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Effect of flavonoid structure on the fluidity of model lipid membranes

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ABSTRACT

We investigated how the structural properties of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) and butylated hydroxytoluene (BHT) correlate with structural changes of phosphatidylcholine plus sphingomyelin (2.4:1) model lipid membranes. Changes were measured by fluorescence anisotropy, electron paramagnetic resonance, and differential scanning calorimetry. Two fluorophores and two spin probes were used to monitor membrane characteristics close to water–lipid interface and in the middle of the bilayer. The data obtained were correlated to the amount of bounded compounds, the number of H-bonds, and the topological polar surface area (TPSA) of the compounds. These correlations reflect the behaviours of (+)-catechin, (−)-epicatechin, EGC, EGCG and BHT. Our results confirm that phenolics studied here are bounded to a membrane surface predominantly *via* hydrogen bonds, while BTH is inserted into the lipid bilayer.

1. Introduction

In vitro, phenolic compounds have been shown to have a broad spectrum of biological effects. The food industry is interested in them particularly, because they are good antioxidants and therefore can prevent oxidation processes and prolong the stability of foods, and also because they can act as antimicrobials. Several flavonoids from herbs (Abram & Donko, 1999) or teas were tested for such effects (Friedman, 2007).

As part of the human diet, phenolic compounds might also provide health benefits associated with reduced risk of chronic diseases, potentially due to their ability to reduce oxidising agents but their bioavailability is reportedly very low in mammals. Molecular mechanisms of their action are still poorly understood (Passamonti et al., 2009). Using four different mammalian cell lines, we have recently shown that bilberry and blueberry anthocyanins can act as powerful intracellular antioxidants, even at very low concentrations (<1 μg/L; i.e. in the nM range) (Bornšek et al., 2012). The fate of such phenolic compounds in the human body is important to be known when trying to explain their *in vivo* effects. Several studies on bioavailability can be found in the literature with some controversial results regarding the transport of a phenolic compound through the cell membrane. Sirk, Brown, Sum, and Friedman (2008); Sirk, Brown, Friedman, and Sum (2009) tried to explain phenolics behaviour using molecular dynamics simulations and noted that all of the tea catechins have strong affinities for the lipid bilayer, with some of them (e.g. catechin, epicatechin and epigallocatechin-3-gallate (EGCG)) incorporated into the lipid bilayer very fast (in 50 ns) (Sirk et al., 2009; Sirk et al., 2008). However, EGC remained mostly in the aqueous phase, and had the lowest affinity for the lipid bilayer, reportedly due to the additional 4′-OH group in ring B (see Fig. 1).

How such behaviour can be explained? We propose that a structure of a compound determines its behaviour in the case of membrane transport and that data based on its polarity, number of H-bonds and topological surface area (TPSA) can predict how a flavonoid can penetrate cell membrane (by passive transport or by a transporter protein). In order to prove such hypothesis we have investigated in our previous work the influence of four flavonoids with liposomes composed of POPC/POPE/POPS/CH (Ulrih, Ota, Šentjurc, Kure, & Abram, 2010). We have shown that flavonoids can affect cell membrane fluidity and consequently a transport through the membrane and that the observed changes can be correlated to the total number of –OH groups and TPSA. Among

Abbreviations: BHT, butylated hydroxytoluene; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, *N,N,N*-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium p-toluenesulphonate; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DSC, differential scanning calorimetry; EGC, epigallocatechin; EGCG, epigallocatechin gallate; EPR, electron paramagnetic resonance; POPC, 1-palmitoyl-2-oleoyl-sn-glicero-3-phosphoholine; POPE, 1-palmitoyl-2-oleoyl-sn-glicero-3-phosphoethanolamine; POPS, 1-palmitoyl-2-oleoyl-sn-glicero-3-phospho-L-serine; CH, cholesterol.

