

Sum Frequency Generation Spectroscopic Studies on Phase Transitions of Phospholipid Monolayers Containing Poly(ethylene oxide) Lipids at the Air–Water Interface

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Vibrational sum frequency generation (SFG) spectroscopy was applied to study the phase transitions of the mixed monolayers of L- α -distearoyl phosphatidylethanolamine (DSPE) and DSPE covalently coupled with poly(ethylene oxide) at the amino head group (DSPE-EO₄₅, DSPE with 45 ethylene oxide monomers) at the air–water interface. The SFG spectra were measured for the mixed monolayers with the mole fractions of DSPE-EO₄₅ of 0, 1.3, 4.5, 9.0, 12.5, and 16.7% at the surface pressures of 5, 15, and 35 mN/m. The monolayer compression isotherms indicated that the mixed monolayers at 5, 15, and 35 mN/m are mainly in the so-called “pancake”, “mushroom”, and “brush” states, respectively. The SFG spectra in the OH stretching vibration region give rise to SFG bands near 3200 and 3400 cm⁻¹. The mean molecular amplitude of the former band due to the OH stretching of the “icelike” water molecules associated mainly with the hydrophilic poly(ethylene oxide) (PEO) chains, exhibits appreciable decrease on compression of the mixed monolayers from 5 to 15 mN/m. The result corroborates the model for the pancake–mushroom transition, which presumes the dissolution of the PEO chains from the air–water interface to the water subphase. Further compression of the mixed monolayers to 35 mN/m causes a slight decrease of the line amplitude, which can be explained by considering a squeezing out of water molecules from the hydrophilic groups of DSPE-EO₄₅ in the brush state, where the PEO chains strongly interact with each other to form a tight binding state of the hydrophilic groups. The relative intensities of the SFG bands due to the CH₃ asymmetric and symmetric vibrations were used to estimate the tilt angles of the terminal methyl group of DSPE, indicating that the angle increases with increasing the mole fraction of DSPE-EO₄₅. The angles almost saturate at the mole fraction larger than 10%, the saturation angle being nearly 90° at 5 mN/m, ca. 60° at 15 mN/m, and ca. 47° at 35 mN/m. Then, the introduction of the hydrophilic PEO head group causes a large tilting of the alkyl groups of DEPE in the mixed monolayers.

Introduction

The presence of terminally grafted water soluble polymers such as poly(ethylene oxide) (PEO) at the surface of colloidal particles and biomaterials has been attracting attention in recent years, especially regarding sterically stabilized liposomes and vesicles used for drug delivery systems.¹ The grafting of the water-soluble polymer enhances circulation time of liposomes from a few hours to days in vivo.^{2–5} The enhancement has been ascribed to the protection of the sterically stabilizing sheath of the polymer chains extending from the liposome surfaces, preventing their close approach to cell membranes, which is necessary to prevent adhesion, fusion, and lysis of the liposomes by immune systems.

One of the most frequently used grafted phospholipids to elucidate the stabilization mechanism is a grafted L- α -distearoyl phosphatidylethanolamine, where the head group of the lipid is covalently bound to the PEO chains. (DSPE-EO₄₅ with 45 ethylene oxide monomers, see Figure 1.) The PEO chain has a molecular weight of about 2000, and the grafted DSPE may be considered as a phospholipid having an extra large hydrophilic head group. Interactions between the grafted polymer chains protruding from the monolayers at the air–water interface can

be quantitatively controlled by changing the mole fraction of DSPE-EO₄₅ to the total lipids and the surface pressures or the surface area per molecule. Previous works on the DSPE-EO₄₅ system have been focused on measuring the extension of the grafted polymer from membrane surfaces consisting of DSPE-EO₄₅ itself or DSPE-EO₄₅/DSPE mixtures by using a variety of techniques, e.g., X-ray scattering of the lamellae of the mixtures,⁶ light scattering of DSPE-EO₄₅ liposomes,⁷ force profile measurement of supported bilayers consisting of DSPE-EO₄₅ and DSPE,⁸ and neutron reflectometry of the mixed monolayers at the air–water interface.⁹ Phase behaviors of the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface have also been studied by measuring monolayer compression (π -A) isotherms.^{8–11} Although all the experimental results lack information at the molecular level, general consensus seems to have been obtained about the structures of the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface, as summarized in the following. At lower mole fractions (less than 13%) and lower surface pressures (e.g., ca. 5 mN/m) the PEO chains are adsorbed to a water surface, forming a flat so-called “pancake”-like structure (Figure 2A).¹⁰ On increasing the surface pressure (e.g., from 5 to 15 mN/m), the polymer chains desorb from the water surface, changing their conformation from the pancake to a hemispherical or “mushroom”-like structure (Figure 2B).^{12,13} The polymer chains in the mushroom regime are not perturbed

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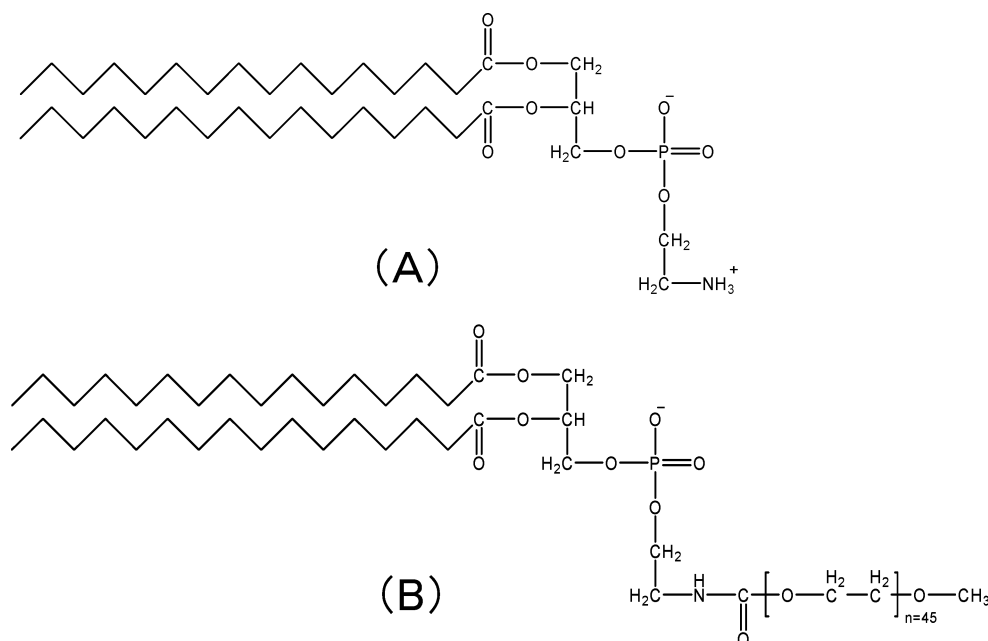


Figure 1. Chemical structures of DSPE (A) and DSPE-EO₄₅ (B).

by their neighbors. On increasing further the surface pressures (e.g., from 15 to 35 mN/m), the polymer chains are converted from the mushroom structure to a so-called “brush” structure, where the polymer chains are strongly interacting with each other (Figure 2C). The mushroom to brush transition has been generally observed for the surfaces of grafted polymer chains.^{12–14}

