

Design of a Lipid Bilayer Electrical Device. Strong Chemical Structure Dependence and Molecular Mechanisms on the Phase Transition-Dependent Electrical Impedance Responses of the Device in Air

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This paper describes the design and fabrication of a lipid film-modified electrical device and the relation between the chemical structure of the lipids and the impedance responses of the device in air. Eight different synthetic poly(ion-complexed) lipids including quaternary ammonium lipids, glutamic acid (Glu)-based lipids with a short or a long methylene spacer chain, and diethanolamine-based quaternary ammonium lipids with a long or a short spacer chain were synthesized. Transparent multilayer films with crystalline-to-liquid crystalline phase transition were formed, and impedance responses for interdigitated array electrodes coated with cast films of these lipids were examined. Complex plane plot analyses together with quartz crystal microbalance and FTIR experiments have revealed molecular mechanisms for the unique impedance responses which could be classified as the following three types. Type I is the phase transition-dependent impedance responses, where the impedance changes dramatically near the phase transition temperatures of the lipid bilayer films on the electrodes. The change is derived from the increase in the mobility of the ion-conducting carrier (protons) coupled with the phase transition. The lipid devices coated with cast films of quaternary ammonium lipids with no spacer or a short spacer belong to this type. Type II is a device coated with a cast film of a diethanolamine-based quaternary ammonium lipid with a long spacer. *T_c*-dependent impedance response similar to that in type I is observed, but the molecular mechanism of the response is different. Type III exhibits a phase transition-independent impedance response. Electrical devices using Glu-based primary ammonium lipids and a Glu-based quaternary ammonium lipid with a long spacer as electrode modifiers give this type of response. Conformational mobility of the hydrophilic head group moieties of these lipid bilayers is maintained rigid through hydrogen bonding even at temperatures higher than the phase transition which results in this response.

Introduction

Synthetic bilayer membranes possess fundamental physico-chemical properties^{1,2} similar to those of biomembranes and liposomes^{3,4} and can be immobilized as multilayer films by several means;⁵ the polyion-complexing method and the casting method of polyion-complexed lipids which are formed from the combination of ammonium lipids and anionic polymers are especially simple and useful.⁶ We have reported that electrodes modified with synthetic lipid films provide unique electrochemical behavior based on the properties of bilayers.^{7–15} Our goal is to design and prepare molecular electrical devices using lipid membrane film materials and to examine their electrical properties in air. Studies on the development of molecular electrode lipid bilayer transducing devices and their interfacial electric signals are quite interesting for the application and utilization of biomembranes. Despite the considerable attention on the potential use of molecular lipid bilayer membranes as novel electrode modifiers,^{16–38} studies in this field have been very limited.

The combination of the chemistry of synthetic bilayer membranes and of modified electrodes (both have been extensively developed independently in recent years) would be expected to produce a new field in chemistry and also be highly useful. We report here the design of a molecular bilayer electrical device

of interdigitated array electrodes (Figure 1) using polyion-complexed lipid films and the relationship between the chemical structure of lipids and the molecular mechanisms of ac impedance responses of the electrodes under relative humidity control and in ambient air atmosphere.³⁹ Chart 1 shows the chemical structure of synthetic lipids used in this study. All of these can form transparent thin films that exhibit a phase transition between crystal and liquid crystal phases, which is one of the fundamental properties of lipid bilayer membranes.

Experimental Section

Materials. Dioctadecyldimethylammonium bromide ($2C_{18}N^+Br^-$) was available from previous studies.^{9,12} Double-chain ammonium synthetic lipids, didodecyl glutamate-HCl ($2C_{12}Glu(NH_3^+)Cl^-$), dihexadecyl glutamate-HCl ($2C_{16}Glu(NH_3^+)Cl^-$), $2C_{16}GluC_mN^+Cl^-$ (or Br^-) ($m = 2, 11$), and $2C_{14}DeaC_2N^+Cl^-$ were synthesized according to the literature.^{40,41} The intermediates and the final products were identified by IR, ¹H NMR, and thin-layer chromatography. The lipid, $2C_{14}DeaC_{11}N^+Br^-$, was synthesized by the following procedure. The reaction of *O,O'*-(ditetradecanoylimino)diethanol-HCl (0.012 mol) and 11-bromoundecanoic acid (0.024 mol) in the presence of triethylamine (0.024 mol) and diethyl phosphocyanidate (0.020 mol) in chloroform (170 mL) gave *N*-(*ω*-bromoundecanoyl)-*O,O'*-(ditetradecanoylimino)diethanol (8.2 g, yield 88%, mp 52.5–54.0 °C) which was quarternarized with trimethylamine in THF to obtain *N*-(*ω*-trimethylammonio-

