Korean J. Ginseng Sci.
Vol. 20, No. 1, 23~29(1996)

Effect of Triterpenoidal Glycosides of Dammarane Series and Their Aglycones on Phase Transitions of Dipalmitoylphosphatidylcholine

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(Received March 3, 1996)

Abstract : The effect of ginseng glycosides and their aglycones on the thermodynamic characteristics of membranes from dipalmitoylphosphatidylcholine (DPPC) was investigated. Total saponins (TS) from Korean red ginseng, *Panax ginseng* C.A. Meyer, interacted with the gel phase of lipid in the polar region and did not penetrate the deeper glycerol backbone of lipid molecule. From the all investigated components of TS (aglycons and ginsenosides), only 20-(S)-panaxadiol (PD) had an effect similar to TS. High concentration of TS penetrated in hydrophobic C1-C8 region. The presence of cholesterol did not influence the interaction of TS with DPPC. An elimination of transition, however, took place at 10~100 μg/m*l* of TS. DPPC had a low ability to interact with cholesterol (CHL) as compared with other lecithins except ethanolamine. From our results, only TS and PD, at high concentrations (100 mol%), influenced the phase transition of mixture of DPPC:CHL.

Key words : ginseng, DPPC, cholesterol, phase transition, thermodynamic parameter.

Introduction

The regulation of different biophysical and biochemical processes in cells is connected with phase states of lipid bilayers of membranes. The abilities of various drugs to change the phase properties of lipid bilayers underlie on the base of their effects. The interaction with lipid bilayer and determination of the location of substance in model bilayer conditions will, therefore, allow to understand the effects of substance on the higher biological level.

The pharmacological action of preparations from ginseng is explained in the context of structure-functional level of cell membranes.[1,2] In particular, these substances influence on the barrier function of membranes changing their permeability.[3,4] The pattern of changes in structural states of membrane bilayers by ginseng preparations as well as their locations in membrane is, however, not enoughly investigated.

In our work, the effects of glycosides and their aglycons from Korean red ginseng on the thermodynamic characteristics of model membranes from DPPC were investigated.

Materials and Methods

The L-dipalmitoylphosphatidylcholine (DPPC) (Fluka, German) and cholesterol (CHL) (Serva, German) were used without further purification for the preparation of aqueous multilamellar suspension. Both CHL and DPPC were dissolved in chloroform and were mixed from those stock solutions.[5] Mixtures were dried under argon gas and then evaporated to dryness in a vacuum overnight. After the dried mixtures were dispersed and then resuspended with deionized water, they

were heated to approximately 10~20℃ above the phase transition temperature and were vortexed to give a multilamellar suspension. Glycosides and their aglycones were added in the prepared multilamellar suspension and were incubated for 25~30 minutes at room temperature.

The calorimetric analysis was perfomed on a high-sensitivity differential scanning microcalorimeter (DASM-4, USSR). The scan rate was 1 K/min in all experiments. The concentration of DPPC was 0.1~0.15 μg/m*l*.

The total saponin (TS), 20-(S)-protopanaxadiol (PD), 20-(S)-protopanaxatriol (PT), and ginsenosides Rg_1 and Rb_1 were offered from the analytic center of Korea Ginseng & Tobacco Research Institute (Taejon, Korea).

Results

Pure DPPC multilamellar suspension exhibited two endothermic (pretransition and main) transitions upon heating. The first pretransition occured at low temperature and had small enthalpy and low cooperativity. The pretransition arises from the conversion of a lamellar gel ($L_{\beta'}$) phase to the rippled gel ($P_{\beta'}$) phase. The main phase transition took places at 41℃ and had large enthalpy and high cooperativity. This transition arises from a conversion of the rippled ($P_{\beta'}$) gel phase to the liquid-crystalline (L_{α}) phase. The typical thermogram of DPPC is presented in Fig. 1 (curve 1). The parameters of thermogram in our experiments were not different from published values.[6,8] Pretransition had Tm=34.6℃, dH =1.1 kcal/mol (enthalpy) and $dT_{1/2}$=2.6℃ (half-height). The main transition has dH=8.4 kcal/mol, Tm=41.7℃ and dT1/2=0.6℃.