In the present paper, we applied a sum frequency generation (SFG) spectroscopy to study the phase transitions of the mixed monolayers at the air–water interface. SFG is a nonlinear optical technique, in which two laser beams, one in the visible region and of fixed frequency and the other in the infrared region and of tunable frequency, are spatially and temporally overlapped at an interface to produce a vibrational spectrum specific to the interfacial species. Since the SFG spectroscopy was experimentally demonstrated by the Shen laboratory,^{15,16} it has been established as one of the most efficient methods to extract information on surface structures from small molecules such as water to large molecular systems such as self-assembled monolayers of lipids and proteins at various interfaces.^{15–23} The important points to be solved by applying the SFG spectroscopy on the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface are as follows: (i) participation of the water molecules associated with the hydrophilic groups of DSPE and DSPE-EO₄₅ to the pancake–mushroom and mushroom–brush transitions; (ii) the orientation and conformation changes of the alkyl groups of the DSPE moieties in the mixed monolayers during the transitions.

SFG studies have been performed on poly(ethylene glycol) (PEG) and its related polymers at various interfaces.^{24–29} Goates et al.²⁴ applied the SFG spectroscopy to study monolayers of nonionic surfactants poly(ethylene oxide) monododecyl ethers (C₁₂EO_m; *m* = 2–8) at the air–water interface, indicating an appreciable increase in conformational disorder of the alkyl chain with increasing area per molecule. According to Tyrode et al.,²⁹ who reported the SFG spectra of the C₁₂EO_m (*m* = 4 and 8) monolayers at the air–water interface, the ssp-polarized spectra give prominent bands at 3250 and 3400 cm^{–1}, indicating a significant ordering of strongly and weakly hydrogen-bonded water molecules associated with the PEO head group. To the best of our knowledge, however, the SFG study on the DSPE-

EO₄₅/DSPE mixed monolayers at the air–water interface has not been performed yet.

Experimental Section

Materials. DSPE and DSPE-EO₄₅ were obtained from Avanti Polar Lipids, Inc., and used as received. Water used in the experiment was purified with a Millipore Inc. Milli-A Plus, and its resistivity was measured to be higher than 18 MΩ.

Preparation of Monolayers. The DSPE-EO₄₅/DSPE mixtures with various mole fractions (mol of DSPE-EO₄₅ to mol of the total lipids) were prepared by mixing appropriate amounts of DSPE-EO₄₅ (1.40 mg/mL) and DSPE (0.37 mg/mL) solutions. The mixed 1.3, 4.5, 9.0, 12.5, and 16.7% monolayers were spread in the form of methanol/chloroform (1/4) solutions on a pure water in a MiniMicro trough of KSV Instruments Ltd., which allows us to change surface pressure and to keep the pressure at a constant value by using a PC control system. At each surface pressure the sample was kept at least 15 min to stabilize the monolayer samples. The trough was used also for the measurements of monolayer compression (π -A) isotherms.

SFG Spectral Measurements. SFG spectra were measured by using the apparatus, which is virtually identical with that used in the previous study.³⁰ Briefly, the spectra were recorded by combining 3-ps pulses of 800 nm (1 kHz, ca. 20 μ J/pulse) and 3-ps pulses of infrared light (1 kHz, ca. 4 μ J/pulse at 3 μ m) tunable from 2800 to 3600 cm^{–1} at the interface in a co-propagating geometry with the angle of incidence of 43.1° from the surface normal for the visible light and the angle of 53.2° for the IR beam. The 800-nm light is a part of the amplified output of a Ti:sapphire system, which consists of a mode-locked Ti:sapphire laser (Spectra Physics, Tsunami Model 3950) pumped by a cw green laser (Spectra Physics, MILLENNIA DPSSL, 532 nm, 5 W), a Ti:sapphire regenerative amplifier (Quantronix 4813) pumped by the second harmonic of a Nd:YLF laser (Quantronix 527DP-H, 1 kHz, 9.1 mJ/pulse), and a stretcher-compressor (Quantronix 4823). Approximately 600 μ J of the output at 800 nm was introduced to an OPA/OPG system (Light Conversion, Model ps-TOPAS-800/DF) to produce the tunable IR light. The sum-frequency beam was introduced to a monochromator (Acton, SP-308) equipped with a liquid-

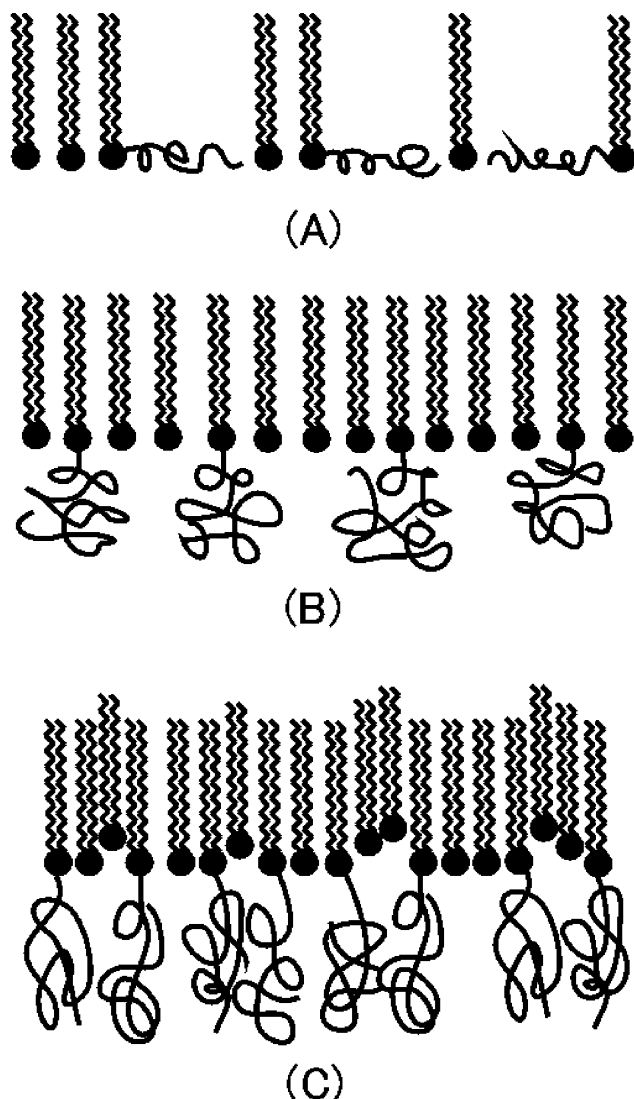


Figure 2. Schematic representation of the pancake (A), mushroom (B), and brush (C) states of the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface.

nitrogen-cooled charge-coupled device detector (Princeton Instruments, LN-CCD HRBN) to record SFG spectra. All spectra were measured at $21 \pm 1^\circ\text{C}$ and taken with the ssp polarization combination, which refers to the sum frequency, visible and infrared beams, respectively.

