UV and DSC Analyses of Suspensions of Mixtures of Phosphatidylcholine and a Fatty Acid Having a Diene Group

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Synopsis. The aqueous suspensions of the mixtures of a phosphatidylcholine (1,2-Bis[(2*E*,4*E*)-2,4-octadecadienoyl]-sn-glycero-3-phosphocholine (**1b**) and 1,2-dioctadecanoyl-sn-glycero-3-phosphocholine (**1a**)) with a fatty acid ((2*E*,4*E*)-2,4-octadecadienoic acid and octadecanoic acid), having a diene chromophore were analyzed by DSC and UV measurements. The phase transition was dependent on the composition of the lipid mixtures. The UV spectral change of the diene chromophore of a fatty acid for the **1a** mixtures corresponded well with the phase transition determined by DSC analyses, but not for the **1b** mixtures.

Liposomes composed of phosphatidylcholine and other lipids have been studied as biological membrane models.[1] However, liposomes are often unstable and tend to aggregate and precipitate during storage. To overcome these problems, charged compounds such as fatty acid, phosphatidic acid, dihexadecyl hydrogenphosphate, and octadecylamine, which can control the surface potential of liposomes, are incorporated in phosphatidylcholine bilayers. Among them, fatty acids are interesting because they are one of the components of biological membranes and are present at high concentrations in certain membranes: for example, 8% in rat liver plasma.[2] They control various membrane-mediated cellar functions,[3-6] such as the enzyme activity, membrane fluidity, permeability, and fusion. A mixture of fatty acid and phosphatidylcholine is a simple model membrane used for understanding the roles of fatty acids in biological membranes.

Studies on the mixtures of a fatty acid and a phosphatidylcholine having saturated acyl chains have revealed:[7-10] (1) fatty acids eliminate the pretransition; (2) mixing is good at the 1:2 molar ratio of saturated phosphatidylcholine and fatty acids having the same carbon numbers (1:2 complex formation); (3) the thermal phase transition is a lamellar-to-nonlamellar phase, and (4) the apparent pKa of fatty acid in the lipid bilayer is surprisingly large.

A phase-transition analysis of lipid bilayer membranes is normally carried out by DSC, NMR, and ESR methods. In addition to these types of measurements, a simple method using UV measurements has been reported: for example, on lamellar aggregates of single-chain amphiphiles containing a chromophore, such as an azobenzene residue.[11] The shift of λ_{max} of the chromophore is useful for a phase-transition analysis. A phosphatidylcholine having polymerizable groups (dienes) in acyl chains is also used as a UV probe of bilayer transitions.[12]

We describe here an application of a diene-containing fatty acid to bilayer transition analysis of fatty acid–phosphatidylcholine mixtures by a UV measurement, and compare the results with those of a DSC measurement.

Experimental

Materials. 1,2-Bis[(2*E*,4*E*)-2,4-octadecadienoyl]-sn-glycero-3-phosphocholine (**1b**), (2*E*,4*E*)-2,4-octadecadienoic acid (**2b**), and 1,2-dioctadecanoyl-sn-glycero-3-phosphocholine (**1a**) were purchased from Nippon Oil & Fats Co., Ltd. 1-Hexadecanoyl-(2*E*,4*E*)-2,4-octadecadienoyl-sn-glycero-3-phosphocholine (**1c**) was prepared.[13] The purity was confirmed by thin-layer chromatography on silica gel. Octadecanoic acid was purchased from Kishida Chem. Co., Ltd. **2b** was recrystallized once from hexane. Benzene was distilled from calcium hydride. Phosphatidylcholine and fatty acid mixtures were prepared by freeze-drying from dry benzene.

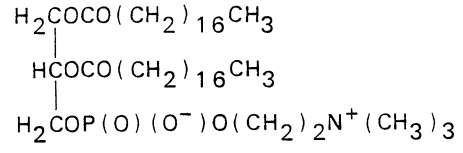
Lipid suspensions were prepared by adding a prescribed amount of distilled water to the freeze-dried powder. They were suspended with glass beads (diameter: 2—3 mm) on a Vortex mixer at a temperature above the transition temperature under a nitrogen atmosphere for 15 min.

DSC was performed by using DSC 10 (Seiko I&E). Samples were placed into silver microtubes, and measurements were run with temperature scanning at 2 °C min^{-1} under a nitrogen atmosphere.

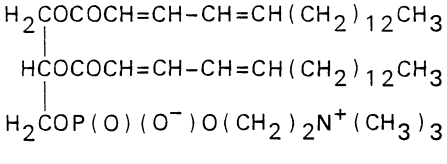
The temperature-dependence of the absorption maximum corresponding to the diene groups of lipid suspension was analyzed in a quartz cell (path-length: 10 mm) with UV spectrometry (Shimadzu MPS-2000) with a heating rate of 1 °C min^{-1}.

