Effects of Metal Cations on the Dynamic Characteristics of a Phospholipid Thin Film at an Air/Water Interface

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(Received May 31, 1996)

We measured the time-dependent surface pressure of a dioleoylphosphatidylcholine film at an air/water interface. Our results indicate that: (i) the surface pressure of the thin film traces a closed hysteresis loop accompanied by repetitive cycles of compression and expansion, and (ii) the area of the hysteresis loop markedly increases in the presence of sodium ions. We propose a kinetic model for this phenomenon which includes cooperative aggregation/dissociation at the air/water interface. The experimental findings have been successfully reproduced with the aid of a computer simulation based on this theoretical model.

Although, in the past, there have been numerous studies on the dynamic surface pressure (π)–surface area (A) curve of lipid monolayers at an air/water interface,[1—5] the viscoelastic properties with a periodic change in the surface area have not yet been fully clarified. This may be due to a difficulty in obtaining reproducible characteristics. The poor reproducibility has been attributed to such factors as that along with a periodic change in the surface area some lipid molecules at the air/water interface leak to the subphase[6] and stick to a blade and/or a trough,[6] and that the contact angle against a Wilhelmy plate increases.[7] In fact, most previous reports concerning the dynamic π–A curves have shown a time-dependent shrinkage of the π–A curve. In other words, a spiral loop has been observed.[6,8]

Recently, we reported on steady closed π–A curves with compression and expansion in the presence of taste compounds, such as nicotine (bitter), sodium chloride (salty), sucrose (sweet), and citric acid (sour) under appropriate experimental conditions.[9,10] We found that the π–A curve traces a single closed hysteresis loop in the shape of a somewhat distorted ellipsoid. The π–A curve changes in a characteristic manner depending on the taste category of the chemicals added. The shape of a hysteresis loop is expected to reflect the viscoelastic properties of a lipid thin film.[11] If the lipid thin film is a linear viscoelastic-body, the dynamic π–A curve traces a genuine elliptical loop. However, most curves are in the form of a distorted ellipsoid, due to the nonlinearity of a lipid thin film. This distortion, or nonlinearity, has been ignored in previous studies on the dynamic π–A characteristics.

To evaluate this nonlinearity in a quantitative manner, we adapted an analytical method to perform a Fourier transformation on a time-dependent change in pressure. In this method, the first-harmonic real and imaginary components correspond to the linear (ideal) elasticity and viscosity of the lipid thin film, respectively. The higher-harmonic components reflect the nonlinearity of the viscoelasticity. To perform such an analysis, it is necessary to accurately control the surface area. We have constructed a novel experimental apparatus for this purpose.

In this paper we report on the dynamic surface behavior of an unsaturated phospholipid thin film at the air/water interface in the presence of metal cations with the help of our new experimental apparatus. We have found that Na^+ has a marked effect on the dynamic behavior of a phospholipid thin film. We discuss our experimental findings in relation to the cooperative aggregation and dissociation of phospholipid aggregates at the air/water interface.

Experimental

Materials. A synthetic phospholipid, 1,2-dioleoyl-3-*sn*-phosphatidylcholine (DOPC), was obtained from Avanti Polar Lipid Inc. All other reagents were analytical-grade chemicals, and were used without further purification (Wako Pure Chemical Industries Ltd.). Before each experiment, water was purified using a Millipore-MilliQ Labo filtering system at a resistivity above 18 MΩ cm, and then distilled with an acid permanganate medium. Chloroform was purified by three consecutive distillations. DOPC (0.133 mM, $1\ M = 1\ mol\ dm^{-3}$) in chloroform was prepared as a spreading solution.

Dynamic Pressure–Area Curves. The experimental apparatus was designed by us and manufactured by Kyowa Interface Science Co. Ltd.[12] The surface area was changed repeatedly from 25 to 100 $Å^2\ molecule^{-1}$ in a sinusoidal cycle with a period of 60 s.[13] The obtained dynamic surface-pressure data were successively stored in an NEC PC-9801 personal computer (NEC, Japan). We observed the dynamic surface pressure after four cycles of compression and expansion, as the π–A curves began to trace a single-closed loop within four cycles of compression and expansion. Thus, the surface pressure at the maximum surface area was taken as a reference, i.e., $\pi = 0$ at the maximum of A (see e.g. Figs. 2 and 4). The time-course in the dynamic surface pressure was then Fourier-transformed to a frequency domain.