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TABLE 1: Phase Transition Temperatures (T_c) for Cast Films of Poly(ion-complexed) Lipids

lipid	$T_c/^\circ\text{C}$ ($\Delta H/\text{kJ mol}^{-1}$)	
	in air	in water
$2\text{C}_{16}\text{N}^+\text{PSS}^-$	26 (23)	29 (24)
$2\text{C}_{18}\text{N}^+\text{PSS}^-$	42 (25)	42 (24)
$2\text{C}_{12}\text{Glu}(\text{NN}_3^+)\text{PSS}^-$	35 (15)	28 (15)
$2\text{C}_{16}\text{Glu}(\text{NN}_3^+)\text{PSS}^-$	58 (54)	55 (20)
$2\text{C}_{16}\text{GluC}_{11}\text{N}^+\text{PSS}^-$	64 (14)	44 (23)
$2\text{C}_{16}\text{GluC}_2\text{N}^+\text{PSS}^-$	44, 59 (9.6) (4.6)	44 (23)
$2\text{C}_{14}\text{DeaC}_{11}\text{N}^+\text{PSS}^-$	43 (25)	25 (24)
$2\text{C}_{14}\text{DeaC}_2\text{N}^+\text{PSS}^-$	51 (7.1)	29 (13)

mational change from trans to gauche conformation in the long alkyl chains similar to those observed for aqueous lipid bilayer membranes.^{43,44}

Phase Transition-Dependent Impedance Responses of Lipid Film-Coated Patterned Electrodes. (a) Responses under 75% Relative Humidity. As we reported previously,⁹ an interdigitated array electrode coated with a lipid bilayer film of $2\text{C}_{18}\text{N}^+\text{PSS}^-$ at 1 kHz showed drastic change in ac electrical parameters due to the phase transition of the lipid bilayer. Used material, however, was contaminated with a small amount of Br^- ion;⁹ therefore, the contribution of the halide ion conductivity to the impedance responses could not be excluded. For simplicity in considering the mechanisms of impedance responses in this paper, halide ion-free poly(ion-complexed) lipids were prepared and used as the lipid materials (for details, see Experimental Section).

Figure 2 shows complex plane plots and Bode line plots for an electrode coated with the cast film of $2\text{C}_{18}\text{N}^+\text{PSS}^-$. At lower temperatures, the electrical responses at frequencies lower than 250 Hz could not be measured because of their high impedance. The sizes of the circular arches have a strong temperature dependence. Bode line plots of the electrode at lower temperatures (25–35 °C) show almost no temperature dependence, where the impedance is largely governed by the capacitive component. Bode line plots of a nonmodified (bare) interdigitated array electrode at this temperature range are almost identical with that of the modified electrode, indicating that the electrical signals from the lipid film device cannot be separated from that of the background. However, at temperatures above 45 °C, a drastic decrease in resistance is evident. Complex impedance analyses for modified electrodes using other lipids, $2\text{C}_{16}\text{N}^+\text{PSS}^-$, $2\text{CnGlu}(\text{NH}_3^+)\text{PSS}^-$ ($n = 12, 16$), $2\text{C}_{16}\text{GluCmN}^+\text{PSS}^-$ ($m = 2, 11$), and $2\text{C}_{14}\text{DeaC}_m\text{N}^+\text{PSS}^-$ ($m = 2, 11$), were also conducted (data not shown). Figure 3 shows the temperature dependence of resistance (R) which is obtained from the intercepts of the circular arches with the real axis at lower frequencies. The observed temperature dependence was reversible. Bilayer devices of $2\text{C}_{16}\text{N}^+\text{PSS}^-$, $2\text{C}_{18}\text{N}^+\text{PSS}^-$, and $2\text{C}_{16}\text{GluC}_2\text{N}^+\text{PSS}^-$ give clear breaks at temperatures of 27, 44, and 48 °C, respectively, which correspond to the phase transition temperatures of each cast bilayer film under air atmosphere. When the temperatures were cycled back to 20 °C, we could see strong T_c -dependent impedance responses with little hysteresis. Similar T_c dependence is observed for $2\text{C}_n\text{GluC}_2\text{N}^+\text{PSS}^-$ ($n = 14, 18$)-coated bilayer electrode devices (data not shown), indicating that the change in the lipid alkyl chain length does not influence on the T_c -dependent impedance response. Figure 4 shows resistance obtained from the complex plane plots for