Results and Discussion

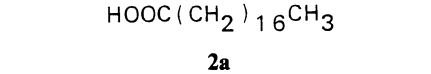
A saturated phospholipid (1,2-dioctadecanoyl-sn-glycero-3-phosphocholine (**1a**)) and an unsaturated one (1,2-bis-[(2*E*,4*E*)-2,4-octadecadienoyl]-sn-glycero-3-phosphocholine (**1b**)) were used and mixed with a saturated fatty



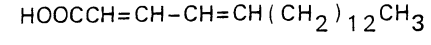
1a



1b



2a



2b

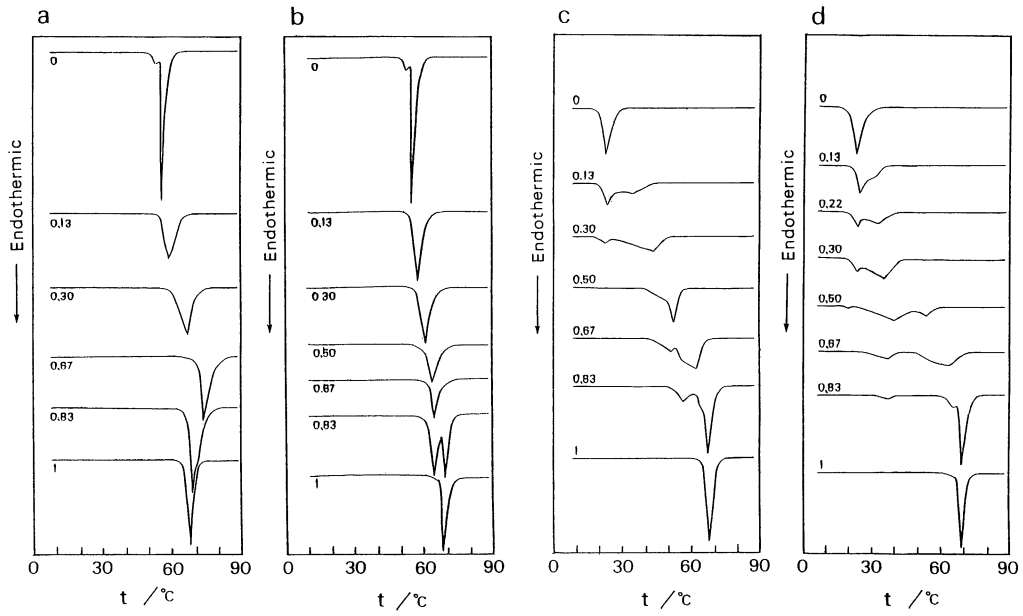


Fig. 1. DSC thermograms for the various mixtures of the phospholipids and the fatty acids with different molar ratios. (a): **1a**/**2a**, (b): **1a**/**2b**, (c): **1b**/**2a**, (d): **1b**/**2b**. Mole fraction of fatty acid is indicated beside each thermogram.

acid (octadecanoic acid (**2a**)) or an unsaturated one ((2*E*,4*E*)-2,4-octadecadienoic acid (**2b**)). All of the compounds have acyl chains of the same carbon numbers (18), since it has been reported that mixing strongly depends on a combination of the chain length of a phospholipid acyl chain and the fatty acid.[8] The phase-transition behaviors were analyzed by DSC measurements and by observing the UV spectral change of the diene chromophores upon increasing the temperature at a constant rate.

The DSC analyses were performed on lipid suspensions packed in silver microtubes, but not on aluminum ones, due to the presence of an acid compound. Figure 1 shows DSC curves for various mixtures of the phospholipids and the fatty acids with different molar ratios. As shown in Fig. 1b, the mixture of **1a** and **2b** showed a single peak upon increasing the fraction of the fatty acid component up to 0.67 and the transition temperature increased, as in the case of mixtures of **1a** and **2a** (Fig. 1a). This means that two components mix well under mole fractions of the fatty acid up to 0.67. An increase of the fatty acid component to more than 0.67 causes the formation of a domain of excess fatty acid, in addition to a homogeneously mixed domain of **1a** and **2b** with a molar ratio of 1:2. This can be seen by the appearance of two peaks.

Apart from these two mixtures, those related to unsaturated phospholipid **1b** showed complexed behaviors, as indicated in Figs. 1c and 1d. The mixtures showed three transitions in the DSC curves with increasing the mole fraction of the fatty acid component. This indicates that the good mixing found in mixtures of the saturated phospholipid **1a** with fatty acids did not occur in those of the unsaturated phospholipid **1b** mixtures. Figures 1c and 1d indicate that the phase transitions at lower temperatures change to transitions at higher temperatures with an increase in the fatty acid fraction.

