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Hydroperoxide and carboxyl groups preferential location in oxidized biomembranes experimentally determined by small angle X-ray scattering: Implications in membrane structure

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ABSTRACT

We report small angle X-ray scattering (SAXS) data from large unilamellar vesicles as model membranes composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC) and two oxidized species, namely its hydroperoxidized form POPC-OOH and 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC) lipid that has a carboxyl group at the end of its truncated *sn*-2 chain. The replacement of POPC by either POPC-OOH ($POPC\text{-}OOH_xPOPC_{1-x}$) or PazePC ($PazePC_xPOPC_{1-x}$), with oxidized lipid molar ratio x varying from 0.00 up to 1.00, permits to experimentally inspect changes in the membrane structural properties due to oxidation. The volume fraction distribution of each lipid chemical group along the bilayer is determined. The results quantify that 95% of the hydroperoxide group lies in the membrane polar moiety, near the carbonyl and phosphate groups, whereas just 5% of OOH group experiences the polar/apolar interface, for all values of x studied. In the case of PazePC up to $x = 0.33$, a bimodal distribution of the carboxyl group in the interior and polar regions of the lipid membrane is obtained, probably due to a dynamic movement of the shortened alkyl chain towards the water interface. The mean molecular area A gradually increases from 65.4 ± 0.4 Å^2 for POPC bilayers to 78 ± 2 Å^2 for pure POPC-OOH bilayers, whereas POPC-OOH membrane thickness resulted to be 20% thinner than the non-oxidized POPC membrane. For PazePC up to $x = 0.33$, A increases to 67 ± 2 Å^2 with 10% of membrane thinning. The SAXS results thus demonstrate how the lipid oxidation progress affects the membrane structural features, thus paving the way to better understand membrane damage under oxidative stress.

1. Introduction

Lipids of biological membranes are prone to chemical and photo-induced oxidation because they contain high amounts of mono-unsaturated and polyunsaturated fatty acids. By turn, lipid oxidation leads to the formation of new molecular entities, which resemble lipids but have hydrophilic groups hanging on the alkyl chains or often on the shortened acyl oxidized lipid tails [1,2].

A controlled amount of oxidized lipid products (referred to herein as OxL) is required for cell signaling, maturation and differentiation, as well as apoptosis. However, the production of OxL, if uncontrolled, can have a deleterious effect on the functioning of the cell, and also be involved in a variety of diseases [3,4]. Indeed, methodological advances at the molecular-level detection and identification of oxidative species have provided insights into oxidative lipid modification and its involvement in cell signaling as well as in major diseases and inflammation processes [5-9]. Extensive evidence suggests a correlation between lipid peroxidation and degenerative neurological disorders such as Parkinson's and Alzheimer's diseases [10-12], as well as type 2 diabetes [13,14]. Aging and carcinogenesis induced by UV and many physiological processes have been also related to the formation of oxidative species [15,6,13]. However, in spite of the obvious relevance of understanding the molecular basis of several diseases, the exact modes of action of oxidized lipids on membranes remain elusive.

Usually, lipid oxidation reactions start with lipid hydroperoxide formation and progress to lipids with truncated alkyl chains [16]. Several molecular dynamics (MD) studies have been carried out during the last decade (reviewed by Jurkiewicz et al. [17] and more recently by Siani et al. [1]) with different oxidized lipid species inside the model lipid bilayer systems. In particular, simulation results show for hydroperoxized lipids an increase in membrane lateral area accompanied by a decrease in the bilayer thickness and order parameters [18,1,19]. The presence of hydroperoxide groups in the lipid bilayer does not promote pore formation regardless of the water model and force field employed.

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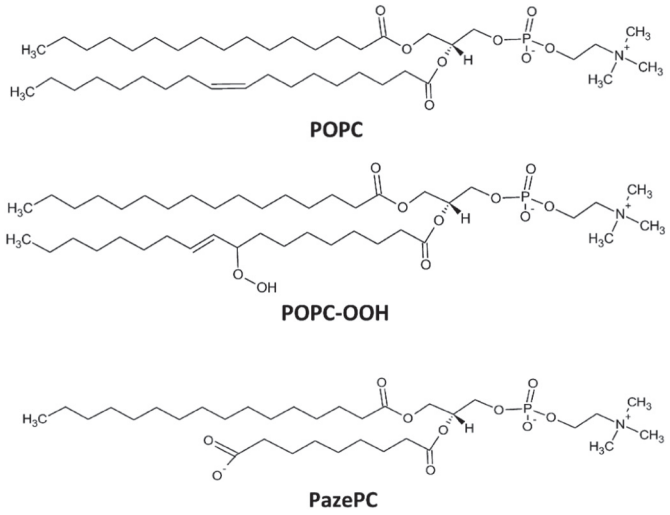

Fig. 1. Chemical structures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and two oxidatively modified phospholipids: the hydroperoxidized form of POPC (POPC-OOH) and 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC).

However, there is a controversy in the literature concerning the hydroperoxized group location in the membrane predicted by MD. Some MD results point out that hydroperoxized groups of OxL tend to be preferentially located near the headgroup region [18,20,1], whereas a bimodal distribution of the hydroperoxized groups in the membrane interior and polar moiety has also been reported [19]. Therefore, results from computing simulations are not unique in terms of hydroperoxized group location in the lipid bilayer but, in fact, depend on the used force field and methodology approach.

