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A Scanning Calorimetric Study of the Effect of Clover Saponin on Liposomal Phospholipid Membrane

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Abstract □ The effect of clover saponin on the phase transition of liposomal lipid bilayers of dimyristoyl phosphatidylcholine was investigated with differential scanning calorimetry. The thermograms of the liposomal bilayers incorporated with the clover saponin were obtained, and the enthalpy changes and the sizes of cooperative unit of the transition were calculated. The results showed that incorporation of the clover saponin into the liposomal bilayers effectively reduced the transition temperature at which the transition from solid state to liquid-crystalline state occurs, and broadened the thermogram peaks. It also reduced the size of cooperative unit of the transition. These results indicate that the clover saponin might have significant effect on the fluidity of biological membranes.

Keywords □ dimyristoyl phosphatidylcholine, differential scanning calorimetry, clover saponin, size of cooperative unit, fluidity of biological membranes.

The chemical composition and pharmacological activities of saponins have been extensively studied by many workers[1-5]. Comparing with the large volume of these works, studies on the physical properties of these components of herb medicines have been relatively neglected. Saponins are amphiphatic in nature, and moderately surface active. They may penetrate the lipid bilayers of biological membrane, and are expected to exert a great effect on the physiological properties of the biological membrane. The fluidity of the lipid bilayers of biological membrane have been known to play an important role in the physiological functions of biological membrane[6-8], and saponins might exert their pharmacological activities through their effect on the fluidity of the biological membrane.

The interaction of drug molecules with liposomal lipid bilayers serving as model membranes have received much attention in recent years[9,10]. Among these effects resulting from such interaction are alterations in the thermotropic behaviors of the lipid. They include changes in the enthalpies and temperature of the phase transition, and in the shape of the transition curves as observed by differential scanning calorimetry (DSC). Although it is not always possible to give direct correlation of these effects of drugs on the model membranes with their pharmacological activities, it is nevertheless useful to pursue studies along this line in attempts to broaden our knowledge of pharmacological action on the molecular basis.

The present study was undertaken to examine the possible effect that such interaction of saponin with biological membrane might have on the properties of lipid bilayers of biological membrane. Multilamellar liposomes were prepared from L-α-dimyristoyl phosphatidylcholine (DMPC), and the the transition from gel to liquid-crystalline state was examined in the presence of clover saponin by high resolution DSC.

EXPERIMENTAL METHODS

Materials

Synthetic L-α-dimyristoyl phosphatidylcholine was purchased from Antipolar Lipids, Inc. (Birmingham, Lot No. 850345). The clover saponin was isolated and identified according to the method described in ref.[9]. The saponin employed in this experiment was the mixture of the clover saponin A and B, and the chemical structures are shown in Fig. 1. The organic solvents used in preparation of the lipid suspensions were of spectral grade. Disodium phosphate, potassium chloride and sodium

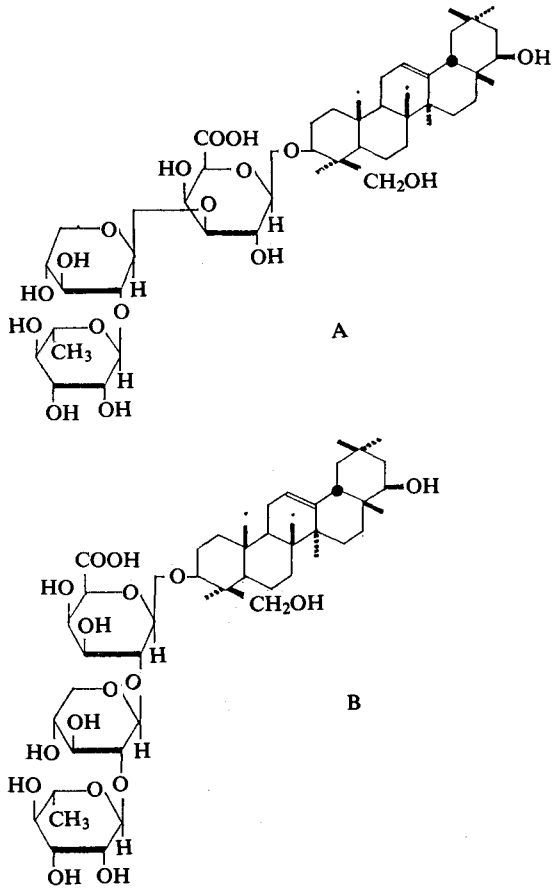

Fig. 1. The chemical structures of clover saponin A and B.

chloride used for buffer solution were of reagent grade, and doubly deionized water was used.

