Diene-Containing Lipids as Probes for Phase-Transition Behavior of Lipids in Liposomes[1)]

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The absorption maximum (λ_{max}) of 1,2-bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC), dispersed in an aqueous medium as liposomes, showed blue shift from 256.8 nm to 241.8 nm when these liposomes were cooled down from liquid crystalline state to gel state. The blue shift is explained to be due to stronger interaction between diene groups at gel state. This diene-containing lipid is examined to be an excellent membrane probe because it is typical phospholipid and miscible with several lipids. Furthermore, the dienoyl groups are known to be settled in the definite positions in bilayer membrane by suitable chemical modification. Phase transition behaviors of various 2,4-octadecadienoyl-containing phosphorylcholines as analogs for DODPC have also been investigated. Although they had similar spectral shifts at gel-to-liquid crystalline phase-transition temperature, their phase-transition temperatures and magnitude of λ_{max} shifts were revealed to be related directly and therefore be governed by the different packing of the diene groups in bilayer membrane. DODPC was mixed with 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine, or cholesterol, and phase behaviors of these mixed liposomes were also examined. Phase-transition of the mixed liposomes was detected easily by the shift of its λ_{max} with high sensitivity. Results were quite comparable to those obtained from other measurements, such as DSC, NMR, and so on.

We have already clarified various membrane properties through polymerization behaviors of polymerizable lipids as liposomes.[2-11)] 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC), which contains one diene group in every acyl chain, is effectively polymerized as liposomes with radical initiators. The polymerization profile was revealed to deeply depend on the characteristics of the applied radical initiators.[3,6)] However this monomeric lipid had polymerizable groups in the same 2,4-position in the 1- and 2-acyl chains, polymerization of diene groups in the 1-acyl chains was selectively initiated by azobis(isobutyronitrile) (AIBN) which was incorporated into a hydrophobic region of the bilayer membrane. On the contrary, the diene groups in the 2-acyl chains were polymerized by the addition of azobis(2-amidinopropane)dihydrochloride (AAPD), water-soluble radical initiators, suggesting that the diene groups in the 2-acyl chains were faced an aqueous phase.[3)] We also developed a new polymerization technique with which liposomes could be polymerized at any temperature.[10)] Polymerization was performed with AIBN and/or AAPD as photosensitizers and irradiating the UV-light of 360 nm at temperatures below or above the gel-to-liquid crystalline phase-transition temperature (T_c). Water-soluble AAPD penetrated lipid membrane at temperatures above T_c but not below.[10)] We prepared liposomes which possessed polymerized outer- or inner-half of bilayer with advantageous characteristics of impermeability of AAPD below the T_c.[10)] Change of microviscosity of lipid membrane at T_c was also analyzed with water-insoluble AIBN incorporated in hydrophobic region of the membrane. Initiation efficiency of the polymerization was affected by membrane microviscosity.[10)] We found that UV spectral shift for polymerizable lipids was attributed to the changes in lipid packing in the membrane. Here we describe this shift behavior in detail.

Experimental

Materials. 1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC) was purchased from Nippon Oil & Fats Co., Ltd. 1-Palmitoyl-2-(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylchloline (POPC) was a gift from Toyo Soda Co., Ltd. 1,3-Bis(2,4-octadecadienoyl)-*rac*-glycero-2-phosphorylcholine (1,3-DPC) was synthesized according to the method as reported previously.[11)] 1,2-Dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) and cholesterol were purchased from Sigma. The purity of lipids was confirmed with thin-layer chromatography (Merck, silica-gel plates) with chloroform/methanol/water (65:35:5 by vol) to show a single spot.[3)]

***rac*-1-*O*-(2,4-Octadecadienoyl)-2-*O*-octadecylglycerol:** 2-Phenyl-1,3-dioxan-5-ol was obtained according to the procedure of Hibbert et al.[12)] The product was then allowed to react with 1-bromooctadecane yielding 2-octadecyloxy-1,3-propanediol according to the procedures of Arnold.[13)] 2-Octadecyloxy-1,3-propanediol (5.00 g, 14.5 mM; 1 M=1 mol dm^{-3}) and 0.95 g pyridine (12 mM) were dissolved into 250 ml dry dichloromethane, and added dropwise to 40 ml dry dichloromethane containing 2,4-octadecadienoyl chloride (10 mM) at room temperature. After addition of 4-(dimethylamino)pyridine, the mixture was stirred overnight at room temperature under nitrogen atmosphere. The mixture was washed successively with 1M HCl aqueous solution, then with saturated NaCl aqueous solution. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The purification of the residual product was carried out by passing through a silica gel column with benzene/ether (10/1 by vol.) as an eluent. The desired fraction was collected and solvents were removed under reduced pressure. *rac*-1-*O*-(2,4-Octadecadienoyl)-2-*O*-octadecylglycerol (3.74 g) was obtained with 62% yield. R_f=0.25 (benzene/

