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Structure-dependent interactions of polyphenols with a biomimetic membrane system

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ABSTRACT

Polyphenols are naturally-occurring compounds, reported to be biologically active, and through their interactions with cell membranes. Although association of the polyphenols with the bilayer has been reported, the detailed mechanism of interaction is not yet well elucidated. We report on spatio-temporal real-time membrane dynamics observed in the presence of polyphenols. Two distinct membrane dynamics, corresponding to the two classes of polyphenols used, were observed. Flavonoids (epi-gallocatechin-3-gallate, gallocatechin, theaflavin and theaflavin-3-gallate) caused lipid membrane aggregation and rigidification. As simple structural modification through opening of the aromatic C-ring into an olefin bond, present in trans-stilbenes (resveratrol and picead), completely changed the membrane properties, increasing fluidity and inducing fluctuation. There were differences in the membrane transformations within the same class of polyphenols. Structure-dependent classification of membrane dynamics may contribute to a better understanding of the physicochemical mechanism involved in the bioactivity of polyphenols. In general, an increase in the number of hydrophilic side chains (galloyl, hydroxyl, glucoside, gallate) increased the reactivity of the polyphenols. Most notable was the difference observed through a simple addition of the gallate group. Unraveling the importance of these polyphenols, at a functional group level further opens the key to tailored design of bioactive compounds as potential drug candidates.

1. Introduction

Polyphenols are a group of naturally occurring secondary metabolites derived from shikimate-derived phenylpropanoid and polyketide pathways. They feature more than one phenolic ring and are classified based on the nature of their carbon skeleton: phenolic acids, flavonoids, stilbenes and lignans [1,2]. The most common sources of polyphenols for humans are green tea, red wine, fruits and vegetables [3–5]. Polyphenols exhibit beneficial traits such as anti-inflammatory, anti-cancer and antioxidant activities [6–8]. The dietary intake of polyphenols is remarkably high in comparison with other dietary antioxidants such as vitamins C and E, carotenoids [9] and selenium [10]. They also chelate highly redox-active metal ions [11–13] giving them an even stronger protective effect against oxidative damage. This is attributed to the presence of aromatic OH groups, where the OH groups are located on the aromatic rings, the oxidation state of the C-ring, and the overall number of OH groups present. Polyphenols, along with antioxidant vitamins and enzymes, may help protect against cancer, atherosclerosis, aging, and neurodegenerative diseases such as Parkinson's and Alzheimer's which have been linked to oxidative stress [14–18]. A report by van Acker et al. [19] suggested that some polyphenols could replace vitamin E as a chain-breaking antioxidant in liver microsomal membranes.

Although there are many reports on beneficial effects of polyphenols, their mechanisms are yet to be well understood. Some recent studies have suggested that polyphenols may interact with membranes and that those interactions may form the basis by which polyphenols confer their beneficial effects [20,21]. Green tea catechins have been found to be biologically active through their interactions with cell membranes [22]. With the use of plant decoctions as beverages (such as green tea) in daily diet, particular attention has been paid in this respect to their consumption and their action on the mucous membrane of the mouth and alimentary tract [23]. Sirk and colleagues proposed that polyphenols showed affinity for the lipid bilayer by binding to the lipid head groups near the bilayer surface (adsorption) and penetration into the bilayer interface (absorption). Their results showed that polyphenols form hydrogen bonds with membranes, with phenolic hydroxyl groups serving as the hydrogen bond donors and oxygen atoms on

Abbreviations: DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; EGCg, Epi-gallo catechin gallate; GC, gallocatechins; Rhodamine DHPE, Lissamine™ Rhodamin B 1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine, Triethylammonium salt; TF, theaflavins; TF-2, theaflavin-3-gallate

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the phospholipid as the hydrogen bond acceptors [24]. The presence of a gallate moeity is especially important, as polyphenols with gallate moeity formed 40% more hydrogen bonds than those without it. EGCg (Epi-gallo catechin gallate), one of the most studied catechins (derived from green tea) readily forms hydrogen bonds with the lipid bilayer. They indicated that the presence of the gallate moeity and its cis configuration with ring B promotes hydrogen bond formation, thus suggesting that configuration also plays an important role in bioactivity of polyphenols.

