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Fourier transform infrared-attenuated total reflection spectra of dipalmitoylphosphatidylcholine monomolecular films

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Single monomolecular films of 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) were transferred onto a germanium plate by the Langmuir-Blodgett technique at various surface pressures encompassing a plateau region in a surface pressure(π)-area(A) isotherm. Molecular orientation and structure of the monomolecular films were investigated by the Fourier transform infrared-attenuated total reflection spectroscopy. Appreciable changes in frequency and halfbandwidth of the antisymmetric and symmetric CH_2 stretching bands of the palmitoyl chains were found at the onset of the plateau region in the π-A isotherm. By analyzing polarized infrared-attenuated total reflection spectra, the palmitoyl chains of DPPC films were proved to be oriented vertically to the germanium surface with all-*trans* conformation, irrespective of the surface pressure on the film transfer. This finding suggests the existence of islands or surface micelles on the surface throughout the surface pressure examined. The changes in the spectral parameters mentioned above were explained by changes in the state of packing of these islands. The plateau is ascribable to a transition process from a close-packed island phase to a molecularly homogeneous phase.

Introduction

It has been known that there appears a plateau region in the surface pressure(π)-area(A) isotherms of various kinds of monolayers, such as (a) long-chain fatty acids like myristic acid [1] and 12-hydroxy stearic acid [2], and (b) synthetic polypeptides like poly-γ-benzyl-L-glutamate [3], poly-γ-methyl-L-glutamate [4] and poly-ε-benzylcarbonyl-L-lysine [5]. The plateau observed for 12-hydroxy stearic acid has been interpreted as due to the change in molecular conformation on water [2]. On the other hand, the plateau observed for polypeptides has been found to be characteristic of the α-helix form and ascribed to a transition process from monolayer to bilayer [3–5].

A plateau region has also been found for phospholipid monolayers such as 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) in the process of the liquid-expanded to liquid-condensed phase transition [6–10]. A number of theoretical works including statistical mechanical [11–14], Monte Carlo [15,16] and molecular dynamic calculations [17,18] have been performed to explain this transition (see Ref. 19 for a thorough review of early theoretical works). One of the prevailing interpretation is that the liquid-expanded/liquid-condensed transition is due to a first-order liquid crystal-gel transition which accompanies the increase in the number of *trans* conformers in the acyl chains [10,12,14,16,17]. Another is that it is due to a second-order transition [11,13] from an orientationally disordered phase to a state with long-range orientational order, the main change being not conformational but orientational. Both of these interpretations state that the plateau region originates from the dis-

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order-order transition. However, Von Tscharner and McConnell [20] have recently suggested the possibility that the transition from fluid to solid monolayers occurs in the liquid-condensed region from the result of a fluorescent-probe experiment [20]. It has been also argued that the rigidity of thermodynamic discussions in the system of 'non-equilibrium' spread films is doubtful; only the equilibrium spreading pressure provides the information for a complete thermodynamic analysis of the transition [21]. As stated above, the molecular mechanism so far proposed for the liquid-expanded/liquid-condensed transition is highly controversial.

To obtain more detailed information on the structural aspect of this liquid-expanded/liquid-condensed transition in a plateau region, Fourier transform infrared-attenuated total reflection spectroscopy was applied for single DPPC monomolecular films transferred to a germanium plate at various surface pressures covering the plateau region. The molecular orientation of DPPC films was investigated by polarized infrared-attenuated total reflection spectra. Furthermore, the change in spectral parameters (frequency, bandwidth and intensity) of the CH_2 stretching bands with surface pressure was also discussed in relevance to the state of molecular packing in DPPC monolayers on the water surface.

Materials and Methods

DPPC was obtained from Sigma and used without further purification. Substrate water was purified by a Mitamura Riken model PLS-DFR automatic lab-still consisting of a reverse osmosis module, an ion-exchange column, and a double distiller. Spreading solutions of 1 $mg \cdot ml^{-1}$ were prepared by dissolving DPPC in chloroform (specially prepared reagent, UVS-26) purchased from Nakarai Chemicals.