ether=9/1 v/v); MS: 606 (M^+); IR in cm^{-1} (KBr): 1720 (ester carbonyl), 1640, 1620 (diene), 3400 cm^{-1} (OH); Elementaly Anal. (calcd value for $C_{39}H_{74}O_4$ in parenthesis): C 77.17 (77.17), H 12.87 (12.29%); ^{13}C NMR ($CDCl_3$, TMS) (in ppm): 62.7, 62.0, 77.8 (glycerol backbone), 70.6 (-C-O-C-), 118.5, 128.3, 145.4, 145.9 (diene), 167.2 (ester carbonyl); mp: 44.5 °C.

1-*O*-(2,4-Octadecadienoyl)-2-*O*-octadecyl-*rac*-glycero-3-phosphorylcholine (OOPC): *rac*-1-*O*-(2,4-Octadecadienoyl)-2-*O*-octadecylglycerol (3.60 g, 5.93 mM) and trimethylamine (0.72 g, 7.1 mM) were dissolved in 50 ml of dry benzene. 2-Chloro-1,3,2-dioxophosphorane 2-oxide (1.01 g, 7.1 mM) in 5 ml of dry benzene was slowly added to the solution at 10 °C. After the mixture was stirred at room temperature in the dark for 12 h, formed precipitates were removed by filtration. The filtrate was evaporated and the residue was dried in vacuo. It was allowed to react with dry trimethylamine (20 ml) in the mixture of *N,N*-dimethylformamide and benzene (15 ml/30 ml) in a pressure bottle at 60 °C for 4 h. Distilled acetone (0.2 ml) was then added to the mixture, the precipitate formed was collected, washed with dehydrated acetone and dried in vacuo. The residue was purified by passing through the zwitterion-type ion-exchange chromatography (Bio-Rad, AG501-x8(D)) with chloroform/methanol (20 ml/20 ml) as an eluent. The fractions having the same R_f value as that of egg york lecithin were collected. Solvent was removed and the residual product was lyophilized from dry benzene/dry methanol (5/1 v/v) to give 2.70 g of 1-*O*-(2,4-octadecadienoyl)-2-*O*-octadecyl-*rac*-glycero-3-phosphorylcholine (OOPC): yield 59%; FAB-MS: 772 (M+1); elementary anal. (calcd value for $C_{44}H_{86}NO_7P$ in parenthesis): N: 1.70 (1.81%); IR in cm^{-1} (KBr): 1710 (ester carbonyl), 1640, 1620 (diene), 1250 (P=O), 1090 and 970 (phosphorylcholine); ^{13}C NMR ($CDCl_3$, TMS) (in ppm): 54.3 (choline methyl), 64.4, 76.6 (glycerol backbone), 59.5, 66.3 (choline ethyl), 70.6 (-C-O-C-) 118.8, 145.6, 128.2, 145.1 (diene), 167.1 (ester carbonyl).

Methods. A total of 0.20 g of lipids was dissolved in dehydrated chloroform and was slowly evaporated in a rotating sample tube to prepare thin films on the inner surface of the tube, then 20 ml of distilled water was added. Small unilamellar liposome suspension was prepared by sonication (Tip type, Tomy Seiko UR-200P) at 60 W for 20 min under nitrogen atmosphere.[4] As disordered lipid packing perturbs accurate measurement on the characteristics of the oriented lipids in liposomes, freshly prepared liposomes were incubated at 8 °C to increase liposome size and to minimize the disordered molecular packing through liposomal fusion.[8,14] Accurately diluted liposome suspension was put into quartz cell, and the absorption maximum corresponding to the diene groups was analyzed with UV spectrometry (Shimadzu MPS-2000) under heating or cooling (1.0 °C min^{-1}).

