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Very Large Cooperative Phenomena on the Main Phase Transition of L-α-Dipalmitoylphosphatidic Acid During the Cooling Process

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Gel/liquid crystal phase transition of concentrated water suspension of L-α-dipalmitoylphosphatidic acid was examined by using differential scanning calorimeter. During the cooling process, very sharp exothermic curve was obtained compared with endothermic one during the heating process. This sharp peak is a good evidence of high cooperativity among alkyl chains of lipid molecules during the transition from liquid crystalline phase to gel phase.

Gel/liquid crystal phase transition behavior of phospholipids has been studied in very dilute water suspension.[1-3] However, in concentrated water suspension, van der Waals interaction among alkyl chains of phospholipids is expected to be much stronger, and so phase transition behavior is expected to be different from dilute suspension. In this paper, 1 part of L-α-dipalmitoylphosphatidic acid was added to 14 part (by weight) of water, and the mixture was ultrasonicated at 73 °C for 10 min. Then the thermal behavior of the suspension was examined by using Du Pont 910 differential scanning calorimeter. Calibration of the temperature and heat flow of the instrument was done with standard Indium metal. Results are summerized on Table 1 and a typical example is shown in Figs. 1 and 2.

The most characteristic result is that the curve of the exothermic enthalpy change during the cooling process is very sharp. As shown in Fig. 2c, when the phase transition begins, heat flow reaches the maximum value within several seconds. This transition accompanys rapid release of heat, so the temperature of the sample rises rapidly in spite of the cooling process (Fig. 2d). Half width of the peak is very small (around 20 seconds). These characteristics do not depend on the cooling rates. In all cooling rates, once phase transition begins, it seems to be accelerated autocatalytically. Phase transition temperature (Tp or Tm) during the cooling process is several degrees lower than that during the heating process. Besides, exothermic enthalpy change of the phase transition (and also the endothermic one) is extraordinarily large (around 105 kJ mol^{-1}, Table 1) compared with the values of phospholipids already reported.[1]

All these results show that very large cooperativity operates among alkyl chains of lipid molecules during the process of the phase transition, especially

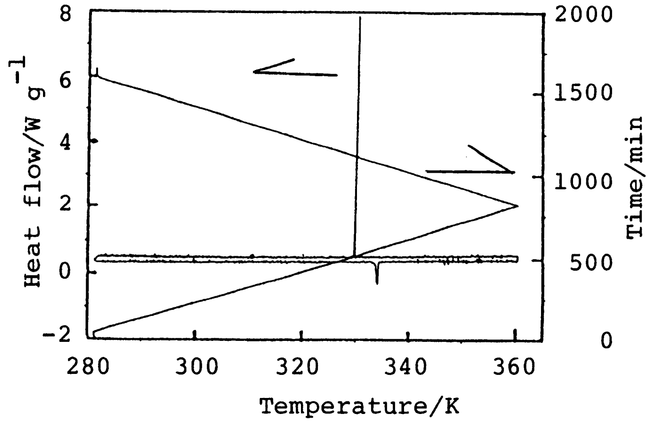

Fig. 1. DSC curve of L-α-dipalmitoylphosphatidic acid with water (1:14 in weight). Heating and cooling rate is 0.1 K min^{-1}.

