Chemistry and Physics of Lipids 200 (2016) 74–82

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Influence of oxidized lipids on palmitoyl-oleoyl-phosphatidylcholine organization, contribution of Langmuir monolayers and Langmuir–Blodgett films

Christine Grauby-Heywang[a,*], Fabien Moroté[a], Marion Mathelié-Guinlet[a], Ibtissem Gammoudi[b], Ndeye Rokhaya Faye[a,1], Touria Cohen-Bouhacina[a]

[a] Laboratoire Ondes et Matière d'Aquitaine (LOMA), UMR CNRS 5798, Université de Bordeaux, 351 cours de la libération, 33405 Talence Cedex, France
[b] Cellule de transfert NanoPhyNov, Université de Bordeaux, 351 cours de la libération, Talence Cedex 33405, France

ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form 1 July 2016
Accepted 11 July 2016
Available online 12 July 2016

Keywords:
Oxidized lipid
Langmuir monolayer
Langmuir–Blodgett film
Surface pressure measurements
Atomic force microscopy

ABSTRACT

In this work, we studied the interaction of two oxidized lipids, PoxnoPC and PazePC, with POPC phospholipid. Mean molecular areas obtained from (π–A) isotherms of mixed PoxnoPC–POPC and PazePC–POPC monolayers revealed different behaviors of these two oxidized lipids: the presence of PoxnoPC in the monolayers induces their expansion, mean molecular areas being higher than those expected in the case of ideal mixtures. PazePC–POPC behave on the whole ideally. This difference can be explained by a different conformation of oxidized lipids. Moreover the carboxylic function of PazePC is protonated under our experimental conditions, as shown by (π–A) isotherms of PazePC at different pH values. Both oxidized lipids induce also an increase of the monolayer elasticity, PoxnoPC being slightly more efficient than PazePC. These monolayers were transferred from the air–water interface onto mica supports for a study by AFM. AFM images are on the whole homogenous, suggesting the presence of only one lipid phase in both cases. However, in the case of PazePC–POPC monolayers, AFM images show also the presence of areas thicker of 7 nm to 10 nm than the surrounding lipid phase, probably due to the local formation of multilayer systems induced by compression.

1. Introduction

Oxidation of membrane lipids can occur via different processes, such as oxidative stress responsible for the formation of reactive oxygen species (ROS) damaging biomolecules (Deigner and Hermetter, 2008), photodynamic therapies (Girotti, 2001; Castano et al., 2004) or enzymatic reactions (Greig et al., 2012). Damages due to oxidation can be dramatic, since it leads to the formation of deeply modified lipids, such as phospholipids containing hydroxyl or hydroperoxy groups on the unsaturated chain, phospholipids containing a truncated chain with a terminal carbonyl or hydroxyl group, or lysophospholipids (O'Donnel, 2011). The main consequence of these modifications is that oxidized lipids are more polar than the intact ones. Chemically, polyunsaturated phospholipids are more sensitive to oxidation, and their polar head group is very often phosphatidylcholine (PC), PC being the major phospholipid in mammalian membranes (Catala, 2012).

Effects of these oxidized lipids are multiple. For instance, they can be recognized by specific receptors which activate different signaling pathways leading to various cellular responses (Fruhwirth et al., 2007; Greig et al., 2012). In this context, it has been shown that lipid oxidation plays a role in inflammatory processes observed in various diseases, such as atherosclerosis, cardiovascular diseases or cancers (Greig et al., 2012; Fruhwirth et al., 2007; Koppaka and Axelsen, 2000). Oxidized lipids could also stimulate the formation of amyloid plaques, responsible for the Alzheimer disease (Koppaka and Axelsen, 2000; Volinsky and Kinnunen,

Abbreviations: π, surface pressure; A, mean molecular area; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; OPPC, 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine; PoxnoPC, 1-palmitoyl-2-(9′-oxo-nonanoyl)-sn-glycero-3-phosphocholine; PazePC, 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine; PC, phosphatidylcholine; PS, phosphatidylserine; ROS, reactive oxygen species; AFM, atomic force microscopy; LB, Langmuir–Blodgett.