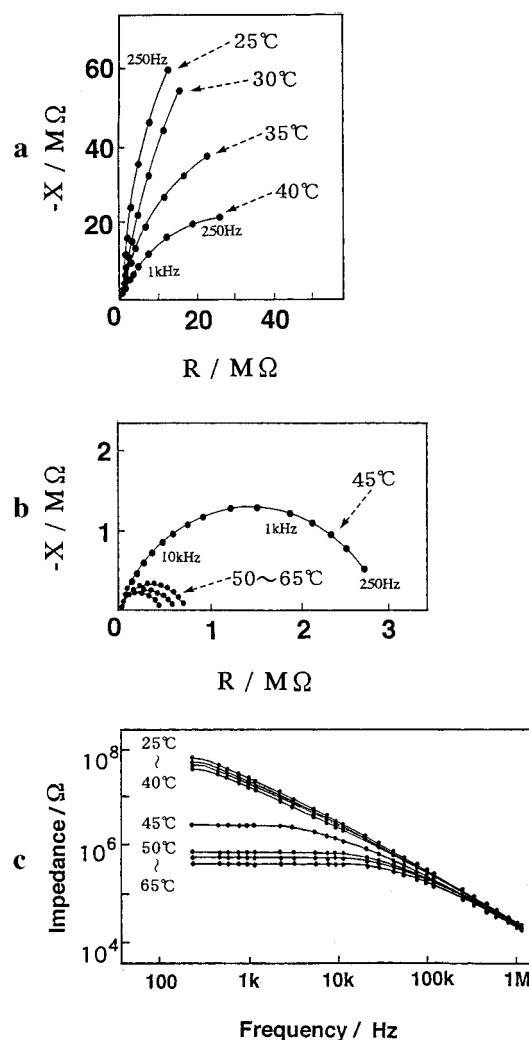


Figure 2. Impedance plane plots (a, b) and Bode line plots (c) for a $2\text{C}_{18}\text{N}^+\text{PSS}^-$ -modified interdigitated array electrode under 75% relative humidity at various temperatures.

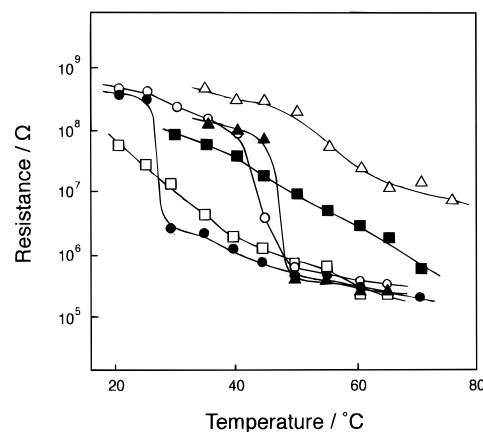


Figure 3. Temperature dependence of the resistance of interdigitated array electrode coated with cast films of $2\text{C}_{18}\text{N}^+\text{PSS}^-$ (open circles), $2\text{C}_{16}\text{N}^+\text{PSS}^-$ (closed circles), $2\text{C}_{12}\text{Glu}(\text{NH}_3^+)\text{PSS}^-$ (open squares), $2\text{C}_{16}\text{Glu}(\text{NH}_3^+)\text{PSS}^-$ (closed squares), $2\text{C}_{16}\text{GluC}_{11}\text{N}^+\text{PSS}^-$ (open triangles), or $2\text{C}_{16}\text{GluC}_2\text{N}^+\text{PSS}^-$ (closed triangles).

a $2\text{C}_{14}\text{DeaC}_{11}\text{N}^+\text{PSS}^-$ -modified electrode at given atmosphere as a function of temperature. Increased relative humidity is found to cause the shift of break temperatures to lower temperatures, which would reflect the shift of phase transition temperatures of the bilayer film.