Results and Discussion

Figure 1 shows the π–A isotherm of DOPC monolayers, which were slowly compressed at a constant speed with 0.3125 Å^2 molecule^{-1} min^{-1}. In previous studies, the π–A isotherm of a lipid thin film at an air/water interface was very often measured under quasi-static compression. In this condition, the limiting area corresponds to the area of the cross-section of a surfactant molecule, and the slope reflects the elastic property of the monolayer at the air/water interface.[11,14)] The observed limiting area increases in the order control <KCl<$MgCl_2$ <NaCl, and the slope in the vicinity of the limiting area becomes small in the presence of NaCl. We observed the dynamic surface behavior in order to elucidate the viscoelastic properties of a DOPC thin film as the surface area was changed sinusoidally.

Figures 2A and 2B show the dynamic π–A curve and Fourier-transformed spectra of the dynamic surface pressure for the time trace of the surface pressure ($\pi(t)$). Generally, periodic functions, no matter how complicated, can be represented as an expansion of sine and cosine terms, known as a Fourier series,

$$\pi(t) = \pi_0 + \sum_{n=1}^{\infty} a_n \cos(n\omega_0 t) + \sum_{n=1}^{\infty} b_n \sin(n\omega_0 t). \tag{1}$$

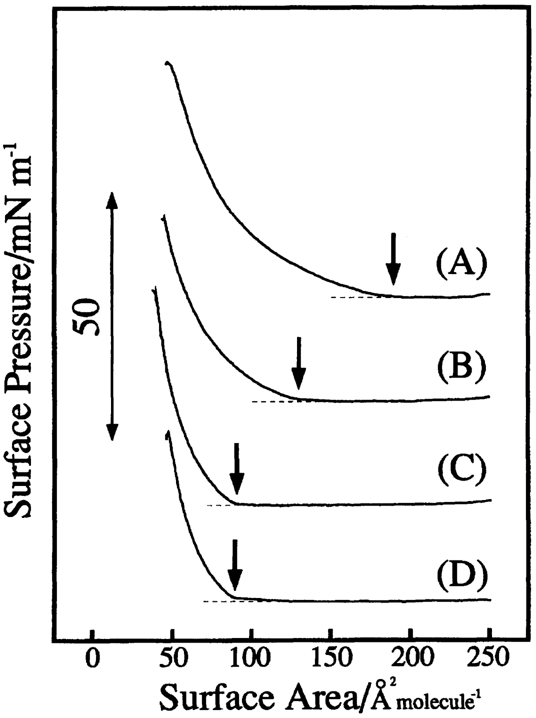

Fig. 1. Quasi-static π–A isotherms of a DOPC monolayer at an air/water interface. The arrows indicate the limiting area. The subphase temperature was 13 °C. The surface pressure at the starting point of the compression is 0 mN m^{-1}. (A) 1 M NaCl, (B) 1 M $MgCl_2$, (C) 1 M KCl, (D) distilled water.

For a viscoelastic-body, it has been well established that the cosine (real) and sine (imaginary) parts in the Fourier spectra correspond to the energy accumulated and dissipated terms, respectively.[15)] Therefore, it is clear that the real and imaginary parts indicate the elastic and viscous components of the DOPC thin film. Note that the presence of higher harmonics in the frequency spectra implies that these viscoelastic characteristics have non-negligible nonlinear components. Figure 2C shows the inverted Fourier transformation of the frequency spectra in Figure 2B. With this data-processing method, the elastic and viscous terms can be evaluated separately.

We then examined the dynamic π–A curves by changing the metal cations. In this experiment, to clarify the effect of cations, we used only chloride salts, such as LiCl, KCl, and NaCl. It is noted that the anions in the subphase also contribute to the change in the area of the hysteresis loop.[16)] Figure 3A shows the changes in the relative intensity (R_1/R_1° and I_1/I_1°) of the fundamental harmonic components of the Fourier-transformed spectra. The ideal (linear) elasticity increased in the order control <NH_4Cl, KCl<LiCl<NaCl<$MgCl_2$, while the viscosity increased in the order control <KCl<LiCl, $NH_4Cl \ll MgCl_2$ <NaCl. These results are roughly consistent with the previously established view that divalent metal cations tend to stabilize the lipid monolayer at the air/water interface rather than monovalent metal cations.[11,17)] However, Na^+ ion enormously increased the linear viscous property of the DOPC thin film. In order to elucidate the effect on the higher harmonic components in the Fourier spectra, the relative intensity ratios were derived as follows:

$$R_n^r = \frac{\dfrac{R_n}{R_1}}{\dfrac{R_n^\circ}{R_1^\circ}}, \tag{2}$$

$$I_n^r = \frac{\dfrac{I_n}{I_1}}{\dfrac{I_n^\circ}{I_1^\circ}}, \tag{3}$$

where R_n° and I_n° are the intensities of the Fourier n^{th}-harmonic components, obtained in a control experiment (see Fig. 2B). R_n and I_n are the intensities of the components in the presence of inorganic salts. As shown in Fig. 3B—F, the intensity ratio markedly decreased in the presence of NaCl. In particular, R_3^r became a negative value, while I_2^r and I_3^r showed little change compared to R_2^r and R_3^r with the addition of inorganic salts.