Results and Discussion

π -A Isotherms of DSPE-EO₄₅/DSPE Mixed Monolayers at the Air–Water Interfaces. Figure 3 shows the compression surface pressure–molecular area (π -A) isotherms of DSPE and DSPE-EO₄₅/DSPE mixtures with various mole fractions of DSPE-EO₄₅ measured at 21°C . (The isotherm for the 16.7% mixed monolayer is not shown in the figure, since it is similar to that of the 12.5% mixed monolayer.) The isotherms are almost identical with those already reported.^{8,9} The isotherm of the DSPE monolayer indicates a direct transformation from a gaslike surface phase to a stable solid gel phase, and at high surface pressures, $\pi > \text{ca. } 30 \text{ mN/m}$, the isotherms of the mixed monolayers are similar to that of DSPE, suggesting that the PEO chains are almost submerged into the water subphase, as schematically shown in Figure 2C. At a constant surface pressure lower than 20 mN/m , the area per DSPE molecule increases with the mole fraction of DSPE-EO₄₅, which is ascribable to

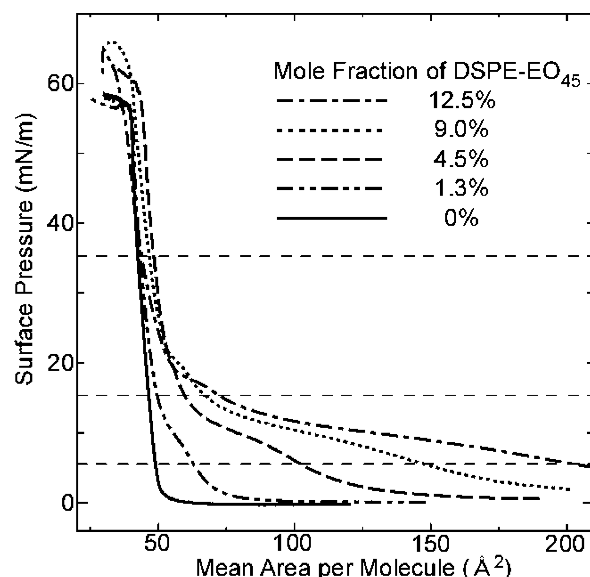


Figure 3. Monolayer compression (π -A) isotherms of the mixed DSPE-EO₄₅/DSPE monolayers with various mole fractions of DSPE-EO₄₅ (0–12.5%) measured at 21°C .

the repulsive lateral interaction between the PEO chains. For the mixed monolayers, two conformational transitions are suggested by breaks in the isotherms. The onset of the first break is observed at a surface pressure of about 8 mN/m . The second break occurring at pressures in the region of 18 – 20 mN/m is discernible for the mixed monolayers with the mole fractions larger than or equal to 4.5% . The first transition has been ascribed to a compression-induced desorption from the water surface of the initially adsorbed PEO chains, as schematically depicted in Figure 2, parts A and B.^{8,9} Here the polymer goes from a so-called “pancakelike” shape to a “mushroomlike” shape. The pancake–mushroom transition has been predicted to grafted polymer chains by de Gennes¹² and Alexander.¹³ On further compression of the mixed monolayers there occurs the second transition, where the PEO chains strongly interact with each other, resulting in a so-called “brushlike” structure.^{8,9} As shown in Figure 2C, upon transformation from the mushroom to brush state, the PEO chains are presumed to be tightly packed blobs with a diameter much smaller than that of the mushroom.¹⁴

The SFG spectra reported in the present paper were measured for the DSPE and mixed monolayers at the surface pressures of 5 , 15 , and 35 mN/m , which are indicated by the horizontal dashed lines in Figure 3. The isotherms indicate that the mixed monolayers are mainly in the pancake state at 5 mN/m , in the mushroom state at 15 mN/m , and in the brush state at 35 mN/m .

SFG Spectra in the OH Stretching Vibration Region of the DSPE-EO₄₅/DSPE Mixed Monolayers at the Air–Water Interface. Figure 4 exhibits the surface pressure dependence of the SFG spectra in the 3600 – 3000-cm^{-1} region observed for DSPE and DSPE-EO₄₅/DSPE mixed monolayers spread on pure water. The SFG spectra of water surface and surfactants at the air/water interface measured under the ssp polarization (corresponding to SFG, visible and IR lights) in this region generally give rise to broad bands centered around 3200 and 3450 cm^{-1} .^{20,29,31} The 3200-cm^{-1} band has been assigned to a continuum of OH symmetric stretching vibrations of tetrahedrally coordinated (or hydrogen-bonded) water molecules near the surface and has been referred as “icelike” region because of its closeness in frequency to the OH stretching of bulk ice. The 3450-cm^{-1} band has been assigned to more weakly

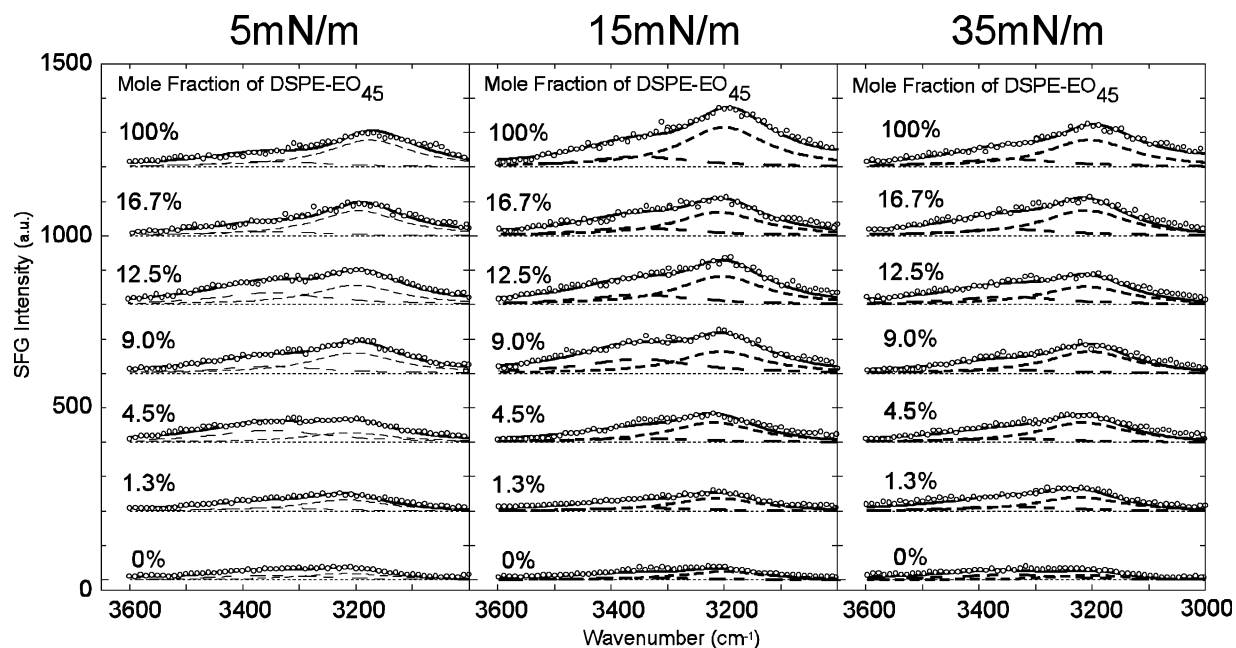


Figure 4. SFG spectra in the OH stretching region measured under the ssp polarization for the mixed DSPE-EO₄₅/DSPE monolayers with various mole fraction of DSPE-EO₄₅ at surface pressures of 5, 15, and 35 mN/m (21 °C). The spectra, except for the bottom ones, are offset by certain values to exhibit the spectra clearly. The dashed curves indicate the contributions of the component bands calculated by using estimated parameters and the solid lines the simulated spectra. See text.