The parameters of main transition were dependent on the concentrations of TS, glycosides (Rb_1, Rg_1) and their aglycons (PT, PD) both in the absence and presence of cholesterol (Figs. 1

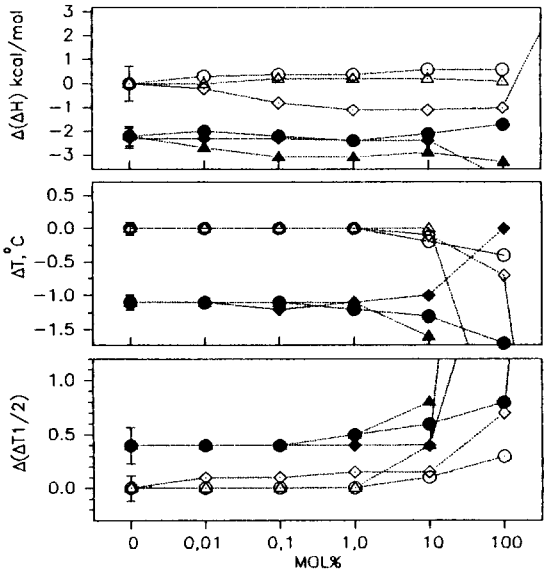

Fig. 1. The influence of TS, PD and PT on the phase transition parameters of pure DPPC and DPPC : CHL (5 mol%) mixture. The values are presented as deviation from values of pure DPPC (see Results). ○ : DPPC+PT; ● : DPPC+PT+CHL (5 mol%); △ : DPPC+PD; ▲ : DPPC+PD+CHL (5 mol%); ◇ : DPPC+TS; ◆ : DPPC+TS+CHL (5 mol%).

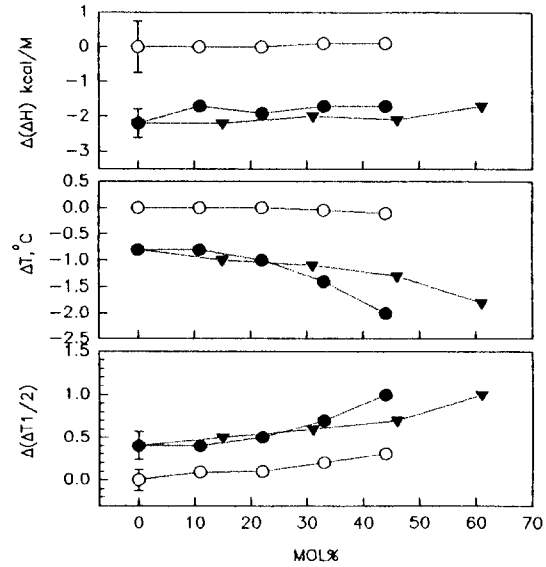

Fig. 2. The influence of ginsenosides Rb_1 and Rg_1 on the phase transition parameters of pure DPPC and DPPC : CHL (5 mol%) mixture. The values are presented as deviation from values of pure DPPC (see Results). ○ : DPPC+Rb_1; ● : DPPC+Rb_1+CHL (5 mol%); ▼ : DPPC+Rg_1+CHL (5 mol%).