Results

1,2-Bis(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (DODPC), which possesses 2,4-diene groups in both 1- and 2-acyl chains, shows the absorption maximum (λ_{max}) at 262.3 nm in chloroform solution at 25 °C. The λ_{max} was almost independent of temperature as shown in Fig. 1. When DODPC lipids were dispersed into water by sonication, they assembled to form liposomes. Though the λ_{max} of DODPC liposomes was found at 256.8 nm at 25 °C, a blue shift from 256.8 nm to 241.8 nm was observed when the suspension was cooled from 19 °C to 12 °C as also shown in Fig. 1. The maximum λ_{max} shift was found at about 16 °C corresponding to the gel-to-liquid crystalline phase-transition temperature (T_c) of DODPC (18 °C: with DSC).[8] The T_c is known to be influenced by liposome size especially for small unilamellar liposomes,[15] and λ_{max} shift behavior was observed in related to the liposome size (data not shown here). Small unilamellar liposomes (SUVs) prepared with

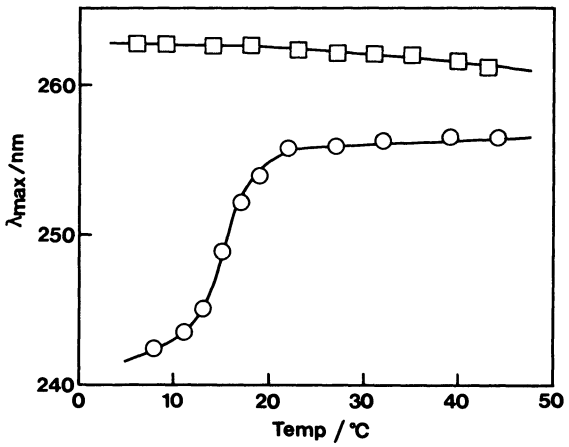

Fig. 1. Temperature dependence of wavelength at the absorption maximum for diene groups of DODPC. □: in chloroform. ○ in liposomes.

Table 1. λ_{max} and ε_{max} of Various Diene-Containing Lipid Liposomes at Temperatures below and above the Phase-Transition Temperature (T_c)

Lipid[a]	λ_{max}/nm		$\varepsilon_{max}\times10^{-4}$		T_{max}/°C	T_c/°C
	below T_c	above T_c	below T_c	above T_c		
DODPC	241.8	256.8	2.95	3.40	16.0	18.9
1,3-DPC	246.0	257.0	2.07	2.75	24.0	27.3
	(241.5	257.0	—	—	25.0)[b]	—
	(236.8	257.0	—	—	20.0)[c]	—
POPC	253.0	259.3	1.27	1.43	28.0	29.2
OOPC	245.0	255.0	1.48	1.70	39.0	40.1

a) Liposomes incubated at 8 °C for 12 h were used. b) Measured for the liposomes incubated for 7 days with elevating temperature. c) With lowering temperature.

sonication method were incubated at temperatures below T_c to prepare large unilamellar liposomes (LUVs) through liposomal fusion.[8,16] The LUVs with average radius of more than 35 nm are known to show almost the same phase-transition behavior.[8] The following measurements were therefore performed for LUVs with average radius of more than 35 nm. Those liposomes could be prepared by incubation at 8 °C for 12 h.

Spectral characteristics of other phospholipids having 2,4-octadecadienoyl group were also examined and summarized in Table 1. The temperature (T_{max}) which gave the maximum value of $d\lambda_{max}/dT$ was compared with a gel-to-liquid crystalline phase-transition temperature (T_c) obtained from DSC measurement. The blue shift of λ_{max} occurred at gel-to-liquid crystalline phase-transition temperature. The temperature dependence on the λ_{max} was normalized for all diene-containing lipids by drawing the relationship between $T-T_{max}$ and λ_{max} as summarized in Fig. 2. The absorption maxima of 1-palmitoyl-2-(2,4-octadecadienoyl)-*sn*-glycero-3-phosphorylcholine (POPC)[7] and 1-*O*-(2,4-octadecadienoyl)-2-*O*-octadecyl-*rac*-glycero-3-phosphorylcholine (OOPC) were measured in the temperature range from 5 °C to 60 °C. Though POPC or OOPC had one diene group in either 2- or 1-acyl chain, respectively, their λ_{max} shift behavior was quite different. In case of POPC, the λ_{max} shifted gradually from 253.0 nm to 259.3 nm on heating. The maximum shift was observed at 28 °C. While, OOPC showed a drastic shift from 245.0 nm to 255.5 nm at 39 °C. 1,3-Bis(2,4-octadecadienoyl)-*rac*-glycero-2-phosphorylcholine (1,3-DPC), an isomer of DODPC,[9] has 2,4-diene groups in 1- and 3-acyl chains. This 1,3-DPC also showed the λ_{max} shift from 246.0 nm to 257.0 nm at around 24 °C. However, 1,3-DPC liposomes were semistable and slowly changed their structure within a few days. When 1,3-DPC liposomes were incubated at 8 °C for 7 days, opalescent liposome dispersion was turbid indicating aggregation and fusion. In this system, λ_{max} changed from 241.5 nm to 257.0 nm (T_{max}=25 °C) with elevating temperature and from 257.0 nm to 236.8 nm (T_{max}=20 °C) with lowering temperature showing a hysteresis. This result was unique for 1,3-DPC liposomes.