On the contrary, the impedance of $2\text{C}_n\text{Glu}(\text{NH}_3^+)\text{PSS}^-$ ($n = 16, 18$) bilayer devices decreases almost monotonically with

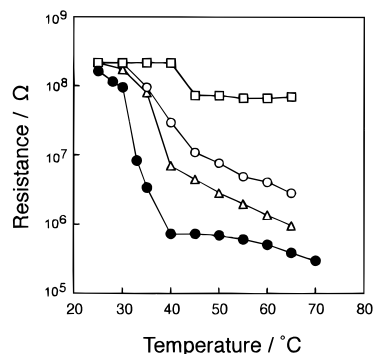


Figure 4. Temperature dependence of the resistance of a $2C_{14}$ -Dea $C_{11}N^+PSS^-$ -coated interdigitated array electrode for the following atmospheric conditions: in ambient air (squares), 50% relative humidity (open circles), 60% relative humidity (triangles), and 75% relative humidity (closed circles).

temperature; no drastic change in resistance is observed at the phase transition temperature of each bilayer film. An electrode coated with a cast film of $2C_{16}GluC_{11}N^+PSS^-$, a glutamate lipid with the longer methylene spacer chain possessing a phase transition at 64 °C under air and 44 °C in H_2O , also shows no break in resistance at the phase transition region.

Together, these results show that the impedance responses of lipid bilayer devices depend on (1) hydrophilic head group moieties (i.e., primary ammonium or quarternary ammonium), (2) connector groups, and (3) chain lengths of methylene spacers in the lipids. The lengths of long double alkyl chains and the thickness of the lipid cast films do not influence the responses. Molecular mechanisms for these impedance responses will be discussed later.

(b) Impedance Responses of the Bilayer Devices under Usual Air Atmosphere. As we reported before,¹² despite the existence of the bilayer phase transition, the impedance responses of a $2C_{18}N^+PSS^-$ bilayer device under usual air atmosphere without relative humidity control⁴⁵ were not influenced by the change in the membrane fluidity, where the intensities of impedance were very large at all measured temperatures (25–60 °C). This is in sharp contrast with that under 75% relative humidity. Bilayer devices modified with other synthetic lipids, except $2C_{14}DeaC_{11}N^+PSS^-$, gave similar impedance responses. A $2C_{14}DeaC_{11}N^+PSS^-$ -modified electrode, on the contrary, was found to give a clear phase transition dependence; evidently, intensities of circular arches of the complex plane plots decrease near 42 °C, which is close to T_c of the bilayer films of the lipid under air atmosphere (see Figure 4).

Quartz Crystal Microbalance Measurement. Impedance responses described above might relate to the adsorption or desorption of water molecules to/from the lipid bilayer films. In order to know how amounts of water molecules adsorb to the lipid films at given condition, QCM experiments^{46,47} were conducted.

Figure 5 show the QCM frequency change of quartz crystal resonators modified with the lipid films. Under 75% relative humidity, no frequency change is observed for the QCM coated with $2C_{18}N^+PSS^-$, $2C_{16}Glu(NH_3^+)PSS^-$, or $2C_{16}GluC_{11}N^+PSS^-$ at the temperature range corresponding to the phase transition of each bilayer film. Under usual air atmosphere, the increase in the resonance frequency of the QCM resonators due to the desorption of weakly hydrated water molecules from the lipid films is seen at temperatures of 10–30 °C, but the phase transition dependence on frequency is not seen. Crane and Fischer⁴⁸ described the influence of fluidity or viscoelastic change of coating materials on the resonance frequency of the shear mode quartz crystal. Okahata and Ebato^{49,50} have reported that QCM can detect the phase transition of lipid multibilayers

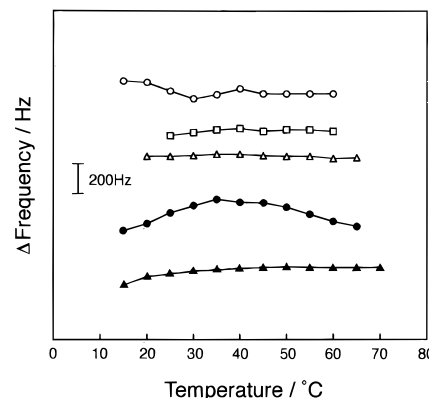


Figure 5. Temperature dependence of frequency changes (ΔF) of piezoelectric crystals coated with cast films of $2C_{18}N^+PSS^-$ (open circles), $2C_{16}Glu(NH_3^+)PSS^-$ (open squares), and $2C_{16}GluC_{11}N^+PSS^-$ (open triangles) under 75% relative humidity and of $2C_{18}N^+PSS^-$ (closed circles) and $2C_{16}GluC_{11}N^+PSS^-$ (closed triangles) in air.

