

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