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Structure-dependent interactions of polyphenols with a biomimetic membrane system

Huong T.T. Phan [a,1], Tsuyoshi Yoda [a,1], Bindu Chahal [a,b], Masamune Morita [a], Masahiro Takagi [a], Mun'delanji C. Vestergaard [a,*]

[a] School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi City, Ishikawa 923-1292, Japan
[b] M. Tech. Chemical Synthesis and Process Technologies, Department of Chemistry, University of Delhi, Delhi 110007, India

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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

* Corresponding author. Tel.: +81 761 51 1993.
E-mail address: munde@jaist.ac.jp (M.C. Vestergaard).

[1] Phan and Yoda are joint first authors.

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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