Figure 4 shows the dynamic π–A curves and inverted Fourier spectra. By adding NaCl to the aqueous subphase, the limiting area was shifted to the right and the slope of the surface pressure became small in the vicinity of the limiting area. Such a trend is consistent with that of the experimental results with a quasi-static π–A isotherm, as exemplified in Fig. 1. In contrast, in the control experiment and also with KCl, the slope of the π–A curve was steep and the area of the hysteresis loop was small. It is therefore clear that Na^+ makes the DOPC thin film more flexible than do other monovalent

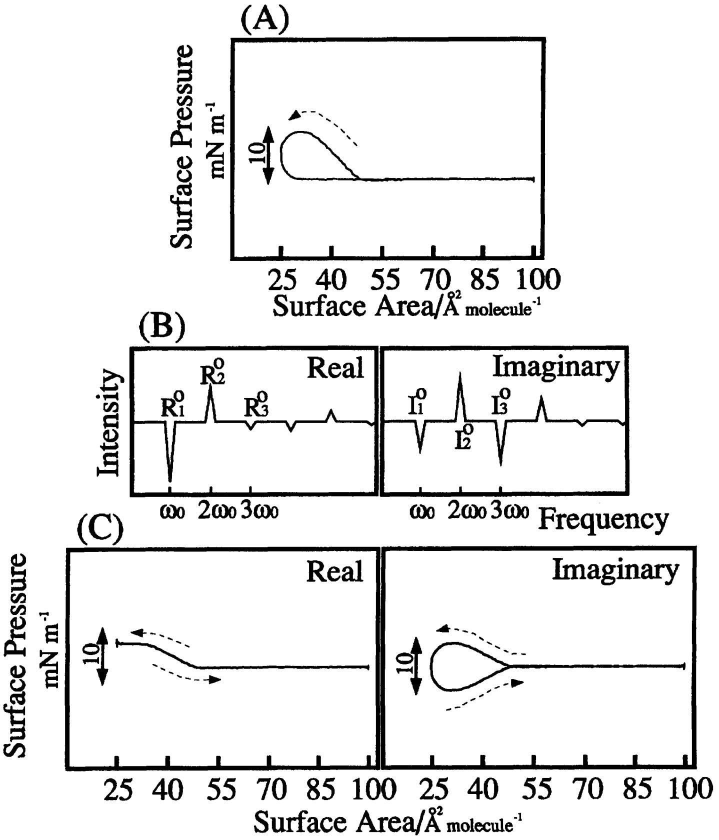

Fig. 2. Dynamic surface behavior of a DOPC thin film at an air/water interface, as the response to the sinusoidal change of A with a period of 60 s. (A) Dynamic π–A curve under steady state. (B) Fourier spectra. The real and imaginary parts indicate the functions of cosine and sine terms, respectively. (C) Inverted Fourier spectra.