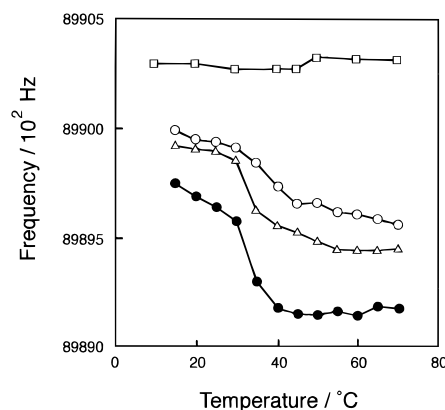


Figure 6. Temperature dependence of frequencies of a piezoelectric crystal coated with a cast film of $2C_{14}DeaC_{11}N^+PSS^-$ for various atmospheric conditions: in ambient air (squares), 50% relative humidity (open circles), 60% relative humidity (triangles), and 75% relative humidity (closed circles).

in water, but not in an air phase. Frequency enhancements at the phase transition in water were explained by slipping between hydrated fluid layers.

A QCM resonator coated with a cast film of $2C_{14}DeaC_{11}N^+PSS^-$ was found to give different responses (Figure 6); i.e., under relative humidity control atmosphere, the frequency decreased drastically near 35–45 °C. This is arising from the sorption of water molecules to the hydrophilic moieties of the lipid bilayer films. The break temperatures observed in the QCM responses are close to those of the impedance responses shown in Figure 4.

Molecular Mechanisms of Impedance Responses. (a) Under 75% Relative Humidity. The impedance of electrodes modified with the bilayer films of $2C_{18}N^+PSS^-$, $2C_{16}GluC_2N^+PSS^-$, and $2C_{14}DeaC_{11}N^+PSS^-$ shows a drastic phase transition dependence (see Figure 3). What are the molecular mechanisms for these impedance responses? Ionic conductivity is a function of the mobility and the concentration of ionic conducting carriers. In this system, the main conducting carrier should be protons.^{51,52} Therefore, two factors are suggested for the decrease in the impedance of the prepared lipid bilayer film devices: one is the increase in the mobility of protons (factor I), and the other is the increase in the adsorption of water molecules onto the hydrophilic head moieties in the multibilayer films (factor II). The number of water molecules adsorbed per single molecule of $2C_{18}N^+PSS^-$ at 20 °C is reported to be 3.7 by the thermogravimetric analysis.⁵³ Other lipids used in this study contain similar

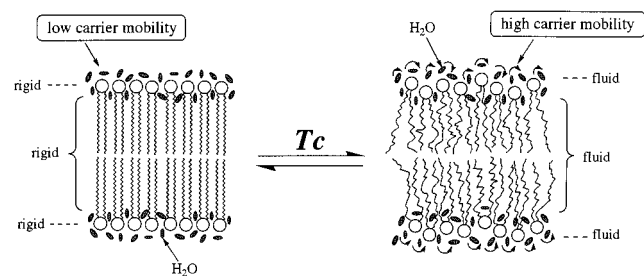


Figure 7. A schematic model for the phase transition of a bilayer membrane of $2C_{18}N^+PSS^-$ which explains the T_c -dependent impedance response.

amounts of water. All together, the following molecular mechanisms for the impedance responses of the bilayer film electrodes are proposed.

Both of the devices coated with the cast films of $2C_{18}N^+PSS^-$ and $2C_{14}DeaC_{11}N^+PSS^-$ show drastic T_c dependence on ac impedance. The QCM responses for these two lipid films, however, are quite different; i.e., QCM of $2C_{18}N^+PSS^-$ gives almost no T_c dependence whereas a $2C_{14}DeaC_{11}N^+PSS^-$ QCM resonator shows strong T_c dependence. These suggest that the molecular mechanisms for the ac impedance of these two electrode devices are not the same. The decrease in resistance of a $2C_{18}N^+PSS^-$ electrode at the phase transition region may be due to the enhancement of the ion-conducting mobility. The same mechanism can be proposed for the electrode devices modified with the films of $2C_{16}N^+PSS^-$ and $2C_{16}GluC_2N^+PSS^-$. Accompanying the bilayer phase transition, a change in conformational mobility at the hydrophilic head group of these bilayers occurs, which leads to a dramatic decrease in resistance. This behavior is schematically drawn in Figure 7.