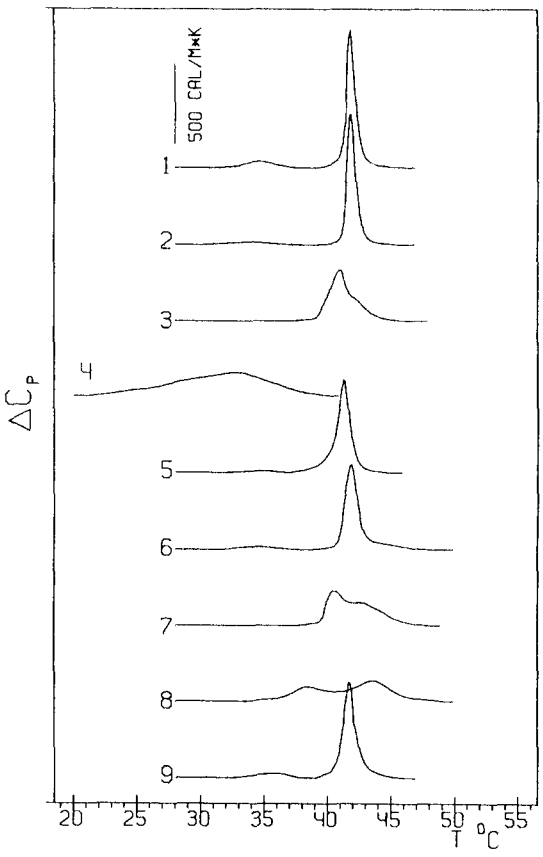

Fig. 3. Temperature dependence of excessive molar heat absorption of pure multilamellar suspensions of DPPC (1), in the presense of 10 μg/m*l* TS (2), 100 μg/m*l* TS (3), 1 mg/ml TS (4), 100mol% PT (5), 10 mol% PD (6), 80 mol% PD (7), 100 mol% PD (8) and 100 mol% Rb_1 (9).

and 2). All parameters are presented in kinds of deviations from foregoing values of dH, $dT_{1/2}$ and Tm of pure DPPC.

1. The Influence of TS on Phase Behavior of Pure DPPC

Increasing the concentrations of TS up to 10 μg/m*l* (weight ratio was TS/DPPC=1 : 15) caused the elimination of pretransition but did not influence on dH, Tm, $dT_{1/2}$ and shape of the main transition. Increasing concentrations up to 100 μg/m*l* (TS/DPPC=1 : 1.5) resulted in the disappearance of pretransition and a further decay of intensity of the main transition. Tm was decreased by 0.7℃. As it is visible from thermogram, an additional peak appeared in the kind of shoulder on the right slope of the main transition. This additional peak arose from melting of the new (second) phase in the gel L_β phase. Further increase of concentrations, up to 1 mg/m*l* (TS/DPPC=6.7 : 1), completely eliminated the main transition. A new broad transition with Tm=32.7℃, $dT_{1/2}$=8.6℃ and dH about 13 kcal/mol was, thus, formed ranging from 20℃ to 39℃.

2. The Influence of PT

PT, up to 10 mol%, poorly influenced on the parameters of main transition and only reduced the enthalpy of pretransition (Fig. 1). Increasing the concentrations up to 100 mol% (see thermogram 5 in Fig. 3) caused the increase of pretransition Tm to 35.3℃, reduced dH even more, and did not change $dT_{1/2}$. The enthalpy of the main transition did not change. $dT_{1/2}$ was increased by 0.3℃ and Tm fell off by 0.4℃. As it is visible from thermogram 5 in Fig. 3, the basis of main transition appears asymmetrical because of the presence of additional transition with small intensity at 40℃.

3. The Influence of PD

PD, up to 100 mol%, did not change the enthalpy of main transition (Fig. 1). At concentration of 10 mol%, PD increased $dT_{1/2}$ by 0.5℃ (Fig. 3, thermogram 6). On the right front of slope at 43℃, the transition of low intensity occurred. Further increase of concentrations up to 80 mol% (Fig. 3, thermogram 7) was followed by the increase of new transition at 43℃. Decreases of intensity and Tm (to 40.5℃) of the main transition were also followed. At concentration of 100 mol% of PD, two separate peaks, with Tm of 38.4℃ and 43.7℃, and $dT_{1/2}$ with 3℃ occurred.