Preparation of lipid Suspensions

Appropriate volume of stock solutions of DMPC in chloroform and the saponin in methyl alcohol were mixed to provide the desired mole ratio for making lipid suspensions. The organic solvents were then removed by a stream of dry nitrogen to make a thin film of the lipid, and it was dried in a vacuum oven at 30°C for 1 hour. The sample was redried with nitrogen gas, and then dried in a vacuum oven at 30°C for 1 hour. The dried thin film was suspended in 2m*l* of phosphate buffer saline (PBS) at pH 7.4 by shaking on a vortex mixer for 1 min., and then left in a thermobath at a temperature above its phase transition temperature for 1 min.

Differential scanning calorimetry

All DSC experiments of the lipid suspensions were performed with a DASM-IM differential microcalorimeter operating at a scan rate of 0.25K $min.^{-1}$. The lipid concentration of all samples was maintained at 1 mg/m*l*. Instrumental base line was obtained by doubly deionized water.

RESULTS AND DISCUSSION

DSC thermograms of multilamellar liposomes of DMPC alone and in the presence of differential concentrations of the clover saponin in PBS (pH 7.4) are shown in Fig. 2. A sharp transition peak was obtained with DMPC liposomes centered at 23, 98°C without any detectable pretransition peak. It means that the transition of the DMPC bilayers from gel to liquid-crystalline state is a highly cooperative process. The transition peak was broadened and shifted to lower temperature at the saponin concentrations up to 8.4 μM. The broadening and the shift of the peaks were proportional to the saponin concentration. The phase transition occurred over a wide range of temperature in the presence of the clover saponin; it started at 20°C, and finished at near 25°C at the concentration of

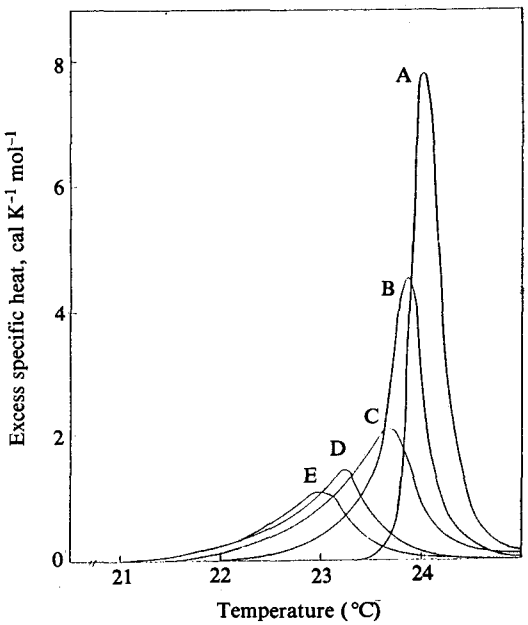

Fig. 2. Variation of excess specific heat capacity with temperature during the main transition of DMPC liposomes incorporated with the clover saponin. The concentration of the added saponin was none for curve A, 12.6μM for B, 25.4μM for C, 29.6 μM for D, and 42.1μM for E, respectively.

