Effects of sample preparation methods on DSC thermograms of phospholipid model membranes

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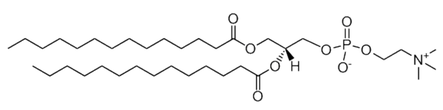
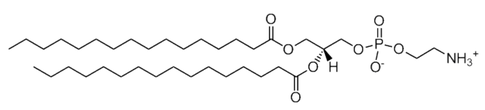
Abstract

The biological cell membrane protects the cytosol of a cell from the surrounding environment and is composed of various lipid molecules, proteins, and cholesterol. In the biophysical studies of membrane-peptide/protein interactions, various model membranes composed of PCs, PEs, and PGs are widely used. DSC is a relatively simple technique used in the study of phase behaviors of the model bilayers due to the easy interpretation of DSC thermograms for phase transition temperature and identification of heat changes accompanied by the phase changes. However, we noticed that, depending on how the model membrane is prepared, there were noticeable amounts of deviation in thermograms from multiple experiments. In this study, we repeatedly conducted DSC experiments to find the optimal condition to provide consistent DSC thermograms. The phase transition temperatures (T_{pre} & T_m) and enthalpy change (ΔH_{pre} & ΔH_m) of DMPC and DPPE multilamellar vesicles(MLVs) were measured using Nano DSC(TA instruments,). From the various sample preparation procedures, we found the optimal procedural condition and minimized errors in the thermograms. Additional details are discussed in the poster.

Materials

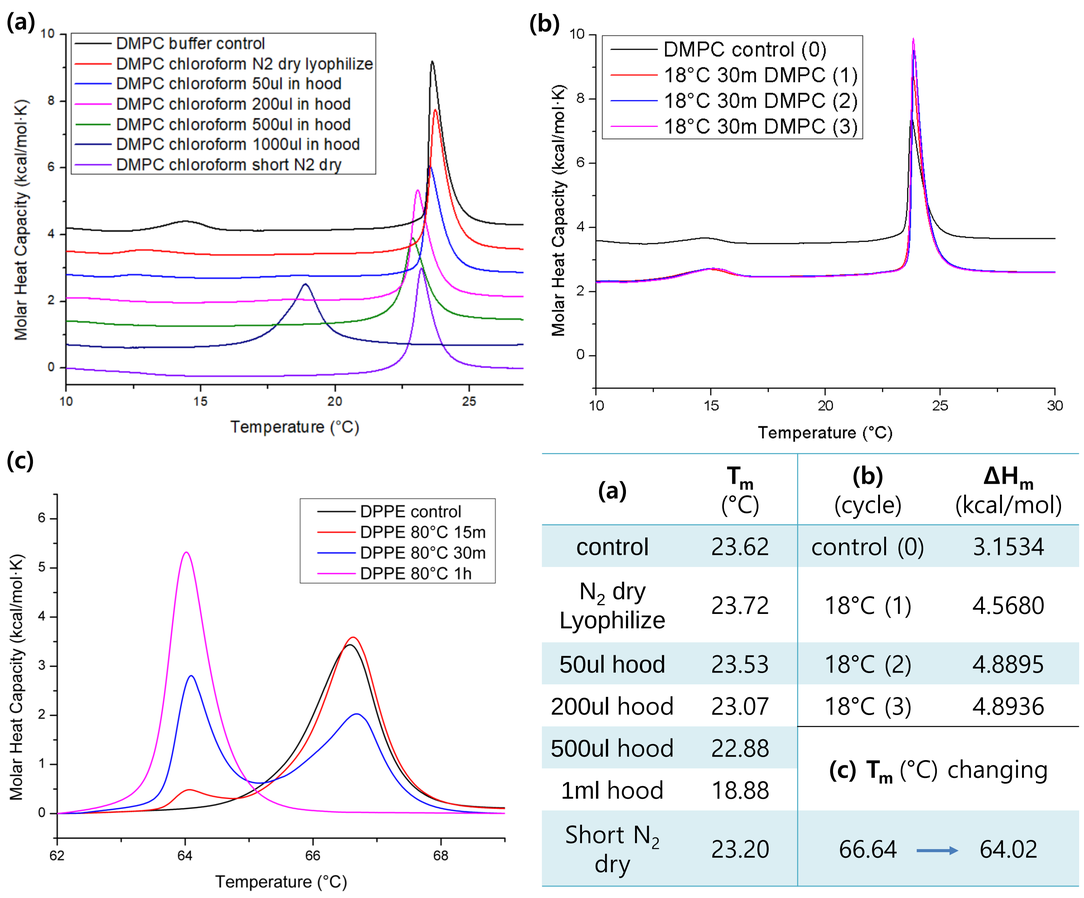


(a) DMPC



(b) DPPE

Sampling variables



(a)	T_m (°C)	(b) (cycle)	ΔH_m (kcal/mol)
control	23.62	control (0)	3.1534
N_2 dry Lyophilize	23.72	18°C (1)	4.5680
50ul hood	23.53	18°C (2)	4.8895
200ul hood	23.07	18°C (3)	4.8936
500ul hood	22.88	(c) T_m (°C) changing	
1ml hood	18.88		
Short N_2 dry	23.20	66.64 → 64.02	

Figure.1(a) DSC thermograms of DMPC vesicles prepared from various methods (b) of DMPC control sample stabilized at a given temperature and time (c) DSC thermograms of DPPE vesicles after heat treatment at 80°C for a given time.