To further our understanding of the association of polyphenols with membranes and how they relate to structure and bioactivity, we evaluated the interactions between a model membrane system and two classes of polyphenols used namely flavonoids and stilbenes. We have used giant unilamellar vesicles (GUVs) prepared from unsaturated phospholipids DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine), a major kind of glycerophospholipids which constitutes the major composition of membrane lipids (40–60%) [25]. GUVs have been actively studied as cell models because they are similar to natural cell structures with regard to size and membrane composition, thus enabling the direct observation of the morphological changes in cell membranes upon external stimuli such as temperature [26,27], oxidative stress [28], and amyloid beta [29–31]. Polyphenols and membranes are thought to mainly interact with one another are via i) hydrogen-bonding and ii) hydrophobic interactions [22,32]. A phenyl ring bearing a hydroxyl group (PhOH) constitutes an amphiphilic moiety which can act as either a hydrogen-bond donor or an acceptor. On the other hand, the hydrophobic character of the planar aromatic nucleus of phenol, function as π stacking (van der Waals) interactions supporting hydrophobic models for polyphenols-membrane interactions [1]. In order to further our understanding of the way various functional groups, and their relative locations on the aromatic rings and chains influence the bioactivity of polyphenols, it is important to study the bioactivity of structurally different polyphenols. We have therefore selectively chosen flavonoids and stilbenes classes of polyphenolic compounds. Please refer to Scheme 1 in SI (supporting information) for the structures of the stilbenes and flavonoids that we used in this study. In the flavonoid class of compounds we have chosen green tea gallocatechins; (GC) gallocatechin and its galloylated derivative EGCg and black tea theaflavins; (TF) theaflavin and its galloylated derivative TF-2 (theaflavin-3-gallate). For stilbenes we have chosen resveratrol and its derivative piceid both of which are found in red grapes. Piceid is a stilbenoid glucoside and is a major resveratrol derivative in grape juices. The gallate side chains in flavonoids or the glucoside moiety in stilbenes could provide essential insights about the hydrogen bonding interactions of structurally different polyphenols with lipid vesicles.

Our results have showed that the two classes of polyphenols induced opposite biophysical changes (phospholipid re-packing) to the membrane system studied. Briefly, flavonoids majorly caused membrane aggregation whereas stilbenes mediated membrane fluctuation. We also observed the difference in frequency and intensity of membrane transformation within the same class of polyphenols.

2. Materials and methods

2.1. Materials

Polyphenols of >97% purity were purchased: TF and TF-2 from Wako Pure Chemicals (Japan); piceid from LKT Laboratories (Japan); resveratrol, GC and EGCg from Sigma-Aldrich Co. (USA). DOPC, chloroform, and methanol were obtained from Avanti Polar Lipids (USA), Kanto-Chemical (Japan), and Nacalai Tesque (Japan), respectively. Fluorescent label of DOPC, rhodamine DHPE (Lissamine™ Rhodamin B 1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine, Triethylammonium salt) ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 580$ nm) was obtained from Invitrogen. All other reagents were purchased from Wako Pure Chemical (Tokyo, Japan) and were of analytical grade. Deionized water obtained from Millipore Milli-Q purification system (Millipore, Bedford, MA, USA) was used for reagent preparation and for cleaning of glassware.

2.2. Preparation of lipid vesicles

Cell-sized lipid vesicles (giant unilamellar vesicles (GUVs; model membranes/liposomes) were prepared from DOPC following the natural swelling method by dissolving in chloroform/methanol (2:1 (v/v)) in a glass tube [26–28]. The final concentration of DOPC was 0.2 mM. The organic solvent was then evaporated under a nitrogen flow and dried under vacuum to make a dry film at the bottom of the test tube. The tube was placed in a desiccator for 3 h to remove the organic solvent. The film was then swollen with Milli Q for 24 h at room temperature.

2.3. Preparation of polyphenol solutions

EGCg and piceid were prepared by dissolving in Milli-Q. TF, TF-2, GC and resveratrol were prepared by dissolving in aqueous methanol (14.25% (v/v)). All stock solution was made at the concentration of 1 mM and stored at −25 °C. When they were used for experiments, in all of them methanol was diluted 10 times with Milli Q water. The final working solution was 100 μM.

2.4. Interaction of polyphenols with cell-sized liposome

5 μL of the liposome solution and 5 μL of 100 μM polyphenol solution were poured into a test tube and gently mixed by soft tapping. Then, 5 μL of resultant mixture was used for microscope observation to detect membrane dynamics induced by polyphenols. The final concentration of polyphenol was 50 μM. Observation of the vesicular dynamics was within 2 min of polyphenol solution introduction to the lipid vesicles. Since experimental procedures affect the interaction between our considered membrane system and the polyphenols, we carefully followed the exact same experimental conditions and procedures for each analysis, and conducted at least 30 replicates for each polyphenol/membrane interaction.

2.5. Microscopic observation

A liposome solution (5 μL) prepared above was placed in silicon well (0.2 mm) on a slide glass and covered with a small cover slip. This well provides a space between glass slide and glass cover for liposomes, thus preventing the disruption of liposome integrity caused by their direct contact with the slide or cover [33]. Changes in membrane morphology were observed using a phase-contrast microscope (Olympus BX50; Olympus, Japan), at RT.

2.6. Image processing

During observation, images of changes in membrane morphology were recorded on a hard-disc drive at 30 frames s^{-1}. The images was then processed using Image J software [26,33] We analyzed membrane fluctuation as a function of radius and its distribution $r(\theta,t)$ ($\theta = 2\pi/n$, $n = 0, 1, 2,\ldots, 100$) [34]. When the value $\sigma \leq \mathrm{sqr}(r(\theta) - <r>)^2>/<r>$ is equal to and more than 1.3%, liposome is considered to be fluctuating [26].