4. The Influence of Ginsenosides Rb_1 and Rg_1

Ginsenosides, Rb_1 up to 30 mol% and Rg_1 to 40 mol%, did not strongly influence on the parameters of phase transition (Fig. 2). The increase of Rb_1 up to 44 mol% (thermogram 9 in Fig. 3) did not eliminate the pretransition. Tm and $dT_{1/2}$

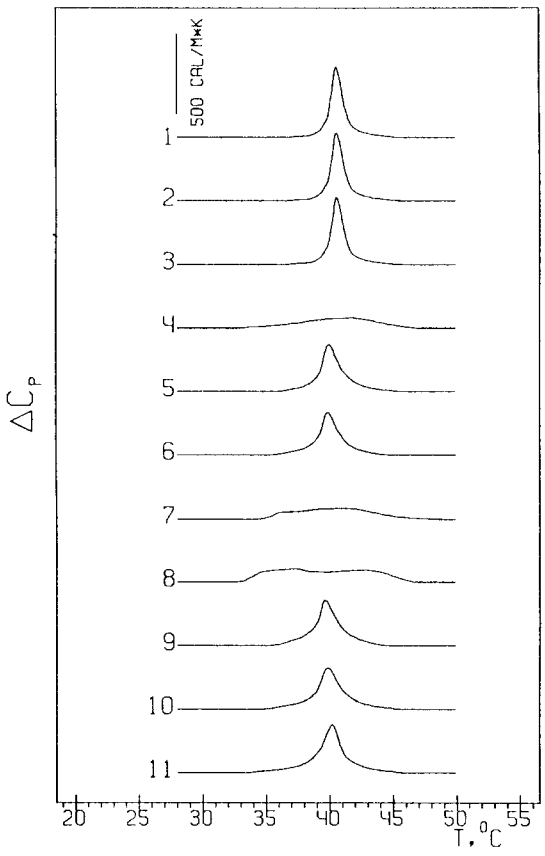

Fig. 4. Temperature dependence of excessive molar heat absorption of multilamellar suspensions of DPPC : CHL, 5 mol% (1), in the presense of 0.1 μg/m*l* TS (2), 10 μg/m*l* TS (3), 100 μg/m*l* TS (4), 100 mol% PT (5), 10 mol% PD (6), 100 mol% PD (7), 100 mol% PD and 1 mol% CHL (8), 100 mol% Rb_1 (9), 100 mol% Rg_1 (10) and mixture of Rb_1 and Rg_1 with total concentration of 60 mol% (11).

were increased by 1.5℃ and 0.5℃, respectively. The main transition did not change. Only $dT_{1/2}$ increased slightly by 0.3℃ at concentrations more than 40 mol%.

5. The Influence of Aglycons on Phase Behaviour of DPPC : CHL Mixture

It is known that CHL strongly changes the thermotropic properties of DPPC.[5,6] CHL at 5 mol% eliminated pretransition, reduced dH to 6.5 kcal/mol, Tm to 40.6℃ and increased $dT_{1/2}$ to 1.1 ℃ (see thermogram 1 in Fig. 4). TS, at concentration of 10 μg/m*l*, did not change the thermotropic phase behaviour of DPPC : CHL mixture. The increase of TS concentrations resulted in a gradual elimination of main transition. At 100 μg/m*l* of TS, a broad component of endotherm only remained with dH=4.6 kcal/mol, Tm=42℃ and $dT_{1/2}$=6.6℃. In the presence of CHL, TS, at 100 μg/m*l*, did not induce the formation of the second phase as in the case of pure DPPC.

