Phase Transition Dependent Fourier Transform Infrared Spectra of Polyion-Complexed Lipid Films in Air

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Temperature-dependent Fourier transform infrared spectra of polyion-complexed synthetic lipid films of dioctadecyl dimethyl ammonium poly(styrene sulfonate), dihexadecylglutamate poly(styrene sulfonate) and two other glutamate-based and diethanolamine-based lipids with a long spacer chain were measured in air and compared with those of liposomal and synthetic bilayer membranes. The antisymmetric CH2 stretching band of the films showed strong phase transition dependence, indicating that the fundamental conformational changes associated with the phase transition of the lipid films are close to those of aqueous bilayer membranes. However, the films displayed some differences in the spectra; the symmetric CH2 stretching and carbonyl(ester) stretching band of the films of the glutamate-based lipids did not exhibit phase transition dependence, in sharp contrast with the results of aqueous bilayer systems. This may be due to the retention of higher ordering in the long alkyl chain even at temperatures above the phase transition.

Keywords Fourier transform infrared spectroscopy, synthetic lipid film, phase transition

Aqueous molecular bilayers can be immobilized in several ways.1 Casting (solvent evaporation) of polyion-complex lipids prepared from the mixing of synthetic lipid bilayers with positive (or negative) charge and an anionic (or cationic) polymer has been reported to give lipid multi-bilayer films.1 Considerable attention is being given to this novel material possessing biomembrane-mimetic functions. Lipid bilayer membranes are "wet materials" which function in aqueous solutions. Immobilization of these aqueous bilayers facilitates their use as "dry material". A variety of physical methods have been used so far to characterize the material: electrochemical methods including membrane potential measurement2, impedometry3, voltammetry4 and potential step chronocoulometry5, quartz crystal microbalance6, ESR spectroscopy7, moduli of elasticity measurements8 and thermogravimetric analyses.8 However, information on molecular conformations and frequency modes of these lipid films in air has been very limited; no report has described the characterization of the lipid films using FT-IR or Raman spectroscopy. In this article, the influence of phase transition on FT-IR spectra of the films of the following four polyion-complexed lipid 1 - 4 in air is reported. Lipid 1 is a simple double-chain ammonium lipid and lipids 3 - 4 possess both ester and amide groups, which allow us to explore the effect of the
molecular structure on the molecular conformation and orientation by the IR technique. Raman spectroscopy has been used to characterize the nature of C–C and C–H stretching of phospholipids in biological and model membranes, but carbonyl stretching is inactive in this method. Phase transition-dependent FT-IR spectra of aqueous bilayers of biological and synthetic lipids and FT-IR of the films of ammonium surfactants containing 21% water have already been described.

**Experimental**

**Materials**

Preparation of polyion-complexed lipid 1 was described elsewhere. Lipids 2–4 were prepared in a similar way, and were characterized by 'H-NMR and elemental analysis. Anid. Lipid 2: Calcd. for C_{45}H_{89}NO_{7}S•H_{2}O. C, 67.71; H, 10.48; N, 1.76; S, 4.02%. Found. C, 67.92; H, 10.85; N, 1.74; S, 3.45%. Lipid 3: Calcd. for C_{59}H_{108}N_{2}O_{8}S•2H_{2}O. C, 68.04; H, 10.84; N, 2.69; S, 3.08%. Found. C, 67.84; H, 10.55; N, 2.57; S, 3.06%. Lipid 4: Calcd. for C_{54}H_{98}N_{2}O_{8}S•3H_{2}O. C, 65.55; H, 10.59; N, 2.83; S, 3.24%. Found. C, 66.21; H, 10.36; N, 2.79; S, 3.53%. The molar ratios of dialkyl ammonium cation/poly(styrene sulfonate) anion determined by S/N ratios of the elemental analyses are: 1.1, 0.96 and 0.92 for 2, 3 and 4, respectively.

**FT-IR measurements**

A typical procedure for sample preparation is as follows. Forty microliters of a chloroform solution of a lipid (30 mg/ml) was spread on a CaF_{2} plate. Solvent evaporation gave a transparent film; this was annealed in hot water (60°C) for 30 min and then air-dried overnight. Hot water-annealing is important to obtain lipid films with fundamental physico-chemical properties of molecular bilayers. Each modified plate thus obtained was assembled in a temperature-controlled flow-through cell (Harrick Scientific Corporation). Temperatures were maintained to be constant within ±0.1°C (instrument, NESLAB INSTRUMENTS, Inc. Circulator RTE-100). FT-IR spectra were measured on a Perkin Elmer Spectrophotometer Model 1600; 256 scans were accumulated for each spectrum. The accuracy in the frequencies of the machine is ±0.01 cm⁻¹.