Surface pressure measurements were carried out at 20.0 ± 0.1°C. Spread monolayers were compressed continuously with a Teflon-coated brass barrier, and the surface pressure was measured by the Wilhelmy method using a Shimazu model ST-1 surface tensometer equipped with a ground-glass plate. Monolayers on the water surface were transferred onto a germanium attenuated total reflection plate (Fig. 1) by the Langmuir-Blodgett technique [22] at various surface pressures below, around and above the plateau region in the π-A isotherm. The plate was immersed in the water substrate immediately after cleaning, and then the monolayer was spread on the water surface. The germanium plate was withdrawn at 10 $mm \cdot min^{-1}$ with its large faces (the xy plane in Fig. 1) vertical and parallel to the direction of compression, the direction of withdrawal being parallel to the y axis. The transfer ratios were found to be 0.97 ± 0.05, irrespective of the surface pressure.

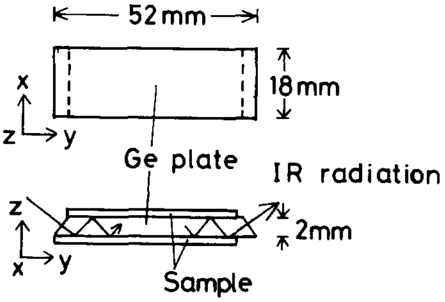

Fig. 1. The germanium attenuated total reflection plate and its coordinate system. Ge, germanium; IR, infrared.

Polarized infrared-attenuated total reflection spectra were recorded on a Nicolet model 6000 Fourier transform infrared spectrophotometer equipped with an InSb detector and a wire grid polarizer. A Perkin-Elmer multiple attenuated total reflection attachment was used. The angle of incidence was 45° and the number of internal reflections determined by the length of deposited films along the y-axis of the attenuated total reflection plate was fixed to 23. Five thousand interferograms, collected with an optical velocity of 1.2 $cm \cdot s^{-1}$ and a maximum optical retardation of 0.25 cm, were coadded, apodized with the Happ-Genzel function, and Fourier-transformed with one-level of zero-filling to yield a resolution of 4 cm^{-1}. The frequency reading was accurate to within ± 0.5 cm^{-1}.

Results

Surface pressure dependence of infrared attenuated total reflection spectra

The π-A isotherm of a DPPC monolayer on the water surface at 20°C is given in Fig. 2, in the

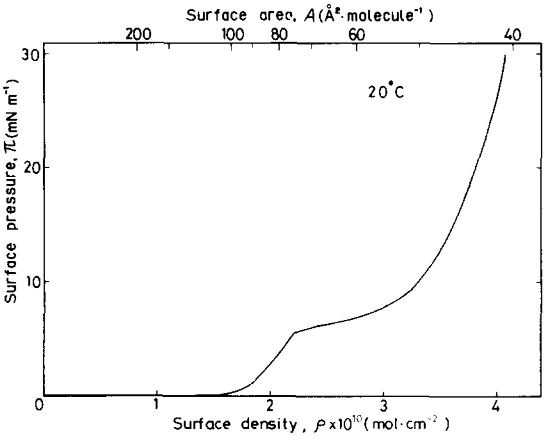

Fig. 2. The surface pressure(π)-surface density(ρ) isotherm of a DPPC monolayer on the water surface at 20°C.

form of π-surface density(ρ) plot, being in good agreement with those previously reported [6–8]. As mentioned above, the first and second steep rises in the isotherm correspond to the liquid-expanded and liquid-condensed phases, respectively.

Fig. 3 represents infrared-attenuated total reflection spectra in the CH_2 stretching region of DPPC monomolecular films prepared at various surface pressures. The bands at 2920 and 2850 cm^{-1} are assigned to the antisymmetric and symmetric CH_2 stretching vibrations of the palmitoyl chains, respectively [23–25].

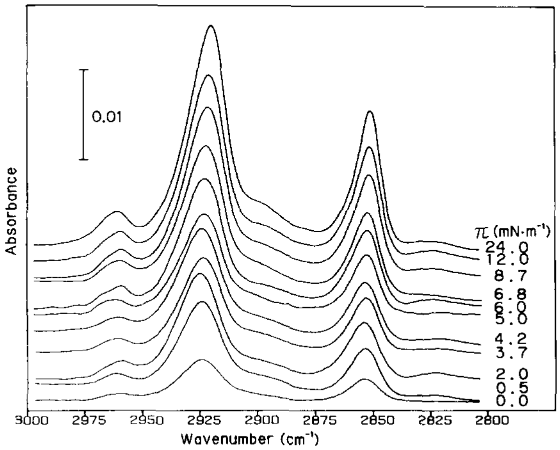

Fig. 3. Surface-pressure dependence of infrared attenuated total reflection spectra of DPPC monomolecular films on a germanium plate. The bottom-most spectrum is for $A = 190$ $Å^2 \cdot molecule^{-1}$.