correlated hydrogen-bonded OH stretching modes of water molecules in an asymmetric hydrogen-bonded environment and usually labeled “liquidlike” in analogy to the position of the strongest band observed in the bulk Raman spectra of liquid water.³² In addition to the above-mentioned bands the air/water interface gives a rather sharp SFG band located near 3700 cm⁻¹, which has been attributed to the free OH bond of surface water molecules.^{20,29,31} The ssp-polarized SFG spectra of DSPE and the mixed monolayers do not show any discernible feature in the free OH stretching vibration region. As can be seen in Figure 4, the SFG spectra exhibit the “liquidlike” band around 3400 cm⁻¹ and the “icelike” band around 3200 cm⁻¹. The DSPE monolayer gives only weak intensities to these bands, as in the case of the monolayers of the zwitterionic phospholipids such as dipalmitoylphosphatidylcholine (DPPC). It has been observed that the SFG spectra of negatively charged phospholipids such as dipalmitoylphosphatidylcholine (DPPG) give an appreciably strong “icelike” band.^{19,20,30} In contrast to the zwitterionic lipid monolayers, the negatively charged hydrophilic groups form an electric double layer, which causes an increased amount of regularly aligned water molecules associated with the hydrophilic groups to give the enhanced “icelike” band. As can be seen from Figure 4, on increasing the mole fraction of DSPE-EO₄₅, the 3200- and 3400-cm⁻¹ bands increase their band intensity, indicating the increase in a net ordering and/or the amount of hydration shell around the PEO head groups. From Figure 4, it is also noted that the intensity increase of the 3200-cm⁻¹ band is much more pronounced than that of the 3400-cm⁻¹ band. Then, the amount of the “icelike” water is much larger than that of the “liquidlike” water in the hydration shell. According to Tyrode et al.,²⁹ who reported the SFG spectra of the monolayers of tetra(ethylene oxide) and octa(ethylene oxide) *n*-dodecyl ethers (C₁₂E₄ and C₁₂E₈) at the air–water interface, the ssp polarized spectra of the monolayers give the 3250- (due to the “icelike” structure) and 3400-cm⁻¹ bands (due to the “liquidlike” structure), where the intensities of the latter band are similar to (in the case of C₁₂E₄) or larger than (in the case of C₁₂E₈) those of the former band. In contrast to the neutral alkyl poly(ethylene oxide) ethers, DSPE-EO₄₅ has the negative

charge on the phosphatidyl group (see Figure 1B). So, in addition to the PEO head group the negative charge also contributes to induce an ordered array of water molecules, resulting in the more pronounced feature associated with the “icelike” structure.

In order to estimate the line amplitudes of the SFG bands due to the “icelike” and “liquidlike” structures in Figure 4, the spectra were simulated by using the following equation^{33–35}

$$I_{\text{SFG}} \propto (S/\sigma)^2 |\chi^{(2)}|^2 = (S/\sigma)^2 |\chi_{\text{NR}}^{(2)} + \sum_q \frac{A_q e^{i\theta_q}}{\omega_{\text{IR}} - \omega_q + i\Gamma_q}|^2 \quad (1)$$

, where S is an overlapping area of the laser beams, σ a mean molecular area measured by the π -A isotherms, and the term (S/σ) corresponds to the number of molecules at the air–water interface monitored by the SFG spectroscopy. $\chi^{(2)}$ is an average molecular surface nonlinear susceptibility, and $\chi_{\text{NR}}^{(2)}$ is its nonresonant contribution. A_q in eq 1 is the mean molecular line amplitude of the q th vibrational mode with the phase angle, θ_q , the resonant frequency, ω_q , and the damping constant, Γ_q . The mean molecular line amplitude represents an average contribution per one molecule of DSPE or DSPE-EO₄₅ in the mixed monolayer to the observed SFG band intensity for a certain surface pressure and mole fraction, and its relative value was calculated by multiplying the line amplitude determined from the SFG spectra (vide infra) by the corresponding mean molecular surface area, σ , under the assumption that the area of the cross section, S , is constant. Vibrational modes of nearby or overlapping bands can constructively and/or destructively interfere with each other through eq 1 because of the coherent nature of SFG spectroscopy. The parameters in eq 1 were determined to get the best fits between the calculated and observed SFG spectra in Figure 4 by using a nonlinear regression procedure (built-in algorithms in the software package IGOR (version 4.0.5.1) were used for the calculation) under the assumption that (i) $\chi_{\text{NR}}^{(2)}$ is negligibly small and (ii) the difference in the phase angles (θ_q) between the 3200- and 3400-cm⁻¹ bands is zero. During the regression procedure it was

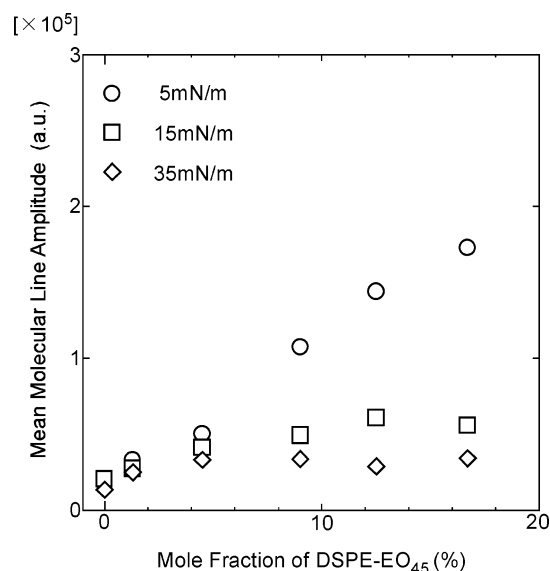


Figure 5. Mean molecular amplitude of the SFG band at 3200 cm^{-1} as a function of the mole fractions of DSPE-EO₄₅ measured for the mixed monolayers at 5, 15, and 35 mN/m. See text.

noticed that the following additional assumptions are necessary to get the overall fits between the calculated and observed spectra, i.e., (iii) the actual frequency corresponding to the 3400 cm^{-1} is fixed at 3350 cm^{-1} , (iv) the frequency corresponding to the 3200-cm^{-1} band are varied in the region of $3224\text{--}3175\text{ cm}^{-1}$, and (v) the damping constants (Γ_q) of the 3400- and 3200-cm^{-1} bands are fixed at 91 and 118 cm^{-1} , respectively. The solid curves in Figure 4 exhibit the simulated spectra and the dashed curves the contribution of the component bands. The mean molecular line amplitude of the 3200-cm^{-1} band are plotted against the mole fractions of DSPE-EO₄₅ for each surface pressure in Figure 5.

