A niosomal bilayer of sorbitan monostearate in complex with flavones: a molecular dynamics simulation study

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Abstract

Bilayers prepared from sorbitan fatty acid esters (Span) have been frequently used for delivery of drugs including flavonoids. We applied molecular dynamics simulation to characterize the structure of a sorbitan monostearate (Span 60) bilayer in complex with three representative flavones, a subclass of flavonoids. At a low concentration, unsubstituted flavone, the most hydrophobic member, was able to flip over and cross the bilayer with a large diffusion coefficient. At a high concentration, it was accumulated at the bilayer center resulting in a phase separation. The leaflets of the bilayer were pushed in the opposite directions increasing the membrane thickness. Order parameter of the stearate chain of Span 60 was not affected significantly by unsubstituted flavone. In contrast, chrysin with hydroxylated ring A was lined up with the acyl chains of Span 60 with its hydroxyl group facing the membrane surface. Neither flipping nor transbilayer movement were allowed. Diffusion coefficient was only 15–25% of that of unsubstituted flavone and order parameter decreased with the concentration of chrysin. Luteolin, the most hydroxylated member, interacted mainly with the headgroup of Span 60 and assumed many different orientations without crossing the bilayer. Unlike chrysin and unsubstituted flavone the bilayer integrity was disrupted at 50 mol% luteolin. These behaviors and structures of flavones in a Span 60 bilayer can be accounted for by their hydrophobicity and sites of hydroxylation.

Keywords

Flavones, flavonoids, molecular dynamics simulation, niosome, Span 60

Introduction

Flavonoids are a group of phytochemicals derived from benzo-γ-pyrone. They have attracted much attention due to their biological activity against oxidative stress, inflammation, and cancer (Romano et al., 2013). Chemical properties of flavonoids and their interaction with the active site of an enzyme have been studied extensively to understand the mechanisms of action. Association of flavonoids with biomembranes is another important subject in relation to antioxidant activity and cellular absorption of flavonoids. Interaction of flavonoids and phospholipid membranes have been studied by a variety of experimental techniques including X-ray scattering (How et al., 2014; Raghunathan et al., 2012; Sun et al., 2009), fluorescence (Abram et al., 2013; Chaudhuri et al., 2007; Ulrih et al., 2010), infrared absorption (How et al., 2014; Pawlikowska-Pawlega et al., 2013), nuclear magnetic resonance (NMR) (Pawlikowska-Pawlega et al., 2013; Scheidt et al., 2004; Sinha et al., 2011, 2014a,b), electron spin resonance (ESR) (Abram et al., 2013; Pawlikowska-Pawlega et al., 2013; Ulrih et al., 2010), differential scanning calorimetry (DSC) (Abram et al., 2013; How et al., 2014; Saija et al., 1995; Sinha et al., 2011, 2014a,b), and isothermal titration calorimetry (ITC) (Sun et al., 2009). In addition, molecular dynamics (MD) simulation has been applied to yield an atomistic view of flavonoid–membrane interaction (How et al., 2014; Košinová et al., 2012; Raghunathan et al., 2012; Saija et al., 1995; Sinha et al., 2011, 2014b; Sirk et al., 2008a,b).

Flavones, a subclass of flavonoids, have a backbone structure shown in Figure 1(b). Hydroxylation of the aromatic rings produces naturally occurring flavones such as 6-hydroxyflavone, chrysin, apigenin, baicalein, and luteolin. Using magic angle spinning NMR Scheidt et al. (2004) showed that ring B of luteolin is close to the POPC membrane surface whereas unsubstituted flavone has no orientational preference. Chrysin lines up with the membrane normal with its ring A close to the surface. Apigenin rigidifies a DPPC bilayer and is preferentially located near the surface (Pawlikowska-Pawlega et al., 2013). Interaction of chrysin with a DPPC bilayer decreases phase transition temperature (Sinha et al., 2014b). Sinha et al. (2014a) examined six flavones with DSC and NMR to show that ring A lies near the membrane–water interface of a DPPC bilayer whereas ring B partitions into the fatty alkyl chain. Flavone without a hydroxyl group penetrates deep into the hydrophobic region.