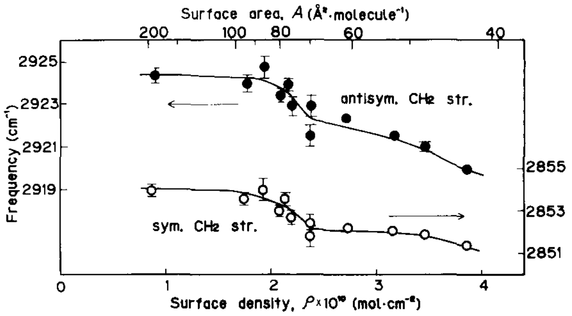

Fig. 4. Surface-pressure dependence of the antisymmetric (●) and symmetric (○) CH_2 stretching frequencies of a DPPC monomolecular film on a germanium plate.

The surface density(ρ) dependences of frequency and halfbandwidth of the antisymmetric and symmetric CH_2 stretching bands were obtained and given in Figs. 4 and 5, respectively. Both bands show the trend to shift to lower frequency sides with increasing surface density (Fig. 4), indicating apparent inflections at the surface density of approx. $2.2 \cdot 10^{-10}$ $mol \cdot cm^{-2}$, which is just the onset of the plateau region in Fig. 2. The values of halfbandwidth are almost constant in the smaller density region but start to decrease at the above surface density value (Fig. 5). These results suggest some structural change in DPPC monomolecular films at the onset of the plateau region in the π-ρ isotherm on compression. The surface-density dependence of integrated intensity of both the CH_2 stretching bands is shown in Fig. 6. The intensity

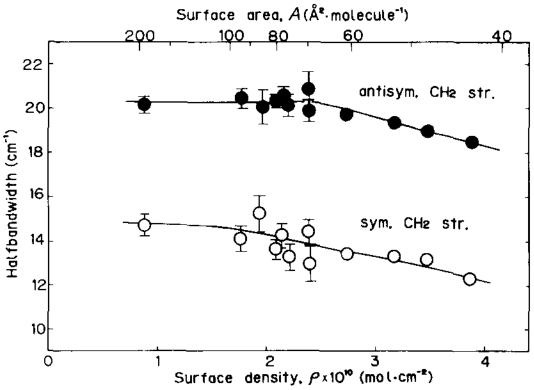

Fig. 5. Surface-pressure dependence of the antisymmetric (●) and symmetric (○) CH_2 stretching halfbandwidths of a DPPC monomolecular film on a germanium plate.

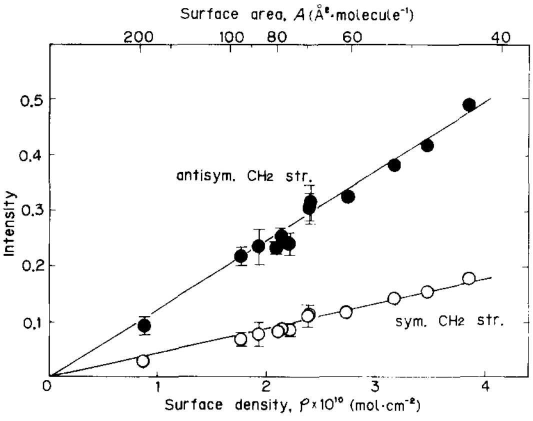

Fig. 6. Surface-pressure dependence of the antisymmetric (●) and symmetric (○) CH_2 stretching intensities of a DPPC monomolecular film on a germanium.

increases almost linearly with the surface density as expected, and any appreciable change in the increment cannot be seen at the surface density of approx. $2.2 \cdot 10^{-10}$ mol · cm^{-2}. Thus, the above-mentioned structural change is thought to be the one which does not accompany the intensity change of the CH_2 stretching bands.

Molecular orientation in the films

In order to investigate the surface-pressure dependence on the molecular orientation of DPPC monomolecular films, polarized infrared-attenuated total reflection spectra were recorded for the films prepared at various surface pressures. Fig. 7 shows representative spectra of the film transferred at the surface pressure of 24.0 mN · m^{-1}. The dichroism is very small in both the antisymmetric and symmetric CH_2 stretching bands. The features of the dichroism were almost the same for the films prepared at all surface pressures examined.