The increase in the mean molecular amplitude of the 3200-cm^{-1} band compared to the amplitude of DSPE itself for each surface pressure is a measure to estimate the amount of the “icelike” water molecules associated with the hydrophilic groups consisting of the PEO chain and the negatively charged phosphate group. On incorporation of DSPE-EO₄₅ to the DSPE monolayer up to the mole fraction to 4.5% , the line amplitude shows a slight increase irrespective of the surface pressures. The change in the line amplitude observed on further increase in the mole fraction strongly depends on the surface pressures. At 5 mN/m the amplitude increases almost proportionately to the mole fraction, while the increases are much suppressed at 15 and 35 mN/m , and the amplitudes almost saturate and keep the values observed for the mole fraction of 4.5% . As explained in the previous section, the π -A isotherms in Figure 3 indicate that the mixed monolayers are in the so-called pancake state at 5 mN/m . The proportionate increase of the amplitude of the 3200-cm^{-1} band corroborates the model proposed to the pancake state, where the PEO head groups adsorb at the air–water interface, as shown in Figure 2A. It is reasonable to consider that the hydrophilic PEO chain at the air–water interface induces the regularly oriented and strongly hydrogen-bonded network of water molecules. In addition, the negative charge of DSPE-EO₄₅ forms an electric double layer under the mixed monolayers, contributing to the formation of an ordered array of water molecules. These two factors cause the proportionate increase in the amplitude of the 3200-cm^{-1} band with the mole fraction of DSPE-EO₄₅. The π -A isotherms indicate that the mixed monolayers are in the so-called mushroom state at 15 mN/m . The pancake–mushroom transition accompanies the dissolution

of the PEO chains into the water subphase, as shown in parts A and B of Figure 2. The dissolution reduces the actual number of the regularly oriented water molecules associated with the PEO head group and/or the number of the oriented water molecules probed by the laser light. In addition, the submerged PEO head groups may block the formation of the electric double layer. These two factors explain the appreciable decrease in the amplitude of the 3200-cm^{-1} band. The saturation may be explained as the result of the segregation of DSPE and DSPE-EO₄₅ in the mixed monolayers. According to Baekmark et al.,¹⁰ who performed the fluorescence micrograph measurements of a mixed monolayer of DMPE (1- α -dimyristol phosphatidylethanolamine)(89%), DSPE-EO₄₅ (10%), and a fluorescence-labeled DSPE (1%), the mixed monolayers in the mushroom and brush regimes consist of condensed DMPE domains with bright peripheral regions attributed to the labeled DMPE and DSPE-EO₄₅ expelled from the DSPE domains. The result suggests that the DSPE-EO₄₅/DSPE mixed monolayers at 15 and 35 mN/m mainly consist of the segregated domains of DSPE-EO₄₅ and DSPE. Presumably, the “icelike” as well as “liquidlike” water molecules are excluded from the hydrophilic groups of DSPE-EO₄₅ in the segregated state, resulting in the saturation behavior of the mean molecular line amplitude. The slight increase in the line amplitude indicates that a small portion of DSPE-EO₄₅ (less than 4.5%) in the mixed monolayers are still in a state similar to that of the pancake state. In the brush state at 35 mN/m the PEO chains interact strongly with each other (Figure 2C), squeezing out the “icelike” water molecules in the mushroom state, explaining the further decrease in the mean molecular line amplitude accompanied by the mushroom to brush transition.

Thus, the analyses of the SFG spectra of DSPE and the DSPE-EO₄₅/DSPE monolayers at the air–water interface provide direct evidence at the molecular level for the models proposed to the pancake–mushroom and mushroom–brush transitions.

SFG Spectra in the CH₃ and CH₂ Stretching Vibration Region of the DSPE-EO₄₅/DSPE Mixed Monolayers at the Air–Water Interface. Figure 6 exhibits the surface pressure dependence of the SFG spectra in the $3000\text{--}2800\text{-cm}^{-1}$ region observed for DSPE and DSPE-EO₄₅/DSPE mixed monolayers spread on pure water. Although the band intensities in the SFG spectra of the pure DSPE-EO₄₅ monolayer (the top spectra) are very weak because of the larger molecular area compared to those of the mixed monolayers, they exhibit broad bands at 2950 and 2870 cm^{-1} , which are due to an overlap of CH₃ asymmetric stretching ($\nu_{\text{as}}(\text{CH}_3)$) and CH₃ Fermi resonance ($\nu_{\text{F}}(\text{CH}_3)$) bands and a CH₃ symmetric stretching ($\nu_{\text{s}}(\text{CH}_3)$) band, respectively,^{19–23} associated with the alkyl group of the DSPE moiety. It is noteworthy that, as can be seen in the inserted spectra, the spectrum measured at 15 mN/m gives a band at 2850 cm^{-1} ascribable to a CH₂ symmetric stretching mode of the alkyl group ($\nu_{\text{s}}(\text{CH}_2)$), which is absent in the spectrum measured at 35 mN/m . The appearance of the $\nu_{\text{s}}(\text{CH}_2)$ band reveals the existence of gauche isomers in the alkyl chains of DSPE-EO₄₅ in the monolayer at 15 mN/m .^{19,24} According to the π -A isotherm, measurements on the DSPE-EO₄₅ monolayer at the air–water interface,^{9,10} the monolayer is in the mushroom state at 15 mN/m and in the brush state at 35 mN/m . Then, the SFG measurement indicates that, the mushroom–brush transition accompanies the conversion of the alkyl chain from an irregular structure containing the gauche isomers to the all-trans structure. Baekmark et al.,³⁶ who performed infrared spectral measurements on a pure DSPE-EO₄₅ monolayer at the air–water interface as a function of surface pressures, also reported that

