New Phase Formation of Dimyristoylphosphatidylglycerol Bilayer-Assembly with Sodium or Ammonium Ions: Gelation Phenomena in the Lamellar Liquid Crystals Aged in Water

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The thermal properties of lamellar phases of dimyristoylphosphatidylglycerol (DMPG) bilayer-assemblies with counter ions of Na^+ and NH_4^+ are remarkably different. However, these lamellar phases of NaDMPG and NH_4DMPG in water have been found to undergo a new gelation in common when they are aged for a couple of weeks below 40 °C. The new gel phases named Gel-2 reversibly change to the corresponding liquid crystal phases (LC-2) at transition temperatures (T_I) of 40 °C for NaDMPG and 44 °C for NH_4DMPG, respectively.

The phase diagram of dimyristoylphosphatidylcholine (DMPC)-water system was elucidated by the XRD and DSC studies.[1] Recently, a new phase transition was found at 29.0 °C (named T^* temperature) for the lamellar phase of DMPC on the basis of the surface chemical[2] and ESR data.[3] Further, Tajima *et al.* have found that stability and properties of hexadecane emulsions prepared with DMPC markedly change at T^*.[4,5]

On the other hand, dimyristoylphosphatidylglycerol (DMPG) bilayers having an anionic character seem to be very interesting in the fields of practical use on account of its molecular structure similar to DMPC. However, the use of DMPG has been quite rare, probably because unexpected behavior of DMPG bilayers due to hydrolysis or biodegradation has been suggested.[6]

Recently, we have found that sodium salt of DMPG bilayers in water takes a new jelly state above 31.7 °C (the T^* temperature of NaDMPG), and such a jelly state that has a nematic structure changes isothermally into a new gel state (Gel-2) during about two week-aging below 40 °C.[7] This Gel-2 state is reversibly transformed into a new liquid crystal state (LC-2) at 40 °C (named T_I temperature). However, we have not yet elucidated why NaDMPG bilayers take a nematic state when heated over T^*, and how a change of counter ions affects the thermal stability of DMPG bilayers. Thus, the present study has been attempted to make clear the differences in the thermal transitions between Na^+ and NH_4^+ salts of DMPG bilayer-assemblies, and particularly to elucidate aging effects on the thermal properties of these bilayer-assemblies in water.

Synthetic phospholipids of NaDMPG and NH_4DMPG were purchased from Avanti Polar Lipids Co., and were used without further purification. Preparation of the phospholipid dispersions and measurements of DSC and XRD were noted elsewhere.[4,7]

In order to compare the thermal properties of NaDMPG and NH_4DMPG dispersions, DSC measurements were made for these dispersions incubated overnight at various temperatures as shown in Figures 1a and 1b. The DSC charts for NaDMPG obviously depend on the incubation temperatures. Namely an endothermic peak was observed at the T^* temperature (31.7 °C) for the dispersions incubated below T^*, while no peak was observed for the dispersions incubated above T^*. In this regard, we have thought that NaDMPG bilayers take a nematic liquid crystal state above T^* (31.7 °C).[7] In the case of NH_4DMPG, the charts did not exhibit endothermic peak at T^* at any incubation temperatures, though the gel (Gel-1) – liquid crystal (LC-1) transition at 23.5 °C (T_m) was observed. These aspects for NH_4DMPG are similar to DMPC. Thus, the results in Figures 1a and 1b indicate that a difference in counter ions of DMPG bilayers brings about obviously different thermal properties.

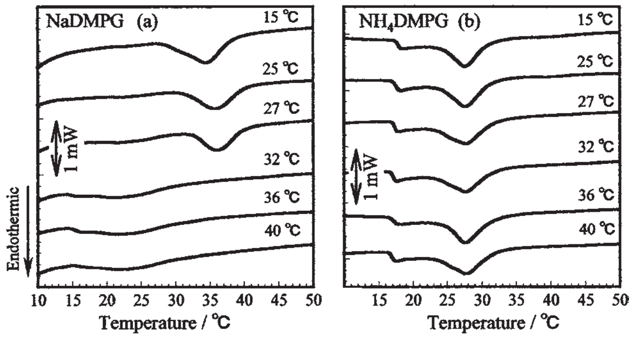

Figure 1. DSC charts for NaDMPG (a) and NH_4DMPG (b) dispersions incubated overnight at various temperatures.