3. Results and discussion

In this work, we have investigated the effect of two classes of polyphenols: trans-stilbenes and flavonoids. The choice of representative candidates was not accidental. EGCg has been reported extensively in literature, thus providing us a good reference point for our current study [22–24] as has resveratrol [1]. Resveratrol first came into

prominence during the course of research into the so-called "French Paradox", which unveiled a lower incidence of coronary heart diseases in France as a consequence of the regular drinking of wine, and this, despite a high dietary intake of fat [1,35]. As far as we are aware, TF and picead have been reported only once [36,37], and its galloylated derivative TF-2 has not been while catechin gallate has received limited attention. Having structures that are closely related (between group comparison) and closely related at functional group level (within class comparison) has enabled us to draw a structure-dependent relationship between the polyphenols and their interaction with the lipid bilayer.

3.1. Spatio-temporal membrane dynamics induced by flavonoids

We introduced the four flavonoids to the lipid model membrane solution, at final concentrations of 50 μM and 0.1 mM, respectively. Real-time observation of the mixture using an optical microscope revealed three distinct spatio-temporal lipid membrane transformations (Fig. 1). The lipid vesicles either burst with subsequent release of daughter vesicles (A); or burst with subsequent release of small lumps (B); or formed small endo-buds (C). Formation of small lumps leading to vesicle bursting in the presence of EGCg has previously been reported [38]. The authors detected hole formation a step before the formation of a lump. In our study, Movie SII (in SI) clearly shows the opening of the vesicle prior to release of pre-formed lumps. We did not observe hole-formation prior to lump formation. All the three membrane transformation pathways underwent an aggregation as the first step. Some of the aggregation rate was so fast that we could not clearly capture the transformation process. Aggregation was followed either by the formation of (i) endo-buds, (ii) small vesicles or (iii) small lumps, resulting in a visible decrease in membrane diameter (see Movies SI and SII in SI). Some liposomes aggregated relatively slowly and were still undergoing aggregation by the end of the 30 min observation period. This pathway is most likely an intermediate step toward one of the three pathways discussed above. We termed this membrane transformation simply as aggregation. These results indicated that flavonoid-induced dynamics of model membranes was characterized by the appearance of aggregation which rendered the membranes more rigid. Membrane rigidification in the presence of a flavonoid, genistein, has been reported [39]. The mechanism can be explained by possible changes in membrane phospholipids upon their binding with flavonoids. There are two oppositely-acting major forces existing among phospholipids in the interfacial region of lipid bilayer and controlling the surface area (a) per molecule. They are hydrophobic attraction at the hydrocarbon–water interface and repulsion of the phospholipid headgroups. The former force, which is generated from interfacial tension among identical molecules, causes the molecules to assemble and decreases the surface area, a (Fig. S3). In contrast, the latter, which includes steric repulsive interaction, hydration force, and electrostatic double-layer contribution, tends to disaggregate the molecules and increases a [40]. Changes in the balance between the two forces significantly influence membrane area and dynamics. NMR and FTIR spectroscopic investigations have shown that some flavonoids such as genistein and green tea catechins interact with membrane phospholipids at the interface [22,39]. In this study, we propose that hydrogen atoms of polyphenolic hydroxyl groups in EGCg, GC, TF, and TF-2 formed hydrogen bonds with oxygen atoms of phospholipid headgroups. This interaction may reduce the number of water molecules surrounding the phosphate and carbonyl groups of the headgroups, thus decreasing hydration force between them. In addition, the binding of phosphate groups with the external compounds may induce the headgroup of phospholipids to change its orientation from nearly parallel to perpendicular with respect to the membrane surface. This conformational change decreased the space occupied by headgroups [41]. Thus, flavonoids/membrane lipids interaction reduced repulsive force among phospholipid headgroups, resulting in (i) the aggregation of phospholipids and (ii) a smaller surface area per molecule. Because of their big and globular shape, flavonoids may not be able to penetrate into the interior of lipid bilayers. They are mainly associated with the headgroup region of the outer leaflet and induced a big decrease in area of this leaflet compared to the inner. When the area difference between the two leaflets reached a critical unstablility point, buds, small vesicles or small lumps were formed toward the inside of mother vesicles. In the first and second transformation pathways (Fig. 1), the number of daughter vesicles and small lumps was noticeably