Table.1 T_m(°C) and ΔH_m (kcal/mol) of Figure.1

Experiment

DMPC or DPPE 0.45mg/0.45ml phosphate buffer (pH7.4 no salt)

1. In order to minimize the weighing error of lipid samples, prepared lipids in a control group and an experimental group at once.

2. For each experiment, degassed buffer or organic solvent is added and vortexed in an Eppendorf tube. (If two or more types of lipids are mixed, use organic solvent, but if one type of lipid is used, buffer was added directly to the powder form of lipids.). If the lipids are not well dispersed in buffer at distribution, modify the concentration or distribute it into an organic solvent at step 2')

(2') When organic solvent such as chloroform is added.
2'-1. Use high purity nitrogen gas to blow away the solvent as soon as possible.
2'-2 Dry it more than 8 hours using a lyophilizer.
2'-3. Fill the degassed buffer with the desired concentration.
(In the case of organic solvent that vaporizes quickly at room temperature, Store an Eppendorf tube at low temperature and take out immediately before distribution.)

(2'') When buffer is put in right away.
2'-1. Fill the degassed buffer with the desired concentration.

3. Heat the lipids for 45 to 60 minutes above the phase transition temperature using water bath. (If the lipids don't dissolve and maintain the film phase, do Freeze & thaw once in advance)

4. Do Freeze & Thaw 3 times. (MLVs)
(In the thaw process, melted slowly while performing vortexing.)

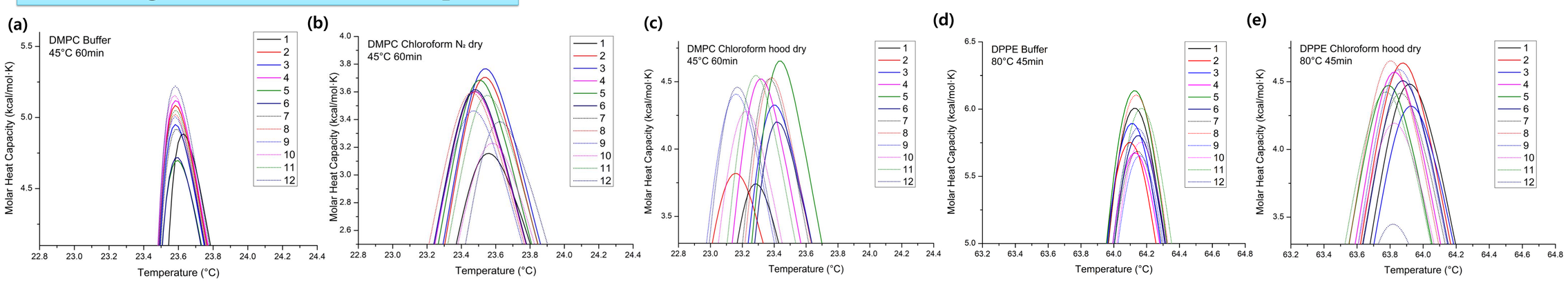
5. Store it in 4°C refrigerator for more than 8 hours to perform thermal stabilization.

6. Vortex the lipids and distribute them.

7. After degassing the sample for 10 minutes, fill the sample chamber and operate nano DSC

8. After the experiment, wash it 15 times or more with distilled water at higher temperature than the lipid phase transition temperature and wash it again with a buffer.

Checking for errors between samples



	(a)	(b)	(c)	(d)	(e)
T_m (°C)	23.58 ± 0.007	23.42 ± 0.053	23.30 ± 0.106	64.14 ± 0.022	63.85 ± 0.163
ΔH_m (kcal/mol)	3.948 ± 0.077	3.923 ± 0.163	4.084 ± 0.162	5.209 ± 0.049	4.560 ± 0.104

Figure.2 DSC Thermograms of DMPC and DPPE vesicles showing variations among samples. (a)&(d): Buffer was added without chloroform treatment. (b): Chloroform was dried with N_2 gas and lyophilized. (c)&(e): Chloroform was dried for 2 days in the hood.

Table.2 T_m(°C), ΔH_m (kcal/mol) and standard deviation of <Figure.2>

Conclusion

DMPC and DPPE are lipids that cause phase transition of lipid membranes within an appropriate temperature range, so they are often used in DSC experiments. The phase transition temperature (T_{pre}, T_m) of the lipid membranes and the amount of enthalpy change (ΔH) required for phase transition provide important information for understanding the properties of the material interacting with the lipid membranes. In this experiment, DSC Thermograms were compared and analyzed for DMPC and DPPE vesicles prepared by several different ways. In the case of DMPC, the phase transition temperature (T_{pre}, T_m) decreased further as the removal time of chloroform used as a solvent took longer (Fig. 1.a). Furthermore, even if the same DMPC sample was repeatedly measured with sufficient stabilization, a decrease in the heat capacity of the solution and an increase in ΔH were observed when the samples were treated with heat prior to the DSC measurement (Fig. 1.b). In the case of DPPE stored at -20°C, the phase transition temperature decreased as the heat treatment time at 80°C increased (Fig. 1.c). In both DMPC and DPPE, the slower the removal of organic solvents, the lower the T_m, and the standard deviation of T_m and ΔH increased. Therefore, heat treatments and thermal stabilization on the lipid vesicles should be carefully applied before DSC measurement. Additionally, for an organic solvent, the sooner it is removed, the more reproducible DSC thermogram is provided.