However, when NH_4DMPG dispersions were aged for a long period at respective temperatures, the peaks at T_m shifted toward higher temperatures with time, and finally reached 44.0 °C (the T_I temperature of NH_4DMPG). These results are summarized in Figure 2. The dotted lines are charts for the dispersions incubated overnight, and the solid lines are those for the dispersions in equilibrium after about two-week aging. The small peak at 53 °C in the bottom curve may be identified with myristic acid produced by the hydrolysis of NH_4DMPG after further aging (15 days at 40 °C.

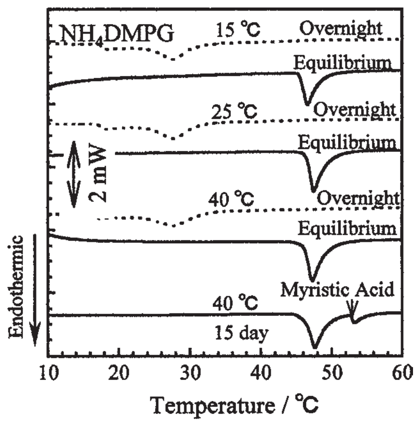

Figure 2. DSC charts for NH_4DMPG dispersions. Dotted lines: incubated overnight, solid lines: equilibrium (Gel-2).

Then, we have thought that the lamellar phases of NH_4DMPG dispersions (Gel-1 below T_m and LC-1 above T_m) are isothermally transformed into a new gel phase (Gel-2) only by a long time aging, irrespective of incubation temperatures. Namely, this Gel-2 phase melts at T_I into a new liquid crystal phase (LC-2). Such a new gel phase formation has never been reported for NH_4DMPG dispersion.

Figure 3 shows XRD patterns for the lamellar phases of NH_4DMPG incubated overnight at respective temperatures (dotted lines) and the patterns for resulting Gel-2 phases (solid lines). These data indicate two features; (1) the bilayer distances of Gel-1 (15 °C) and LC-1 (25 °C, 40 °C) are almost the same as corresponding Gel-2 ($d = 5.33 \pm 0.05$ nm); (2) the d values do not vary with temperature, in contrast to NaDMPG[7] or DMPC.[8] Thus, we suppose *ad hoc* that the bilayer distances observed for the NH_4DMPG dispersions are almost constant.

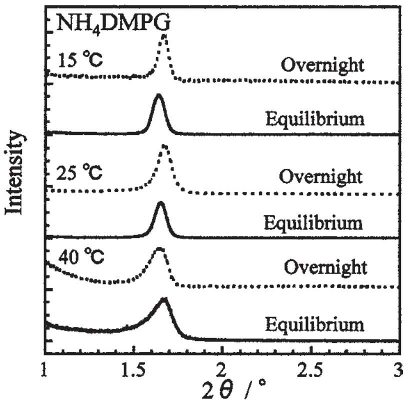

Figure 3. XRD patterns for NH_4DMPG dispersions. Dotted lines: incubated overnight, solid lines: equilibrium (Gel-2).

As for NaDMPG dispersion, we have reported on a similar gelation phenomenon at 40 °C,[7] and on the properties of Gel-2 phase as an emulsifier.[9] Thus, thermal characters of NaDMPG dispersion below 40 °C were further investigated. Figure 4 shows the time dependence of DSC charts for the dispersions incubated at 15 °C and 25 °C, together with the previous data at 40 °C.[7] While, at the initial stage of aging, the peak at T^* (31.7 °C) was observed for the dispersion incubated at 15 °C or 25 °C, after a couple of days, the peak at T^* diminished gradually. Concurrently, a small peak emerged at lower temperature, grew, shifted to higher temperatures, and finally converged at 40 °C after about 2-week aging. These results indicate that NaDMPG dispersions incubated at 15 °C, 25 °C, and 40 °C are all transformed isothermally with time into a new gel phase (Gel-2), the T_I temperature of which is 40 °C.