From the experimental point of view, results using giant unilamellar vesicles (GUVs) as model membranes revealed that the hydroperoxide groups (Fig. 1) have a significant impact on membrane features as, for instance, increase in lateral area [21] in good agreement with MD data, fluidity [22], elastic modulus [23] and rafts organization [24]. However, there is not any experimental data reported on the literature that demonstrates either a decrease in the bilayer thickness or the most probable location of the hydroperoxide group in the membrane as theoretically predicted by MD.

Subsequent oxidation leading to lipids with shortened chains also alters membranes' properties and may lead to an increase in membrane permeability, pores formation [25,26] and membrane rupture [27]. Khandelia and Mouritsen [28] simulated a system comprising 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC) lipid that has a carboxylate group at the end of its truncated *sn*-2 chain (Fig. 1). According to MD results, the shortened tail of PazePC completely reverses orientation, thus exposing its carboxyl group to aqueous phase, in very good agreement with Förster-type resonance energy transfer (FRET) results obtained between cytochrome-c and fluorescently labeled PazePC model membranes [29] and electron density profiles extracted from X-ray reflectivity on supported lipid bilayers containing up to 20 mol% of PazePC [30]. Interestingly, PazePC may have implication in atherogenesis [31].

Therefore, either as a result of a physiological process or derived from photo-oxidation, the influence of OxL on the physical properties of membrane is not fully understood. In fact, the complexity of the studied processes and the limitations of the tools necessary to characterize the overall membrane structural modifications imposed by the presence of OxL hinder our current understanding of this phenomenon. Therefore, in spite of the fact that MD can describe qualitatively some lipid bilayer properties, it is very important the use of proper experimental techniques to access structural information from membranes in a quantitative manner. On this ground, small angle X-ray scattering (SAXS) technique is a powerful tool to investigate lipid bilayer structure. In particular, the experimental determination of the volume fraction distribution of each chemical group of the lipid molecules along the bilayer has been obtained by analyzing SAXS data with the well-known scattering density profile (SDP) model [32-37], which we have modified and entered in the Genfit software data analysis package [38]. Specifically, SAXS curves of different mixtures of lipids are jointly analyzed by refining common structural parameters. Here, we take advantage of SDP model to obtain the preferential sites of oxidized groups inside the membrane from SAXS data. Our experimental approach consists in incorporating oxidized phospholipid species into the vesicles bilayer. They are POPC-OOH, the hydroperoxidized form of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and PazePC (Fig. 1). By replacing defined amounts of the unsaturated lipid with a corresponding oxidized product, the oxidation process can be mimicked, yielding vesicles of varying oxidized lipid concentration. Understanding and measuring how oxidation of the lipid bilayer affects its structural architecture is key to comprehend how it impacts on cell destabilization and damage.

2. Materials and methods

2.1. Materials

The phospholipids POPC and PazePC were purchased from Avanti Polar Lipids. POPC-OOH was synthesized by our group according to the protocol described elsewhere [39]. Fig. 1 shows the chemical structures of POPC, POPC-OOH and PazePC.

2.2. Large unilamellar vesicles (LUVs) preparation

The investigated LUVs consisted of $POPC_{1-x}POPC\text{-}OOH_x$ (with the oxidized lipid molar ratio $x = 0.00$, 0.33, 0.67 and 1.00) and $POPC_{1-x}PazePC_x$ (with $x = 0.00$, 0.10 and 0.33), respectively. The lipids mixtures were dissolved in chloroform, which was evaporated under N_2 stream, to deposit a thin lipid film on the wall of a glass tube. The final traces of residual solvent were removed under vacuum at room temperature for 1 h. 10 mM of total lipids for each LUV composition were suspended in ultra-pure Milli-Q water, followed by extrusion through two-stacked polycarbonate membranes of 100 nm diameter pores (Mini-Extruder system, Avanti Polar Lipids Inc, Alabaster, Alabama, USA). This process was repeated 31 times, and the LUVs dimension (100 nm) was checked by dynamic light scattering (DLS). All liposomal preparations were freshly prepared and used in the same day. Of note, samples prepared with molar ratio of PazePC larger than 0.33 were not characterized as monodisperse solutions of LUVs by both SAXS and DLS and, hence, discarded from our data analysis.

2.3. Small angle X-ray scattering (SAXS)

SAXS experiments were performed at the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, Brazil). The scattering vector modulus $q = 4\pi \sin\theta/\lambda$ (2θ being the scattering angle and λ the X-ray wavelength fixed to 1.548 Å) ranged from 0.012 and 0.453 $Å^{-1}$. All experiments were carried out at room temperature of 22 ± 1 °C.

SAXS data analysis was based on the SDP model that has been successfully applied to extract from MD simulations of lipid bilayers the number densities and hence the volume fraction distributions of the different chemical groups along the bilayer normal [40,37]. Since X-ray or neutron scattering length densities (SLDs) can be easily calculated from volume fraction distributions, the same model has also been exploited to analyze SAXS as well as small-angle neutron scattering (SANS) data of lipid bilayers. The basic concept of the model is to describe the volume fraction distribution of lipid chemical groups with elementary analytic functions, such Gaussians and error functions. The water volume fraction distribution is then obtained as the complement to unity with respect to the total lipid volume fraction. However, since

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