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Niosomes are lamellar vesicles prepared from nonionic surfactants. With a low cost of production and a good chemical stability, they are widely used as vehicles for drug delivery. Among nonionic surfactants sorbitan fatty acid esters (Span) are highly biocompatible and thus niosomes of Span have been used in the delivery of drugs including flavonoids (Sarkar et al., 2002; Song et al., 2014; Waddad et al., 2013). In this respect, it is important to understand the interaction of flavonoids with niosomal bilayers of Span on a molecular level but the subject has not been studied extensively either by experimental or theoretical methods. As an extension of our previous work on niosomes (Han, 2013a; Yeom et al., 2014), we performed MD simulation of sorbitan monostearate (Span 60) bilayers in complex with three representative flavones, e.g. unsubstituted flavone, chrysin, and luteolin.

Methods

Structures of flavones studied in this work are shown in Figure 1. Chrysin has hydroxyl groups only on ring A whereas luteolin has them on rings A and B. They are naturally occurring phytochemicals. Unsubstituted flavone was also included as a hydrophobic flavone. Atom numbering of Span 60 and flavones are shown in Figure 1(a) and (b), respectively. Two angles, $\alpha$ and $\beta$, are defined in Figure 1(e) to describe the orientation of a flavone in the bilayer.

The Gromos 43a1-s3 force field (Chiu et al., 2009) was used in the calculation because it has been extended to include several phospholipids and cholesterol and successfully reproduced the physical properties of lipid bilayers (Braun et al., 2013; Han, 2013a,b). Topology and parameters for the stearate and glycerol fragments of sorbitan monostearate (Span 60) were transferred from DPPC whereas those for sorbitan moiety were obtained from similar structures listed in the Gromos 43a1-s3 force field. Parameters for flavones were obtained from ProDrG (Schüttelkopf & van Aalten, 2004) but the atomic charges were reassigned by looking up the similar structures listed in the Gromos 43a1-s3 force field (structure and topology files are given in Appendix).

Gromacs software package (version 4.5.2) was used for MD simulation (van der Spoel et al., 2005). To construct an initial structure, desired number of molecules of Span 60, cholesterol, and flavone were placed in a $12 \times 12$ array of grid boxes (size 0.5 nm) to make a layer of 144 molecules. The layer was then duplicated, inverted, and combined with the first layer to produce a bilayer. Finally Packmol (Martinez et al., 2009) was used to generate a water box of 1.5 nm in thickness above and below the bilayer.

The initial structure was subjected to an energy minimization and subsequently equilibrated for 100 ps at 1 bar and 298 K. A production MD simulation was carried out until the size of the simulation box reached an equilibrium. Particle mesh Ewald method (Essman et al., 1995) was used in the calculation of Coulomb interactions. Cutoff values for the Coulomb and van der Waals interactions were 1.0 and 1.6 nm, respectively. Bond lengths were constrained by using the LINCS algorithm (Hess, 2008) to allow a 2 fs integration. The temperature of the bilayer (lipids and flavone) and solvent (water) were coupled separately using a V-rescale thermostat (Bussi et al., 2007). A barostat (Parrinello & Rahman, 1981) was used to couple the pressure semi-isotropically. The results were reproducible in that the equilibrium properties were not dependent of the initial structures.