The dichroic ratio defined by

$$r = \Delta A_{\parallel} / \Delta A_{\perp} \qquad (1)$$

was calculated and plotted against the surface density, ρ, in Fig. 8 (bottom). Here ΔA is the change in reflection absorbance defined by

$$\Delta A = \log(R_0/R) \qquad (2)$$

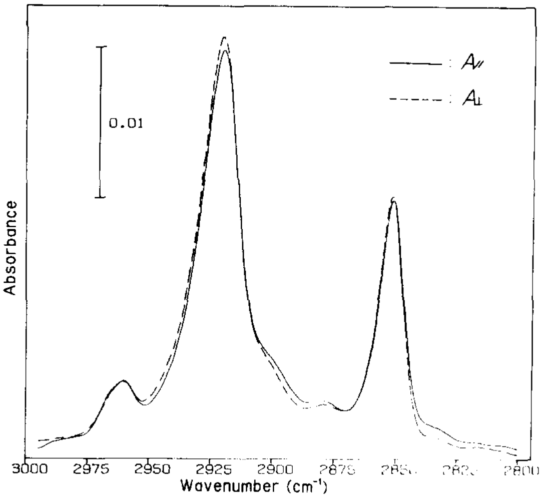

Fig. 7. Polarized infrared-attenuated total reflection spectra of a DPPC monomolecular film on a germanium plate transferred at 24.0 mN·m^{-1}. $A_{\parallel}$ and $A_{\perp}$ refer to the direction of electric vector parallel and perpendicular to the plane of incidence, respectively.

where R and R_0 are the reflectivities in the presence and the absence of a thin film, respectively [26].

Hansen [26,27] has derived general approximate equations for reflectivity changes due to the presence of a very thin film. In previous works [3–5,28], we have applied the Hansen's equation to the

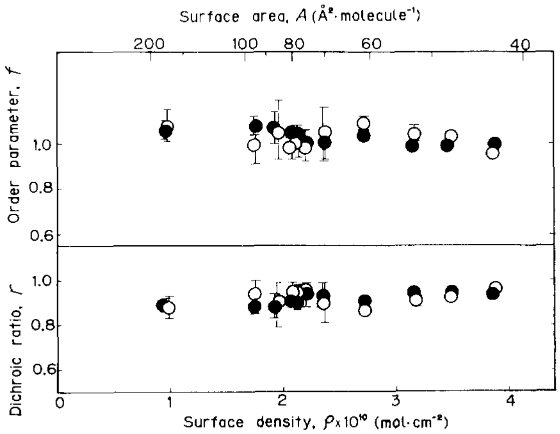

Fig. 8. The dichroic ratio (r) and the order parameter (f) of DPPC monomolecular films transferred to a germanium plate. Symbols ● and ○ refer to the antisymmetric and symmetric CH_2 stretching modes, respectively.

attenuated total reflection from a three-phase plane-bounded system with germanium (phase 1), an anisotropic monomolecular film (phase 2) and air (phase 3). According to them, the expressions for the perpendicular and parallel reflectivity changes are given by

$$\Delta A_{\perp} = 0.236\alpha_x hN \tag{3}$$

$$\Delta A_{\parallel} = (0.220\alpha_y + 0.252\alpha_z)hN \tag{4}$$

Here, α_x, α_y, and α_z are the absorption coefficients of the thin film along the x, y, and z axes, respectively, N the number of total reflections (23 in this experiment), and h the film thickness.

In order to relate the values of α_x, α_y, and α_z to parameters of orientation, we assume the following model. First, the two palmitoyl chains of a DPPC molecule behave independently, and the chain axes make uniaxial orientation with respect to the surface normal (z-axis). Second, the transition moments of the antisymmetric and symmetric CH_2 stretching modes are uniformly distributed with the angle of 90° around each chain axis. Then, the order parameter F of the transition moment of the CH stretching modes with respect to the z-axis can be expressed as follows [29]

$$F = f\cdot\frac{3\cos^2 90° - 1}{2} = \frac{\alpha_z - \alpha_x}{\alpha_z + 2\alpha_x} \tag{5}$$