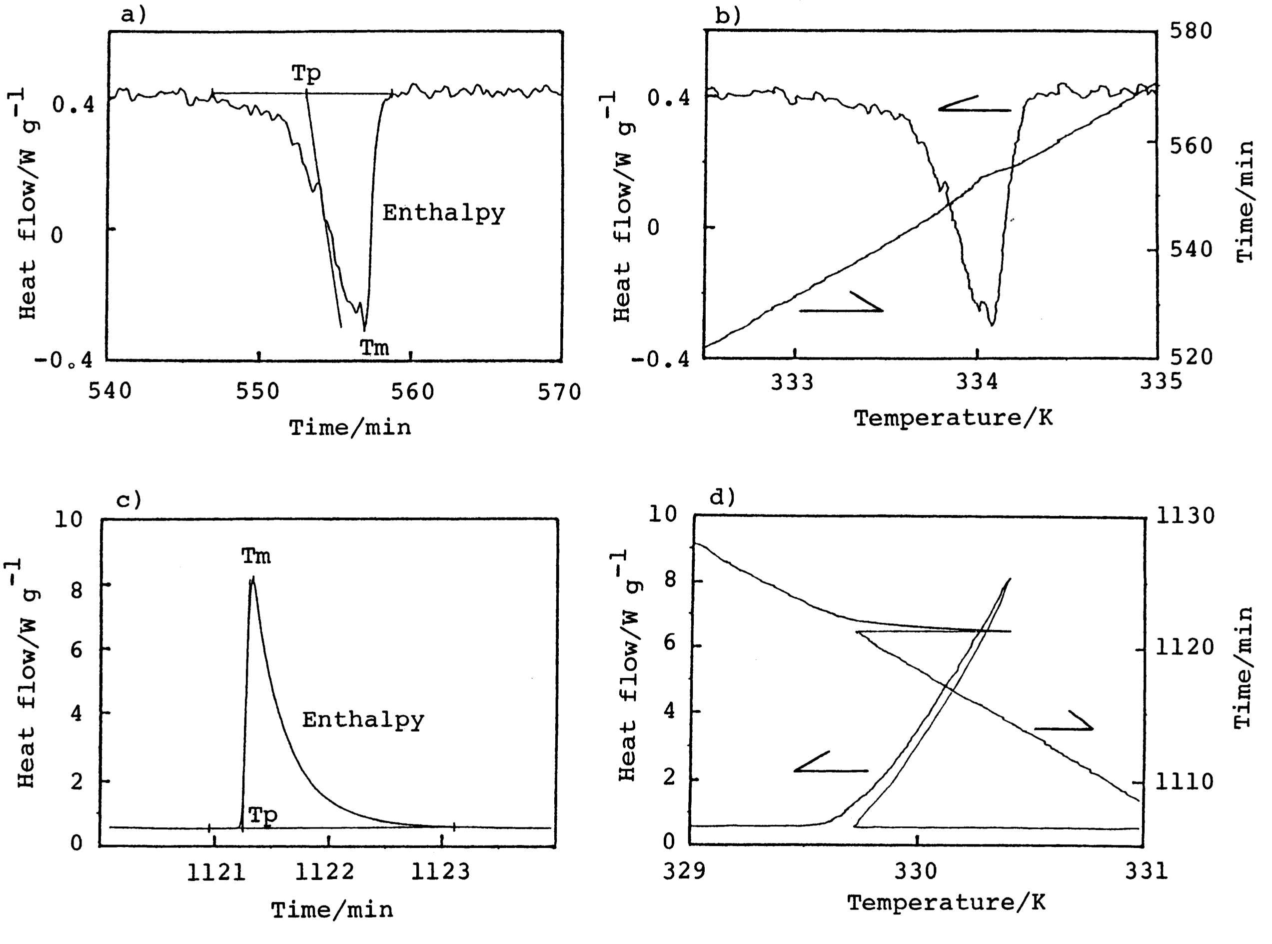

Fig.2. Magnified DSC curves of Fig.1.

a) Endothermic, heating rate of 0.1 K min^{-1}, plotted against time.

b) Endothermic, heating rate of 0.1 K min^{-1}, plotted against temperature.

c) Exothermic, cooling rate of 0.1 K min^{-1}, plotted against time.

d) Exothermic, cooling rate of 0.1 K min^{-1}, plotted against temperature.