Figure 1. Structures of (a) Span 60, (b) unsubstituted flavone, (c) chrysin, and (d) luteolin. Numbering of the atoms and rings of a flavone is shown in (b). In (e), $\alpha$ is defined by the angle between the vector from $C_5$ to $C_2$ (the long molecular axis; blue arrow) and the membrane normal, which is $z$-axis. Also defined is $\beta$, which is the angle between the normal to the rings A and C (red arrow) and the membrane normal.
Appropriate tools provided by the Gromacs package were used to calculate the density profiles along the normal to the membrane and the order parameter. To obtain a diffusion coefficient the mean squared displacement was plotted against time and the slope from the linear part was divided by 4 and 6 for a lateral (Span 60) and three-dimensional diffusion (flavone), respectively. Some bilayers took a very long simulation time (>300 ns) to reach equilibrium, a state with a constant size of the simulation box. In all analyses only the data from the last 20 ns of simulation were used.

**Results**

Span 60 bilayers studied in this work were consisted of equimolar concentrations of Span 60 and cholesterol, a composition that has been widely used in drug delivery. Cholesterol is known to be essential for the physical stability of the bilayer. In addition to a pure Span 60/cholesterol bilayer, we constructed bilayers containing 3, 25, and 50 mol% flavone, which had a composition of Span 60/cholesterol/flavone in a ratio of 140:140:8, 108:108:72, and 72:72:144, respectively. Chrysin molecules in the initial structures were aligned with its hydroxylated ring A pointing toward the membrane surface. As both rings A and B are hydrophobic and hydrophilic in unsubstituted flavone and luteolin, respectively, no orientational preference was presumed in positioning these flavones within the membrane. To avoid confusion we use “flavone” only for the group which “unsubstituted flavone”, chrysin, and luteolin belong to.

Figure 2. Equilibrium structure of a Span 60 bilayer containing (a) 3 mol% and (b) 50 mol% unsubstituted flavone. Span 60, cholesterol, and flavone are in gray, green, and orange, respectively.
Interaction of unsubstituted flavone with a Span 60 bilayer

Figure 2 shows the equilibrium structures of Span 60 bilayers at the end of MD simulation. At a concentration of 3 mol%, unsubstituted flavone stayed in the hydrophobic region of the bilayer (Figure 2a). Some molecules were located close to the surface while others near the bilayer center. The molecules appeared to prefer lining up with the lipid chains although they were allowed to flip over to the other orientation. When the concentration was increased to 25 mol%, some molecules of unsubstituted flavone formed clusters resulting in an inhomogeneous distribution within the membrane (not shown). If the concentration was increased further to 50 mol%, the majority of the molecules were lumped together at the bilayer center pushing the two leaflets in the opposite direction and thus making the membrane thicker (Figure 2b). Small number of molecules stayed in the hydrophobic region of the lipids.

In order to analyze the structure in greater detail, we calculated electron densities of Span 60, cholesterol, and unsubstituted flavone and the results are presented in Figure 3. Span 60 had a characteristic of a typical bilayer with its two maxima separated by 4.08 nm. The C1, O\upsilon, and O\upsilon atoms of Span 60 were located, respectively, at 1.87, 2.10, and 2.28 nm from the bilayer center with a well-defined distribution (not shown). The maximum of the electron density of Span 60 coincided with that of O\upsilon, which is close to the center of the sugar (sorbitan) ring. Cholesterol was positioned with its oxygen atom close to the ester oxygen of Span 60. Inclusion of 3 mol% flavone did not have a significant effect on the electron density of either Span 60 or cholesterol. As shown in Figure 3(a), the electron density of unsubstituted flavone was large in the hydrophobic region of the lipids and its penetration into the headgroup region was negligible (Figure 3a). Its carbonyl oxygen (O\upsilon) distributed throughout the bilayer interior with several preferred locations (not shown). This is consistent with the transbilayer movement of unsubstituted flavone that will be discussed below.