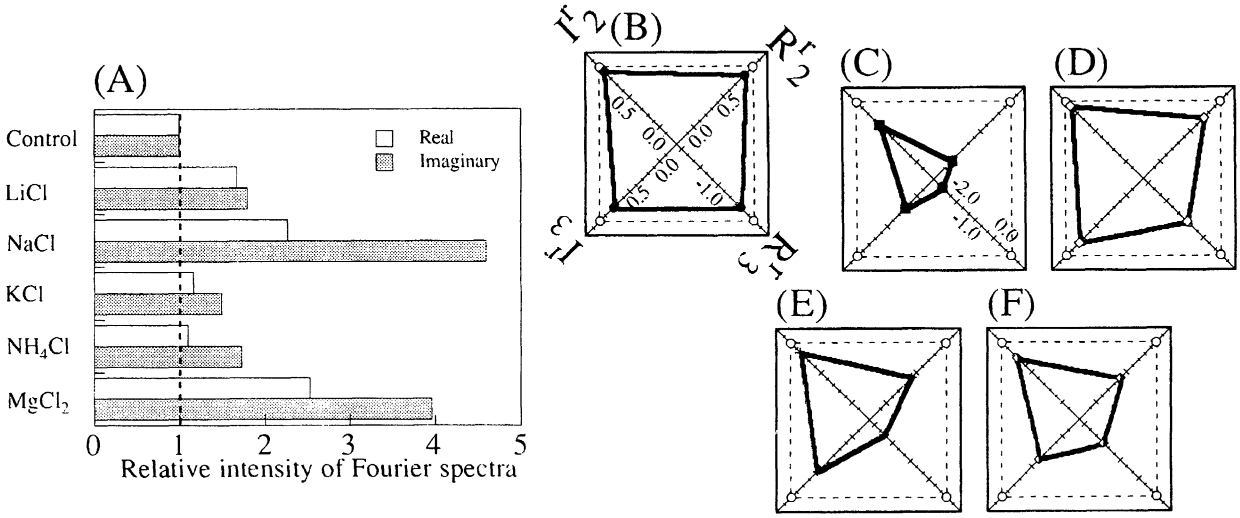

Fig. 3. (A) Change in the intensity of Fourier fundamental harmonic components relative to those in the control experiment. Changes in the relative intensity of higher-harmonic components: (B) 1 M LiCl, (C) 1 M NaCl, (D) 1 M KCl, (E) 1 M NH_4Cl, (F) 1 M $MgCl_2$. Broken lines indicate the results of the control experiment.

cations.

Furthermore, the inverted Fourier real spectra for the control experiment traced a flat plateau from ca. 25 up to 40 $Å^2$ molecule^{-1}. On the other hand, for the same range of surface area, the spectra traced a decreasing curve with compression in the presence of inorganic salts. Generally, as indicated in Fig. 1, the surface pressure does not decrease with compression under quasi-equilibrium. Therefore, the decrease in the surface pressure can be interpreted as a relaxation phenomenon related to the collapse of the DOPC thin film at the air/water interface, judging from the collapse point in Fig. 1. It is clear that the dynamic surface pressure

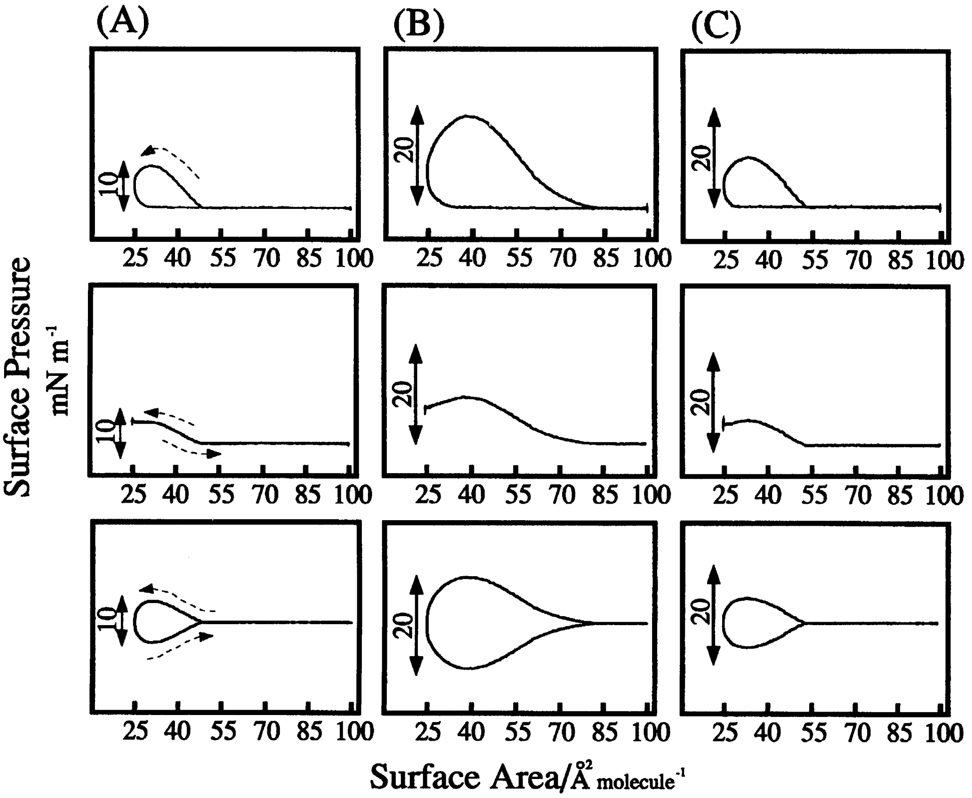

Fig. 4. Inverted Fourier spectra of the dynamic change in surface pressure. (A) Control, (B) 1 M NaCl, (C) 1 M KCl. Top; dynamic *π–A* curves, Middle; inverted real Fourier spectra, Bottom; inverted imaginary Fourier spectra.

observed for the range between 25 and 40 Å^2 molecule^{-1} is markedly decreased in the presence of Na^+. These experimental results suggest that the interaction between Na^+ and phospholipid head groups should be unique compared to that with other ions.