* Corresponding author.
E-mail addresses: christine.grauby-heywang@u-bordeaux.fr (C. Grauby-Heywang), fabien.morote@u-bordeaux.fr (F. Moroté), marion.mathelie-guinlet@u-bordeaux.fr (M. Mathelié-Guinlet), ibtissem.gammoudi@u-bordeaux.fr (I. Gammoudi), rokhaya.faye@ihu-liryc.fr (N.R. Faye), touria.cohen-bouhacina@u-bordeaux.fr (T. Cohen-Bouhacina).
[1] Present address: Centre de Recherche Cardio-Thoracique de Bordeaux—INSERM U1045, IHU-LIRYC, PTIB—Hôpital Xavier Arnozan, Avenue du Haut Lévêque, 33604 Pessac, France.

http://dx.doi.org/10.1016/j.chemphyslip.2016.07.001
0009-3084/

2013). At last, they are involved in apoptosis, since the externalization of oxidized lipids is necessary for the clearance of apoptotic cells by macrophages (Fruhwirth et al., 2007; Volinsky and Kinnunen, 2013).

Concerning molecular mechanisms, lipids being essential components of cells, it is clear that their oxidation can induce strong modification in the cell functioning, oxidized lipids acting as second toxic messengers or inducing structural damages in membranes (Catala, 2012; Volinsky and Kinnunen, 2013). In particular, oxidized lipids can be locally concentrated in membranes, thus influencing their biophysical properties (Kinnunen et al., 2012; Volinsky and Kinnunen, 2013). In this context, a lot of studies have been performed on lipids from the mitochondrial membrane, since the main part of endogenous free radicals, acting as strong oxidants, is produced in mitochondria during aerobic respiration (Gabbita et al., 1998). It has been shown that the accumulation of oxidized lipids in microsomes induces an increase of the lipid acyl chain order (Eichenberger et al., 1982). In the same idea, the lipid oxidation induced by $FeSO_4$ on mitochondria decreases the membrane fluidity (Nepomuceno et al., 1997). On the contrary, Gabbita et al. observed an increase of the membrane fluidity after the oxidation of synaptosomes and mitochondrial membranes, this higher fluidity being assigned to the formation of gaps in the membranes (Gabbita et al., 1998). Disorder in the lipid packing in the presence of oxidized lipids was also reported in supported bilayers made with phospholipids from mitochondria incubated in ROS-generating conditions, even if all the oxidizing conditions are not similarly efficient (Megli and Sabatini, 2003). In particular, in the case of mitochondria exposed to CCl_4, known to increase the oxidative stress in a short term, the bilayer disorder is proportional to the oxidant amount and to the incubation time (Megli and Sabatini, 2004).

However, the analysis of these experiments is difficult, since the diversity of molecules produced by oxidation in an initial complex lipid mixture is wide and the nature of oxidized molecules is not always known in details. However, perfectly defined oxidized lipids are now available, leading to less ambiguous results. This is the case of 1-palmitoyl-2-(9′-oxo-nonanoyl)-sn-glycero-3-phosphocholine and 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine, PoxnoPC and PazePC, respectively, which are oxidized derivatives of 1-palmitoyl-2-oleoyl-phosphatidylcholine or POPC. At the cellular level, it has been shown that PoxnoPC is involved in apoptosis and necrosis processes (Uhlson et al., 2002), whereas PazePC behaves as a weak ligand peroxisome receptor (Davis et al., 2001). The behavior of these lipids or similar ones included in membrane models has been already studied by different experimental methods: electron paramagnetic resonance (Megli et al., 2005), surface pressure and surface dipole measurements coupled with fluorescence microscopy at the air–water interface (Sabatini et al., 2006), fluorescence energy transfer (Mattila et al., 2008), fluorescence correlation spectroscopy z-scan (Beranova et al., 2010; Parkkila et al., 2015), fluorescence solvent relaxation (Volinsky et al., 2011), scattering stopped flow experiments (Lis et al., 2011), differential scanning calorimetry and nuclear magnetic resonance (Wallgren et al., 2012; Wallgren et al., 2013) . . . Molecular simulations have been also successfully applied, giving precious complementary information, such as molecule orientation and conformation (Khandelia and Mouritsen, 2009; Beranova et al., 2010; Cwiklik and Jungwirth, 2010; Khandelia et al., 2014). On the whole, data show that oxidized lipids, in sufficient amount, can induce bilayer/micelle transitions (Megli et al., 2005), or the formation of defects and the increase of lipid flip-flop (Volinsky et al., 2011). On the other hand, oxidized lipids are for instance able to stabilize sphingomyelin/cholesterol domains in ternary PC/sphingomyelin/cholesterol mixtures (Volinsky et al., 2012; Parkkila et al., 2015). Moreover, the oxidized, and thus more polar, chain is able to reverse in a more or less "extended lipid conformation" (Khandelia and Mouritsen, 2009), in order to get closer to the interfacial region or even to point out in water. This particular orientation, described recently in a "lipid whisker model" (Catala, 2012) improving the "fluid mosaic model" of Singer and Nicolson (Singer and Nicolson, 1972), induces important changes in the membrane structure and dynamics (mean molecular areas, bilayer thickness, hydration profile, phase separation . . .), depending on the percentage of the oxidized lipid, the experimental conditions (pH, cations) and the nature of surrounding lipids.

