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Comprehensive portrait of cholesterol containing oxidized membrane

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ABSTRACT

Biological membranes are under significant oxidative stress caused by reactive oxygen species mostly originating during cellular respiration. Double bonds of the unsaturated lipids are most prone to oxidation, which might lead to shortening of the oxidized chain and inserting of terminal either aldehyde or carboxylic group. Structural rearrangement of oxidized lipids, addressed already, is mainly associated with looping back of the hydrophilic terminal group. This contribution utilizing dual-focus fluorescence correlation spectroscopy and electron paramagnetic resonance as well as atomistic molecular dynamics simulations focuses on the overall changes of the membrane structural and dynamical properties once it becomes oxidized. Particularly, attention is paid to cholesterol rearrangement in the oxidized membrane revealing its preferable interaction with carbonyls of the oxidized chains. In this view cholesterol seems to have a tendency to repair, rather than condense, the bilayer.

1. Introduction

Unsaturated phospholipids, abundant constituents of cellular membranes, are prone to being chemically modified due to oxidative stress. Reactive oxygen species (ROS) often disrupt double bonds [1] which is then followed by insertion of oxygen atoms either to the side of the hydrophobic lipid chain in the form of hydroxides or hydroperoxides [2], or the carbon chain (typically *sn*-2) is truncated and terminated by a polar aldehyde or carboxylic group [3]. These oxidized phospholipids (oxPLs) have been related to various patho-physiologies such as inflammation [4–6] or atherosclerosis [5] just to name a few. OxPLs experience increased reactivity which may turn into further propagation of the oxidative reaction among lipids through the mitochondrial membrane, where ROS are mainly generated, to other organelles or cells. Furthermore, the insertion of an aldehyde group enables Schiff base formation [7] with amino groups in various proteins and lipids leading to significant changes of molecular properties of these moieties.

Terminating the *sn*-2 chain with a hydrophilic group is inevitably connected with structural rearrangement of the membrane since the strongly hydrophobic tail region of the membrane is no longer preferable for the oxidized chain. To minimize the unfavorable interaction, the chain has been shown to reverse out to the aqueous phase [8]. This phenomenon alters the membrane biophysics, which is the issue we would like to focus on in this manuscript.

The biophysical properties of membranes containing oxPLs have been addressed in a few papers already [8–12]. In lipid monolayers containing immiscible lipid phases, the presence of truncated phosphatidylcholines stabilizes liquid ordered domains [13]. Further, it has been shown that lipid oxidation increases partitioning of the truncated lipid into the liquid ordered phase and an increased diffusion coefficient of the oxidized moiety has been reported [11]. Molecular dynamics (MD) simulations using coarse grained Martini force field explain the altered diffusion behavior by pulling of the oxPL hydrophilic headgroups out of the DOPC headgroup region. The MD simulations further predicted that oxPLs are drug back inwards after cholesterol had been added. This was experimentally demonstrated by smearing out the difference in diffusion coefficients between the oxidized and non-oxidized lipids upon cholesterol addition [11].

While the diffusion study concentrates on the motion of the truncated lipid, here we address the physical properties of the entire single-phase membrane containing oxPLs. We investigate membrane bilayers consisting of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) where 5 to 10% of POPC molecules were replaced by 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC), with oleoyl residue replaced by truncated carbon chain terminated with a carboxylic acid. By means of atomistic MD simulations, as well as experimentally, we examine the role of cholesterol showing that it fills the void space arisen upon truncation of the lipid chain. On free standing model membranes of giant unilamellar

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vesicles (GUVs) we demonstrate oxidation-promoted changes in diffusion properties of individual membrane components and the effect of cholesterol in minimizing the oxidative bilayer damage. The MD simulations explain the phenomena by revealing a cholesterol population that specifically interacts with the carbonyl oxygen in the truncated lipid chain. The cholesterol carbonyl interaction with the truncated lipid is, compared to the same interaction with non-truncated lipids, further favored by better access to the carbonyl. The subtle structural membrane changes are translated to the lipid mobility characterized by the lateral diffusion coefficient or reorientation of the spin label.

2. Results

2.1. Structure of PGPC containing bilayer as obtained by MD simulations

As we would like to emphasize the role of cholesterol in oxidized membranes, both in simulations as well as in experiments we focus on different effects of cholesterol in oxidized and non-oxidized membranes. Generally, it is accepted that cholesterol causes lateral shrinking of the fluid phospholipid bilayer accompanied by increase of its thickness. This ordering and increased lateral compactness is commonly represented by a reduced area per lipid (APL). Our simulations show a lower cholesterol-dependent drop in APL in the POPC bilayer with 9% POPC replaced by PGPC than in a pure POPC bilayer (a drop of 4.7% and 6.1%, respectively). This suggests that the cholesterol action is suppressed in the oxidized membrane, which is in agreement with the experiments that will follow. APL is however a global parameter that does not directly explain what happens at the level of individual lipid molecules. Therefore, we will now concentrate on the atomistic description of the bilayer to comprehend the origin of the changes.