It is visible from Fig. 1 that PT slightly influenced on the enthalpy, Tm and $dT_{1/2}$ of DPPC : CHL mixture. Only at concentration of 100 mol%, PT shifted Tm of transition by 0.6℃ and increased $dT_{1/2}$ by 0.3℃ as compared to DPPC/CHL mixture (see thermogram 5 in Fig. 4). PD, likely as with pure lipid, is more active than PT. At concentration of 10 mol%, PD decreased the Tm of thermogram by 0.6℃ and increased $dT_{1/2}$ by 0.5℃. The increase of PD concentrations up to 100 mol% eliminated the main phase transition. The formation of two phases seen with pure lipid, however, did not entail. The reduction of CHL concentrations down to 2 mol% resulted in the occurrence of two transitions alreadily seen with pure lipid (Fig. 3) within the same range of temperature.

Ginsenosides Rb_1 and Rg_1 also slightly influenced on the phase transition of DPPC : CHL mixture. The increase of Rb_1 concentrations up to 40 mol% and Rg_1 to 60 mol% resulted in the identical effect: Tm was decreased by 0.8℃ and $dT_{1/2}$ was increased by 0.7℃ (see thermograms 8, 9 and 10 in Fig. 4). It is necessary to note that the similar effect was possible at the action of Rg_1 and Rb_1 when they are mixed together in total concentrations of 55~60 mol% (Fig. 4, thermograms 9, 10 and 11).

Discussion

According to Jain,[6] the thermodynamic parameters and forms of peak of main transition in DPPC depend on the depth of penetration of

substance in the hydrophobic region of bilayer. The changes of enthalpy, Tm and $dT_{1/2}$ were reflected by the amount of lipids participating in transition, packaging densities of hydrocarbon chains, and numbers of molecules in cooperative domain. Our results demonstrate the ability of TS to induce the formation of the second phase in bilayers at 10~100 μg/m*l* concentrations. The first phase is consisted of pure DPPC with slightly decreased packing of chains as is confirmed by a shift of Tm to 41.0℃. Therefore, a size of cooperative domain does not vary as far as the $dT_{1/2}$ of main phase transition does not change.

The disappearrance of pretransition, decrease of enthalpy of main transition at a constant size of domain and invariable general enthalpy may be connected with the interaction of TS with gel phase of DPPC. In results, two phases are formed: the first phase is a pure lipid and a new phase is a mixture of DPPC:TS with more closed package and smaller size of cooperative domain than at pure lipid. TS interacts with gel phase of lipid preferably in polar region of bilayer and does not penetrate the deeper glycerol backbone of lipid molecule. It is possible to expect that the subsequent increase of concentrations should remove the phase of pure lipid and leave only phase of DPPC:TS with constant total enthalpy and Tm at 41~43℃.

The increase of concentration, however, results in the destabilization of bilayer with decreasing Tm and consequently disturbing packing of lipids strongly and decaying sizes of cooperative unit largely. The increase of enthalpy, up to 13 kcal/mol, appears strange enough. This behaviour is possibly explained by the change of partition coefficient by TS at its high concentration. Accordingly, TS components penetrate in hydrophobic C1-C8 region. As a result, high concentration of TS modifies the gel phase and TS penetrates in bilayer during transition. That is explained by a similar increase of enthalpy of transition. The presence of CHL does not influence on the interaction of TS with DPPC. However the elimination of transition takes places without the additional formation of two phases at 10~100 μg/m*l* of TS.

According to Jain,[6] compounds which induce a new phase in the kind of shoulder on the right slope of the main peak are relatively large, disk-shaped, asymmetric and reasonably polar. Such characteristics coincide well with the structure of aglycones. From the all investigated components of TS (aglycons and ginsenosides), only PD had an effect similar to TS. In a given research, just PD possibly attributed to the ability of TS to form two phases. Thermotropic behaviour of DPPC:PD is similar to the behaviour of DPPC:TS. PD induced two phase formation in gels with different parameters of packing and of sizes in cooperative domain at 10 mol%. We believe that PD incorporates in the polar region of bilayer. The DPPC:PD phase is consisted of the domains with small size but with more closed packing of lipids than in pure lipid. Specially we note that PD slightly disturbed the packing density of lipids and sizes of cooperative unit.