In a previous work, we studied the natural oxidation by atmospheric oxygen of Langmuir–Blodgett (LB) films made of two monounsaturated lipids, POPC and OPPC (1-oleoyl-2-palmitoyl-phosphatidylcholine), using atomic force microscopy (AFM) (Faye et al., 2013). AFM is a high resolution scanning probe surface analysis technique, based on the measurement of interaction forces between a fine tip and the sample surface, these forces depending on the tip-sample distance. By scanning the surface it is possible to image it with lateral and perpendicular resolutions of 1 nm and 0.1 nm, respectively. To our knowledge, this powerful imaging technique has not been applied in the case of membrane systems including oxidized lipids, whereas it is perfectly adapted to the non-damaging study of lipid planar monolayers and bilayers (Garcia-Manyès et al., 2007; Garcia-Manyès and Sanz, 2010; Faye et al., 2013). Moreover, AFM presents an important advantage as compared to widespread fluorescence microscopy, since this high-resolution and non intrusive technique does not require the presence of a fluorophore, which can induce itself lipid oxidation (Ayuyan and Cohen, 2006).

AFM images of POPC and OPPC LB films showed that both films are sensitive to oxidation, even if this process is rather low and occurs after a few days. This process, which is not observed if samples are kept under vacuum, is responsible for the appearance of domains regularly distributed on the film surface and higher than the surrounding intact phase, suggesting that oxidation occurs locally, likely in areas presenting a local defect. We assumed that these domains result from the reversal of the oxidized chain, as previously described, leading to the raising of the whole molecule.

Unfortunaly, it was not possible in this first study to define the composition of LB films in terms of oxidized lipids, since this oxidation was not chemically controlled. Therefore, in the present work, we continue to explore the behavior of oxidized lipids in membrane models, by introducing PoxnoPC and PazePC, two known derivatives of POPC, in POPC monolayers. These lipids were added to POPC at a known amount (in the 4–20 mol% range). In a first step, surface pressure measurements enabled us to study the lateral packing of these lipid mixtures, whereas compressibility modulus gave information on the monolayer elasticity. As in the case of AFM, such methods have been relatively rarely applied to the study of oxidized lipids (Sabatini et al., 2006; Volinsky et al., 2012). In a second step, mixed monolayers were transferred on planar supports by the LB technique to be characterized by AFM. Our results show that PoxnoPC and PazePC behave differently in POPC monolayers in terms of mean molecular areas, PoxnoPC inducing an expansion of mixed monolayers, contrary to PazePC. Both oxidized lipids induce an increase of the monolayer elasticity. AFM images do not show any phase separation in mixed monolayers, but reveal the presence of thicker areas in LB films containing PazePC, likely due to the local formation of multilayer systems induced by compression. At last, these images are very different from those previously obtained in the case of POPC LB films submitted to atmospheric oxygen, which confirms that oxidation occurs locally and propagates by chain reaction in pure POPC LB films.