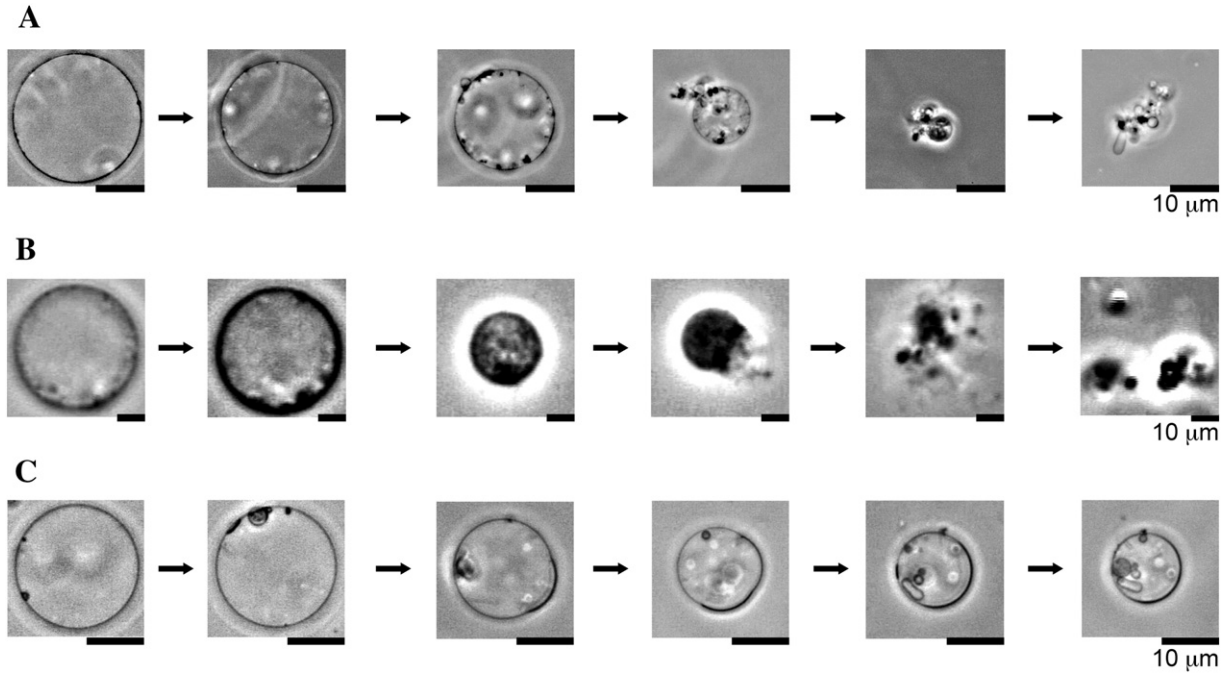

Fig. 1. Typical phase contrast images of spatio-temporal membrane transformation pathways of lipid vesicles in response to the presence of four different types of flavonoids (Epigallocatechin gallate; Gallocatechin; Theaflavin and Theaflavin-3-gallate). The transformations culminated (A) burst with subsequent release of daughter vesicles; (B) or burst with subsequent release of small lumps; or (C) small endo-buds formation. The images were captured in real-time using a phase contrast microscope recorded at 30 frames s^{-1}. They were subsequently processed using Image-J.

high. We hypothesize that the Brownian motion of these small lipid assemblies caused enough pressure against the membrane surface, leading to the burst of the mother vesicle and the eventual release of the small lipid assemblies.

The four studied flavonoids had different effects with respect to the frequency and distribution profile of membrane transformation. Liposomes showed morphological changes with higher percentage in the presence of TF-2 > TF > EGCg > GC (Fig. 2A) corresponding to the number of phenolic gallate groups and hydroxyl groups in polyphenols. Molecular size and shape are also important. They influence how the polyphenols interact with the membrane, following this initial H-bond driven interaction. The latter will be discussed later on in the manuscript. TF induced transformation in most of the vesicles ($n = 30$), with 70% of vesicles undergoing aggregation. Endo-bud formation was observed in 27% of all vesicles. Burst with subsequent release of daughter vesicle release was also clearly captured in some liposomes (3%). The galloyllated derivative of TF, TF-2 caused all vesicles to burst with subsequent release of daughter vesicle release, most likely derived from a higher decrease rate in membrane area. In the case of EGCg, 86% of all vesicles ($n = 30$) underwent three forms of transformation: aggregation (48% of transformation), burst with subsequent release of small lumps (30%), and burst with subsequent release of daughter vesicles (22%). Having no gallate group (compared with EGCg), GC exhibited a significantly less membrane destabilization at 30% compared to the 86% transformation in the presence of EGCg. All the transformed vesicles underwent aggregation (Fig. 2B). The result clearly shows that changes in the hydrophilic side chain (gallate and hydroxyl group) number significantly influence the reactivity of flavonoids. It has been concluded that the presence of a gallate moiety is especially important, as polyphenols with gallate moiety formed 40% more hydrogen bonds than those without it [24]. Erlejman et al. also reported the necessity of hydroxyl groups in flavonoids for the interaction of flavonoids with membranes [42].

3.2. Kinetics of membrane shrinkage caused by flavonoids

In order to increase our understanding on any membrane changes and their possible relationship to flavonoid structure, we investigated the kinetics of membrane shrinkage, which calculates changes in surface area over time, of EGCg and TF as representatives of the flavan-3-ols and the oxidative derivative, respectively. We used TF rather than TF-2 because the membrane transformation caused by the latter was too fast for us to capture clear images. Fig. 3A unequivocally shows that EGCg induced a faster membrane shrinkage than TF. This is interesting when we consider that the latter caused a higher percentage of membrane transformation. As discussed above, phenolic hydroxyl groups play an important role in the interaction between flavonoids and membrane lipids. TF has more hydroxyl groups than EGCg, thus being able to interact with headgroups of membrane lipids to a bigger extent than EGCg. This suggests that the kinetics of membrane shrinkage in the presence of flavonoids may be caused by an interplay between a flavonoid's size and shape as well as the molecular packing properties of the molecules in membranes. It has been shown previously that the shape of lipid vesicles is determined by the correlation among optimal surface area a_0, hydrocarbon volume v and critical chain length l_c, called shape factor v/a_0l_c. The smaller shape factor of phospholipid is, or the more conical the molecule is, the more curved and smaller lipid vesicle is formed [40]. TF has a big and globular shape, while EGCg is smaller and less globular. Therefore, the latter could insert in the headgroup region of outer leaflet of the lipid bilayers while the former only adsorbed onto the surface of the bilayer. As a result, the complex of two phospholipids and a central flavonoid was more conical for EGCg than TF (Fig. 3B). This enabled EGCg to render lipid vesicles more curved and smaller and at a faster rate compared to TF.