When 25 mol% unsubstituted flavone was present in the bilayer, electron density profiles of Span 60 and cholesterol did not change significantly except that the bilayer thickness defined as the C1–C1 distance of Span 60 decreased slightly from 3.73 nm to 3.61 nm (see Table 1). Unsubstituted flavone, however, started to have a large electron density at the bilayer center. Surface area per lipid increased from 0.304 nm\(^2\) to 0.358 nm\(^2\) (Table 1), implying that the flavone molecules in clusters occupied some regions within the membrane. At a concentration of 50 mol%, electron density of Span 60 and cholesterol was depleted from the bilayer center and replaced by unsubstituted flavone. The bilayer thickness increased dramatically to 5.25 nm. Surface area per lipid was 0.318 nm\(^2\), much smaller than the value for 25 mol% and only slightly larger than the value for 3 mol%. As the flavone molecules underwent a phase separation forming a large cluster at the bilayer center, the region occupied by the lipids was largely

![Normalized electron density of Span 60 (black), cholesterol (green), and unsubstituted flavone (orange) in a Span 60 bilayer containing (a) 3 mol%, (b) 25 mol%, and (c) 50 mol% unsubstituted flavone.](image)

Figure 3. Normalized electron density of Span 60 (black), cholesterol (green), and unsubstituted flavone (orange) in a Span 60 bilayer containing (a) 3 mol%, (b) 25 mol%, and (c) 50 mol% unsubstituted flavone.

### Table 1. Physical properties of Span 60 bilayers containing flavones.

| Flavonoid | Mol%\(^a\) | Thickness (nm)\(^b\) | Surface area per lipid (nm\(^2\))\(^c\) | Diffusion coefficient (µm\(^2\)/s) | Span 60\(^d\) | Flavonoid\(^e\) |
|-----------|-------------|----------------------|----------------------------------|-----------------------------------|----------------|----------------|
| None      | –           | 3.74                 | 0.299                            | 6.21                              | –              | –              |
| Flavone (unsubstituted) | 3.3  | 3.73                 | 0.304                            | 4.87                              | 21.9           | –              |
|           | 25          | 3.61                 | 0.358                            | 4.86                              | 6.26           | –              |
|           | 50          | 5.25                 | 0.318                            | 3.08                              | 3.17           | –              |
| Chrysin   | 3.3         | 3.73                 | 0.305                            | 6.03                              | 3.53           | –              |
|           | 25          | 3.29                 | 0.376                            | 3.08                              | 1.93           | –              |
|           | 50          | 2.89                 | 0.525                            | 1.38                              | 0.79           | –              |
| Luteolin  | 3.3         | 3.80                 | 0.298                            | 3.28                              | 3.49           | –              |
|           | 25          | 3.79                 | 0.310                            | 1.10                              | 0.49           | –              |
|           | 50          | n.d.\(^f\)           | 0.413                            | 0.16                              | 0.27           | –              |

\(^a\)Refers to the ratio of a flavone to the total lipid, the latter being Span 60/cholesterol 1:1.
\(^b\)Defined as the distance between the C1 of Span 60 in the opposite leaflets.
\(^c\)Area of simulation box at equilibrium was divided by the total number of lipids (Span 60 and cholesterol) in a single leaflet of the bilayer.
\(^d\)Lateral diffusion.
\(^e\)Three-dimensional diffusion.
\(^f\)Not determined.
devoid of the flavone molecules and thus had a similar property as the pure bilayer without a flavone.

Dynamic properties of the bilayer were examined by the trajectories of the position (center of mass) and orientation (angles $\alpha$ and $\beta$ defined in Figure 1) of flavone molecules during the simulation as shown in Supplementary Figure S1. In the bilayer containing 3 mol% unsubstituted flavone, some flavone molecules traveled from one leaflet to the other. This high mobility was also manifest in the diffusion coefficient ($21.9 \, \text{m}^2/\text{s}$), which is 6–7 times as large as that of chrysin and luteolin (see Table 1). Large fluctuations in the angle $\alpha$ were observed although the flavone molecules spent more time with $\alpha$ of 0 (up) and 180 degrees (down). The angle $\beta$ fluctuated around 90 degrees with a relatively small amplitude suggesting that the molecules rotated about the axis perpendicular to the molecular plane. At a higher concentration of the flavone, the transbilayer motion and rotation of the flavone molecules were more restricted along with diffusion but the fluctuation in $\beta$ was increased to some extent. Interestingly, order parameter, a measure of segmental motion along the fatty acid chain, was not affected significantly by the presence of unsubstituted flavone at all concentrations. As discussed above, the formation of clusters of the flavone molecules and phase separation leave intact the region occupied by Span 60 and cholesterol. Average number (0.67) of hydrogen bonds between the Span 60 molecules was not altered by the presence of unsubstituted flavone in accord with the above interpretation.