The molar extinction coefficients (ε_{max}) of diene-containing lipid derivatives are also summarized in Table 1. ε_{max} for all lipids increased a little at temperatures above their phase-transition temperatures. As expected from the number of diene groups in one lipid molecule. ε_{max} of OOPC was almost identical as that of POPC and half of that of DODPC regardless of temperature.

The λ_{max} shift reflects the phase-transition temperature of diene-containing lipid liposomes. The ability of these lipids was also applied to analyze the phase-transition behavior of natural lipid assemblies. The λ_{max} shift for DODPC/DPPC (1:20 by mol) mixed liposomes was observed at 40 °C as shown in Fig. 3, corresponding to the gel-to-liquid crystalline phase-

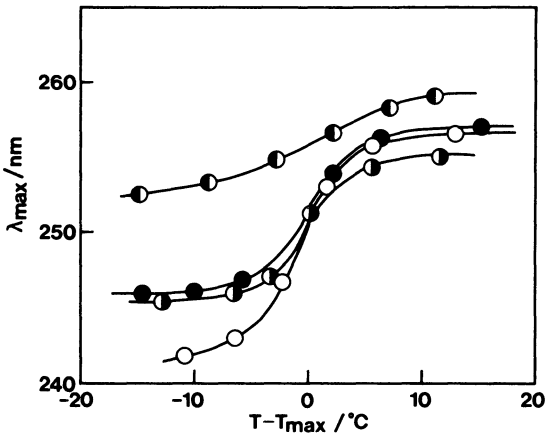

Fig. 2. Relationship betweeen $T-T_{max}$ and wavelength at the absorption maximum of DODPC (○), 1,3-DPC (●), OOPC (◑), and POPC (◐).

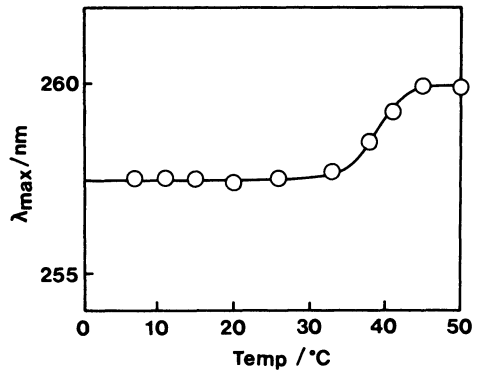

Fig. 3. Temperature dependence of wavelength at the absorption maximum of DODPC in DPPC/DODPC (20:1) mixed liposomes.

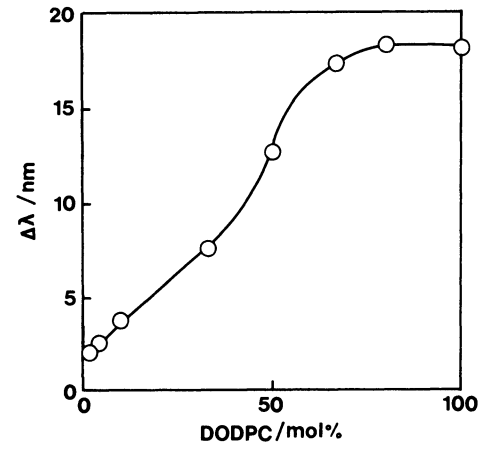

Fig. 4. Effect of DODPC mole fraction on the difference ($\Delta\lambda$) of wavelength between at the absorption maximum of DPPC/DODPC mixed liposomes at 5 °C and 260.0 nm. $\Delta\lambda$ at 0% of DODPC mole fraction corresponded to 258.5 nm.