3.3. Flavonoids mediate phase separation in a mono-composed lipid bilayer vesicle

As seen in Fig. 1A and Movie SII (in SI), there is a distinct formation of black clusters (aggregates) in some lipid vesicles after addition of flavonoids. We were interested in finding out the property(ies) of these dark regions. We therefore, labeled the membrane phospholipid (DOPC) using a fluorescent dye, rhodamine DHPE, that specifically binds to DOPC. Then, we exposed the lipid vesicles to the four flavonoid solutions. First, we were able to confirm that these lumps consisted of phospholipids, although we could not rule out the presence of flavonoids in the small lumps. In the presence of TF, a sudden transition from one-phase to two-phase separated membranes was clearly observed (Fig. 4). This phenomenon was not observed with addition of EGCg and piceid (data was not shown). A real-time observation by both fluorescent and phase-contrast modes showed that most of all non-labeled regions in phase-separated liposomes coincided with the dark areas in aggregated liposomes (Fig. 4). This suggests that TF may exist in these regions and the interaction of the compound with membrane phospholipids affects the binding of phospholipids with their fluorescent probe.

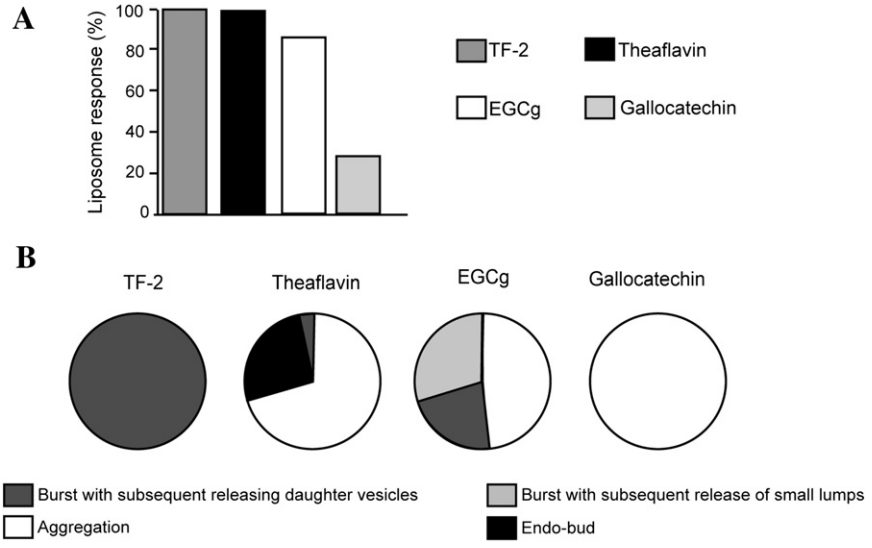

Fig. 2. Flavonoids induced membrane transformations. (A) Percentage of transformed lipid vesicles in the presence of four flavonoids; (B) Distribution of membrane transformation pathways induced by the flavonoids.

