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Antiradical activity of gallic acid included in lipid interphases

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ABSTRACT

Polyphenols are well known as antioxidant agents and by their effects on the hydration layers of lipid interphases. Among them, gallic acid and its derivatives are able to decrease the dipole potential and to act in water as a strong antioxidant. In this work we have studied both effects on lipid interphases in monolayers and bilayers of dimyristoylphosphatidylcholine. The results show that gallic acid (GA) increases the negative surface charges of large unilamellar vesicles (LUVs) and decreases the dipole potential of the lipid interphase. As a result, positively charged radical species such as $ABTS^{\bullet+}$ are able to penetrate the membrane forming an association with GA. These results allow discussing the antiradical activity (ARA) of GA at the membrane phase which may be taking place in water spaces between the lipids.

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1. Introduction

It is well known that gallic acid (GA), 3,4,5-trihydroxybenzoic acid (Fig. 1A) and its derivatives are biologically active compounds widely present in plants [1–3] and beverages such as tea and wine and is one of the anticarcinogenic polyphenols present in green tea [4,5].

GA is a strong chelating agent that forms complexes of high stability with iron(III) [6,7]. It has shown phytotoxicity and antifungal activity against *Fusarium semitectum, Fusarium fusiformis* and *Alternaria alternata* [8].

Free radicals occur as a natural consequence of cell metabolism and have been implicated in the etiology and pathogenesis of numerous disease states including cardiovascular disease, cancer and diabetes [9–11]. They are also produced as a result of oxidative stress [12–14]. Gallate esters present antioxidant capacity against hydroxyl, azide, and superoxide radicals [15–19] and they are able to scavenge hypochlorous acid at a rate sufficient to protect α-1-antiproteinase against inactivation [20–23]. Also, GA is of great interest in arteriosclerosis prevention [24].

One of the targets of free radicals is the lipid molecule in cell membranes [25]. In this regard, it has been shown that GA decreases the peroxidation of ox brain phospholipids [26]. It is known that the association of GA with phospholipid in organic solvents improves its antioxidant potential of GA by enhancing its bioavailability [27]. However, to our knowledge, no data about the activity of GA included in lipid membranes has been reported. Thus, it is of interest to study the interaction of this compound with lipid membranes and its chemical activity in the presence of lipids.

In terms of membrane structure, polyphenols are able to interact with lipid membranes causing a collapse of the water space between bilayers. This has been ascribed to the decrease of the membrane dipole potential induced by polyols and polyphenols. In particular, it has been shown that GA reduces the dipole potential of lipid monolayers spread on an air–water interface in around 30–40 mV [28].

However, there are no systematic studies correlating the antioxidant properties of GA with its interaction with membrane particles. For this reason, the aim of this paper is to analyze the surface changes promoted by GA on lipid interphases of neutral lipids such as phosphatidylcholines (DMPC) and its influence on antiradical activity (ARA).

The radical cation derived from 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) (Fig. 1B) is commonly used to evaluate the antioxidant effectivity of pure compounds and complex mixtures (A. M. Osman et al. and references there in [29]). These radical cations can be generated by enzymatic, chemical, and electrochemical means. Several studies have shown that the kinetics of the reaction between the $ABTS^{\bullet+}$ radical cations and polyphenols in solution is rather complex and the lack of relationship between the rate law and stoichiometric

Abbreviations: GA, gallic acid; DMPC, 1,2-dimyristoylphosphatidylcholine; ABTS, 2,2′-azino-bis-3-ethylbenzo thiazoline-6-sulfonic acid; ARA, antiradical activity

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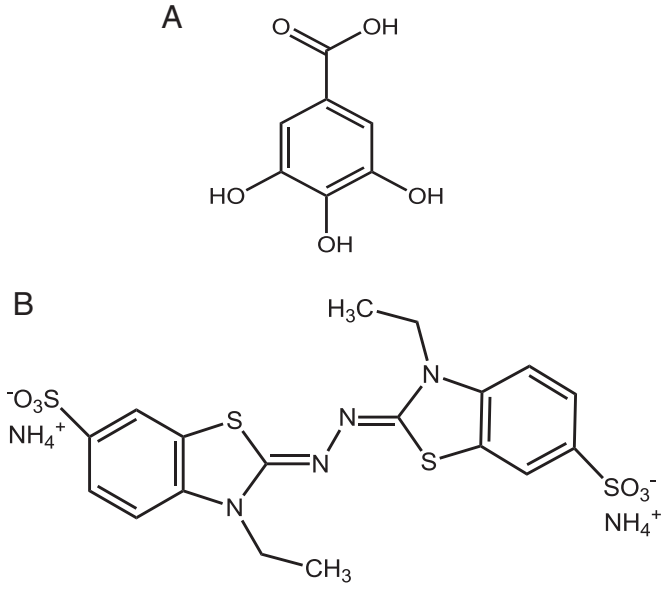

Fig. 1. Molecular structure of (A) GA and (B) $ABTS^{\bullet+}$.

factors has been also reported [28,29,32]. In this paper, we have compared the $ABTS^{\bullet+}$ reaction with GA in solution and in lipid membranes to infer the influence of lipid matrix on the ARA.