At mole fractions of a fatty acid less than 0.3 the mixture showed two peaks, corresponding to the presence of two blocks: one comprising pure **1b**, and the other comprising **1b** and a fatty acid with a molar ratio of 1.6—2.0:1.0. The molar ratio could be calculated from a decrease in the endothermic heat change, compared to that of a pure **1b** suspension. Upon further increases in the mole fraction of a fatty acid component up to 0.67, the first intermediate mixture changed to the second and third mixtures along with a concomitant decrease of **1b**, itself, and the first intermediate mixture. At a much higher content of a fatty acid, the **1b** mixtures form a block of excess fatty acid; this was confirmed by the appearance of an endothermic peak of pure fatty acid **2a** or **2b**, as in the cases of mixtures of **1a** and a fatty acid. Therefore, the third peak may be responsible for the 1:2 homogeneous mixtures that are normally found in the saturated lipid systems described above.

It has been reported that the UV chromophores of phospholipids and synthetic surfactants can be used to determine the phase-transition temperatures of their molecular assemblies.[11,12] The absorption maxima change sharply at the transition temperature. Here, we tried to use the diene chromophore of fatty acid **2b** to analyze mixtures with phospholipids **1a** or **1b**.

Mixtures of **1a**/**2b** gave a spectral change in the UV region with the temperature. Figure 2 summarizes the changes in the absorption maxima of various mixtures of **1a** and **2b** with increased temperature. The changes were sharp in the mixtures (the mole fraction of **2b** up to 0.67) and the phase-transition temperatures could be calculated. Mixtures of **1b** with a fatty acid had only one transition.

Table 1. Comparison of Transiton Temperatures Determined by DSC and UV Measurements[a)]

Lipid mixture	Transition temperature (T_c)/°C							
	Mole fraction of fatty acid							
	0.0	0.13	0.22	0.30	0.50	0.67	0.83	1.00
1a/2a	55	55	—	61	—	72	66	65
1a/2a (2b)	(54)	(57)	—	(62)	(68)	(69)	—	—
1a/2b	55	54	—	58	61	62	62, 69	68
	(54)	(54)	—	(58)	(63)	(63)	—	—
1b/2a	19	17, 26	—	17, 24	49	43, 52	52, 65	65
	(18)	(19)	(22)	(27)	(36)	(35)	—	—
1b/2b	19	21	20, 25	18, 24	16, 26, 47	27, 49	25, 62, 68	68
	(18)	(21)	(21)	(23)	(31)	(27)	—	—

a) The values in the parentheses mean transition temperatures determined by UV measurements.

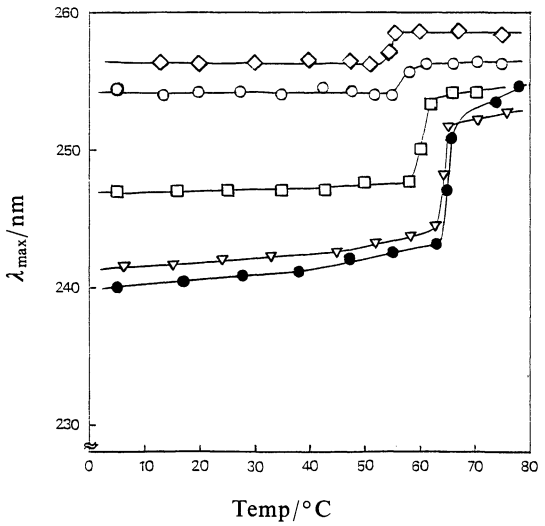


Fig. 2. Temperature dependence of the absorption maxima for diene groups in various mixtures of **1a** and **2b**. Mole fraction of **2b**: 0 (◇), 0.13 (○), 0.30 (□), 0.50 (▽), 0.67 (●).

Table 1 summarizes the transition temperatures (T_c) of various lipid mixtures determined by DSC and UV measurements. For **1a/2b** and **1a/2a** mixtures, both measurements gave the same transition temperatures, respectively, but not for the mixtures of **1b** with a fatty acid. The results show that care must be taken when using a UV probe for the determination of phase-transition temperatures.

These results indicate for the miscibility of phosphatidyl choline with fatty acid that: (1) the (2*E*,4*E*)-diene group containing a fatty acid is capable of mixing well with saturated phosphatidylcholine as does a saturated fatty acid; (2) the presence of the (2*E*,4*E*)-diene moiety in diacyl chains of phosphatidylcholine interferes with the homogeneous mixing with a fatty acid. In addition to these results, a diene-containing phospholipid, 1-hexadecanoyl-2-[(2*E*,4*E*)-2,4-octadecadienoyl]-sn-glycero-3-phosphocholine (**1c**, T_c: 24 °C),[12)] gave a single transition peak (T_c: 57 °C) when it was mixed with **2a** with a molar ratio of 1:2. This indicates that the (2*E*,4*E*)-2,4-octadecadienoyl group at the 2-position of the glycerol backbone is not responsible for non-homogeneous mixing of **1b** with a fatty acid.

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