**Results and Discussion**

IR spectra of aqueous bilayers of biological and synthetic lipids are known to show strong temperature dependence. In this study, FT-IR spectra of multibilayer cast films of the polyion-complexed lipids were measured at several temperatures in air and compared with those of aqueous bilayer systems. As typical examples, IR spectra of the lipid films of lipids 2 and 4 are shown in Fig. 1. Selected bands and their assignments for the bilayer films are summarized in Table 1. The assignments were conducted by referring to those of synthetic ammonium lipids and of phospholipids. Figure 2 shows the temperature dependence of the peak maxima of antisymmetric and symmetric stretching bands based on the CH₂ group in the alkyl chain. Bilayer films of lipid 1 and 4 gave an antisymmetric CH₂ band at 2918 cm⁻¹ at lower temperatures, which shifted to 2922 cm⁻¹ at higher temperatures. The break temperatures observed at 45°C for a 1 film and 57°C for a 4 film are close to those of phase transition temperatures (Tₜ) determined by differential scanning calorimetry (Tₜ of films of 1 and 4 are 42°C and 51°C, respectively), indicating that the bilayer fluidity change causes the spectral shift. Antisymmetric CH₂ bands of
films of lipids 2 and 3 were observed at 2915 and 2915.8 cm\(^{-1}\), respectively. They also showed a temperature-dependent spectral shift; however, in comparison with lipids 1 and 4, the antisymmetric bands in these films showed a shorter wavenumber shift. This result suggests the formation of a higher ordered orientation in the long alkyl chains in the films.

The symmetric stretching band of films of lipids 1 and 4 also showed a clear phase transition dependence. The observed frequencies and \(T_c\) dependence are almost identical with those of phospholipid membranes.\(^{10}\) This indicates that the packing mode or orientation of the synthetic lipid in the films in air is very similar to those of biological lipids in the liposomal membrane in water. The temperature-dependent spectral change is ascribable to the trans-gauche conformational change of the alkyl chain associated with a phase transition between the crystal phase and liquid crystal phase. Snyder and coauthors\(^{15}\) described the theoretical analyses of the conformational change in the crystal-to-liquid transition (melting) of \(n\)-alkanes. The symmetric bands of lipid films of 2 and 3, on the other hand, did not show \(T_c\) dependence, and instead give rise to a shift to lower wavelength by 1-1.5 cm\(^{-1}\) and 1-4 cm\(^{-1}\) at lower and higher temperature regions, respectively. Such a result indicates the formation of higher ordering in the alkyl chains in the films of 2 and 3 based on the well-organized packing of the glutamate lipids.

As we described before, the carbonyl stretching band of aqueous bilayers of lipid 5 and 6 showed phase transition dependence\(^{11}\), whereas the aqueous bilayer of lipid 7, a lipid with a short spacer chain, did not give such \(T_c\) dependence. In the case of the immobilized cast multibilayer films explored in this study, the behavior was different; in contrast with aqueous bilayers\(^{11}\), the carbonyl stretching of lipid 3 possessing a longer spacer chain did not show temperature dependence similar to that of a film of 2. Such a result would mean that, in air, high ordered orientation in the ester moiety is maintained even at temperatures above the phase transition. On the contrary, a film of lipid 4 shows \(T_c\) dependent carbonyl stretching, though the shift is small (Fig. 3). The change in the frequency of carbonyl (ester) suggests the suppression of polarization of the carbonyl group; the ester carbonyl groups in the crystalline state are in a high orientation which enhances their polarization and the loss in ordered conformation leads to lessened polarization, which allows the shift to higher frequency.\(^{16}\)

The amide I band of a film of 3 was observed at 1649 - 1650 cm\(^{-1}\); this was ascribable to the associated amide. The wavenumber shifted by 1 cm\(^{-1}\) with increasing temperature; however, the phase transition did not affect the frequencies, indicating that the lipid molecules in the film formed hydrogen bonds even at temperatures above the phase transition. A similar behavior has been reported for the aqueous bilayer of surfactants 5 and 6.\(^{11}\) As shown in Fig. 3, a film of 4 gave different phenomena; the amide II band of this film exhibited a temperature dependence which reflected the phase transition. The lipid bears a tertiary amide and is expected to associate with water molecules via hydrogen bonding; this tendency is lessened by an increase in the bilayer fluidity. We conclude that intermolecular hydrogen bonding plays the crucial role for the conformational change associated with the phase transition of the lipid bilayers.
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Fig. 3 Frequencies of the ester (above) and amide (below) carbonyl stretching vibration of lipid films of 1 (○), 2 (●), 3 (□) and 4 (■) as a function of temperature.

References


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