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Effect of Xanthone and 1-Hydroxy Xanthone on the Dipole Potential of Lipid Membranes

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ABSTRACT

Xanthone (Xa) and 1-OH Xanthone (1-OHXa) in 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) membranes increase the dipole potential. Results with 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine (Ether PC), lacking carbonyl groups, indicate that the CO dipole of Xa molecule does not contribute to the increase of dipole potential. Molecular Dynamics calculations confirm that CO group of Xa locates parallel to the membrane interphase. Xa decreases the area per lipid in DPPC monolayers and the membrane polarity as measured by GP of Laurdan explaining the dipole potential increase. This rearrangement of water in the CO region is consistent with Fourier Transform Infrared Spectroscopy results indicating that CO of Xa are buried in the membrane phase. In contrast, CO groups of DPPC show an additional population of bound COs that seems to be more exposed to water, which contributes to the increase in dipole potential. These results are also corroborated by MD simulations.

1. Introduction

Xanthones are oxygenated heterocyclic compounds structurally typified by the presence of a dibenzo-γ-pirone system differing in the position and nature of the substituents [1]. Unsubstituted xanthones play an important role as pharmacophores. In turn, substituted xanthones or xanthen-9H-one derivatives are a class of compounds with high therapeutic potential [2].

There are over fifty natural xanthones extracted from *mangosteen* [3–6] among others. The taxonomic importance in xanthone families have aroused great interest not only for the chemosystematic investigation but also from the pharmacological point of view like antimalarial [7], antibacterial [8, 9], antifungal [10] [11], antioxidant, antitumoral [12, 13], antimicrobial and anti-hepatotoxic effects, as well as anti-inflammatory activity [14] and anti-Alzheimer properties [15–17]. Moreover, it is used in the preparation of xanthydrol for the determination of urea in blood and found in essential oils from genciana and other flowers and also isolated from pil in Norway coasts.

Xanthones are present in plants and their specific substituents can act [18] as defence compounds against herbivores and microorganisms. In this regard, it was introduced in the market as an insecticide, ovicida and larvicide for the butterfly *carpocapsa* [19–22].

This diversity of functions makes them interesting in regard to their specific effects on cellular structures. The structures of 9H-Xanthen-9-one (Xa) and 1-hydroxy-9H-xanthen-9-one (1-OHXa) (Fig. S1A and B) are well known by Infrared Spectroscopy, Mass Spectral and Nuclear Magnetic Resonance data [23]. Xanthone skeleton has a hydrophobic core composed of a heterocycle of xanthene oxidized in position 9 and a carbonyl group (Fig. S1A). On the other hand, 1-OHXa (Fig. S1B) shows a certain degree of hydrophylicity due to the presence of an −OH group in position 1.

The molecular features of Xa make it insoluble in water and highly soluble in organic solvents. Hence, due to its non-polar character, it is expected that at least some of its biological effects would take place affecting membrane properties. In this regard, effects of hydroxy-xanthones on the physicochemical properties of dipalmitoylphosphatidylcholine (DPPC) liposomes have been investigated in terms of lipid bilayer phase state, by means of molecular dynamics simulations and surface properties such as, zeta potential studies. In this direction, recently it has been reported that the entrapment of xanthones in

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; EtherPC, 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine; Xa, 9H-Xanthen-9-one; 1-OHXa, 1-hydroxy-9H-Xanthen-9-one.

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biomimetic systems such as liposomes can enhance its absorption through the biological membranes [24].

Xanthene dyes, such as fluorescein, rose bengal B, erythrosine B and eosin Y, have been found to affect the dipole potential of membranes composed of diphytanoylphosphocholine, diphytanoylphosphoserine, and diphytanoylphosphoethanolamine. Adsorption of Rose Bengal on the bilayer interface leads to a reduction of the dipole potential drop at the membrane-solution boundary. The effect was ascribed to their uncharged and charged forms, respectively. In the case of the xanthene dye fluorescein, it was shown that it remains on one side of the BLM, revealing a low partition in the membrane phase and that its action is mainly produced at the interphase [25–27].

However, xanthones localization within the membrane and its effects on the organization of the lipid membrane surface, are not yet clear. Since surface properties, in particular dipole potential, are determinant for adsorption and absorption of peptides and proteins and in adhesion phenomena, it is important to know how these compounds may affect those properties in lipid membrane.

Therefore, the aim of this work is to determine the possible mechanism of Xa to change the surface properties of lipid interphases, considering that these are determinant in adhesion, partition and fusion processes. In particular, the effect of 9H-Xanthen-9-one (Xa) and 1-hydroxy-9H-Xanthen-9-one (1-OHXa) on dipole potential, molecular packing and hydration of lipid membranes was carried out by fluorescent spectroscopy, FTIR and Molecular Dynamics simulations.

