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Effects of Saponins on the Osmotic Behavior of Multilamellar Liposomes

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Abstract □ Effects of total ginseng saponin, 20-S-protopanaxadiol saponin, 20-S-protopanaxatriol saponin and platycodon saponin on the osmotic behavior of liposomes were investigated by optical measurement. These saponins showed different activites on liposomal membrane, and cholesterol in liposomes was an important factor to this variation of saponin activities.

Keywords □ Multilamellar liposomes, Osmotic behavior, Saponins.

Most drugs encounter biological membrane at some points in their interaction with cells prior to reaching their target. Consequently, it is essential to understand the mechanism of the drug and membrane interaction. But complex composition of membrane does not allow a simple analysis of this interaction. A possible way to approach this problem is to use model membrane for testing more specifically the lipid contribution in the drug and membrane interaction[1].

Mutilamellar liposomes are known to be useful model systems for investigating many membrane-related phenomena, especially for studying the permeability properties of lipid barrier of biological membrane[2].

The osmotic behavior of liposomes is very similar to that of an ideal osmometer and the volume change of liposomes can be followed by optical measurements[3-6].

In this study, the effects of saponins on multilamellar liposomes composed of various components were investigated by optical measurements.

EXPERIMENTAL METHODS

Materials

Total ginseng saponin, 20-S-protopanaxadiol saponin and 20-S-protopanaxatriol saponin were obtained from Korean Ginseng and Tobacco Research Institute. Platycodon saponin was generous gift from Professor, Eun Bang Lee, Natural Products Research Institute, Seoul National University. Cholesterol was purchased from Nakarai Chemical Ltd. Japan. Egg phosphatidylcholine was purchased from Merck Co. and purified by alumina column chromatography. Phosphatidic acid was obtained by enzymatic hydrolysis of egg phosphatidylcholine with phospholipase D extracted from Savoy cabbage. All other reagents were commercial and of analytical grade.

Preparation of Liposomes

Two kinds of liposomes were prepared from phospholipids with or without cholesterol. Appropriate amounts of egg phosphatidylcholine (PC), phosphatidic acid (PA) and/or cholesterol (Ch) were mixed in a round bottomed flask in

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the ratio of 96:4:(10) by weight. Chloroform was removed in a rotary evaporator and further dried under vacuum for 2 hours. To this dry lipid film, aqueous solution of 50mM glucose (2mM EDTA, 10mM Tris HCl buffer, pH 7.5) was added and lipid was suspended by a vortex mixer. Lipid concentration of this stock liposome suspension was 12.5 mg phospholipid/ml.

Osmotic Behavior of Liposomes

One hundred μl of stock liposome suspension was diluted with 3ml of glucose solutions of various concentrations to give concentration gradients across the lipid bilayers. The absorbance at 450nm was measured one hour after the dilution at room temperature using a Unicam SP 1750 UV Spectrophotometer.

Initial Swelling Rate of Liposomes in Hypotonic Medium

Appropriate amount of the stock liposome suspension prepared in 50mM glucose solution was added to a 1cm path length cuvette containing 25mM glucose solution to give final volume of 3ml. Right after handly shaking of the cuvette, the absorbance change at 450nm was monitered for 30 seconds.

Effects of Saponins on Liposomes

Appropriate amount of saponin was preincubated with dilution medium and reaction was started by adding an aliquot of stock liposome suspension. Further experimental procedure was the same as described in osmotic behavior of liposomes and initial swelling rate of liposomes in hypotonic medium.

RESULTS AND DISCUSSION

Osmotic Behavior of Liposomes and Effects of Saponins

Fig. 1 shows the osmotic behavior of liposomes composed of various components. The ideal osmotic activity of liposomes was already described with following equation[3-5],

$$V = V_{act}(C_{in}/C_{out}) + V_{dead} \quad [1]$$

where V is the total volume of liposomes and V_{act} and V_{dead} are volumes of osmotically active and non-active parts, respectively. C_{in}/C_{out} is the ratio of solute concentration of inner compartment to that of outer compartment of liposomes, representing the osmotic pressure.

Such a volume change similar to that of chloroplasts and mitochondria, can be monitered by absorbance change according to the equation derived by Yoshikawa et al[6]

$$V = k(1/A)^{3/2} \quad [2]$$

By combination of equations 1 and 2, an equation describing the linear relationship between $(1/A)^{3/2}$ and C_{in}/C_{out} can be derived as follow

$$(1A)^{3/2} = 1/k[V_{act}(C_{in}/C_{out}) + V_{dead}]$$

In this study, liposomes composed of PC/PA (96:4) and PC/PA/Ch(96:4:10), which showed linear osmotic activity in the C_{in}/C_{out} range of 0~2.0 were used as model membrane systems.