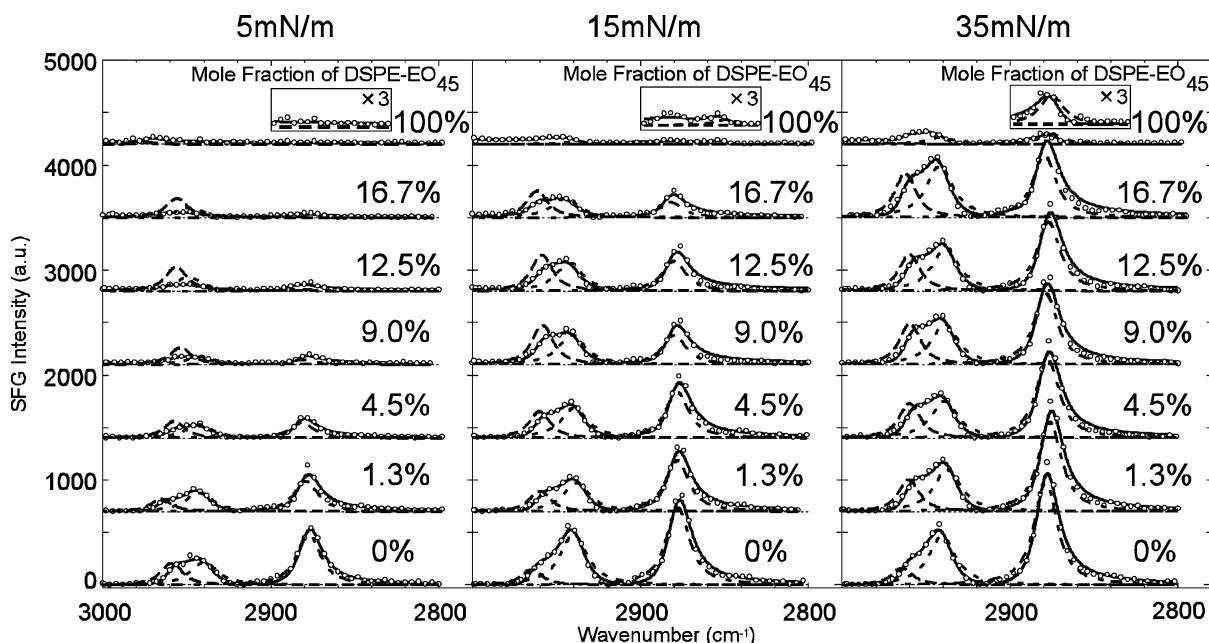


Figure 6. SFG spectra in the CH stretching region measured under the ssp polarization for the mixed DSPE-EO₄₅/DSPE monolayers with various mole fraction of DSPE-EO₄₅ at surface pressures of 5, 15, and 35 mN/m (21 °C). The spectra, except for the bottom ones, are offset by certain values to exhibit the spectra clearly. The dashed curves indicate the contributions of the component bands calculated by using estimated parameters and the solid lines the simulated spectra. See text.

the mushroom–brush transition accompanies the conversion from gauche to trans isomers in the alkyl chains of the DSPE moiety. As Figure 6 shows, the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface give SFG bands with appreciable intensities at ca. 2960 (shoulder), 2940, and 2880 cm^{−1}, which are ascribable to the $\nu_{\text{as}}(\text{CH}_3)$, $\nu_{\text{F}}(\text{CH}_3)$, and $\nu_{\text{s}}(\text{CH}_3)$ bands, respectively. The intensities of these bands, which are ascribable mainly to the terminal methyl groups of DSPE in the mixed monolayers because of the weakness of the corresponding bands in the spectra of the DSPE-EO₄₅ monolayer, increase in the order of the pancake (5 mN/m), mushroom (15 mN/m), and brush (35 mN/m) regimes; this is mainly due to the successive increase in the surface density of the DSPE molecules. In contrast to the case of the DSPE-EO₄₅ monolayer, the SFG band near 2850 cm^{−1} ascribable to the $\nu_{\text{s}}(\text{CH}_2)$ band is absent in the spectra of the mixed monolayers irrespective of the surface pressures and the mole fraction of DSPE-EO₄₅. Then, the alkyl groups of the DSPE molecules in the monolayers take the all-trans conformation. On the basis of the IR spectral measurement on the DSPE-EO₄₅ monolayer, Baekmark et al.³⁶ concluded that the mushroom–brush transition cannot be interpreted in terms of the conformational transition of the PEO head groups, but it should be explained by a process of the alkyl chain condensation coupled with the strong reduction in the number of gauche isomers in the alkyl chain moiety. The SFG measurements indicated that the mushroom–brush transition of the mixed monolayers does not accompany the conversion from an irregular structure containing gauche isomers to all trans structure in the alkyl groups of the DSPE molecules.

The line amplitude of each component band was estimated by the curve resolution procedure based on eq 1. In the procedure the phase angles (θ_q in eq 1) of the component bands were assumed to be 0 except for the phase angle of the $\nu_{\text{as}}(\text{CH}_3)$ component, which was taken to be π , as has been explained based on a symmetry consideration.²² This is necessary to reproduce the broad features centered near 2950 cm^{−1} or the absence of a dip between the $\nu_{\text{as}}(\text{CH}_3)$ and $\nu_{\text{F}}(\text{CH}_3)$ bands (Figure 6). The dashed curves in Figure 6 exhibit the contribu-

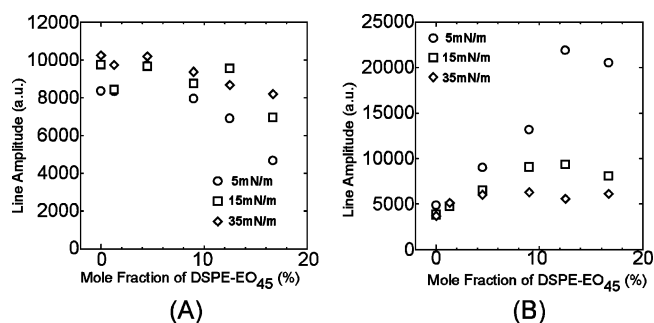


Figure 7. SFG intensities of the $\nu_{\text{s}}(\text{CH}_3)$ band at 2880 cm^{−1} (A) and the $\nu_{\text{as}}(\text{CH}_3)$ band at 2960 cm^{−1} (B) as a function of the mole fractions of DSPE-EO₄₅ measured for the mixed monolayers at 5, 15, and 35 mN/m. See text.

tion of the component bands and the solid curves the simulated spectra. Parts A and B of Figure 7 plot the line amplitude of the $\nu_{\text{s}}(\text{CH}_3)$ and $\nu_{\text{as}}(\text{CH}_3)$ bands, respectively, as a function of the mole fraction of DSPE-EO₄₅. As Figure 7A shows, the line amplitudes of the $\nu_{\text{s}}(\text{CH}_3)$ band measured at 5 mN/m decrease precipitously with increasing mole fraction, contrasting the cases of the amplitudes measured at 15 and 35 mN/m. The result suggests that the terminal methyl groups of DSPE tilt away from the surface normal with increasing mole fraction at 5 mN/m. The average tilt angles of the terminal methyl groups were estimated as a function of surface pressures and the mole fractions of DSPE-EO₄₅ by the procedure proposed by Hirose et al.,^{37–41} in which the tilt angle with respect to the surface normal is calculated from the ratio of the SFG intensity of the $\nu_{\text{as}}(\text{CH}_3)$ band to that of the $\nu_{\text{s}}(\text{CH}_3)$ band determined for each specific experimental condition (surface pressure and mole fraction). In the calculation the refractive indices of air, water, and the lipid monolayers were assumed to be 1.00, 1.33, and 1.50, respectively, and as for the molecular hyperpolarizability tensors, $\beta_{\alpha\beta\gamma}$, of the $\nu_{\text{s}}(\text{CH}_3)$ modes the following ratios were assumed:³⁷ $\beta_{\text{aac}}/\beta_{\text{ccc}} = 4.00$ and $\beta_{\text{cac}}/\beta_{\text{aac}} = 4.21$, where a and c denote the axes perpendicular and parallel, respectively, to

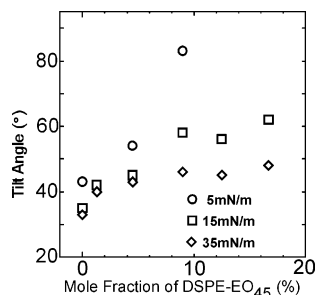


Figure 8. Tilt angles of the CH₃ group as a function of the mole fractions of DSPE-EO₄₅ in the mixed monolayers at 5, 15, and 35 mN/m. See text.

the C_3 symmetry axis of the CH₃ group and the ac plane is taken to include one of the CH bond of the CH₃ group. The results of the calculation are summarized in Figure 8. (In the figure the tilt angles for the mixed monolayers with the mole fraction larger than 10% at 5 mN/m are not given, since the $\nu_s(\text{CH}_3)$ and $\nu_{as}(\text{CH}_3)$ bands of the monolayers are too weak, as can be seen from Figure 6, to determine the intensity ratios.)