PT and ginsenosides poorly influenced on the phase transition of DPPC. PT slightly (by 0.3℃) increased $dT_{1/2}$ transition and decreased sizes of lipid domain at 100 mol%. Rb_1 did not influence on the pretransition with smaller than 40 mol%. Only at 40 mol%, Rb_1 slightly reduced the size of cooperative domain.

It is impatient to make a direct conclusion about abilities of PT, Rg_1 and Rb_1 to interact with bilayer from our experimental data. The absence of strong influence on phase transition testifies that the mentioned substances do not interact with the lamellar ($L_{\beta'}$) gel phase of DPPC.

As it is known, the addition of CHL modifies the lipid bilayer.[5,7,8] But DPPC has a low ability to interact with CHL as compared with other lec-

ithins except ethanolamine.[7] It is visible from the results that only TS and PD at high concentrations (100 μg/m*l* and 100 mol%) influenced on the phase transition of mixture of DPPC:CHL. It is visible that PT (100 mol%), PD (10 mol%), Rg_1 (60 mol%) and Rb_1 (60 mol%) caused very similar effects: they poorly disturbed the packing of lipids and slightly reduced the sizes of cooperative domains of DPPC in mixture with CHL. In the case of high concentration of TS (100 μg/m*l*), biphasic system was not formed. TS, with the growth of concentrations up to 100 μg/m*l*, eliminated the transition with 5 mol% CHL, while the similar result was reached in the case of pure lipid at 1 mg/m*l* of TS. TS acted more effectively in the presence of CHL. As far as we have assumed that the phase separation is presumably connected with effect of PD, its last addition to mixture of DPPC:CHL, as well as in case of TS, did not cause the formation of two phases but eliminated the transition. It is possible to assume that CHL and high concentrations of TS and PD act together in the elimination of phase transition.

It is, however, difficult to generalize about the nature of interaction of CHL with TS or PD in comparison with pure DPPC from our data. On one hand, an availability that CHL interacts more strongly with TS or PD than DPPC may be generated, permitting one's expection that the "effective" concentration of TS or PD is decreased in the presence of CHL, which is confirmed by the elimination of transition at the concentration of 100 μg/m*l* of TS in the presence of CHL. On the other hand, the addition of CHL to mixture of DPPC:PD (100 mol%), causes more rapid elimination of the second phase than pure lipid phase if CHL interacts more strongly with PD than with DPPC. We notice, however, rather the uniform elimination of both phases resulted from uniform affinities of CHL to phases of both pure lipid and DPPC:PD. If the last statement is true, in cases of TS and PD, CHL creates the better sterical conditions for the incorporation of TS and PD in gel phase, as if it promotes their effects.

In the given work, we did not put the purpose to establish the connection between the structure of studied substances with their abilities to penetrate in different regions of lipid bilayers. This is the subject of our further studies.

요 약

Diphosphatidylcholine(DPPC)으로 제조한 인공막에서 열역학적 변수에 미치는 인삼의 glycosides와 그 aglycones의 영향을 조사했다. 인삼의 총사포닌(TS)은 지질막의 극성을 띄는 부분에 작용하여 그곳의 지질분자들의 겔상에 영향을 주었으며, 지질분자의 글리세롤 구조에는 영향을 미치지 않았다. 조사한 물질들 중 파낙사다이올(PD)만이 TS의 작용과 비슷했고, 고농도의 TS는 비소수성인 C1부터 C8까지의 영역을 침투했다. 콜레스테롤(CHL)은 TS와 DPPC의 상호작용에 아무런 영향을 주지 않았으며, TS의 농도가 10~100 μg/m*l*일 때는 2개의 상을 보이는 상전이가 없어졌다. 우리는 이 실험에서 TS와 고농도의 PD(100 mol%)만이 DPPC와 CHL로 이루어진 복합체의 상전이에 영향을 미침을 확인했다.

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