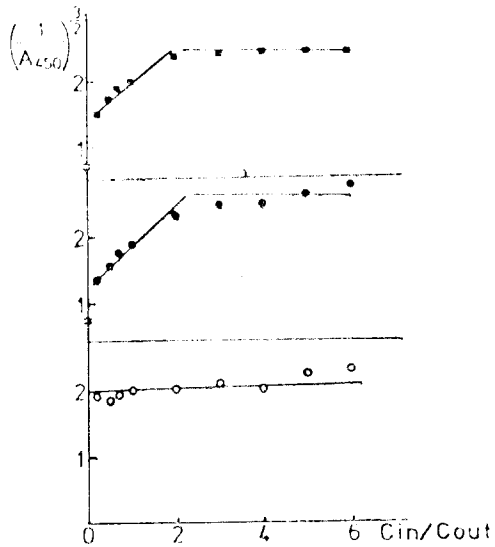

Fig. 1: Osmotic behavior of multilamellar liposomes composed of ●, PC/PA(96 : 4); ■, PC/PA /Ch(96 : 4 : 10): ○, PC.

Under more hypotonic conditions, liposomes cannot act as a perfect osmometer due to the release of solute from liposomes[3,6].

The effects of various saponins on osmotic behavior of liposomes are shown in Fig. 2. Total ginseng saponin and 20-S-protopanaxadiol saponin disturbed the linear relationship between $(1/A)^{3/2}$ and C_{in}/C_{out} in liposomes with or without cholesterol. 20-S-protopanaxatriol saponin also disturbed the osmotic behavior of PC/PA liposomes but this saponin had no effect on PC/PA/Ch liposomes. In contrast to triol saponin, platycodon saponin showed its activity only on PC/PA/Ch liposomes. It can be assumed that platycodon saponin interact cholesterol of liposomal membrane and induce the permeability change of PC/PA/Ch liposomes.

Saponin hemolysis is generally attributed to

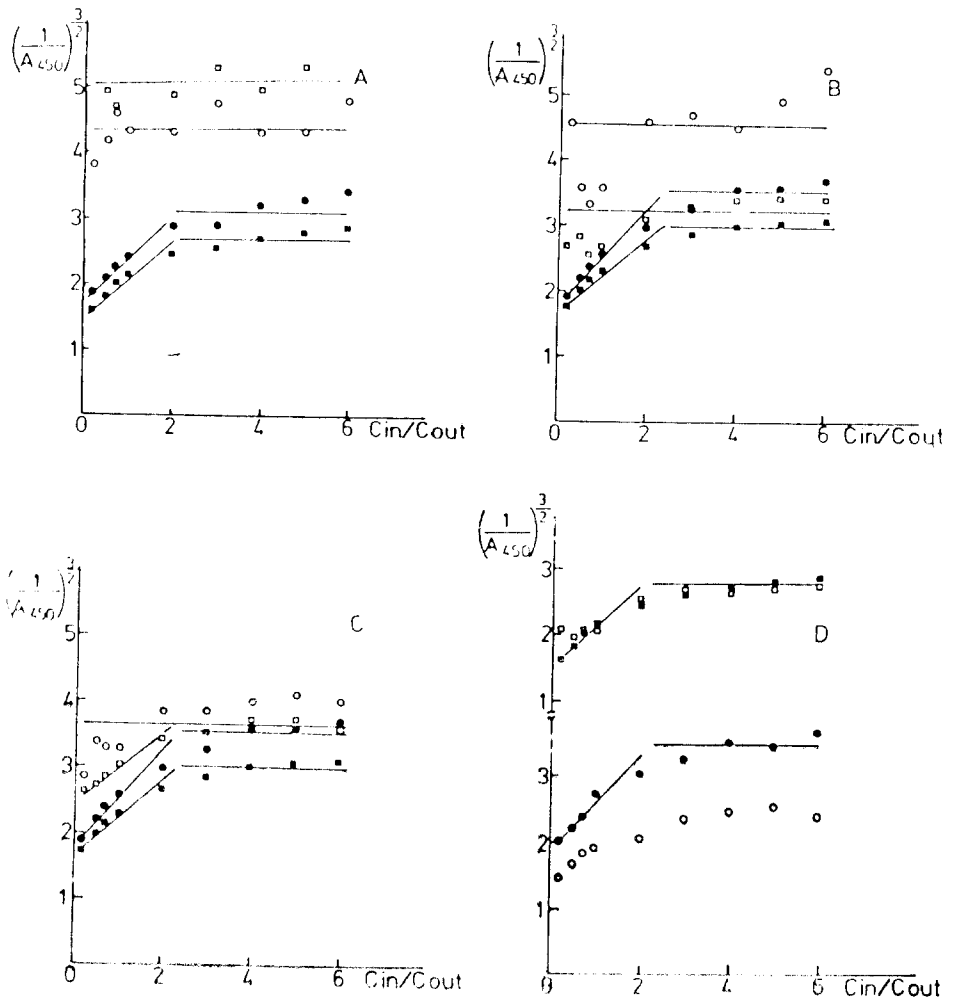

Fig. 2: Effects of saponins on the osmotic behavior of liposomes A, ginseng saponin (1.4mg/ml); B, 20-S-protopanaxadiol saponin (0.7mg/ml); C, 20-S-protopanaxatriol saponin (0.7mg/ml); D, platycodon saponin (1.4mg/ml). Solid circles and squares represent intact PC/PA and PC/PA/Ch liposomes, respectively. Void symbols represent saponin-treated PC/PA and PC/PA/Ch liposomes, respectively.