transition temperature of DPPC, but not that of DODPC. The shift from 260.0 nm to 257.5 nm was different from that of DODPC (256.8 nm to 241.8 nm). This shift (2.5 nm) was very small compared with that of pure DODPC liposomes (15.0 nm). It is important to investigate λ_{max} shift behavior against composition of mixed liposomes. When DODPC fraction decreased, λ_{max} at temperatures below T_c significantly red-shifted while λ_{max} above T_c gradually saturated to 260.0 nm. The wavelength at 260.0 nm was considered to be limited value of longer wavelength. Differences ($\Delta\lambda$) between λ_{max} at gel state and that 260.0 nm against DODPC mole fraction were shown in Fig. 4. Since λ_{max} at 0% of DODPC mole fraction at gel state was 258.5 nm, the $\Delta\lambda$ was 1.5 nm. This increased with the DODPC composition and saturated to 18.2 nm when DODPC fraction exceeded 80%. In this case, T_{max} also reached the T_c of the pure DODPC liposomes. Furthermore, temperature range, at which the λ_{max} shift occurred, changed according to the composition of DODPC/DPPC mixed liposomes. There were two break points in the slope of temperature dependence of λ_{max}, namely, the onset and the offset blue shift. In mixed liposome system, temperatures of these two break points were significantly different. They were plotted against the molar ratio of DODPC to obtain an equilibrium phase diagram of DPPC and DODPC mixed liposome as shown in Fig. 5.[17] The temperature (T_f) of the onset blue shift drew the fluidus curve while the temperature (T_s) of the offset point did the solidus curve.

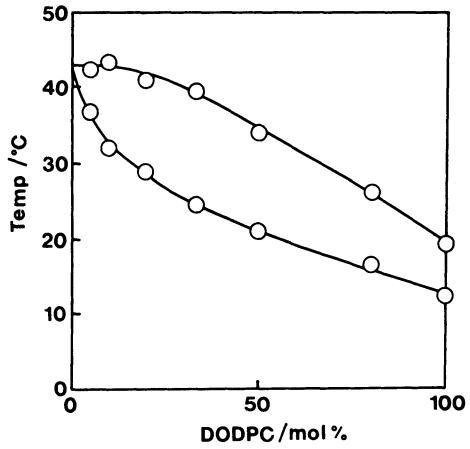

Fig. 5. Phase diagram of DODPC/DPPC mixed liposomes obtained from λ_{max} shift behavior. Upper curve was fluidus curve made of T_f and lower one was solidus curve made of T_s.

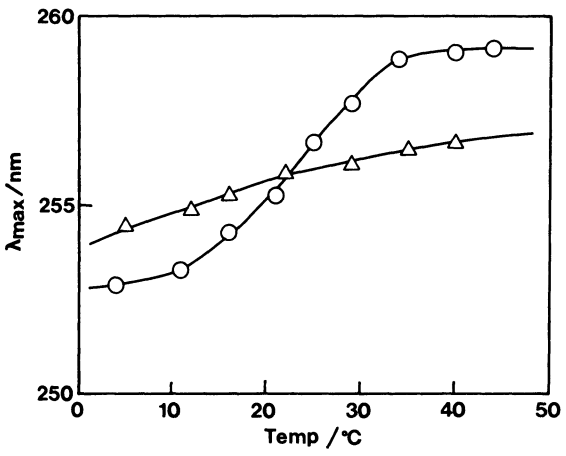

Fig. 6. Temperature dependence of the wavelength at the absorption maximum for diene groups of POPC (○) and POPC/cholesterol (1:1) mixed liposomes (△).

