한국영양식량학회지

The Thermotropic Phase Behaviors of Artificial Phospholipid Liposomes Incorporated with Soyasaponin

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Abstract

The effect of soyasaponin on the liposomal phospholipid membrane was investigated by differential scanning calorimetry (DSC). Soyasaponins were obtained and the enthalpy changes and the sizes of cooperative unit of the transition were calculated. The thermograms of L-α-dimyristoyl phosphatidylcholine (DMPC) incorporated with soyasaponin showed that the phase transition temperature was significantly lowered and the peak was broadened. This was attributed to the possibility that incorporation of soyasaponin into the lipid bilayers reduced the cooperative unit of phospholipid bilayers. These results indicate soyasaponin might have significant effect on the fluidity of biological membrane.

Key words : soyasaponin, phospholipid bilayer, fluidity, differential scanning calorimetry (DSC)

INTRODUCTION

Many physiological activities of saponins have been suggested to originate from ginsenosides[1-3]. But the studies on the physical properties of these saponins have been relatively neglected. Saponins might have a tendency toward hydrophobic self-aggregation in aqueous solution, and have ability to solubilize and emulsify fats and lipids. They may penetrate the lipid bilayers of biological membrane and are expected to exert a great effect on the physical properties of the biological membrane. The fluidity of the phospholipid bilayers of the biological membrane have been known to play an important role in the physiological functions of the biological membrane[4-6], and saponins might exert their physiological activities through their effects on the fluidity of the biological membrane.

The interaction of the incorporated molecules with liposomal phospholipid bilayers serving as model membranes have received much attention in recent years[7,8]. 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 shapes of the transition curves as observed by differential scanning calorimetry (DSC). Although it is not always possible to make direct correlation, between these effects of drugs on the model membranes with their physiological activities, it is nevertheless useful to pursue studies along this line in attempts to broaden our knowledge of physiological action on the molecular basis.

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

MATERIALS AND METHODS

Materials

Synthetic L-α-dimyristoyl phosphatidylcholine was purchased from Antipolar Lipids, Inc. (Birming-

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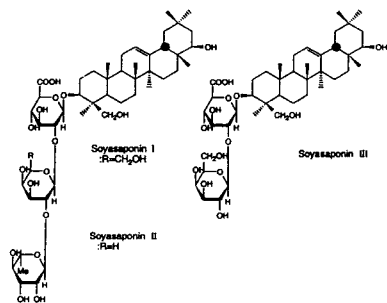[†]To whom all correspondence should be addressed

Fig. 1. The chemical structures of soyasaponin I , II and III.

ham, Lot No. 850345). Saponin employed in this experiment was crude saponin which contains soy-asaponin I, I and, III and the chemical structures of these saponins are shown in Fig. 1. The organic solvents used in preparation of the lipid suspensions were the spectral grade. Disodium phosphate, potassium chloride and sodium chloride used for buffer solution were the reagent grade, and doubly deionized water was used.

Isolation of soyasaponins

The dried soybeans (3kg) were extracted with MeOH twice and removal of the solvent from the combined MeOH solution under reduced pressure gave the MeOH extract (0.5kg). The MeOH extract was partitioned into n-BuOH–H$_2$O(1 : 1) mixture and the n-BuOH layer was evaporated under reduced pressure to give the n-BuOH extract which was dissolved in a small amount of MeOH and poured into a large quantity of diethyl ether. The precipitated crude saponin was collected by filtration and passed through a column of charcoal–celite (1 : 1, 100g) with the aid of MeOH to yield saponin mixture (8g). This saponin mixture was then chromatographed on silica gel several times eluting with CHCl$_3$–MeOH–H$_2$O(7 : 3 : 1, 65 : 35 : 10, lower layer) to give soyasaponin I, I and III. These saponins were identified with the method of Kitagawa et al.[3] and with direct comparison of the authentic specimens.

Preparation of lipid suspensions

An appropriate volume of stock solutions of DMPC in chloroform and saponin in methyl alcohol were mixed to provide the desired concentration 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 within nitrogen gas, and the dried film was suspended in 2ml 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 0.25°C/min. The lipid concentration of all samples was maintained at 2mg/ml instrumental base line was obtained by doubly deionized water.