Here, $\alpha_x = \alpha_y$ was used because of the assumption of the uniaxial orientation around the z-axis, and f is the order parameter of the palmitoyl chain with respect to the z-axis, varying from 1 to $-1/2$ for complete parallel and perpendicular orientation, respectively. For random orientation, $f = 0$. From Eqns. 1, 3, 4 and 5, f is given by

$$f = -2\frac{r - 2.00}{r + 1.20} \tag{6}$$

Therefore, if $r = 0.93$ we have $f = 1.0$, and if $r = 2.0$ we have $f = 0$. In other words, if the palmitoyl chains are perfectly aligned along the z-axis, almost no dichroism will be observed, and if the chains are randomly oriented, $\Delta A_{\parallel}$ is twice as large as $\Delta A_{\perp}$. This is an accidental result caused by the numerical values of the coefficients in Eqns. 3 and 4 and just opposite to the usual sense which we have between the molecular orientation and the dichroism in transmission spectra. The appropriateness of Eqn. 6 was ensured from the polarized attenuated total reflection spectra of isotropic chloroform.

The f values of DPPC monomolecular films are shown in Fig. 8 (top) against the surface density of the films. For both the antisymmetric and the symmetric CH_2 stretching bands, the f values were found to be almost unity at all the surface densities examined, indicating that all of the palmitoyl chains of the DPPC molecules are oriented perpendicularly to the germanium plate (the xy plane), irrespective of the surface pressure of the film preparation. This should be noticed because it is generally presumed that the molecular orientation is remarkably deteriorated at lower surface pressures [15,30].

Discussion

It has been reported that the CH_2 stretching vibrations of DPPC multibilayers exhibit a lower frequency shift following the liquid crystal-gel transition [23,24,31]. The origin of the frequency shift has been ascribed to the increase in the proportion of the *trans* conformation. In the present work, the frequency shift was also observed with increasing surface density. However, it is inadequate to consider that the shift is due to the *gauche-trans* transformation of the palmitoyl chains of DPPC, because the f values are almost unity irrespective of the surface pressure, suggesting that the hydrocarbon chains are oriented perpendicularly to the germanium plate with all-*trans* conformation. This is also supported by the linear dependence of the integrated intensities of the CH_2 stretching bands on surface density shown in Fig. 6. If the *gauche-trans* transformation occurs at approx. $2.2 \cdot 10^{-10}$ mol · cm^{-2}, increment of the integrated intensity must be changed remarkably on that point [24].

On the other hand, the CH stretching vibrations of *n*-octane have been subject to the solvent-induced frequency shift, the amount of which depends mainly on the refractive index of the solvent [32]. Furthermore, remarkable frequency shifts of the CH stretching bands on micelle formation in aqueous solutions of *n*-alkanoates and *n*-alkanesulfonates have been discussed in connection with the change in environments around the hydrocarbon chains [33–36]. All of these frequency shifts reflect the change in intermolecular interaction surrounding the hydrocarbon chain. Therefore, they can be a measure of the change in molecular association or molecular packing. In the present case of DPPC monomolecular films, the change in the surface density may well result in the change in the state of molecular association and intermolecular interaction of DPPC molecules. Thus, the frequency shifts observed here may be interpreted by such changes. As is easily understood from Fig. 4, the most remarkable change in the environment around the DPPC molecules occurs at the onset of the plateau region.

The band narrowing at higher surface densities shown in Fig. 5 may be ascribed to the decreased rotational mobility of the palmitoyl chains. In fact, the narrowing occurs in parallel with the surface viscosity increase reported by Yamanaka [37]. It is a general trend in amphiphilic compounds that the CH_2 stretching bands of the alkyl chain narrow on going from sparse to dense medium [24,25,33,35].

It was concluded in the previous section that the palmitoyl chains of the DPPC molecules are vertically oriented on the germanium plate ($f = 1$) even at low surface densities, that is, low surface pressures on the film preparation. In the low surface density region, however, vertically oriented, isolated molecules are thought to be unstable. Thus, it would be appropriate to assume the existence of isolated small aggregates of molecules, that is, 'islands' or 'surface micelles'. If L-shaped DPPC molecules with the polar headgroups parallel to the water surface are closely packed in the island, the palmitoyl chains of the aggregated molecules would be oriented vertically to the surface [15,30]. Actually, through a electron microscopic study, Ries et al. [38] have pointed out the existence of islands of dipalmitoyl-*rac*-phosphatidylcholine in monolayers transferred at low surface pressures onto a microscopic screen. The presence of two-dimensional surface micelles at low surface densities has also been suggested by Matsumoto et al. [39] in their surface pressure measurements of surfactants. Further, the inhomogeneity of the DPPC monomolecular film in the liquid-expanded state has been reported by the microscopic observation by the use of a fluorescent lipid probe [19,20]. Once we admit the existence of the islands, we can draw features of island packing occurring with the increase in surface pressure as illustrated in Fig. 9, where the π-A isotherm is also shown:

(a) Even in the low surface density region, the small islands of DPPC are formed and each island exists independently.

