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Cholesterol induces surface localization of polyphenols in model membranes thus enhancing vesicle stability against lysozyme, but reduces protection of distant double bonds from reactive-oxygen species

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ABSTRACT

The main scope of the present study was to analyze the membrane interaction of members of different classes of polyphenols, i.e. resveratrol, naringenin, epigallocatechin gallate and enterodiol, in model systems of different compositions and phase states. In addition, the possible association between membrane affinity and membrane protection against both lipid oxidation and bilayer-disruptive compounds was studied.

Gibbs monolayer experiments indicated that even though polyphenols showed poor surface activity, it readily interacted with lipid films. Actually, a preferential interaction with expanded monolayers was observed, while condensed and cholesterol-containing monolayers decreased the affinity of these phenolic compounds. On the other hand, fluorescence anisotropy studies showed that polyphenols were able to modulate membrane order degree, but again this effect was dependent on the cholesterol concentration and membrane phase state. In fact, cholesterol induced a surface rather than deep into the hydrophobic core localization of phenolic compounds in the membranes.

In general, the polyphenolic molecules tested had a better antioxidant activity when they were allowed to get inserted into the bilayers, i.e. in cholesterol-free membranes. On the other hand, a membrane-protective effect against bilayer permeabilizing activity of lysozyme, particularly in the presence of cholesterol, could be assessed. It can be hypothesized that phenolic compounds may protect membrane integrity by loosely covering the surface of lipid vesicles, once cholesterol push them off from the membrane hydrophobic core. However, this cholesterol-driven distribution may lead to a reduced antioxidant activity of linoleic acid double bonds.

1. Introduction

Polyphenols are secondary metabolites with phenolic rings in their structure which are produced mainly by plants and are involved in defense against ultraviolet radiation and pathogens. Actually, phenolic compounds may exhibit a wide range of properties depending on their particular structures. In fact, some of them are pigments such as the anthocyanins, others are related to food flavors. Nevertheless, polyphenols are most commonly associated to radical-scavenging capacity and to the binding of proteins, which has several consequences: the astringency perception, inhibition of several enzymes and formation of precipitates in beverages [12]. Dietary polyphenols can be divided into several classes according to the number of rings and the structural elements connecting the phenolic rings. The main groups are: flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans [16,61]. Among the most studied polyphenols, it can be cited epigallocatechin gallate, which is a major constituent of the green tea [46], naringenin, that is a flavanone mainly found in tomatoes and citrus, specially grapefruits [37] and resveratrol, the most studied polyphenol present in grapes [13]. In addition, some polyphenols can be produced in animals by the action of intestinal bacteria such as the lignan enterodiol [66].

An important property of polyphenols is the ability to interact with membranes [27,42]. In this regard, Hendrich, in a comprehensive review, pointed out that phenolic compounds might interact mainly with the polar region of the phospholipids, although a deeper insertion may also occur, depending on flavonoid structures [27].

On one hand, it has been shown that polyphenols would interact with cell membranes in close contact to membrane proteins, in this way preventing lipid peroxidation and hemolysis [5,22,60]. Han et al. [24] also postulated a specific proteinaceous binding site common to all polyphenols in rat brain. On the other hand, there is convincing evidence that polyphenols might interact not only with membrane-bound proteins but also with lipid membrane. By using various biophysical techniques, it was concluded that polyphenols may insert and modulate the properties of model membranes e.g. dipole potential, permeability, phase transition and thermotropic behavior [28,49,55].

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Pioneering work in our laboratory conclusively demonstrated that the activity of membrane-bound enzymes could be modified by changes in the membrane fluidity [6,7,52]. In this regard, Meyer dos Santos et al. [36] showed that the activity of P-glycoprotein, a transporter of the ABC type, is closely related to the physicochemical properties of the membrane. In the same trend, Takahashi et al. [57] suggested that an alteration in the viscosity of the membrane could also impact ABCA1 activity. Interestingly, most polyphenols can increase membrane viscosity while others may exert the opposite effect [59,62,63]. In a previous work, we demonstrated that resveratrol may enhance ATPase activity of reconstituted ABCG1 by modulating the fluidity of proteoliposome membrane [14]. Patra et al. [43] recently suggested that many of the positive effects of epigallocatechin gallate have their origin in the modification of the plasma membrane "rafts". Since the antioxidant activity is thought to be the main beneficial property of phenolic compounds, this work aimed to analyze the membrane interaction of different polyphenols and correlate it with the antioxidant effect displayed by them. Besides, the possible protection of bilayer integrity against membrane-active proteins was studied as well. The phenolic compounds used in this work are resveratrol, epigallocatechin gallate, naringenin and enterodiol; they are representative to the different classes of polyphenols and their chemical structure is shown in Fig. 1.