Interaction of chrysin with a Span 60 bilayer

Chrysin is doubly hydroxylated at ring A and thus more hydrophilic than unsubstituted flavone. Snap shots of the
Chrysin-containing bilayers at the end of MD simulation are shown in Figure 4. Chrysin molecules, when present at 3 mol% in a Span 60 bilayer, stayed parallel with the lipid molecules with their hydroxylated ring A close to the membrane surface and ring B in the bilayer interior. When the concentration was increased to 25 mol%, chrysin molecules were distributed evenly in the lipid molecules without appreciable formation of clusters (not shown). If the concentration was raised to 50 mol%, distribution of chrysin was still homogeneous and most of chrysin molecules were well aligned underneath the membrane surface (Figure 4b). Surface area per lipid increased from 0.305 nm² to 0.525 nm² as the chrysin concentration was raised from 3 to 50 mol% (Table 1). Number of hydrogen bonds between Span 60 decreased from 0.68 to 0.56 per molecule as chrysin molecules in between made Span 60 molecules further apart. Changes in the surface area and number of hydrogen bonds are consistent with a homogeneous mixing of chrysin and lipids.

Figure 5 shows the normalized electron density profiles of Span 60, cholesterol, and chrysin in the bilayer. Again Span 60 exhibited a characteristic of a typical bilayer independent of the chrysin concentration. The bilayer with 3 mol% chrysin showed no electron density of chrysin near the bilayer center (Figure 5a). Distance of O₇ of chrysin from the bilayer center was almost same as C₁ of Span 60, which coincided with the hydroxyl group of cholesterol (not shown). A chrysin molecule formed ~2 hydrogen bonds with water molecules that diffused into the headgroup region. As expected, O₇ was positioned closer to the bilayer center than O₇. When the concentration of chrysin was increased to 25 mol%, the membrane thickness decreased from 3.73 nm to 3.29 nm (Table 1). As a result the electron density of lipids increased around the bilayer. This was more evident when the chrysin concentration was raised to 50 mol%. A careful inspection of the structure revealed that many Span 60 molecules had their stearate chain bent or folded at the end. In addition, cholesterol showed a large extent of interdigitation of its tail. This is consistent with the electron density profiles in Figure 5(c).

Chrysin molecules, unlike unsubstituted flavone, were locked in a z-position and never crossed the bilayer although fluctuations were increased with concentration (Supplementary Figure S2). In a bilayer containing 3 mol% chrysin, the angle α stayed very close to 0 and 180 degrees (these two are the same as the two leaflets are facing opposite to each other) indicating that no flipping is allowed and the long molecular axis is parallel with the lipid chains. The angle β fluctuated around 90 degrees with a relatively small amplitude suggesting that the plane of rings A and C lied parallel to the membrane normal. At a higher concentration of chrysin, orientation of a chrysin molecule within the bilayer stayed unchanged although fluctuations increased with the concentration. Chrysin had a significant effect on order parameter especially in the middle part of the stearate chain (Supplementary Figure S4). Introduction of chrysin apparently disturbed close packing of lipid molecules.