The main structural effect of the oxidized PGPC lipids' presence in the POPC membrane is the reorientation of truncated *sn*-2 chains of PGPC resulting in the occurrence of their termini at the lipid/water interface. As evident from density profiles presented in Fig. 2, oxygen atoms of carboxylate groups at the termini of PGPC *sn*-2 penetrate into the POPC headgroup region and reside between phosphate and choline groups of POPC (the protrusion of the carboxylate groups of PGPC is also visible in the simulation snapshot shown in Fig. S1 in the Supporting information). Hence, the truncated PGPC chains protrude from the membrane into the water phase. This is further evidenced by the distribution of *sn*-2 tilt-angles shown in Fig. 3A which demonstrate that the majority of the truncated chains of PGPC are reoriented towards the water phase and none of them is directed towards the membrane core. This is in accord with previous simulations and experimental results concerning various truncated forms of oxidized phosphatidylcholines [8–10,14]. As was discussed in those studies, reorientation of oxidized chains occurs due to the polar character of their terminal groups which prefer the well hydrated headgroup environment over the non-polar membrane core.

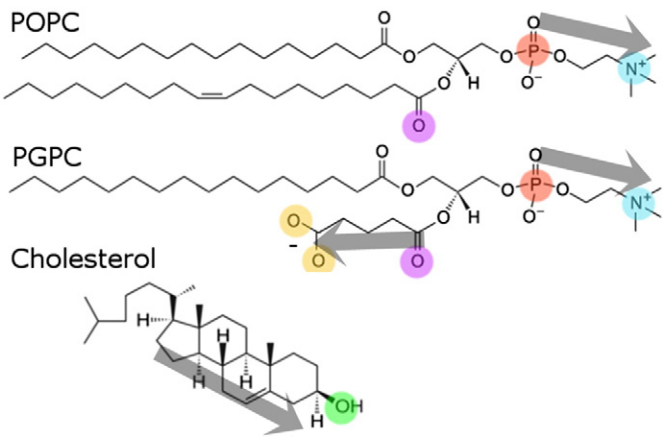

Fig. 1. Chemical structures of lipids considered in this work. The highlighted atoms and vectors are used in the analysis of structural membrane properties in MD simulations (see Figs. 2 and 3).

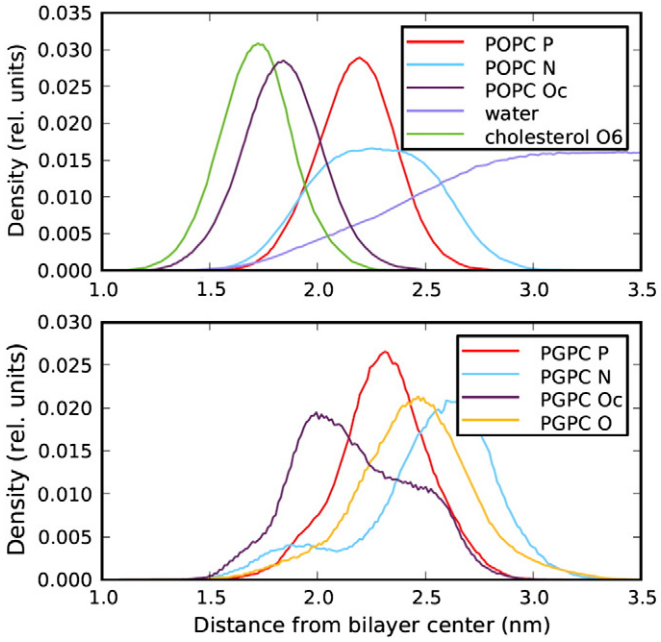

Fig. 2. Density profiles calculated across the membrane based on MD simulation of the POPC + PGPC + chol (116:12:38) bilayer. The following individual membrane components are presented: carbonyl oxygen atoms in *sn*-2 chains (O_c); nitrogen atoms in choline groups (N); phosphorus atom in phosphate groups (P); oxygen atoms in carboxylate groups of the truncated *sn*-2 chain of PGPC ($O_{carboxyl}$); oxygen atoms in hydroxyl groups of cholesterol (O_3) and water. See Fig. 1 for molecular structures. All curves are normalized in order to have the same integral. Density profiles of both membrane leaflets are averaged.

A comparison of tilt-angle distributions calculated in membranes with and without cholesterol, presented in Fig. 3A, shows that the presence of cholesterol has no significant influence on the reorientation of truncated *sn*-2 chains of PGPC. With cholesterol present, most of oxidized chains are reversed and none is directed towards the membrane interior.

Furthermore we analyze in detail the position of lipid headgroups and their orientation in the membrane. In accordance with literature, our MD simulations predict that the presence of 9% of carboxyl-terminated truncated PGPC does not affect the headgroup positions of the prevailing POPC lipid molecules with respect to the membrane center [11]. In the presence of PGPC, in both cholesterol-containing and cholesterol-free bilayers, the distance of the POPC headgroup from the center of the bilayer remains unaffected (see Fig. S2). PGPC headgroups do not coexist in the same position with POPC but shift outwards from the bilayer center, probably due to the looping back of the carboxyl terminated *sn*-2 chains. The orientational freedom of headgroups, which can be associated with the width of the P-N angles distribution, is significantly higher for PGPC than for POPC (see Fig. 3B,C). This can be attributed to two effects. First, the PGPC headgroups shift from the crowded POPC headgroup region towards the water phase, where headgroups have more orientational freedom than in their typical localization. Second, the reversal of PGPC *sn*-2 chains causes many conformational changes in the PGPC molecules and hence affects also the PGPC headgroup orientation. The distribution appears to become bimodal with a population of straight-staying and flat-laying PGPC headgroups (see arrows in Fig. 3C).

When cholesterol is inserted, acyl chains in POPC become more ordered which causes POPC headgroups to shift further from the bilayer center (see Fig. S2 in the Supporting information). The presence of cholesterol has a strong effect on PGPC headgroups (see Fig. 3C). In