2. Materials and Methods

2.1. Lipids and Chemicals

Chemicals: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC); 1,2-di-O-tetradecyl-*sn*-glycero-3-phosphocholine (Ether PC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 9H-Xanthen-9-one (Xa) (MW: 196.19 g/mol) was from Sigma-Aldrich. Purity of lipids and xanthones were higher than > 99% as checked by FTIR and UV spectroscopies. Laurdan (6-dodecanoyl-2-dimethyl aminonaphthalene) was obtained from Molecular Probes and used without further purification. 1-hydroxy-9H-xanthen-9-one (1-OHXa) (MW: 212.20 g/mol) was synthesized from Xa following the protocol of Grover, Shah, Shah (GSS) [28] by reacting equimolecular mixtures of salicylic acid and resorcinol in the presence of zinc chloride at 170 °C for 6 h. Purification was performed by column chromatography using silica gel as adsorbent and hexane/AcOEt as eluent. Partition coefficient experimental values (log P) for Xa base and 1OHXa were determined by applying the ampoule method (Shake-Flask) at room temperature (25 °C) using *n*-octanol as a non-polar phase at pH = 7.4.

2.2. Surface Pressure – Molecular Area (π- A) Isotherms

Surface pressure – molecular area (π- A) isotherms were carried out in a KSV NIMA LB trough (surface area = 240.00 cm^2) using a Pt Wilhelmy plate (39.24 mm^2) as sensor. Accurate volumes of lipids dissolved in chloroform were spread on the surface of 1 mM KCl aqueous solution (pH 5), using a Hamilton micro syringe with an error within ± 0.01 mL. The Platinum probe was flamed until glowed red-hot before each assay. The trough was filled with the barriers in a fully open position. An appropriate volume was added checking that the borders of the meniscus were even in the whole perimeter. Monolayers were allowed to stabilize during 10 min before each measurements. The whole equipment was enclosed in an acrylic box to minimize water evaporation and to avoid contaminations from the environment during the study. Compression curves were carried out at a constant speed (5 mm/min) at 20 ± 2 °C. Data correspond to an average of at least three experimental runs for each lipid Xa or 1-OHXa ratio.

2.3. Dipole Potential Measurements

Dipole potential (ψ_D) was determined in monolayers formed on an air–water interface of a 1 mM KCl aqueous solution by spreading chloroform solutions of each lipid/Xa or 1-OHXa mixture. Data correspond to an average of at least three experimental runs for each mixture. Different aliquots of each mixture were added until constant surface pressure was achieved [29, 30]. In this condition, the interfacial potential was 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 temperature was set at 20 ± 0.5 °C controlled with a calibrated thermocouple immersed in the subphase. Therefore, the reference potential is constant at the temperature of measurement [31].

2.4. ATR-FTIR Spectroscopy

All ATR-FTIR spectra were obtained in a Thermo Scientific 6700 spectrometer with DTGS detector, connected to a system of circulation of dry air to avoid the interference of water vapor and carbon dioxide. The molar ratio of lipid/Xa and lipid/1-OHXa in each film was 70:30. The films were prepared by evaporating the chloroform solvent. Then, they were resuspended in a minimum volume of MQ water at 5 pH. Droplet (50 μL) of each suspension was placed on ZnSe crystal (45° incident angle). Samples were allowed to stabilize at 25 °C and a relative humidity of 35% before each measurement. Spectra were obtained with intervals of three minutes in order to control the water content evolution following the water band intensity and the asymmetric stretching vibration of the methylene groups. Data were obtained after 64 scans per sample at a 4 cm^{-1} resolution and were the average of three independent assays. Spectra were analysed with a Microcal Origin program (version 8.5) applying a combination of Fourier deconvolution and curve-fitting procedures to reconstruct the contours of the original band envelope [32, 33].

2.5. Molecular Dynamics Simulations

Molecular dynamics simulations of DPPC bilayers were performed using AMBER12 simulation package [34] and Lipid11 force field [35]. Initially, the bilayer was composed of 128 DPPC molecules (64 per monolayer in an 8 × 8 arrangement) with a separation between DPPC molecules of 9 Å in a square arrangement. Nine DPPC molecules for each monolayer in alternate places were removed and xanthones were added in the generated free spaces. The membrane was stabilized at $T = 293$ K and solvated with 10,640 TIP3P water molecules [36, 37] (which means around 83 water molecules per lipid) in order to assure that the system was fully hydrated. Solvation was done along the Z axis and the system was subjected to periodic boundary conditions along the (X,Y) plane. The minimization was carried out in two steps, both at constant volume. In the first step solvent were minimized keeping fixed the atoms of the membrane and xanthones, and then releasing it to minimize the entire system. The equilibration of the dimensions and density of the system was achieved after stabilizing the system temperature using the Langevin thermostat [38] and keeping fixed the bilayer. Then, all restraints were removed and the bilayer was free to establish a lipid-lipid equilibrium distance. After 300 ns equilibration, the area per lipid was consistent with experimental values. Both equilibration and final production data used a NPT assembly, with the SHAKE activated for hydrogen bonds, with 10 Å interaction cut off. Production runs typically elapsed 500 ps, but we also performed some larger runs to test the consistency of our results.

2.6. Generalized Polarization with Laurdan

Fluorescence measurements were carried out in a SLM 4800 spectrofluorometer. Multilamellar vesicles (MLV's) were prepared for the