Effect of cholesterol on the phase-transition behavior was also analyzed with the λ_{max} shift. The λ_{max} for POPC/cholesterol (1:1 by mol) mixed liposome increased slightly from 254.2 nm to 256.7 nm when temperature was raised from 5 °C to 40 °C. The slope was smooth and continuous, and no distinct transition was found as shown in Fig. 6. This phenomenon can also be discussed in related to the well-known effect, so-called "Cholesterol Effect".[18,19]

Discussion

All diene-containing phospholipids analyzed here have at least one 2,4-dienoyl group in their acyl chains, and their absorption maximum was 262.3 nm in chloroform solution regardless of their structure. There was no temperature dependence on the λ_{max} in such a molecularly-dispersed homogeneous solution. Once these lipids formed molecular assemblies such as liposomes in an aqueous medium, the absorption maximum was observed at about 257 nm at temperatures above the gel-to-liquid crystalline phase-transition temperature (T_c) of corresponding lipid liposomes as shown in Table 1. All of diene-containing lipid liposomes showed blue shift of λ_{max} at temperatures below the T_c. The magnitude of λ_{max} shift at T_c was revealed to strongly depend on the chemical environment, acyl chain packing and conformation of dienoyl groups. Following three reasons can be presented to explain the blue shift of their λ_{max}.

(1) A structural change of conjugated dienoyl system may be induced at gel-to-liquid crystalline phase-transition temperature. If energy levels of electron orbitals for dienoyl group were changed by its structural change, the shift might occur.

(2) The λ_{max} for dienoyl groups is sensitive to solvent polarity. If polarity around dienoyl groups at temperatures below T_c was different from that above T_c because location of water molecules in bilayer membrane would change at T_c, the λ_{max} might therefore shift.

(3) The shifts in λ_{max} should be caused by the interaction of dienoyl groups in the lipid bilayer. In liquid-crystalline state, segmental motion of dienoyl groups is relatively high and they have more freedom

than those in the gel state. DODPC as liposomes in liquid-crystalline state showed a similar λ_{max} to that in homogeneous solution e.g. in methanol. Diene groups in gel state are oriented more regularly and interacted (stacked) with each other. It should therefore cause the blue shift of λ_{max}.[20]

To clarify the mean factor for this shift behavior, it is important to analyze the spectral characteristics for the diene group in the mixed bilayer membrane, where a little amount of diene-containing lipids is mixed with saturated normal lipids. In case of DPPC/DODPC (20:1 by mol) mixed liposomes (Fig. 3), the gel-to-liquid crystalline phase-transition temperature is almost the same as that of pure DPPC liposomes. However, the difference between λ_{max} below and above the T_c was much smaller than that of pure DODPC liposomes. If the λ_{max} shift was caused by the first and/or second reason, the mixed liposomes should have the same shift as that of the pure diene-containing lipid liposomes. In mixed liposome system, the wavelength of the absorption maxima at gel and liquid crystalline state approached to 258.5 nm and 260.0 nm, respectively in accordance with the increase of DPPC ratio as shown in Fig. 4. The wavelength of 258.5 nm was considered to be attributed to molecularly dispersed DODPC which had only intramolecular interaction of diene groups because the λ_{max} for not interacted diene group in bilayer membrane would be 260.0 nm. It was also supported from the result that the λ_{max} of molecularly-dispersed monodiene-type POPC was found at 260.0 nm at temperature below the T_c. The shift was therefore attributed to the direct interaction with diene groups of lipids. The third one is therefore concluded to be the most suitable explanation for this λ_{max} shift.

Generally, chromophores in stacked state show specific spectra reflecting their interaction. According to the molecular exciton model proposed by Kasha et al.,[13] direction and degree of λ_{max} shift were decided by inclination and distance among the transition dipoles. In bilayer membrane system, λ_{max} of chromophores, such as diene groups which constitute part of chains, is predicted to shift to a shorter wavelength because hydrocarbon chains extend perpendicularly to the membrane plane. The large blue shift however occurred only in gel state of the bilayer membrane as shown in Fig. 1. That was because the diene groups, packed closely with a good orientation manner in gel state, were disordered in liquid crystalline state. The λ_{max} shifted and approached to that of the molecularly dispersed diene-containing lipids (260.0 nm) at temperatures above T_c.