(b) With increasing surface density, the separation between the islands becomes smaller.

(c) On further compression of the films, the islands draw very close to each other and finally come into contact at the onset of the plateau region in the π-A isotherm. In this process, the interaction between the islands gradually increases, surface pressure attaining a final value about 7 $mN \cdot m^{-1}$. Consequently, the appreciable changes in the frequency and halfbandwidth of the CH_2 stretching bands occur as shown in Figs. 4 and 5.

(d) In the plateau region, the islands are crushed by compression, and the gap between the closely packed islands is filled up. During this process, the work per molecule required to crush the islands may remain nearly constant.

(e) At the end of the plateau, filling up of the gap between the islands is completed and thereafter the molecules are uniformly packed to yield a homogeneous liquid-condensed phase.

Thus, the plateau region is ascribed to a transition

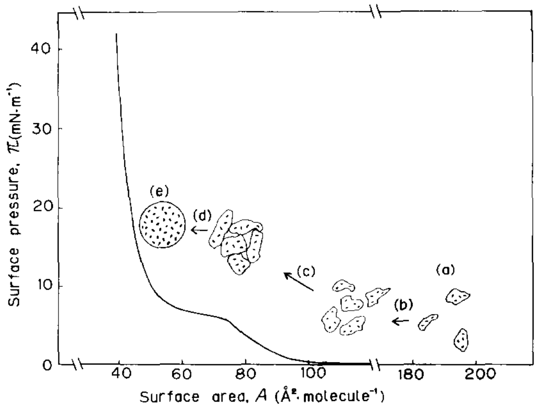

Fig. 9. Schematic representation of various states of DPPC monomolecular films on a germanium plate, and the surface pressure(π)-area(A) isotherm of DPPC on the water surface at 20°C.

process from a close-packed island phase to a homogeneous phase.

The model stated above is inconsistent with the predictions based on the molecular dynamic simulations [17] and Monte Carlo calculations [15] that the order parameters of the acyl chain at low surface densities are fairly smaller than unity. In those calculations, however, the Coulombic attractive forces between fully ionized dipolar headgroups which are parallel to the water surface [9] are completely neglected. The magnitude of this Coulombic interaction manifests itself in the 'critical micelle concentration' as low as $4.6 \cdot 10^{-10}$ M for DPPC in water [40]. The overwhelming preference of DPPC for being in a membrane environment, that is, in an aggregated state, has been pointed out by the unitary free energy of -15 kcal/mol [40].

Thus far, we have drawn pictures based on the vague assumption that the states of molecules on both water and germanium surfaces are the same. Strictly speaking, this might not be the case. It may be possible that the palmitoyl chains of the DPPC monolayer on the water surface contain *gauche* conformations to some extent at surface pressures equal or less than the plateau pressure. Further, the orientation of the headgroups in such molecules could be either parallel (L-shaped molecule) or perpendicular (I-shaped molecule) to the water surface, as deduced from the experimental values of surface dipole moment [9]. The molecule with the headgroup perpendicular to the water surface may act as a repulsive force maker which plays an important role in the liquid-expanded state [41]. In cases where either the *gauche* or the I-shaped conformers exist on the water surface, the transformation to L-shaped *trans* conformers may occur on the film transfer due to the removal of water from the monolayer.

These problems still await further clarification. However, it would be reasonable to consider the presence of the islands at sufficiently low surface pressures, as far as we consider the infrared spectroscopic observations reported here.