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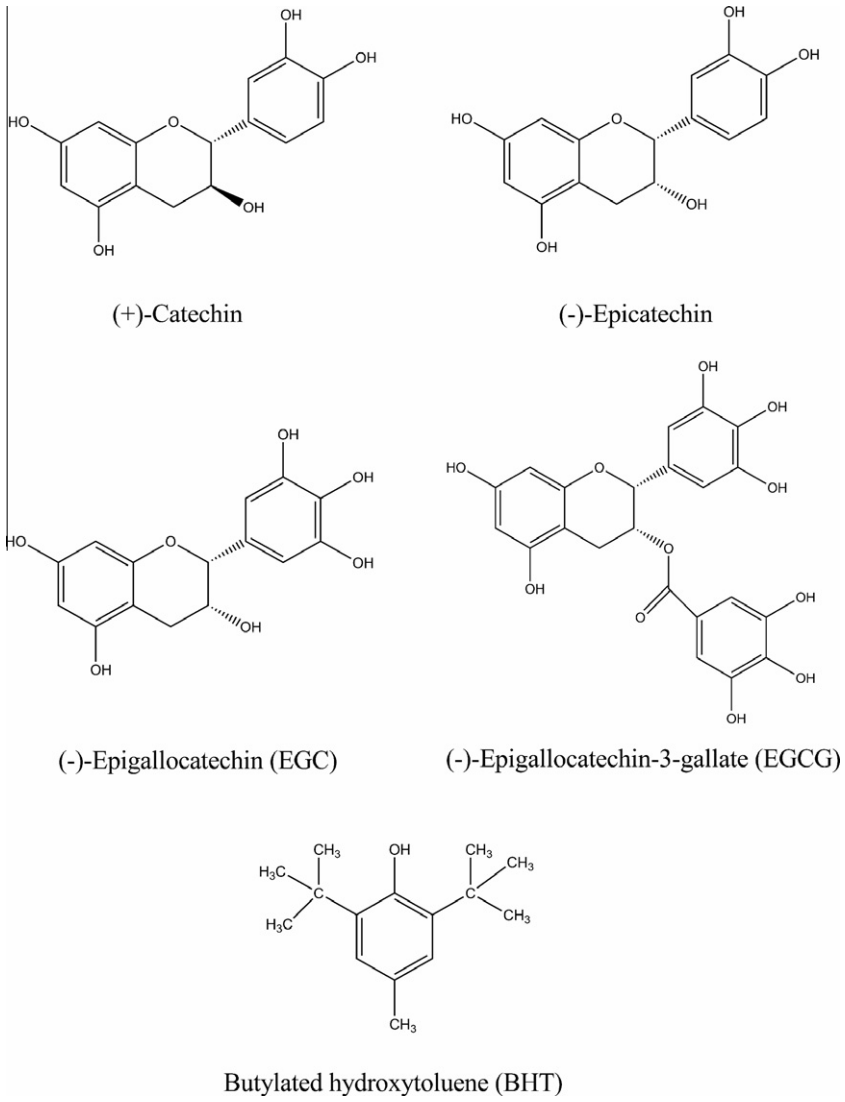

Fig. 1. Structural formulae of the flavonoids and the synthetic antioxidant BHT used in the present study.

them EGCG was the most effective. It affected the membrane fluidity the most although it interacted primarily with the lipid membrane surface.

Being that each cell membrane has different selectivity, as this depends on its composition, and thus modulate the passage of substances into the cell (Šentjurc, Štrancar, & Koklič, 2002) we decided to investigate how absence of cholesterol would affect interactions of the same catechol type flavonoids with model lipid membranes and included in the experiment their derivatives too. All the selected compounds possess powerful antioxidant activity (Terao, 2009). Therefore in the present study we investigated how the structural properties (partition coefficient, number of H-bonds, and TPSA) of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) (Fig. 1) correlate with the structural changes of model lipid membranes prepared from phosphatidylcholine (PC) and sphingomyelin (SM) or 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) only. For comparison, the synthetic antioxidant butylated hydroxytoluene (Fig. 1, BHT) was included, which is a lipophilic compound that is used as an additive in foods, cosmetics and pharmaceuticals (i.e. E321).

The effects of these selected flavonoids and BHT on the structural properties of model lipid membranes was investigated using a combination of fluorescence anisotropy measurements, electron paramagnetic resonance (EPR) spectroscopy, and differential scanning calorimetry (DSC). Two fluorophores and two spin labels were used to monitor the membrane characteristics, two of them close to the membrane water–lipid interface and the other two in the middle of the bilayer.

2. Materials and methods

2.1. Materials

The phospholipids phosphatidylcholine (PC; from egg), sphingomyelin (SM; from egg) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids (USA). The fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH) and *N*,*N*,*N*-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium p-toluenesulphonate (TMA-DPH) were obtained from Aldrich Chemical Company (USA). The flavonoids catechin, epicatechin, EGC and EGCG were from Extrasynthese (France; all $\geqslant$99% purity). BHT was purchased from Fluka (Germany). All other chemicals (e.g. ethanol, buffers) were purchased from Merck (Germany). The spin label methyl esters of doxyl palmitic acid with the doxyl group on