On the other hand, for a $2C_{14}DeaC_{11}N^+PSS^-$ -coated electrode, both factor I and factor II would contribute to the dramatic decrease in resistance at the phase transition region. This is in sharp contrast with that for a $2C_{16}GluC_{11}N^+PSS^-$ film electrode, in which the device shows no T_c dependence. As we have described elsewhere,⁴² the carbonyl stretching bands of the amide of $2C_{16}GluC_{11}N^+PSS^-$ in the FTIR spectra is observed at $1649\text{--}1650\text{ cm}^{-1}$ at temperatures of $20\text{--}70\text{ }^\circ\text{C}$, indicating that this lipid forms hydrogen bonds at temperatures below and above the phase transition. Because of the hydrogen bonding, the conformational mobility of the hydrophilic head group of this lipid bilayer is expected to be rather rigid even at temperatures above T_c , which leads to T_c -independent impedance response. A cast film of $2C_{16}GluC_2N^+PSS^-$ shows the carbonyl stretching bands of the amide at $682\text{--}1682.4\text{ cm}^{-1}$ at temperatures of $20\text{--}70\text{ }^\circ\text{C}$ (data not shown), indicating that hydrogen bonding is not formed at temperatures below and above the phase transition. The results of FTIR show that the difference in the hydrogen-bonding mode leads to the different temperature-dependent impedance responses. Similarity in the T_c -dependent impedance responses between a $2C_{16}GluC_2N^+PSS^-$ electrode and $2C_nN^+PSS^-$ ($n = 16, 18$) electrodes is also understandable. Lipids $2C_nGlu(NH_3^+)PSS^-$ bearing primary ammonium head groups are expected to form hydrogen bonds with hydrated water molecules at the hydrophilic head groups, which would result in the T_c -independent impedance response. We like to emphasize that hydrogen bonding plays an important role for the impedance responses of the lipid-coated electrodes. Drawings depicting the impedance responses of bilayer film electrodes coated with films of $2C_{16}DeaC_{11}N^+PSS^-$ and $2C_{16}GluC_{11}N^+PSS^-$ are shown in Figure 8.

(b) Under Air Atmosphere without Relative Humidity Control. As we described briefly elsewhere, despite the existence of a phase transition, bilayer devices of $2C_{18}N^+PSS^-$ and $2C_{14}DeaC_{11}N^+PSS^-$ gave T_c -independent impedance re-

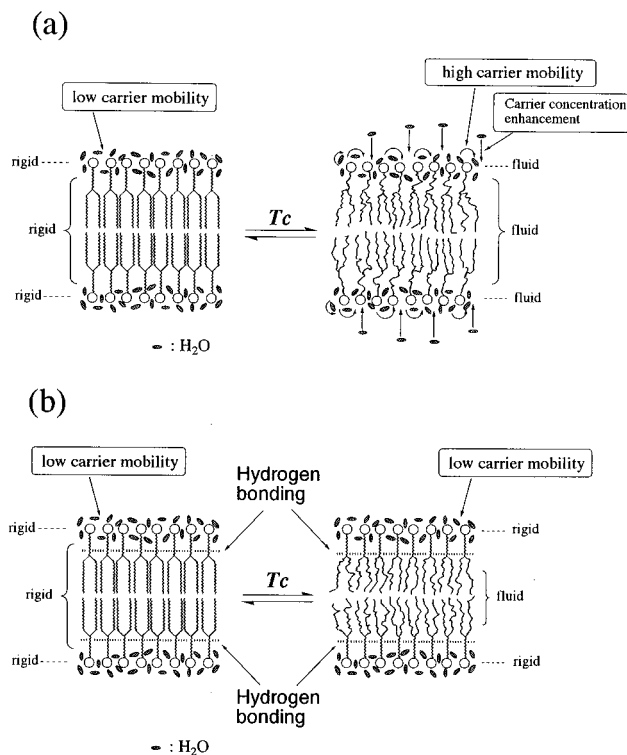


Figure 8. Proposed models for the phase transition of bilayer membranes of $2C_{14}DeaC_{11}N^+PSS^-$ (a) and $2C_{16}GluC_{11}N^+PSS^-$ (b) which explain the T_c -dependent and -independent impedance responses, respectively.