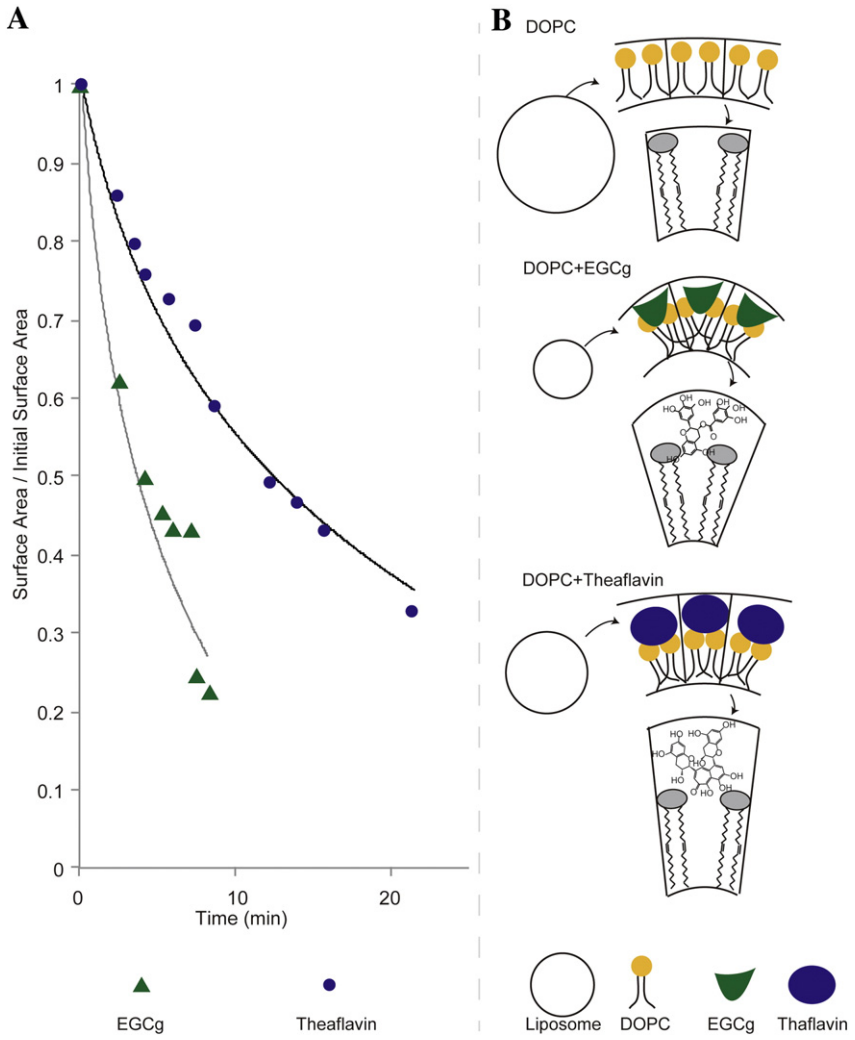

Fig. 3. Kinetics of membrane shrinkage induced by flavonoids. (A) Time-dependent change in membrane surface area during the shrinkage in response to epigallocatechin gallate (green) and theaflavin (blue); (B) Schematic illustration of the interaction of flavonoids with membrane phospholipids and its effect on membrane curvature. This illustration attempts to demonstrate the importance of flavonoid size and shape in influencing how the flavonoid interacts with the membrane surface. It should be noted that the initial interaction, which is H-bond driven (and influenced by the number of phenolic OH groups) is not captured in this illustration.

The phase transition of homogeneous membranes is commonly observed found in two or more-component liposomes induced by changes in temperature, pH, or photo-oxidation [43–45]. However, this is the first time that the phase transition of one-component liposomes has been reported. We are aware that this is an interesting phenomenon but we do not yet currently understand the mechanism. We propose that the relatively strong (compared with EGCg and picead) hydrophilic interactions between TF and the polar headgroups of the lipid play a strong role. We have started to look into this interesting phenomenon and hope to report the findings in a near future.

3.4. The interaction of trans-stilbenes with bilayer lipid membranes

Fig. S4 in SI shows phase-contrast images of membrane transformation induced by resveratrol and piceid. Four transformation pathways were clearly observed, including fluctuation (A), exo-filament (B), stomatocyte (C), and small exo-bud (D). During the transformation process, all lipid vesicles underwent fluctuation. This indicates that the trans-stilbenes inserted into lipid bilayer and cause membrane area to increase. The membrane excess area led to a reduction in volume (V) to area (A) ratio and resultant fluctuation [26,46]. The insertion happened first in the outer leaflet of bilayer, rendering the area of this leaflet lager than the inner. As a result, exo-filaments or small exo-buds could be formed following fluctuation to relieve the membrane excess area. When the external compounds penetrated deeply into the inner leaflet, a bigger change in the area of two leaflets was induced, but the area difference between the inner and outer leaflets decreased. In this case, negatively curved regions (stomatocyte) were formed in order to redress the change which had brought an increase in energy [47].

Similar to flavonoids, we observed a difference in how the two trans-stilbenes, resveratrol and its glucoside derivatives piceid affected membrane dynamics. In the presence of resveratrol, 70% of all vesicles ($n = 30$) underwent transformation through three separate pathways: fluctuation (52%), exo-filament formation (34%), and stomatocyte formation (14%). Piceid mediated transformation of 87% of the vesicles ($n = 30$). More than a half of transformed vesicles underwent fluctuation (69%). Exo-filament and small exo-buds were formed in 15% and 12% of the vesicles, respectively. Stomatocyte was captured in only 4% of the destabilized vesicles (Fig. 5A&B). The rather elongated linear and lypophilic structure of resveratrol suggests that it may insert into the bilayer, intercalating between membrane phospholipids [48]. From our findings, we agree with a previous study which reported that resveratrol inserts a bit more deeply into the lipid bilayer than piceid, although the latter also does insert rather than adsorbing onto the surface of the outer leaflet [37]. The reason for the difference is that picead having the glucoside moiety (four additional hydroxyl