Calculation for cooperative unit

The size of cooperative unit is the number of lipid molecules in the domain of simultaneous transition in such transition. In highly cooperative transition, the cooperative unit is extremely large. The transition shows a sharp peak in the thermogram. When small molecules are incorporated into the lipid bilayers, the cooperative unit should be greatly reduced. In such cases, the transition usually starts at a lower temperature than the phase transition temperature, occurs progressively, and shows broadening of the peak in the thermogram. The size of cooperative unit (ξ) can be calculated by the ratio of van' t Hoff enthalpy (ΔH_{VH}) to calculated enthalpy (ΔH_{cal})[9]. When the latent heats of a small quantity of lipid and a standard substance are measured at the same range and same chart speed, the enthalpy change of the phase transition can be calculated by the following equation[10].

$$\Delta H = \frac{\Delta H_s \times M_s}{A_s} \times \frac{A}{M} \ (kcal/mole)$$

Where ΔH is the heat for sample (kcal/mole), ΔH_s is the heat of fusion of standard substance (kcal/mol), M_s is the quantity of standard substance (mol), M is the quantity of sample (mol), A_s is the peak area for standard and A is the peak area for sample.

The van' t Hoff enthalpy (ΔH_{VH}) of liposomes can

be calculated as follows. If θ is the fraction of the lipid in the liquid-crystalline state, there assuming an equilibrium constant $K=\theta/1-\theta$, one obtains from $dlnk/dT=\Delta H_{VH}/RT^2$,

$$\frac{d\theta}{dT} = (1-\theta)\frac{\Delta H_{VH}}{RT^2}$$

where R is the gas constant and ΔH_{VH} is van' t Hoff enthalpy. From the midpoint transition, $\theta=1/2$, at temperature T_m,

$$\frac{d\theta}{dT} = \frac{\Delta H_{VH}}{4RT_m^2}$$

where $d\theta/dT$ is the midpoint of transition and T_m is the midtransition temperature. Comparison of ΔH_{VH} with ΔH_{cal} provides important information on the cooperativity of the phase transition. For non-cooperative processes $\Delta H_{VH}/\Delta H_{cal}=1$: for cooperative processes $\Delta H_{VH}/\Delta H_{cal}\gg 1$. The number of molecules in the cooperative unit is operationally defined as the ratio of $\Delta H_{VH}/\Delta H_{cal}$.

RESULTS AND DISCUSSION

DSC thermograms of multilamellar liposomes of DMPC alone and in the presence of differential concentrations of soyasaponin 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 saponin concentrations up to 0.0541mg/ml. The broadening and the shift of the peaks were proportional to saponin concentration, that resulted in the increase of half-height width of the transition temperature (HHW, $\Delta T_{1/2}$).

The phase transition occured over a wide range of temperature in the presence of soyasaponin ; it started at 21°C, and finished at near 24°C at the concentration 0.1353mg/ml of saponin. According to the classification proposed by Jain[11], the shape of the thermogram suggests that the possible site of penetration of soyasaponin into phospholipid bilayers would

be the palisade layer. Probably the hydrophilic moiety of saponin might anchor the solute near the surface. The enthalpies of transition, ΔH_{cal} were measured from planimetric intergration 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 calcul-
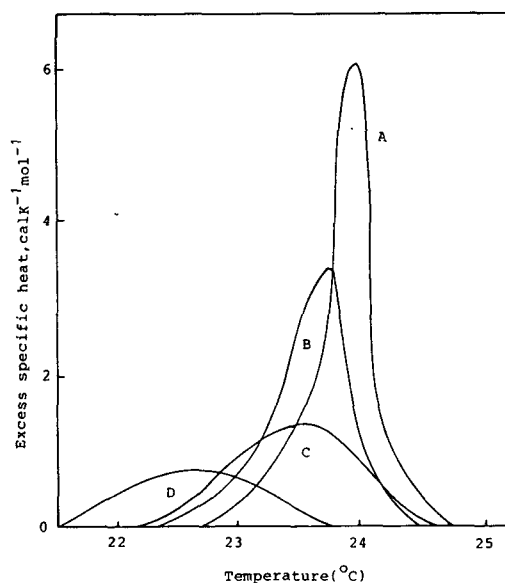