**Interaction of luteolin with a Span 60 bilayer**

Both rings A and B are hydroxylated in luteolin making it even more hydrophilic than chrysin. Figure 6(a) shows a bilayer with 3 mol% luteolin at an equilibrium. Most of the luteolin molecules were trapped in the headgroup region. Only one molecule assumed a vertical orientation with its ring B lying in the hydrophobic interior. When the luteolin concentration was increased to 25 mol%, most of the luteolin molecules were still in the headgroup region with some protruding to the aqueous phase (not shown). Only a few were in the bilayer interior and the bilayer integrity was preserved. Luteolin molecules in the headgroup region interacted with each other to form chain-like clusters. When the concentration was further increased to 50 mol%, luteolin formed larger clusters on the surface and the bilayer was disrupted significantly (Figure 6b). Luteolin on the surface was in contact with water and each molecule formed ~3.8 hydrogen bonds with water.

Figure 7(a) shows electron density profiles of luteolin and lipids in a bilayer containing 3 mol% luteolin. Unlike chrysin and unsubstituted flavone, luteolin had a maximum electron density at a position very close to that of Span 60, which corresponds to the sugar ring. This is consistent with the structure shown in Figure 6, in which luteolin molecules were embedded in the headgroup region. It had little density in the bilayer interior due to its high hydrophilicity. When the luteolin concentration was increased to 25 mol%, the bilayer integrity was disrupted slightly. Small increase in the surface area was observed without a change in the bilayer thickness (Table 1). A large disruption of the bilayer structure was detected at 50 mol% luteolin. There was a large increase in the surface area, but the thickness could not be determined due to disappearance of well-defined maxima in the density profile.

In a bilayer containing 3 mol% luteolin, the center of mass of luteolin stayed further away from the bilayer center than...
chrysin and unsubstituted flavone in accordance with the binding of luteolin to the headgroup region (Supplementary Figure S3). The long molecular axis changed constantly in all directions as reflected in the large fluctuations in $\alpha$. The angle $\beta$ also changed in large quantities suggesting that luteolin, with both rings A and B that are available for the interaction with the polar headgroup and water molecules, constantly switches between the two. At a higher concentration, the transbilayer motion was restricted so that a luteolin molecule was almost trapped at a given $z$-position. The angle $\alpha$ was allowed to have many different values with relatively small fluctuations meaning that the long molecular axis has various orientations that cannot be changed easily. The molecular plane stayed parallel with the lipid chains although tilting was significant at a 50 mol% concentration (Supplementary Figure S4). There was only a small change in order parameter when the luteolin concentration was increased from 3 mol% to 25 mol%. As luteolin molecules interacted mainly with the atoms in the headgroup region, they had little effect on the bilayer interior.

**Discussion**

Interaction of flavonoids with phospholipid bilayers has been studied extensively by experimental and theoretical methods to understand the structure–antioxidant activity relation and bioavailability of bioactive flavonoids (Hendrich, 2006; Selvaraj et al., 2015). To our knowledge, no report has been published on the structure of flavonoids in complex with niosomal bilayers although use of niosomes as vehicles for drug delivery has been increased recently (Sarkar et al., 2002; Song et al., 2014; Tavano et al., 2014; Waddad et al., 2013).
We therefore compare our results with those obtained for phospholipid bilayers.

Unsubstituted flavone is not a naturally occurring flavonoid so that its interaction with a lipid membrane has rarely been studied. A detailed study of unsubstituted flavone complexed with a lipid bilayer has been reported by Sinha et al. (2014a), who used DSC and multinuclear NMR to locate the flavone within a DPPC bilayer. The concentration of the flavone was varied between 5 mol% and 29 mol%, which is comparable to our work. Unsubstituted flavone has a higher affinity toward the membrane than its hydroxylated derivatives suggesting that hydrophobic interaction is predominant for the flavone–membrane association. They also found that unsubstituted flavone penetrates deep into the hydrophobic core of the lipid, has a minimal effect on the headgroup mobility, and does not have a preferred orientation. Similar results were also obtained by others (Scheidt et al., 2004). Our simulation on a POPC bilayer and a Span 60 bilayer also demonstrated a large mobility of unsubstituted flavone within the bilayer interior. Unsubstituted flavone like cholesterol decreased the membrane fluidity of a POPC bilayer (Supplementary Figure S5a) presumably due to a packing effect. However, it had a marginal effect on a Span 60 bilayer (Supplementary Figure S4a), which is already loaded with cholesterol. Order parameter of the Span 60/cholesterol bilayer was much larger than that of the POPC bilayer in the absence of unsubstituted flavone.