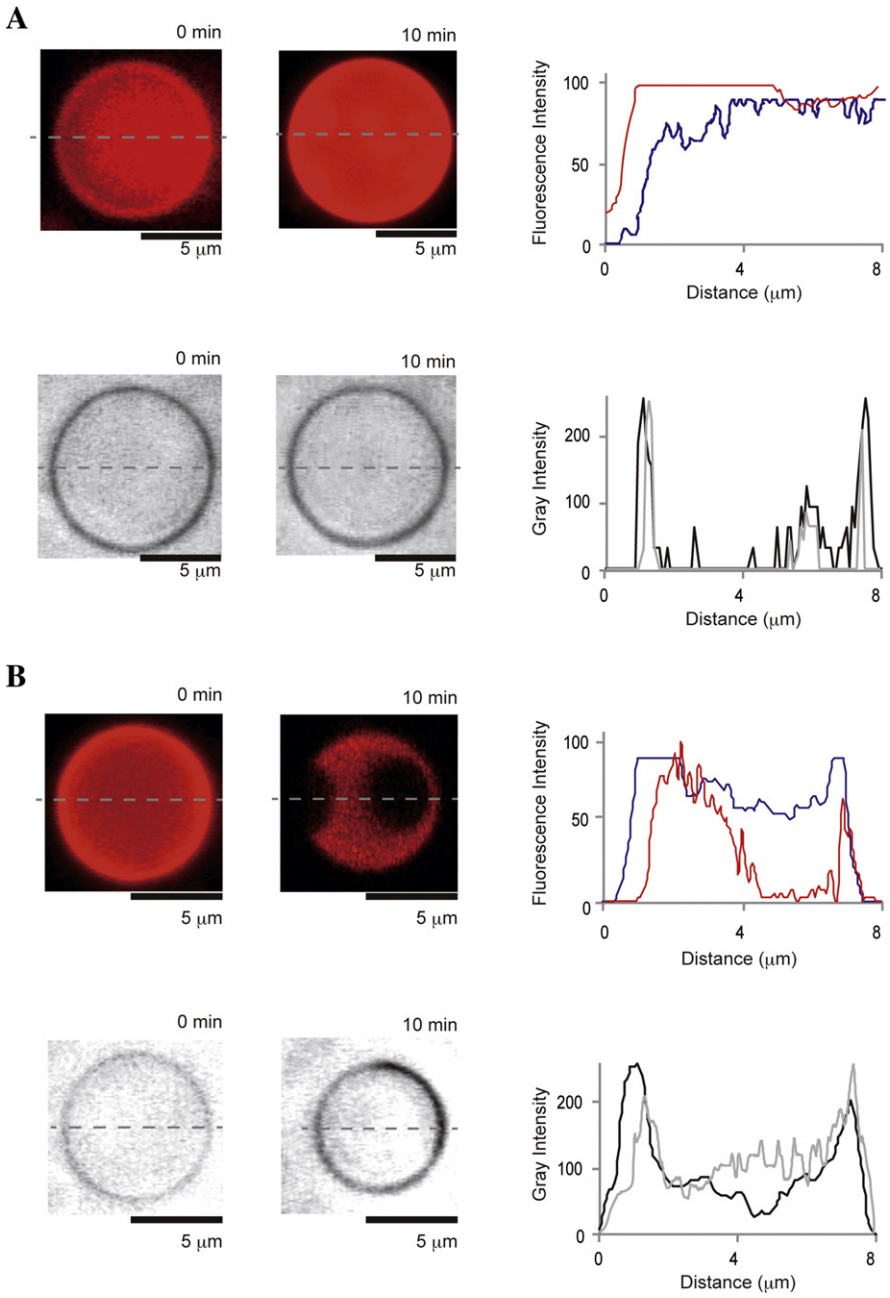

Fig. 4. Phase separation in mono-composed lipid bilayer vesicles induced by theaflavin. (A) Typical fluorescent images of DOPC vesicles and the fluorescent intensity of the vesicles measured at the beginning of observation (blue) and after a 10-min observation period (red) (i), the corresponding phase contrast images of DOPC vesicles and the gray intensity of the vesicles measured at the beginning of observation (black) and after a 10-min observation period (gray) (ii); (B) Typical fluorescent images of phase separation induced in lipid vesicles in the presence of theaflavin and the fluorescent intensity of the vesicles measured before (blue) and after 10 min (red) (i), the corresponding phase contrast images and the gray intensity of the vesicles measured before (black) and after 10 min (gray) (ii). The images were captured in real-time using a fluorescent contrast microscope recorded at 30 frames s^{-1} and subsequently processed using Image-J.

groups) makes it relatively more hydrophilic than resveratrol. In addition, the substitution of a small OH group with a glucoside makes piceid longer, thus producing a larger distance between two membrane phospholipids when it inserts into the bilayer. Therefore, piceid was likely to induce a faster fluctuation rate of vesicles as we have shown in Fig. 5C.

3.5. Unraveling the differences between flavonoids' and trans-stilbenes' interactions with bilayer lipid membranes

It is highly interesting to observe the two differing possible mechanisms of interactions – and their effect on membranes – between the two classes of polyphenols that we have studied in this work. Flavonoids caused lipid membranes to aggregate and a decrease in membrane surface area, leading to membrane rigidification, while trans-stilbenes largely had an opposite effect. Flavonoids, with their more globular structure interacted mainly with the outer leaflet of the membrane, causing area reduction on the outer leaflet. In order to redress this instability, negative curvature occurred leading to formation of either small lumps or small endo-vesicles, which detached from the surface and could be observed migrating into the inner aqueous vesicular space. Stilbenes induced membrane fluctuation caused by increasing excess surface area [46] which in turn caused an increase in membrane fluidity [26]. Why does a simple opening of the flavonoid aromatic C ring into an olefin group change the membrane properties from rigid to fluid? Here, we attempt to discuss this difference. Flavonoid possesses a considerably big, globular structure with many surrounding polar hydroxyl groups