Table 1. DSC data of L-α-dipalmitoylphosphatidic acid with water (1:14 in weight ratio) at different rates of heating and cooling

Rate a)	Heat b) /Cool	Tp c)	Tm d)	ΔH e)	HW f)	DC1 g)	RC1 h)	GR i)	DC2 j)	RC2 k)
K min^{-1}		K	K	kJ mol^{-1}	s	kJ mol^{-1} s^{-1}		s	kJ mol^{-1} s^{-1}	
0.1	Heat	333.8	334.1	105	191.6	0.55		233	0.45	
	Cool	329.8	330.4	106	15.9	6.69	12.2	4.8	22.14	49.1
0.2	Heat	334.0	334.3	107	122.7	0.87		100	1.07	
	Cool	329.5	330.0	109	22.3	4.88	5.6	7.2	15.12	14.2
0.5	Heat	333.8	334.4	103	72.2	1.43		89	1.16	
	Cool	327.9	328.3	105	17.5	6.03	4.2	3.6	29.29	25.3
1.0	Heat	333.8	334.5	106	52.0	2.04		55	1.93	
	Cool	328.0	328.3	108	20.6	5.24	2.6	4.2	25.68	13.3
2.0	Heat	334.1	334.9	105	34.9	3.00		34	3.08	
	Cool	330.0	330.4	106	16.8	6.30	2.1	6.0	17.65	5.7
5.0	Heat	334.6	335.6	109	23.9	4.56		17	6.41	
	Cool	327.9	328.4	108	13.5	7.98	1.8	3.0	35.91	5.6

a) Heating or cooling rate. Each sample was heated from 280 K to 360 K at a constant rate, then cooled at the same rate to 280 K.

b) Heating process or cooling process.

c) Phase transition temperature as exemplified in Fig. 2 a) or c).

d) Peak temperature (minimum or maximum value) of the phase transition curve as exemplified in Fig. 2 a) or c).

e) Phase transition enthalpy between gel and liquid crystal phase.

f) Half width of the phase transition curve plotted with time as abscissa.

g) Degree of cooperativity-1, defined as ΔH/HW.

h) Ratio of cooperativity-1, defined as (DC1 when cooled)/(DC1 when heated).

i) Rising or falling gradient of the phase transition curve, defined as (Time at Tm)-(Time at Tp).

j) Degree of cooperativity-2, defined as ΔH/GR.

k) Ratio of cooperativity-2, defined as (DC2 when cooled)/(DC2 when heated).

during the exothermic process when cooling. Some methods can be considered to express the amount of cooperativity. Here, as semi-quantitative indices, degree of cooperativities (DC1 and DC2) are defined as shown in Table 1. DC1 and DC2 of the cooling process are very large and independent on the cooling rates, while those of the heating process depend largely on the heating rates. In order to clarify the differences of the two processes, ratio of cooperativities (RC1 and RC2) are defined as shown in Table 1. Both RC1 and RC2 are always larger than 1, and the smaller the heating or cooling rate is, the larger the DC1 or DC2 becomes. The ratio of the height of the two peaks is almost equal to the value of RC1 as exemplified in Fig. 1. RC2 seems to be more adequate to express the specific cooperative phenomena during the cooling process. However, as GR value of the cooling process is very small, and so the error of GR is very large (10 to 20 percent), then the values of DC2 or RC2 are rather scattering.

Low concentrations of coexisting H^+, OH^- or other ions did not affect the phase transition at all. However, when the ion concentration exceeded a limit, endothermic or exothermic peak became much smaller abruptly or disappeared completely. In order to compare the results of L-α-dipalmitoylphosphatidic acid with other phospholipids, thermal behaviors of L-α-dipalmitoylphosphatidylethanolamine, L-α-dipalmitoylphosphatidylcholine, and some other phosphatidylcholines were examined under the same experimental condition. No sharp exothermic curve was obtained in all these cases regardless of cooling rates. Tp or ΔH was nearly the same value as already reported1) in both heating and cooling process. And so RC1 and RC2 values were not so different from 1 in all cases. These results show that large cooperative phenomena among lipid molecules occur only in some special lipids with very small hydrophilic head group and in some limited ionic conditions. Further studies are currently in progress. It is interesting to consider the role of cooperativity of lipids in living body.

References

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