A unique property of unsubstituted flavone is that it accumulated as a lump at the bilayer center when present at a high concentration (Figure 2b). In a POPC bilayer, such a phase separation was observed at a higher concentration. A similar phenomenon was also observed for other hydrophobic molecules in a lipid bilayer (unpublished results). In this way, a large quantity of a hydrophobic drug can be loaded in a liposome without disrupting the integrity of the bilayer.

Interaction of chrysin with a DPPC and POPC bilayer has been studied previously. Sinha et al. (2014a) reported that the hydroxylated ring A is located near the DPPC-water interface. They proposed that chrysin lies perpendicular to the membrane normal although baicalein (with one more hydroxyl group on ring A than chrysin) and 6-hydroxyflavone assume a parallel orientation. In contrast, an earlier study by Scheidt et al. (2004) proposed that ring A of chrysin in a POPC bilayer lies close to the membrane surface and ring B penetrates the hydrophobic region. MD simulation of a chrysin-POPC system showed that the orientation of chrysin in the POPC bilayer was in general the same as that in the Span 60 bilayer, but the latter has a less mobility due to a tighter packing of the membrane interior (not shown). Chrysin decreased order parameter of the Span 60/cholesterol bilayer (Supplementary Figure S4b) but increased that of POPC bilayer (Supplementary Figure S5b). Sinha et al. (2014a) reported an increase in order parameter of DPPC by chrysin.

Luteolin has two hydroxyl groups each on rings A and B that interact with the headgroups of Span 60. Using magic angle spinning NMR Scheidt et al. (2004) proposed a structure in which luteolin’s ring B points toward the aqueous phase and ring A stays in the interior of a POPC bilayer. A related compound apigenin, which has one less hydroxyl group on ring B, forms hydrogen bonds between the hydroxyl groups of ring A and oxygen atoms of phosphoester and palmitate ester of DPPC suggesting that it binds to the headgroup region (Pawlikowska-Pawle García et al., 2013). Our result on luteolin in a Span 60 bilayer shows that the long molecular axis can be tilted by many different angles. Our MD simulation showed that, unlike chrysin and unsubstituted flavone, luteolin significantly reduces the thickness of a POPC bilayer (not shown). Quercetin, another flavonoid with multiple hydroxylation on rings A and B, is known to decrease the thickness of a DOPC bilayer (Košinová et al., 2012). In contrast, luteolin up to a 25 mol% concentration did not affect the thickness of a Span 60 bilayer (Table 1).

Conclusions

Interaction of flavones with a Span 60/cholesterol bilayer has been studied by MD simulation. Unsubstituted flavone at a low concentration was able to flip over and cross the bilayer but at a high concentration it was accumulated at the bilayer center increasing the membrane thickness. Chrysin was lined up with the acyl chains of Span 60 with its hydroxyl group facing the membrane surface. Neither flipping nor transbilayer movement were allowed. Luteolin interacted mainly with the headgroup of Span 60 and assumed many different orientations without crossing the bilayer. With a large degree of hydroxylation, luteolin significantly disrupted the bilayer structure at a high concentration. These behaviors of flavones in a Span 60 bilayer can be explained by the hydrophobicity and site(s) of hydroxylation. This work presents the first atomic-level structures of a niosomal bilayer loaded with flavones as a model of Span 60 niosomes for the delivery of flavones. Experimental studies using X-ray scattering and NMR, for example, are needed to test the theoretical models presented in this work.
Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. This study was supported by 2014 Research Grant from Kangwon National University (No. 120140202).

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Supplementary materials available online