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References

1 Langmuir, I. (1933) J. Chem. Phys. 1, 756–776
2 Tachibana, T., Yoshizumi, T. and Hori, K. (1979) Bull. Chem. Soc. Jpn. 52, 34–41
3 Takenaka, T., Harada, K. and Matsumoto, M. (1980) J. Colloid Interface Sci. 73, 569–577
4 Takeda, F., Matsumoto, M., Takenaka, T. and Fujiyoshi, Y. (1981) J. Colloid Interface Sci. 84, 220–227
5 Takeda, F., Matsumoto, M., Takenaka, T., Fujiyoshi, Y. and Uyeda, N. (1983) J. Colloid Interface Sci. 91, 267–271
6 Philips, M.C. and Chapman, D. (1968) Biochim. Biophys. Acta. 163, 301–313
7 Trauble, H., Eibl, H. and Sawada, H. (1974) Naturwissenschaften 61, 344–354
8 Albrecht, O., Gruler, H. and Sackmann, E. (1978) J. Phys. 39, 301–313
9 Vilallonga, F. (1968) Biochim. Biophys. Acta 163, 290–300
10 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44
11 Kaye, R.D. and Burley, D.M. (1974) J. Phys. A. 7, 1303–1313
12 Marcelja, S. (1974) Biochim. Biophys. Acta 367, 165–176
13 Bell, G.M., Mingins, J. and Taylor, J.A.G. (1978) J. Chem. Soc. Faraday Trans. 2, 74, 223–234
14 Caille, A., Pink, D.A., De Verteuil, F. and Zuckermann, M.J. (1980) Can. J. Phys. 58, 581–611
15 Scott, H.L. (1977) Biochim. Biophys. Acta 469, 264–271
16 Georgallas, A. and Pink, D.A. (1982) J. Colloid Interface Sci. 89, 107–116
17 Kox, A.J., Michels, J.P.J. and Wiegel, F.W. (1980) Nature 287, 317–319
18 Van der Ploeg, P. and Berendsen, H.J. (1982) J. Chem. Phys. 76, 3271–3276
19 Baret, J.F. (1981) in Progress in Surface and Membrane Science, Vol. 14 (Cadenhead, D.A. and Danielli, J.F., eds.), pp. 291–351, Academic Press, New York
20 Von Tscharner, V. and McConnell, H.M. (1981) Biophys. J. 36, 409–427
21 Gershfeld, N.L. (1982) J. Colloid Interface Sci. 85, 28–40
22 Blodgett, K.B. (1935) J. Am. Chem. Soc. 57, 1007–1022
23 Asher, I.M. and Levin, I.W. (1977) Biochim. Biophys. Acta 468, 63–72
24 Cameron, D.G., Casal, H.L. and Mantsch, H.H. (1980) Biochemistry 19, 3665–3672
25 Umemura, J., Cameron, D.G. and Mantsch, H.H. (1980) Biochim. Biophys. Acta 602, 32–44
26 Hansen, W.N. (1970) in Optical Studies of Adsorbed Layers at Interfaces (Symposia of the Faraday Society, No. 4), p. 27
27 Hansen, W.N. (1973) in Advances in Electrochemistry and Electrochemical Engineering, Vol. 9 (Delahay, P. and Tobias, C.W., eds.), pp. 1–60, Interscience, New York
28 Higashiyama, T. and Takenaka, T. (1974) J. Phys. Chem. 78, 941–947

29 Takenaka, T. and Nogami, K. (1972) Bull. Chem. Soc. Jpn. 45, 2367–2371

30 Whitlington, S.G. and Chapman, D. (1966) Trans. Faraday Soc. 62, 3319–3324

31 Dluhy, R.A., Mendelsohn, R., Casal, H.L. and Mantsch, H.H. (1983) Biochemistry 22, 1170–1177

32 Cameron, D.G., Hsi, S.C., Umemura, J. and Mantsch, H.H. (1981) Can. J. Chem. 59, 1357–1360

33 Umemura, J., Cameron, D.G. and Mantsch, H.H. (1980) J. Phys. Chem. 84, 2272–2277

34 Umemura, J., Mantsch, H.H. and Cameron, D.G. (1981) J. Colloid Interface Sci. 83, 558–568

35 Kawai, T., Umemura, J. and Takenaka, T. (1983) Bull. Inst. Chem. Res., Kyoto Univ. 61, 314–323

36 Kawai, T., Umemura, J. and Takenaka, T. (1984) Colloid Polym. Sci. 262, 61–66

37 Yamanaka, T. (1982) Membrane 7, 359–365

38 Ries, H.E., Matsumoto, M., Uyeda, N. and Suito, E. (1975) in Advances in Chemistry Series, Vol. 144 (Goddard, E.D., ed.), pp. 286–293, American Chemical Society, Washington, DC

39 Matsumoto, M., Montandon, C. and Hartland, S. (1977) Colloid Polym. Sci. 255, 261–268

40 Smith, R. and Tanford, C. (1972) J. Mol. Biol. 67, 75–83

41 Nagle, J.F. (1976) J. Membrane Biol. 27, 233–250