Loshchilova and Karvaly studied the influence of monovalent cations on phosphatidylcholine bilayers using laser Raman spectroscopy.[18] They found that Na^+ was the most effective in changing the order parameters of the head groups. Uedaira and Suzuki studied the ultrasonic velocity of aqueous solutions of alkali metal chlorides.[19] They reported that the maximum value of the ultrasonic velocity vs. temperature curve was shifted the most in the presence of Na^+.

As for these significant effects of Na^+, Desnoyer and Jolicoeur proposed a possible explanation:[20] these maxima may appear because "the strength of ion–solvent interactions decreases with increasing size of the ions, but the coordination number of the ion increases with increasing size." On the contrary, the coordination numbers of alkali metal ions were formed to be 4 for Li^+, Na^+, and K^+ ions, according to an X-ray analysis and a neutron-scattering experiment.[21] In order to obtain clear insight concerning the significant effect of Na^+, additional work is necessary, including a discussion on the state of the water molecules, especially the water structure in the vicinity of the phosphatidylcholine head groups under kinetically controlled conditions.[22,23]

Based on the above results, we tried to elucidate the origin of the hysteresis loop of the dynamic surface pressure and the effects of monovalent cations, such as Na^+, on a DOPC thin film with the aid of a computer simulation. We previously explained the dynamic surface behavior using the following kinetic model, which includes the cooperative aggregation of surfactant molecules onto the air/water interface (Scheme 1):[24]

$$\mathrm{S+S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}, \qquad \text{(i)}$$

$$\mathrm{S+D'} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{D_L}, \qquad \text{(ii)}$$

Scheme 1.

where S indicates the regular state,[24] D indicates the domains of the DOPC aggregates, $\mathrm{D_L}$ indicates the large domains, such like oil-droplets, formed by collapse of the DOPC monolayer at the air/water interface, and k_1, k_{-1}, k_2, and k_{-2} are the kinetic coefficients of each process, respectively. As expected, that the number of domains increase through collisions between surfactant molecules at the surface, process (i) is most simply approximated as a second-order reaction. However, the growth rate of a large domain is supposed to be expressed as a function of the surface pressure in addition to the collision probability.[24]

Based on this scheme, we use the following system of equations:

