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The interaction of eugenol with cell membrane models at the air–water interface is modulated by the lipid monolayer composition

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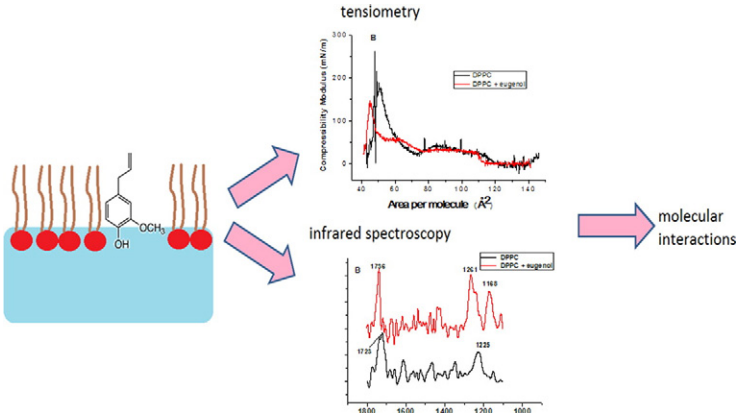
HIGHLIGHTS

- Langmuir monolayers of lipids are formed at the air–water interface.
- Eugenol is incorporated to the monolayer.
- Interactions at the molecular level are identified with tensiometry and PM–IRRAS.
- Intermolecular interactions are modulated by the monolayer lipid composition.

GRAPHICAL ABSTRACT



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ABSTRACT

Eugenol, a natural phenylpropanoid derivative with possible action in biological surfaces as microbicide, anesthetic and antioxidant, was incorporated in lipid monolayers of selected lipids at the air–water interface, representing cell membrane models. Interaction of eugenol with the lipids dipalmitoylphosphatidylcholine (DPPC), dioctadecyldimethylammonium bromide (DODAB), and dipalmitoylphosphatidylserine (DPPS) could be inferred by means of surface pressure-area isotherms and Polarization–Modulation Reflection–Absorption Spectroscopy. The interaction showed different effects on the different lipids. A higher monolayer expansion was observed for DPPS and DODAB, while more significant effects on the polar groups of the lipids were observed for DPPS and DPPC. These results pointed to the fact that the interaction of eugenol with lipid monolayers at the air–water interface is modulated by the lipid composition, which may be important to comprehend at the molecular level the interaction of this drug with biological surfaces.

1. Introduction

Eugenol is an allylphenol, belonging to the class of natural products known as phenylpropanoids. It is extracted from essential oils such as, nutmeg, clove oil, cinnamon, basil and bay leaf [1–3]. This compound has been reported to be used in flavorings, perfumeries, and in medicine as local antiseptics and anesthetics.

Also it is considered as a bactericide, and an antiviral compound [4,5], and can be employed as restorative applications in dentistry, in antioxidants for plastics and rubbers, as an anesthetic, and in some mousetraps. Also it has been reported that eugenol kills certain human colon

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cancer cell lines in vitro and in vivo [6–8]. Furthermore, it is reported that it is hepatotoxic, causing damage to living beings [9].

In this sense, it is of interest to study the interactions of this compound in biointerfaces, such as cell membranes. The understanding of how eugenol interacts with these surfaces may help comprehend its molecular mechanism of action in cell membranes. It is reported that eugenol inserted in liposomes inhibits the enzymatic action of xanthine oxidase–xanthine-iron [10]. Also, it has been shown that eugenol prevents membrane damaging events for which its presence resulted in the inhibition for the formation of malondialdehyde in irradiated liposomes. Fujisawa et al. [11] reported that the cytotoxic activity of eugenol against cells was enhanced by visible-light irradiation due to its high redox potential.

A proper system to be used as a model for cell membranes is the Langmuir monolayers, which is composed by monomolecular films of amphiphilic compounds at the liquid–gas interface. When organic solutions of membrane lipids are spread on the air–water interface, a model for half a membrane is formed [12], and interactions with proteins [13], polysaccharides [14], peptides [15] and drugs [16] can be investigated by means of tensiometry, vibrational spectroscopy and other surface specific techniques. Particularly, the interaction of several drugs with lipids at the air–water interface have been studied in Langmuir monolayers of lipids [16–18], and to the best of our knowledge no report on the interaction of eugenol with cell membrane models represented by a Langmuir monolayer has been reported. However, some studies have been shown the insertion of eugenol in bilayers [10–19], which makes promising the study with Langmuir monolayers as a complementary model. Also, these monomolecular films are useful to understand molecular interactions for other kind of lipid-drug systems, such as in liposomes in drug delivery systems.

In this present work, we studied the interaction of eugenol with Langmuir monolayers composed of selected lipids. In order to better understand the role of the chemical nature of the lipid in these interactions, three different lipids were employed, a zwitterionic one, dipalmitoylphosphatidylcholine (DPPC), a positively charged one, dioctadecyldimethylammonium bromide (DODAB), and a negatively charged lipid, dipalmitoylphosphatidylserine (DPPS).