The λ_{max} shift for LUVs of some diene-containing lipids also reflected their structural characteristics as shown in Fig. 2. In case of DODPC, stacking of diene groups in the 1-acyl chains should be unequivalent with that in the 2-acyl chains because of their unsymmetrical chain packing in bilayer membrane.[4] The stacking between diene groups in the 1- and 2-acyl chains however also exists as described above. The comparison of the stacking of diene groups in the 1-acyl chains and that in the 2-acyl chains should be carried out with corresponding monodiene type lipids such as OOPC and POPC, respectively. λ_{max} of POPC was entirely longer than that of OOPC. This was attributed to the different orientation of diene groups of POPC to those of OOPC. Since diene groups in the 1-acyl chains of OOPC oriented perpendicularly to the membrane plane, stronger interaction was easily expected between these diene groups. While, those of POPC oriented with large inclination, and lots of dipoles aligned in the opposite direction with the same inclination. Interaction of 2,4-diene groups in the 2-acyl chains should therefore be very weak, leading to smaller blue shift of POPC than OOPC as shown in Fig. 2. The different packing of diene groups in the 1- and 2-acyl chains has already been shown from X-ray diffraction and D-NMR analyses on natural phospholipids.[21,22] It was also supported by our results on the selective polymerization of the 1- and 2-acyl chains by the addition of either water-soluble or -insoluble radical initiators.[4,6] Diene-groups in the 2-acyl chains of DODPC faced an aqueous phase while those in the 1-acyl chains located in a hydrophobic phase in consequence of perpendicular orientation of glycerol moieties to the liposome membrane plane. Diene-groups in the 2-acyl chains were therefore selectively polymerized with water-soluble radical initiators such as AAPD. POPC liposomes were accordingly polymerized with only water-soluble radical initiators.[7]

The spectral changes of azobenzene moieties of the assembled single chain amphiphiles have also been reported.[23] These amphiphiles are known to form bilayer structures in an aqueous medium, and the blue shift of λ_{max} was also found below the phase-transition temperature. This phenomenon was explained systematically with this exciton model in the one dimension because of card-like packing of azobenzene moieties in the membrane, and the λ_{max} shift was referred to the results from X-ray analyses.[24,25] In our system, the λ_{max} shift of diene groups was however smaller than those of azobenzene system. In the present paper, we discussed here the shift behavior with the two dimensional exciton model, i.e., molecular lamellar exciton model because diene group could be regarded as arrow.[26] We have to take the interaction of dipoles in two dimension into consideration. The treatment of interaction of dipoles with inclination to membrane plane is complicated because dipoles with the same inclination do not orient with the same direction in membrane plane.

Recently, O'Brien et al.[27] reported the shift of the absorption maxima for diene-containing lipids. They mentioned that the evidence for aggregation of diene

groups of DODPC and POPC in bilayer membrane could not be obtained from those lipids at 22 °C because those liposomes showed not so clear λ_{max} shift at that temperature. They should have taken the phase-transition temperature into account. The λ_{max} of around 260 nm for DODPC at 22 °C surely shifted to 243 nm at temperatures lower than the phase-transition temperature (16 °C) as shown in Fig. 1. In case of POPC, small shift of λ_{max} from 260.0 nm even below the phase-transition temperature (22 °C) occurred due to the different packing manner of diene groups in the 2-acyl chains as described above.

Temperature dependence of the absorption maxima was concluded to be essentially related to the lipid structure as shown in Table 1. Lipids having the same hydrophobic hydrocarbon number can be aligned in the following sequence by their T_c;

$$\text{DODPC}(18.9\,^\circ\text{C}) < \text{1,3-DPC}(27.3\,^\circ\text{C}) < \text{OOPC}(40.1\,^\circ\text{C}) < \text{DSPC}(55.0\,^\circ\text{C})$$

DSPC has two saturated acyl chains. OOPC has one diene group in 1-acyl chain. While DODPC or 1,3-DPC have two dienoyl groups in 1- and 2-acyl, or 1- and 3-acyl chains, respectively. This tendency has already been known for the natural lipids, i.e., the more the number of unsaturated bonds is, the lower the phase-transition temperature is. The difference of T_c between DODPC and 1,3-DPC was apparent to be due to the different lipid packing of these lipids in bilayer membrane. This lipid packing has already been analyzed with polymerization manner under the different point of view.[9] The diene-containing phospholipids are found to be applicable as probe molecules to analyze chemical environment of lipids in liposomes without disturbing the lipid packing. Furthermore, the position to be analyzed in bilayer membrane can be chosen by the location of diene groups in the acyl chains.