its interaction with cholesterol in red blood cell membrane[7-10]. But Segal *et al.*[11-14] suggested that cholesterol do not serve as a specific binding site for saponins and sapogenins. In the case of 20-S-protopanaxatriol saponin tested in this study, cholesterol seems not to be a specific target of saponin and it seems to stabilize liposomal membrane against the saponin activity.

Initial Swelling Rate of Liposomes and Effects of Saponins

The absorbance due to light scattering of liposomes particles is proportional to the number of liposomes per unit volume and to the concentration of lipid in the suspension, which together with the relation, $V \alpha 1/A$, gives the following equation,

$$A=k(L)/V$$

where k is a constant and (L) is the lipid concentration[2]. Differenciating the reciprocal of this equation gives the following equation,

$$d(1/A)/dt=1/k\cdot dV/dt\cdot 1/(L).$$

$1/k\cdot dV/dt$, the slope of plot of $d(1/A)/dt$ vs. $1/(L)$, is indicative of initial swelling rate of liposomes because k is a constant.

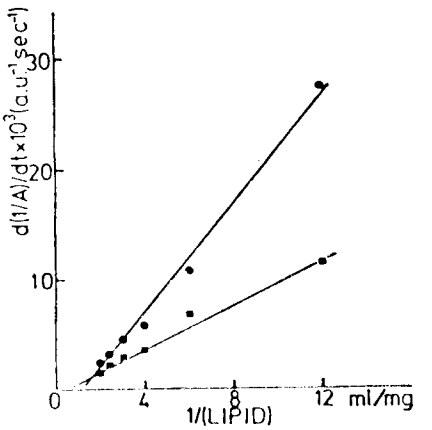

Fig. 3: Initial swelling rate of liposomes composed of ●, PC/PA and ■, PC/PA/Ch.

Fig. 3. shows these plots of PC/PA and PC/PA/Ch liposomes. The difference of slopes of these two plots indicates that water permeation is more rapid in liposomes without cholesterol. The ability of cholesterol to decrease the liposomes permeability was already reported by several authors(15-18).

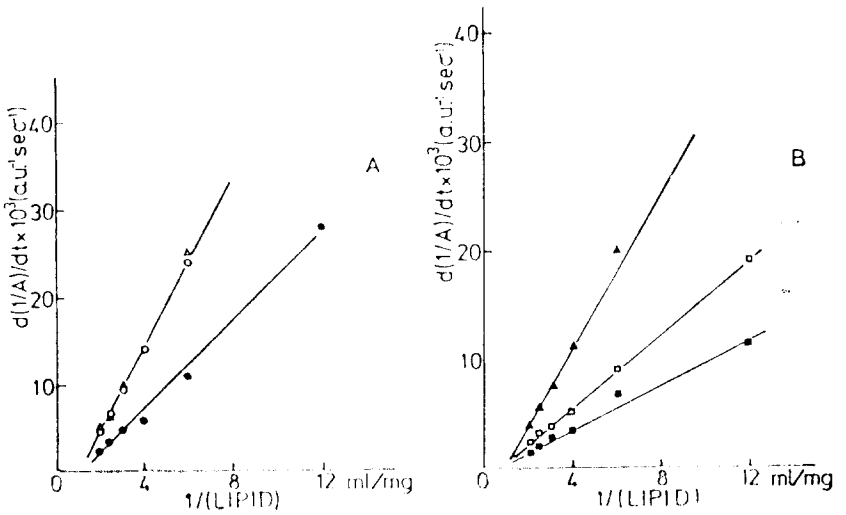

Fig. 4: Effects of saponins on initial swelling rate of liposomes A, Effects of ○, diol and △, triol on ●, PC/PA liposomes; B, Effects of □, diol and ▲, triol on ■, PC/PA/Ch liposomes. Concentration of each saponin was less than 0.6mg/ml.

The effects of ginseng saponins at very low concentration were examined and the results are shown in Fig. 4. Both diol and triol saponins increased the slope of plots of d(1/A)/dt vs. 1/(L) in liposomes with or without cholesterol. These result suggests that ginseng saponins interact with liposomes even at very low concentration.

From the results of this study that 20-S-protopanaxadiol saponin, 20-S-protopanaxatriol saponin and platycodon saponin show different activities, it can be concluded that saponin activity is dependent on its structure. Another important observation is that cholesterol in liposomal membrane plays a different role in the interaction between liposomes and saponins depending on the saponin structure. That is, cholesterol in some cases is a target of saponin activity and in other cases prohibits saponin activity.

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