sponses under air atmosphere without relative humidity control. Similar results are obtained for the electrodes coated with the cast films of $2C_nGlu(NH_3^+)PSS^-$ ($n = 16, 18$), $2C_{16}GluC_mN^+PSS^-$ ($m = 2, 11$), in which the observed impedance responses are almost the same with that for an unmodified electrode. This indicates that the electrical signals are mainly derived from the capacity of the electrode surfaces, and the bilayer films on the electrode do not reflect the electrical signals. Two explanations are suggested for these results; (i) the mobility of the ion-conducting carriers in the lipid films does not accelerate accompanying bilayer phase transition, or (ii) carrier mobility enhancement coupled with phase transition is canceled by a decrease in the carrier concentration in the films. QCM measurements for these films on electrodes indicate that at lower temperatures heating causes the desorption of adsorbed water molecules from the films (Figure 5), which does not couple with the phase transition. Therefore, the latter possibility is negligible. Despite the fluidity enhancement of the bilayers at temperatures above T_c , the head groups of the films are still rigid, which results in very high impedance.

In contrast, an electrode coated with a cast film of $2C_{14}DeaC_{11}N^+PSS^-$ gives a clear phase transition-dependent impedance response which could be explained by the enhancement of the carrier mobility (factor I). Note again that the chemical structure of the lipids and their impedance responses are closely related. $2C_{14}DeaC_{11}N^+PSS^-$ is a lipid with the longer spacer chain which contains a tertiary amide bond; therefore, intermolecular hydrogen bonding in the lipid via the amide group is impossible. Hence, fluidity enhancement of the spacer chain coupled with the phase transition causes the conformational mobility change at the bilayer head groups, followed by the impedance change.

Conclusions

We have designed and fabricated lipid bilayer devices and examined their temperature-dependent ac impedance charac-

teristics under 75–50% relative humidity atmosphere and in usual air without relative humidity control. Under the relative humidity control atmosphere, the molecular mechanisms of the impedance responses of the bilayer modified electrical devices can be classified into following three types.

Type I: The phase transition of bilayer films on the electrodes influences impedance responses, and the change in impedance is derived from a change in mobility of the ion-conducting carrier. The devices modified with quarternary ammonium lipid with no spacer ($2C_{18}N^+PSS^-$, $2C_{16}N^+PSS^-$) and short spacer ($2C_{16}GluC_2N^+PSS^-$, $2C_{14}DeaC_2N^+PSS^-$) are classified as this type. The conformational mobility of the hydrophilic head group of the bilayers of these lipids is suggested to be rather flexible at temperatures above the phase transition temperatures.

Type II: The phase transition-dependent impedance responses is similar to those in type I. In addition to the change in mobility of the conducting carrier, a drastic increase in the adsorption of water to the hydrophilic head group of the lipid bilayer at the phase transition region is expected to contribute to the drastic impedance change. An electrode coated with the diethanolamine-based lipid with the long methylene spacer chain ($2C_{14}DeaC_{11}N^+PSS^-$) belongs to this type.

Type III: The phase transition does not influence the impedance responses. Primary ammonium lipids and the glutamate-based lipid with the long spacer chain are the lipids that show this type. In these lipid bilayer films, the conformational mobility of the bilayer head groups is maintained rigid even at temperatures above T_c , where assembled hydrophobic double long chains form a fluid bilayer phase.

Under air atmosphere without relative humidity control, only electrodes coated with $2C_{14}DeaC_{11}N^+PSS^-$ show phase transition-dependent impedance responses. Despite the existence of phase transition, no temperature dependence is observed in the electrical impedance of the devices using other lipid films as modifiers.

The strong chemical structure dependence on the impedance responses arises from the difference in the conformational mobility of the hydrophilic head groups which influence directly the mobility of the ion conducting carrier. Hydrogen bond formation between the lipids and/or lipids with water molecules plays an important role in the phase transition-dependent impedance responses of the lipid bilayer devices. This study opens possibilities for the development of lipid bilayer electrode devices whose properties can be regulated by the nature of self-assembled lipid bilayer membranes and for the use of lipid films as novel materials for "solid state electrochemistry".

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