The molar extinction coefficient (ε_{max}) also shifted at gel-to-liquid crystalline phase-transition temperature. The ε_{max} decreased when temperature was set below the T_c. That was because the molar extinction coefficient of chromophore in a stacked stage was different (generally smaller) from that in the dispersed stage. The ε_{max} of POPC as liposomes was found to be smaller than that of OOPC as liposomes, though the ε_{max} values of POPC and OOPC were completely the same in organic solvents. It can be comprehended by the polarity around diene groups.[28] The absorption spectrum of diene group varies with solvent polarity. When the solvent polarity was higher, the ε_{max} tended to be smaller. The smaller ε_{max} of POPC indicated that the polarity of diene groups of POPC was higher than that of OOPC. The polarity of diene group in the 2-acyl chain is considered to be a little higher than that in the 1-acyl chain. These results strongly supported that 2,4-diene group of the 2-acyl chain faced an aqueous phase which had already been suggested from the analyses of polymerization behaviors of those lipids.[3,7] Furthermore, these spectroscopic analyses were quite suitable to measure the phase-transition of very dilute liposome suspension because the ε_{max} was large.

The λ_{max} for DPPC/DODPC (20:1 by mol) mixed liposomes shifted at 40 °C, corresponding to the T_c of this mixed liposome. The phase-transition temperature was almost the same as that of DPPC liposomes. Though diene-containing lipids can be used as membrane probe which detect the phase-transition temperature of lipid membrane, this probe itself is a lipid which has own phase-transition temperature. It is easy to reach the following conclusion that the present lipid system has effective to detect the T_c of the matrix membrane, it however has inevitable concentration dependence. It is favorable to use 5 mol% of the DODPC in order to detect the phase-transition of the matrix membrane because 5 mol% of DODPC is considered not to affect the phase-transition of the major component.[12] The shift for the mixed liposomes, however, was significantly smaller than that of pure diene-containing lipid liposomes. At this ratio, the shift was almost due to interaction of diene groups in 1- and 2-acyl chains of the lipid. Monodiene-type lipids therefore cannot be used as probes for phase-transition of matrix lipids. The lipid, having two diene groups which locate at the same position in bilayer membrane, is the best for this purpose because it is expected to be intramolecular interaction of diene groups.

When more than 5 mol% of DODPC was mixed to DPPC liposomes, λ_{max} shift also reflected the phase-transition of DODPC itself and it became broad.[29] It was a typical characteristic of mixed liposome systems. Two transition temperatures at which the onset and the offset of the blue shift occurred were related to T_f and T_s, respectively.[17] At temperatures above T_f, DODPC showed a constant value of λ_{max} because all of DODPC were in a liquid crystalline state. When the mixed liposome dispersion was cooled below T_f, gel phase of DODPC, which shows smaller λ_{max}, was formed in mixed liposomes. The portion of gel phase and the ratio of DODPC in the gel phase became larger by lowering temperature to T_s. It resulted that the value of λ_{max} gradually shifted to shorter wavelength. At temperatures below T_s, the mixed liposomes were totally in a gel state, showing a constant λ_{max} depending on the initial feed composition.

The phase-transition temperature is known to disappear by the addition of cholesterol, and slight temperature dependence of the λ_{max} was observed for cholesterol/POPC (1:1 by mol) mixed liposomes as shown in Fig. 6. The same phenomenon has already

been found with other measurements such as DSC,[18] NMR,[29] fluorescence spectroscopy[30] and so on. Cholesterol/POPC equimolar mixture system at 5 °C showed its λ_{max} at 254.2 nm which was longer wavelength than that for POPC (253.0 nm). Cholesterol shows an effect to fluidize the lipid membrane at temperatures below the T_c. It caused the decrease in the interaction between dienoyl groups of POPC below the T_c. The λ_{max} of cholesterol/POPC equimolar mixture liposomes (256.7 nm) at 40 °C, above the phase-transition temperature of POPC liposomes, was shorter than that of POPC liposomes (259.3 nm). That is considered to be the existence of some interaction of dienoyl groups due to less mobility of POPC in cholesterol containing lipid membrane. These spectral changes must be useful to detect the phase state of any lipid systems without disturbing their assembled structure.

Conclusion

The absorption maxima of diene-containing lipids as liposomes showed blue shift at gel state. The shift was resulted from the interaction of dienoyl groups, and it was explained by the exciton model. The different acyl chain packing of various diene-containing lipids was revealed to be the major factor to cause the temperature dependence of the absorption. The λ_{max} shift also reflected the phase-transition temperature of mixed liposomes. Besides the phase-transition temperature, the information about mobility and packing of diene groups were also obtained at any temperatures, which could not be observed by DSC measurement. The diene-containing lipids were concluded to be unique and quite potential compounds as membrane probes.

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