Comparison of the tilt angles at a certain mole fraction of DSPE-EO₄₅ indicates that, upon increasing the surface pressure, the tilt angle decreases appreciably, as observed for the dipalmitoyl phosphatidylglycerol (DPPG) and dipalmitoyl phosphatidylcholine (DPPC) monolayers at the air–water interface.³⁰ As the surface pressure increases, the alkyl groups of the phospholipids are converted to more and more closely packed state, resulting in the decrease in the tilt angle of the terminal methyl group. As can be seen from Figure 8, the tilt angles of the DSPE monolayer are estimated to be ca. 43° at 5 mN/m, ca. 35° at 15 mN/m, and ca. 33° at 35 mN/m. Upon increasing the mole fraction of DSPE-EO₄₅ up to 1.3%, the tilt angles slightly increases, irrespective of the surface pressures. In the range of the mole fraction larger than or equal to 4.5% the tilt angle increase strongly depends on the surface pressure. At 5 mN/m the tilt angle increases appreciably, approaching nearly 90° at mole fractions larger than 10%. Thus, the incorporation of DSPE-EO₄₅ in the pancake state results in a large orientation change of the alkyl groups, which may cause a more or less random distribution in the azimuth of the alkyl groups in the mixed monolayers. On the other hand, the tilt angle increase is much suppressed at 35 mN/m, characterizing the brush state of the mixed monolayers. In this regime it has been considered that the hydrophilic PEO groups are totally submerged into the water subphase and interacting with each other to form a more or less tight binding state of the alkyl groups of DSPE and DSPE-EO₄₅. Then, it is reasonable that the effect of the PEO groups on the orientation of the alkyl groups in the brush state is much suppressed compared to that in the pancakes state. The appreciable increase of the tilt angle with increasing the mole fraction of DSPE-EO₄₅ observed at 15 mN/m suggests that the PEO chains give some effects on the orientation of the alkyl groups of DSPE in the mushroom state.

According to Majewski et al.,⁹ who measured the density distribution of the mixed DSPE-EO₄₅/DSPE monolayers at 40–45 mN/m (the brush regime) using neutron reflectometry, reported that there is a decrease in the density and thickness of the alkyl chains with increasing the mole fraction of DSPE-EO₄₅. The above-mentioned SFG experimental results suggest that the decrease in the thickness can be explained by the tilting of alkyl chains induced by the increase in the mole fraction at 35 mN/m, although the tilting is much reduced compared to those in the pancake (at 5 mN/m) and mushroom (at 15 mN/m) states.

Conclusion

The compression isotherms measured for the DSPE-EO₄₅/DSPE mixed monolayers with the mole fraction of DSPE-EO₄₅ in the range of 1.3–16.7% indicated that the mixed monolayers at 5 mN/m are mainly in the so-called pancake state, the monolayers at 15 mN/m in the mushroom state and the monolayers at 35 mN/m in the brush state. The SFG spectra in the OH stretching (3600–3000 cm^{−1}) and CH stretching (3000–2800 cm^{−1}) vibration regions observed for the mixed monolayers in each state gave direct information at molecular levels regarding the so-called pancake–mushroom and mushroom–brush transitions.

The proportionate increase in the mean molecular line amplitude of the 3200-cm^{−1} band due to the “icelike” water molecules with increasing mole fraction of DSPE-EO₄₅ in the pancake state presented a clear evidence for the model where the PEO chains spread at the air–water interface (Figure 2A). The hydrophilic PEO chain at the air–water interface induces the regularly oriented and strongly hydrogen-bonded network of water molecules. In addition, the negative charge of DSPE-EO₄₅ forms an electric double layer under the mixed monolayers, contributing to the formation of an ordered array of water molecules. These two factors contribute to enhance the line amplitude of the 3200-cm^{−1} band. The suppression of the proportionate increase of the “icelike” water band observed at 15 mN/m corresponds to the mechanism proposed to the pancake–mushroom transition, which presumes the dissolution of the PEO chains from the air–water interface to the water subphase (parts A and B of Figure 2). The dissolution reduces the number of water molecules forming the ordered hydrogen-bonded network associated with the PEO group and/or the number of water molecules forming the ordered hydrogen-bonded network probed by the laser light. Presumably, the PEO chains submerged into the water subphase shield the negative charge to block the formation of the electric double layer. The slight decrease of the line amplitude observed for the compression of the mixed monolayers from 15 to 35 mN/m can be explained in terms of the brush model, where the PEO chains strongly interact with each other to form a tight binding state, i.e., the formation of the state squeezes out water molecules from the hydrophilic groups of DSPE-EO₄₅ in the mixed monolayers.

The SFG spectra of the DSPE-EO₄₅/DSPE mixed monolayers in the CH stretching vibration region give rise to the bands at ca. 2960 (shoulder), 2940, and 2880 cm^{−1}, which are ascribable to the $\nu_{as}(\text{CH}_3)$, $\nu_F(\text{CH}_3)$, and $\nu_s(\text{CH}_3)$ bands, respectively. In addition to the CH₃ stretching bands, the spectrum of the DSPE-EO₄₅ monolayer measured at 15 mN/m give the SFG band near 2850 cm^{−1} ascribable to the $\nu_s(\text{CH}_2)$ band, which is absent in the spectrum of the monolayer at 35 mN/m; the result confirms that the mushroom–brush transition accompanies the conversion of the alkyl group from an irregular structure containing the gauche isomer to the all-trans structure. In contrast to the case of the DSPE-EO₄₅ monolayer, the SFG band near 2850 cm^{−1} ascribable to the $\nu_s(\text{CH}_2)$ band is absent in the spectra of the mixed monolayers, indicating that the alkyl groups of DSPE in the mixed monolayers are in the all-trans conformation irrespective of the surface pressures and the mole fractions (1.3–16.7%) of DSPE-EO₄₅.

The average tilt angles of the terminal methyl groups in the DSPE-EO₄₅/DSPE mixed monolayers at the air–water interface were estimated from the ratios of the SFG intensities of the $\nu_s(\text{CH}_3)$ and $\nu_{as}(\text{CH}_3)$ bands as a function of surface pressures and the mole fractions of DSPE-EO₄₅. The tilt angles of the

DSPE monolayer are estimated to be ca. 43° at 5 mN/m, ca. 35° at 15 mN/m, and ca. 33° at 35 mN/m. The average tilt angles increase with increasing the mole fractions and almost saturate at the mole fraction larger than 10%, giving the values near 90° at 5 mN/m, near 60° at 15 mN/m, and near 47° at 35 mN/m. The pronounced tilting of the methyl group in the pancake regime indicates that the alkyl groups of DSPE have a large orientation flexibility. The largest suppression of the tilt angle change at 35 mN/m seems to be consistent with the model proposed to the brush state, where the PEO chains are in a tightly packed state and the alkyl groups form an ordered two-dimensional array.