Fig. 2. Variation of exess specific heat capacity with temperature during the main transition of DMPC liposomes incorporated with soyasaponin.
The concentration of the added soyasaponin was none for curve A, 0.0541mg/ml for curve B, 0.1353mg/ml for curve C and 0.2920mg/ml for curve D, respectively.
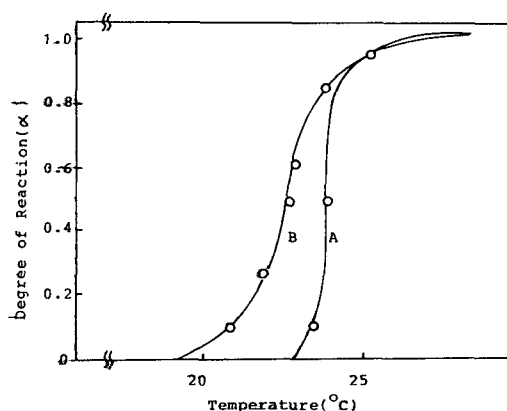

Fig. 3. Reaction degree of the gel to the liquid-crystalline transition vs the temperature of DMPC liposomes.
The concentration of the added soyasaponin was none for curve A, and 0.2920mg/ml for curve B.

Table 1. Parameters for DSC transition curves of DMPC liposomes incorporated with soyasaponin

Saponin conc. (mg/ml)	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{VH}	Cooperativity (ξ)	$\Delta T_{1/2}$
0	23.98	4.37	513.98	118	0.31
0.0541	23.64	3.93	376.92	96	0.63
0.1353	23.24	3.62	219.29	61	1.31
0.2920	22.52	3.23	115.13	36	1.34
0.3640	22.36	3.06	107.59	35	1.36

ated 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}. The main transition temperature, the enthalpies of transition, the van' t Hoff enthalpies of the transition and the cooperative unit in the presence of various concentrations of soyasaponin were compiled in Table 1. The table shows that soyasaponin has a great effect on the thermograms of phospholipid bilayers. The penetration of saponin into phospholipid bilayers reduced the phase transition temperature of the lipid and broadened the thermogram. It is noteworthy that the broadened thermogram peaks by saponin were observed even at very low saponin concentrations in contrast to other chemical substances[11]. This means that soyasaponin is markedly effective in permeating into phospholipid bilayers, and altering the properties 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[12]. 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 phospholipid bilayers at low concentrations of small solutes. However, the molecules of soyasaponin are large, rigid and bulky, and penetration of these molecules into the lipid might induce loose packing of phospholipid 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 intergration. The van' t Hoff enthalpies of the transition and the ratios of ΔH_{cal} to ΔH_{VH} were also reduced in the presence of soyasaponin. The decreasing tendencies of the enth-

alpies, 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 soyasaponin is very effective in modifying the thermotropic behaviors of the liposomal phospholipid bilayers of DMPC and in fluidizing phospholipid bilayers, and its physiological activities might have some correlation with this kind of unspecific interaction.

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대두사포닌이 침투된 인공 인지질 생체유사막의 열에 의한 상변화에 관한 연구

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요 약

인공 생체유사막을 인지질인 dimyristoyl phosphatidylcholine(DMPC)로 제조하여 인지질 막에 미치는 대두사포닌의 영향을 시차열량 분석계로 연구하였다. 대두 사포닌이 침투되기 전, 후의 인지질 막의 상그림을 얻어 상 전이시 엔탈피의 변화와 협동단위수를 상법에 따라 계산하였다. 대두 사포닌이 침투된 인지질 막의 상그림은 순수 인지질 막의 상 그림에 비해 넓적하게 변하였으며 특이하게 상전이 온도를 낮추었다. 이것은 인지질막의 이중층에 침투된 대두사포닌이 인지질 이중층의 협동단위를 감소시킨 것으로 추정된다. 이러한 결과로 보아 대두사포닌은 인지질 막 이중층의 유동성에 중요한 영향을 미친다고 사료된다.