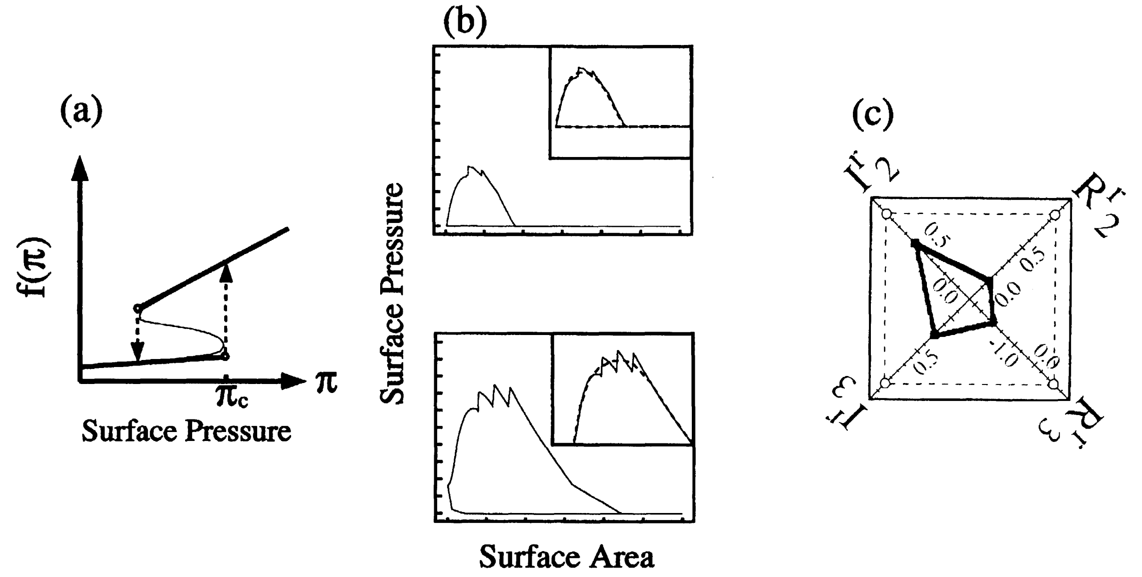

Fig. 5. Computer simulation based on the cooperative aggregation/dissociation model. (a) The feature of the discontinuous function, $f(\pi)$. $\pi<\pi_c; f(\pi)=0.002\pi+15$, $\pi\geq\pi_c; f(\pi)=\pi+50$. (b) Computer simulation of the observed dynamic π–A curves. Top; $\pi_c=2.5$, Bottom; $\pi_c=5.5$. Parameters; $k_1=17.5$, $k_{-1}=1.0\times10^{-1}$, $k_2'=1.0\times10^{-2}$, $k_{-2}=1.0\times10^{-5}$. (c) Simulated relative Fourier peak intensities which were calculated from (b) in Fig. 5.

$$\frac{\mathrm{d[S]}}{\mathrm{d}t}=-k_1[\mathrm{S}]^2+k_{-1}[\mathrm{D}]-k_2'f(\pi)[\mathrm{S}][\mathrm{D}]+k_{-2}[\mathrm{D_L}]^{1/2}+\mathrm{N_S}\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{1}{A}\right), \tag{4}$$

$$\frac{\mathrm{d[D]}}{\mathrm{d}t}=k_1[\mathrm{S}]^2-k_{-1}[\mathrm{D_L}]-k_2'f(\pi)[\mathrm{S}][\mathrm{D}]+k_{-2}[\mathrm{D_L}]^{1/2}+\mathrm{N_D}\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{1}{A}\right), \tag{5}$$

$$\frac{\mathrm{d[D_L]}}{\mathrm{d}t}=k_2'f(\pi)[\mathrm{S}][\mathrm{D}]-k_{-2}[\mathrm{D_L}]^{1/2}+\mathrm{N_L}\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{1}{A}\right), \tag{6}$$

where k_2 is equal to $k_2'f(\pi)$, $\mathrm{N_S}$ and $\mathrm{N_D}$ are the number of regular molecules and domains, and $\mathrm{N_L}$ is the number of oil-droplets at the air/water interface. We assume that the reversal kinetics in step (ii) can be represented as the 1/2 power of $[\mathrm{D_L}]$. Because the rate of two-dimensional aggregation/dissociation should be proportional to the length of its surrounding, rather than the concentration,[25] based on the model that the growth of DOPC domains at the air/water interface is considered as an edge-growth.[25] Here, it should be pointed out that the viscoelastic properties in this work are attributable to the kinetic process of growth and a dissociation of the lipid domain rather than a process involving a change in a homogeneous monolayer at an air/water interface.

$f(\pi)$ is a discontinuous function which includes a critical value (π_c). Such a discontinuous function is considered to be a simple model for a first-order phase transition in a lipid film;[26,27] the drastic change of the rate-coefficient (k_2) is attributed to a cooperative factor for aggregation/dissociation between the DOPC domains. To simplify the numerical analysis and also to reduce the number of "adjustable" parameters, we adapt the approximation so as to express the discontinuity with a combination of two straight lines, as in Fig. 5a. In addition, we assume that the surface pressure (π) is given as the summation of two contributions due to S and D.

To explain the above effects of $\mathrm{Na^+}$, it is necessary to assume that the slope of $f(\pi)$ is small at a low surface pressure, and that the discontinuity in $f(\pi)$ and the critical surface pressure (π_c) are enhanced, considering the experimental results that the limiting area was shifted and the dynamic surface pressure decreased with compression in the range from 25 to 40 Å^2 molecule^{-1}.

Figure 5 shows the relation between $f(\pi)$ and π, the simulated dynamic surface pressure, and the relative peak-intensities obtained as the Fourier transformation on the time-change of the simulated dynamic surface pressure. It is clear that the peak-intensities in the simulation correspond well to the experimental trend given in Fig. 3. Here, the calculated surface pressure oscillates near to π_c, since the damping factor of the dynamic surface pressure was not included in this study. By including the viscous factor, the surface pressure should follow the dotted line, which corresponds to the experimental trends.

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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