References and Notes

- (1) Woodle, M.; Lasic, D. *Biochim. Biophys. Acta* **1992**, *1113*, 171.
- (2) Klibanov, A. L.; Maruyama, K.; Torchilin, V. P.; Huang, L. *FEBS Lett.* **1990**, *268*, 235.
- (3) Lasic, D.; Martin, F.; Gabizon, A.; Huang, S.; Papahadjopoulos, D. *Biochim. Biophys. Acta* **1991**, *1070*, 187.
- (4) Allen, T.; Hansen, C.; Martin, F.; Redmann, C.; Yau-Young, A. *Biochim. Biophys. Acta* **1991**, *1066*, 29.
- (5) Papahadjopoulos, D.; Allen, T.; Gabizon, A.; Mayhew, E.; Matthey, K.; Huang, K.; Lee, K.; Woodle, M.; Lasic, D.; Redemann, C.; Martin, F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11460.
- (6) Kenworthy, A. K.; Hristova, K.; Needham, D.; McIntosh, T. J. *Biophys. J.* **1995**, *68*, 1921.
- (7) Uster, P. S.; Allen, T. M.; Daniel, B. E.; Mendez, C. J.; Newman, M. S.; Zhu, G. Z. *FEBS Lett.* **1996**, *386*, 243.
- (8) Kuhl, T.; Leckband, D.; Lasic, D.; Israelachvili, J. *Biophys. J.* **1994**, *66*, 1479.
- (9) Majewski, J.; Kuhl, T. L.; Gerstenberg, M. C.; Israelachvili, J. N.; Smith, G. S. *J. Phys. Chem.* **1997**, *101*, 3122.
- (10) Baekmark, T. R.; Elender, G.; Lasic, D. D.; Sackmann, E. *Langmuir* **1995**, *11*, 3975. Correction: *Langmuir* **1996**, *12*, 4980.
- (11) Rosilio, V.; Albrecht, G.; Okumura, Y.; Sunamoto, J.; Baszkin, A. *Biophys. J.* **1996**, *12*, 2544.
- (12) de Gennes, P. G. *J. Phys. France* **1976**, *37*, 983.
- (13) Alexander, S. *J. Phys. France* **1977**, *38*, 983.
- (14) Milner, S.; Witten, T.; Cates, M. *Macromolecules* **1988**, *21*, 2610.
- (15) Zhu, X. D.; Shur, H.; Shen, Y. R. *Phys. Rev. B* **1987**, *35*, 3047.
- (16) Guyot-Sionnest, P.; Hunt, J. H.; Shen, Y. R. *Phys. Rev. Lett.* **1987**, *59*, 1597.
- (17) Shen, Y. R. *Nature* **1989**, *337*, 519.
- (18) Hunt, J. H.; Guyot-Sionnest, P.; Shen, Y. R. *Chem. Phys. Lett.* **1987**, *133*, 189.
- (19) Bain, C. D. *J. Chem. Soc. Faraday Trans.* **1995**, *91*, 1281.
- (20) Richmond, G. L. *Chem. Rev.* **2002**, *102*, 2693.
- (21) Kim, G.; Gurau, M.; Kim, J.; Cremer, P. S. *Langmuir* **2002**, *18*, 2807.
- (22) Watry, M. R.; Tarbuck, T. L.; Richmond, G. L. *J. Phys. Chem. B* **2003**, *107*, 512.
- (23) Kim, G.; Gurau, M. C.; Lim, S. M.; Cremer, P. S. *J. Phys. Chem. B* **2003**, *107*, 1403.
- (24) Goates, S. R.; Schofield, D. A.; Bain, C. D. *Langmuir* **1999**, *15*, 1400.
- (25) Dreesen, L.; Humbert, C.; Hollander, P.; Mani, A. A.; Ataka, K.; Thiry, P. A.; Peremann, A. *Chem. Phys. Lett.* **2001**, *333*, 327.
- (26) Chen, C.; Even, M. A.; Wang, J.; Chen, Z. *Macromolecules* **2002**, *35*, 9130.
- (27) Casford, M. T. L.; Davies, P. B. *Langmuir* **2003**, *19*, 7386.
- (28) Kim, J.; Opdahl, A.; Chou, K. C.; Somorjai, G. A. *Langmuir* **2003**, *19*, 9551.
- (29) Tyrode, E.; Johnson, C. M.; Kumpulainen, A.; Rutland, M. W.; Claesson, P. M. *J. Am. Chem. Soc.* **2005**, *127*, 16848.
- (30) Ohe, C.; Ida, Y.; Matsumoto, S.; Sasaki, T.; Goto, Y.; Noi, M.; Tsurumaru, T.; Itoh, K. *J. Phys. Chem. B* **2004**, *108*, 18081.
- (31) Superfine, Q.; Du, R.; Freysz, E.; Shen, Y. R. *Phys. Rev. Lett.* **1993**, *70*, 2313.
- (32) Carey, D. M.; Korenowski, G. M. *J. Chem. Phys.* **1998**, *108*, 2669.
- (33) Kim, G.; Gurau, M. C.; Lim, S. M.; Cremer, P. S. *J. Phys. Chem. B* **2003**, *107*, 1403.
- (34) Walker, R. A.; Gruetzmacher, J. A.; Richmond, G. L. *J. Am. Chem. Soc.* **1998**, *120*, 6991.
- (35) Smiley, B. L.; Richmond, G. L. *J. Phys. Chem. B* **1999**, *103*, 653.
- (36) Baekmark, T. R.; Wiesenthal, T.; Kuhn, P.; Albersdörfer, A.; Nuyken, O.; Merkel, R. *Langmuir* **1999**, *15*, 3616.
- (37) Watanabe, N.; Yamamoto, H.; Wada, A.; Domen, K.; Hirose, C.; Ohtake, T.; Mino, N. *Spectrochim. Acta* **1994**, *50A*, 1529.
- (38) Hirose, C.; Akamatsu, N.; Domen, K. *J. Chem. Phys.* **1992**, *96*, 997.
- (39) Hirose, C.; Akamatsu, N.; Domen, K. *Appl. Spectrosc.* **1992**, *46*, 1051.
- (40) Hirose, C.; Yamamoto, H.; Akamatsu, N.; Domen, K. *J. Phys. Chem.* **1993**, *97*, 10064.
- (41) Akamatsu, N.; Domen, K.; Hirose, C. *J. Phys. Chem.* **1993**, *97*, 10070.
- (42) Baekmark, T. R.; Wiesenthal, T.; Kuhn, P.; Bayerl, T. M.; Nuyken, O. *Langmuir* **1997**, *13*, 5521.