2. Materials and methods

2.1. Chemicals

Lysozyme and the polyphenols enterodiol, epigallocatechin-3-gallate (EGCG), naringenin and resveratrol were purchased from Sigma-Aldrich. Dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), cholesterol (Cho) and linoleic acid (LA) were from Avanti Polar Lipids. 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH), 1,6 diphenyl-1,3,5-hexatriene (DPH) and calcein were purchased from Molecular Probes-Thermo. All other reagents were of the highest purity available.

Stock solutions of the four polyphenols and the fluorescent probes DPH and TMA-DPH were prepared in methanol and kept at −20 °C. Appropriate dilutions for experimental procedures were performed in the same solvent. Cho and phospholipids were kept in a nitrogen atmosphere, at −20 °C as chloroform-methanol solutions in Teflon capped glass tubes. Concentration of phospholipids was routinely checked by the method of Ames [1], while cholesterol concentration was assessed by the cholesterol oxidase method (Sigma).

2.2. Langmuir monolayer experiments

The surface activity of polyphenols was assayed in a NIMA 102 M Langmuir microbalance (KSV NIMA, Finland) equipped with a custom made PTFE trough of 7 mL for constant area experiments. Methanolic stock solutions (1–10 mM) of polyphenols were injected into the 145 mM NaCl sub-phase under constant stirring and the surface pressure was measured with a Wilhelmy platinum plate, as a function of time and up to a constant value of surface pressure was reached, typically 15 min. The surface of exclusion of polyphenols from lipid interfaces was calculated by measuring the increment in surface pressure produced by the different polyphenols when injected into the subphase of lipid monolayers, standing at different surface pressure.

Monolayers of DPPC and DMPC standing at 20, 25 and 30 $mN \cdot m^{-1}$ were studied for polyphenol adsorption from the subphase in order to assess the interaction of the different compounds with liquid condensed and liquid expanded monolayer phase, respectively. DPPC at low surface pressure was not studied as this lipid presents an isobaric monolayer phase transition at around 10 $mN \cdot m^{-1}$ at room temperature, which could interfere when surface pressure increments are intended to measure. Thus, experiments of polyphenol adsorption into lipid monolayers at surface pressures lower than 15 $mN \cdot m^{-1}$ in the absence of cholesterol were carried out only with DMPC [44,47]. Furthermore, we aimed to determine the effect of the presence of cholesterol on the adsorption of polyphenols in both systems ($X_{Cho} = 0.4$). In cholesterol-containing monolayers there is a typical "ordered liquid" phase that has a high degree of mobility, similar to fluid phases but at the same time highly ordered hydrocarbon chains [31,47]. Results are the average of at least five independent experiments.

2.3. Polyphenol-lipid bilayer partition

Multilamellar vesicles (MLV) were prepared by drying organic solutions of lipids under a nitrogen stream, followed by resuspension in buffer (Tris-HCl 25 mM pH 7.4) and vortexing. Afterward, MLV were diluted to a final concentration of 100 μM and 1 μM of polyphenol was added. Mixtures were incubated at 37 °C for 10 min and centrifuged at 10,000 ×*g* for 15 min. Polyphenol concentration in the supernatant

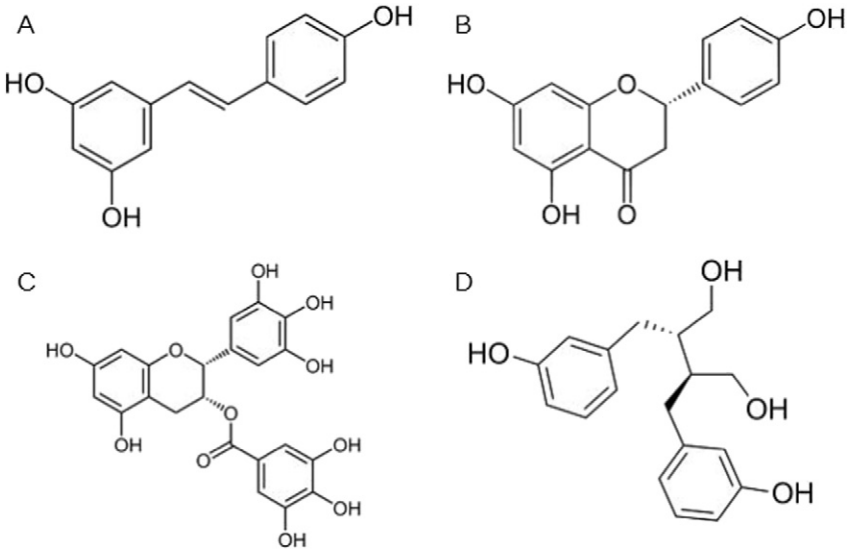

Fig. 1. Chemical structure of the polyphenols resveratrol (Resv, A), naringenin (Nar, B), epigallocateccin gallate (EGCG, C) and enterodiol (Ed, D).