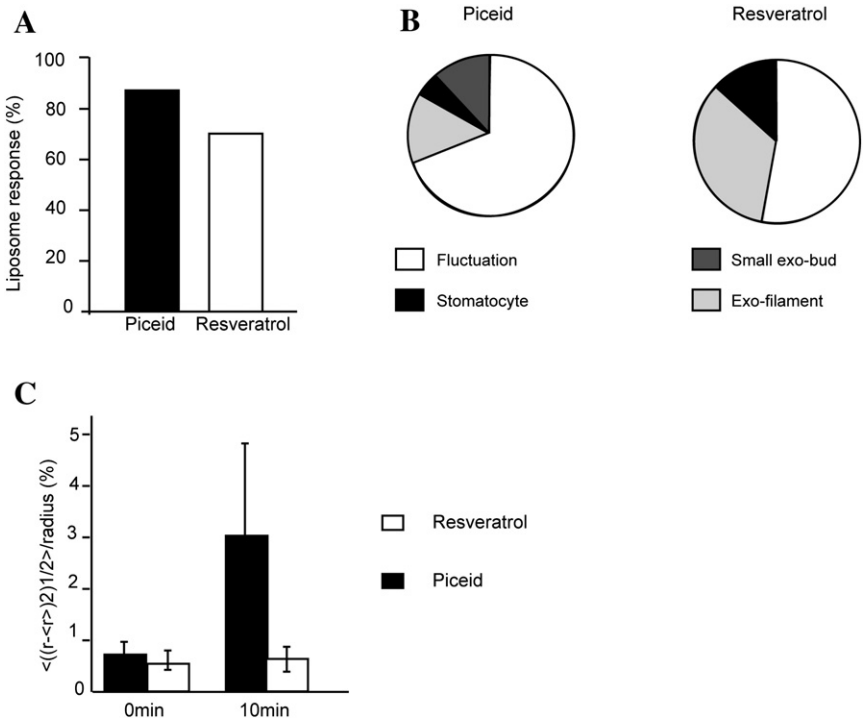

Fig. 5. Membrane transformation induced by trans-stilbenes. (A) Percentage of transformed lipid vesicles upon introduction of resveratrol and piceid; (B) Distribution of membrane transformation pathways induced by resveratrol and piceid; (C) The degree of membrane fluctuation caused by resveratrol and piceid. Membrane fluctuation was calculated as a function of radius and its distribution $\sigma \leq \mathrm{sqr}(r - \langle r \rangle)^2 \rangle / \langle r \rangle (\theta = 2\pi/n, n = 0, 1 \ldots 100)$. A lipid vesicle is considered to be fluctuating when the value σ is equal to or more than 1.3%.

(Fig. S2), thus rendering it more difficult to penetrate into the hydrophobic region within the lipid bilayers, confining interaction at the hydrophilic interface, and inducing an assembly of phospholipid molecules. Conversely, the opening of benzyl ring forms a linear shape of trans-stilbenes with two hydrophilic ends and the central hydrophilic region (Fig. S2). This structure enables the compounds to insert into lipid bilayers. Fabris and colleagues suggested that the insertion is perpendicular to the membrane surface [37]. We propose that the stilbenes orient themselves at an angle near parallel to the membrane surface, where the polar ends can interact with hydrophilic head groups of the adjacent phospholipids and the non-polar region locates into the hydrophobic side chain interior of lipid bilayers (Fig. S4). This configuration enabled trans-stilbenes to expand the membrane area.

4. Conclusions

We have shown that polyphenols interact with model membranes in a structure-dependent manner. Two classes of polyphenols, flavonoids and trans-stilbenes, induced transformation of lipid vesicles by changing membrane properties by two completely opposite means. Due to the presence of gallate, galloyl and hydroxyl groups, flavonoids were able to form hydrogen bonds with head group of membrane phospholipids. They mainly affected hydrophilic region of lipid bilayers, mediated phospholipid aggregation, thus causing a decrease in membrane area and rendering membrane more rigid. Trans-stilbenes, with an opening benzyl ring structure tended to insert deeply into hydrophobic interior of lipid bilayers and increased membrane area. As a consequence, membrane became more fluid and underwent fluctuation. The penetration of trans-stilbenes into model membrane was slightly inhibited by the addition of a glucoside moiety which makes the compound more hydrophilic, rendering the bioreactivity of piceid closer to the membrane surface than resveratrol. Further, the presence of the glucoside group increased the interaction of the stilbene. For all the polyphenols, the higher number of hydrophilic side chains (hydroxyl, gallate, galloyl, glucoside), the more interactive the polyphenol was with the membrane. These results are useful for understanding the mechanism by which polyphenols influence cell membranes and play beneficial roles in many biological processes. Small changes and/or additions of a side chain provided profound difference. The most notable was the difference through addition of the gallate group (comparing GC with EGCg). Unraveling the importance of these polyphenols, at a functional group level further opens the key to tailored design of bioactive compounds as potential drug candidates.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2014.07.001.

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