2. Materials and methods

2.1. General

DPPC, DODAB and DPPS were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in chloroform (Synth, Diadema, Brazil) to a concentration of 0.5 mg/mL. The monolayer subphase approximated physiological conditions and consisted of a 50 mM phosphate buffer and 150 mM NaCl at a pH of 7.4. The water employed was purified using a MilliQ-Plus System (resistivity 18.2 MΩ cm, pH 5.5). LREIMS was measured in MS-QP-5050A mass spectrometer, operating at electron impact (70 eV). ^{1}H and ^{13}C NMR spectra were recorded, respectively, at 300 and 75 MHz in a Bruker Advance II 300 spectrometer using $CDCl_3$ (TediaBrazil) as solvent and TMS (tetramethylsilane) as internal standard. Chemical shifts (δ) are reported in ppm and coupling content (*J*) in Hz.

2.2. Isolation of eugenol

Aiming the isolation of eugenol, twigs of *Nectranda leucantha* were collected in the municipality of Cubatão, São Paulo State, Brazil in November 2013. The dried and powdered plant material (300 g) was exhaustively extracted with n-hexane, and its concentration was reduced under pressure, obtaining about 9 g of n-hexane extract. Part of this crude material (7 g) was subjected to column chromatography over silica gel using increasing amounts of EtOAc in n-hexane to afford six fractions (A to F). Fraction D (76 mg) was purified by Sephadex LH-20 using MeOH as eluent to afford 13 mg of pure eugenol.

Eugenol was characterized by NMR (300 and 75 MHz, $CDCl_3$) and follows: δ_H 6.67 (d, $J = 2.0$ Hz, H-2), 6.84 (d, $J = 8.1$ Hz, H-5), 6.70 (dd, $J = 8.1$ and 2.0 Hz, H-6), 3.32 (d, $J = 6.6$ Hz, H-7), 5.93 (m, H-8), 5.06 (m, H-9), 3.87 (s, 3-OC$\underline{H}_3$). δ_C 131.9 (C-1), 111.1 (C-2), 146.5 (C-3), 143.9 (C-4), 121.2 (C-5), 114.3 (C-6), 39.9 (C-7), 137.8 (C-8), 115.5 (C-9), 55.9 (3-OCH_3). LREIMS *m/z* (rel. int.) 164 (M^+, 100), 149 (47), 137 (31), 131 (40), 121 (20), 103 (25), 91 (20), 77 (21).

2.3. Preparation of monolayers

The Langmuir monolayers were obtained by spreading a chloroform solution of DPPC, DPPS or DODAB on the surface of an aqueous buffer solution. For preliminary tests, eugenol solutions, dissolved in chloroform to a concentration of 0.5 mg/mL, were also spread alone at the air–water interface in order to test the surface activity of this compound. For mixed eugenol-lipid monolayers, first the lipid was spread on the air–water interface. Then, 20 min was allowed for chloroform evaporation, and aliquots of eugenol to render a 2% in mol-lipid were carefully injected in the aqueous subphase. Other proportions were essayed and this value represents a limit for the effect of this drug in terms of expansion of the monolayer. For higher amounts the isotherms do not present reproducibility probably because effects related to the formation of aggregates. Also, considering that drugs interacting with membranes are inserted in relative small amounts, this proportion must approximate conditions in vivo.

Surface pressure-area (π-A) isotherms were obtained in a mini-KSV Langmuir trough equipped with a surface pressure sensor (Wilhelmy method), with an interface compression rate of 5 $Å^2$ $molecule^{-1}$ min^{-1}. After allowing 20 min for evaporation of chloroform, the monolayer was compressed until the collapse is reached. Each isotherm shown in this paper was repeated at least three times for checking the reproducibility, and no variations higher than 0.1 mN/m was allowed. Then for each graph, a representative isotherm is shown. For PM–IRRAS studies, the monolayer was compressed until the desired surface pressure (30 mN/m). The surface pressure was maintained at the desired surface pressure by moving the barriers, and the stabilization of the monolayer was monitored until no additional movement of the barriers was needed. The PM–IRRAS measurements were then taken using a KSV PMI 550 instrument (KSV Instruments, Ltd., Helsinki, Finland) at a fixed incidence angle of 80°. Each spectrum shown in this paper was repeated at least three times for checking the reproducibility and a representative spectrum is shown.

All experiments were carried out at a controlled room temperature (25 °C).

3. Results and discussion

After isolation of eugenol from twigs of *N. leucantha*, its structure was defined by analysis with ^{1}H, ^{13}C NMR and LREIMS data and compared with data previously described in the literature [11,20].

Eugenol spread alone on the air–water interface in the concentration used in this work does not present any surface activity, i.e. its spreading on the air–water interface does not decrease the surface tension of water. When the interface is compressed, thus increasing the surface molecular density, the surface pressure does not raise more than 1 mN/m, which confirms that this compound does not form Langmuir monolayers. As they are insoluble in water and cannot leave the interface, this fact can be attributed to the low spreading coefficient of eugenol, which must cause its aggregation at the air–water interface.

When incorporated in lipid monolayers, the surface activity of eugenol is detected, as inferred by means of analysis of the surface pressure-area isotherms. Fig. 1A shows the action of eugenol in Langmuir monolayers of DPPC. This lipid presents a typical π-A isotherm [21], with a plateau at approximately 14 mN/m, representing the transition between the states liquid-expanded (LE) and liquid-condensed (LC). The monolayer collapses at 55–60 mN/m in molecular areas of 45–50 $Å^2$.