42.1 μM of the saponin. According to the classification proposed by Jain[10], the shape of the thermograms suggests that the possible site of penetration of the clover saponin into the lipid bilayers would be the palisade layer. Probably the hydrophilic moiety of the saponin might anchor the solute to near the surface. The enthalpies of the transition, ΔH_{cal} were measured from planimetric integration of the thermograms. From the fraction of the area under curves of the thermograms, the reaction degrees versus temperature for the transition of the DMPC bilayers were calculated and illustrated in Fig. 3. Since the temperature was scanned at a constant rate, the slope of the curve of this plot could be used to calculate the van't Hoff enthalpy of the transition, ΔH_{vH} using the van't Hoff equation[11],

$$\Delta H_{vH}=4RT_m^2\left(\frac{d\alpha}{dT}\right)_{T_m}$$

where α is the fraction of the lipid in the liquid-crystalline state, and T_m, the main transition temperature. The main transition temperature, the enthalpies of the transition, and the van't Hoff enthalpies of the transition in the presence of various concentrations of the clover saponin were compiled in Table I. The ratios of ΔH_{cal} to ΔH_{vH} were calculated and included in this table. This ratio is usually interpreted as the size of the cooperative unit of the transition[12,13].

The table clearly shows that the clover saponin has a great effect on the thermograms of the lipid bilayers. The penetration of the saponin into the lipid bilayers reduced the phase transition temperature of the lipid and broadened the thermogram. It is noteworthy that the broadening and the shift of the thermogram peaks by the saponin were observed even at very low saponin concentrations in contrast to other drug substances[14,15]. This means that the clover saponin is markedly effective in permeating into the lipid bilayers, and altering the properties of the lipid bilayers. The clover saponin reduced the enthalpy of the transition in proportion to its concentration. This is contrary to the results of other works along this line that reported that the small solutes did not change the enthalpy of the phase transition significantly[15-17]. This means that the ideal solution theory has a limitation in applying it to this system. The ideal solution theory is fairly well applicable for the transition from gel to liquid-crystalline state of lipid bilayers at low concentrations of small solutes. However, the molecules of clover saponin are large, rigid, and bulky, and penetration of these molecules into the lipid might induce loose packing of the lipid in the gel state, and probably reduce the enthalpy of the phase transition. Another possibility is the uncertainty in taking the base line in the graphic integration. The van't Hoff enthalpies of the transition and the ratios of ΔH_{cal} to ΔH_{vH} were also reduced in the presence of the clover saponin. The decreasing tendencies of the anthalpies, van't Hoff enthalpies of the transition, and the sizes of cooperative unit continued within the concentration range examined in this experiment. All these results suggest that the clover saponin is very effective in modifying the thermotropic behaviors of the liposomal lipid bilayers of DMPC and in fluidizing the lipid bilayers, and its pharmacological activities

Table I. Parameters for DSC transition curves of DMPC liposomes incorporated with the clover saponin

Saponin Conc. (μM)	T_m (°C)	ΔH_{cal} (Kcal mole^{-1})	ΔH_{vH} (Kcal mole^{-1})	$\frac{\Delta H_{vH}}{\Delta H_{cal}}$
0	23.98	4.37	513.98	118
8.5	23.89	3.56	414.20	113
12.6	23.83	3.37	370.70	110
16.9	23.68	3.08	312.42	101
20.9	23.49	2.96	293.34	99
25.4	23.43	2.89	236.65	82
29.6	23.23	2.23	178.12	80
33.8	23.15	2.17	159.07	73
42.1	23.05	1.88	137.40	73

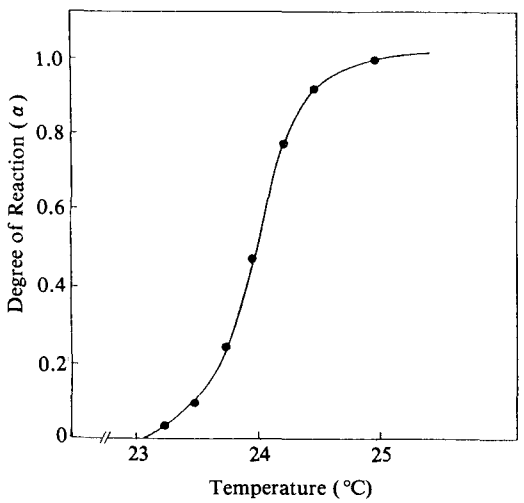

Fig. 3. Reaction degree of the gel to the liquid-crystalline transition vs. the temperature of DMPC liposomes.

might have some correlation with this kind of unspecific interaction.

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