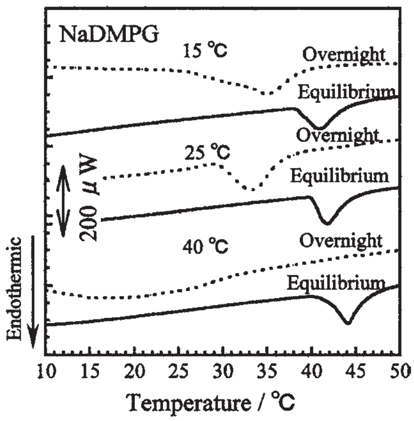

Figure 4. DSC charts for NaDMPG dispersions. Dotted lines: incubated overnight, solid lines: equilibrium (Gel-2).

Table 1 summarizes the transition temperatures for NaDMPG and NH_4DMPG bilayer-assemblies, together with their zeta-potential at various states. Remarkably different characters between NaDMPG and NH_4DMPG bilayers described above may be explained in terms of the zeta-potential that primarily reflects the

Table 1. Thermal properties for DMPG bilayer-assemblies.

Counter ions	Transition temperature/℃			Zeta-potential/mV		
	T_m	T^*	T_I	15	25	40/℃
Na^+	23.5	31.7	40.0	–	-48	-55
NH_4^+	23.5	n.d.	44.0	-38	-38	-38

degree of counter ion dissociation from DMPG bilayers. For example, the incredibly large zeta-potentials for NaDMPG bilayers at 40 °C shown in Table 1 seem to cause so much disturbance in bilayer arrangement that no peak could be detected in XRD measurement at 40 °C,[7] while we could observe XRD peak for NH_4DMPG bilayers, regardless the incubation temperatures and aging times (Figure 3).

The molecular mechanism for gelation of both Gel-2 phases of Na^+ and NH_4^+ salts may be explained in terms of changes in conformation of the terminal glycerol moiety of DMPG molecules. The hydrogen bonds in or between terminal glycerol groups are more stabilized by taking an extended structure induced by hydration of solvent water. As a result of enhanced lateral interactions between hydrocarbon chains, the lamellar phase of DMPG bilayers would start to change into Gel-2 phase so long as the incubation temperature is below T_I. DMPG bilayers that had reached Gel-2 state will be reversibly transformed into a new liquid crystal state (LC-2) at T_I.

We must note that Gel-2 phases could not come out immediately, even though the dispersions of DMPG were heated over T^*. The reason may be that the rearrangement of DMPG bilayer assembly into Gel-2 state needs a long period after the glycerol groups are hydrated with water. In other words, when temperature of the dispersion rises, each DMPG molecule should quickly take the most favorable extended conformation due to thermal motion, but the rearrangement of entire bilayer assembly into the thermodynamically most stable bilayer structure may need a long period. Since the phospholipid bilayer structure in water may be spontaneously altered with time into more stable structure by itself, we have to pay more attention to Gel-2 formation, especially when we argue the time and temperature dependencies of stability of emulsions or vesicles prepared with phospholipids.

In conclusion, we have the following results: (1) The thermal properties of NH_4^+ and Na^+ salts of DMPG bilayer-assembly after overnight aging are remarkably different. (2) The T_m temperature of bilayer-assemblies that is independent of counter ions increases with aging time up to the T_I temperature indicating the formation of a new gel phase (Gel-2). (3) The gelation from lamellar LC states is caused by change in hydration of the terminal glycerol moiety in each molecule followed by entire rearrangement of the bilayer-assembly. (4) The transitions of Gel-2 to LC-2 at the T_I temperatures are reproducible for two weeks, and then myristic acid was observed as a result of hydrolysis of DMPG molecules.

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