Thus, we have characterized the association of GA to DMPC LUVs measuring the zeta potential, the dipole potential and surface pressure and its effects on the kinetics of the antioxidant reaction with $ABTS^{\bullet+}$ of GA adsorbed on lipid surfaces.

2. Methodology

2.1. Materials

1,2-Dimyristoylphosphatidylcholine (DMPC) was obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and its purity was checked by thin layer chromatography using a chloroform/methanol/water mixture. A single spot was found; therefore, it was used without further purification (>99% pure). Chloroform and KCl were of analytical grade. 2,2′-azino-bis-3-ethylbenzo thiazoline-6-sulfonic acid (ABTS) (Fig. 1B) and GA were purchased from Fluka and Anedra, respectively and potassium persulfate was from SIGMA (Saint Louis, MO). Water was of ultrapure quality (0.009 μS/cm) obtained in an OSMOION 10.2 equipment.

2.2. Liposome preparation

DMPC multilamellar liposomes (MLVs) were prepared by dispersing the lipids by vortexing in 1 mM KCl at temperatures higher than that of the phase transition for 60 min. Large unilamellar vesicles (LUVs) were prepared by extruding the liposome dispersions through a polycarbonate membrane (pore size 1000 nm) above the transition temperature of the lipids. Then, the samples were cooled down to working temperature. After LUV preparation, solutions of similar ratios of GA and DMPC were prepared to determine the zeta potential (ζ) and antiradical activity (ARA).

2.3. Zeta potential

The zeta potentials (ζ) of DMPC LUVs were determined in Zeta-Meter System 3.0 equipment. All measurements were done at 22 ± 2 °C and pH = 4–4.5.

The voltage was fixed at 75 V. In this method, individual particles are visualized under the microscope and the mobility is determined automatically particle by particle. The total lipid concentration in all cases was 33 μM and the GA concentration was increased from 0 up to 0.83 mM. A total of 20 measurements were carried out focusing different particles for each sample. Data reported are the average of measurements done for each condition with, at least, three different batches of liposomes.

The size and number of vesicles in each sample were determined using the software provided in an Olympus CKX 41 inverted fluorescence microscope with a magnification of 40×.

2.4. Dipole potential

Dipole potential (Ψ_D) was determined in monolayers formed on an air–water interface by spreading chloroform solutions of lipids on an aqueous subphase (KCl 1 mM) as described before [30,31].

The values of the interfacial potential were determined through a circuit of high impedance, connecting a vibrating electrode above the monolayer and a reference Ag/AgCl electrode in the aqueous subphase. The zero of the potential was achieved with the aqueous solution after extensive cleaning by vacuum.

Lipids were added in carefully measured aliquots of a solution in chloroform of known concentration. After each addition the potential was allowed to stabilize. The dipole potential reached a saturation value after subsequent additions.

The values of areas with this method were obtained following a procedure previously described [31]. Temperature was set at 22 ± 2 °C

2.5. Formation of lipid monolayers. Measure of surface pressure and area per lipid calculation

The saturation point of monolayers with and without GA was monitored by measurements of the surface pressure of the lipid monolayers in a Kibron μtrough S equipment, at constant temperature and area.

Aliquots of a chloroform solution of lipids were spread on a clean surface of 1 mM KCl or aqueous solutions with 10 mM GA and left to reach constant surface pressures, until no changes were observed with further additions of lipids (saturation). Results of surface pressure were expressed in mN/m. With these criteria, areas per lipid were calculated with the first point of the saturation plateau of a curve of monolayer surface pressure vs nmol of lipids added to a constant area of the trough.

2.6. Antiradical activity determination (ARA)

The method used to monitor the antiradical activity (ARA) was the radical cation method ($ABTS^{\bullet+}$) (Fig. 1B). It consists of monitoring by spectrophotometry the $ABTS^{\bullet+}$ absorbance reduction at 734 nm after antioxidant addition. $ABTS^{\bullet+}$ solution (initial absorbance = 1.00) was placed into a cuvette and mixed with aliquots of different GA/DMPC ratios to a final volume of 1 mL. The radical inhibition percent by GA was calculated, applying Eq. (1), [32,35]

$$\%\mathrm{ARA} = 100 \times \left[1 - \frac{A_{ss}}{A_0}\right] \quad (1)$$

where A_0 is the absorbance of $ABTS^{\bullet+}$ solution before adding the antioxidant and A_{SS} is the absorbance of the solution at the steady state. Origin 8.0 software was used to estimate the A_{SS} values by mathematical fitting of kinetic curves according to Eq. (2).

$$A_{(t)} = A_{ss} + A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \quad (2)$$

where $A_{(t)}$ is the absorbance in time (t); A_1 is the maximum absorbance at the first step; k_1 is the kinetic constant at the first step and k_2 is the kinetic constant at the second step.

In order to express ARA in equivalent μmol of this compound, calibration curve was prepared with GA.

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