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Conformations of double-headed, triple-tailed phospholipid oxidation lipid products in model membranes

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ABSTRACT

Products of phospholipid oxidation can produce lipids with a carbonyl moiety at the end of a shortened lipid acyl tail, such as 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC). The carbonyl tail of POVPC can covalently bond to the free tertiary amine of a phosphatidylethanolamine lipid in a Schiff base reaction to form a conjugate lipid (SCH) with two head groups, and three acyl tails. We investigate the conformations and properties of this unique class of adduct lipids using molecular dynamics simulations, and show that their insertion into lipid bilayers of POPC increases the average cross-sectional area per lipid and decreases bilayer thickness. Significant increase in acyl tail fluidity is only observed at 25% SCH concentration. The SCH occupies a larger area per lipid than expected for a lipid with three acyl tails, owing to the interfacial location of the long spacer between the two head groups of the SCH. Schiff base formation of lipids can alter the concentration, homeostasis and localizations of phosphatidylserine and phosphatidylethanol lipids in membranes, and can therefore influence several membrane-associated processes including fusion and budding. The current work provides the first detailed structural model of this unique new class of lipids that may have important roles to play in modulating membrane properties and cell physiology.

1. Introduction

The polyunsaturated *sn*−2 fatty acyl chains of glycerophospholipids in lipoproteins and membranes are prone to modification by reactive oxygen species. Extensive oxidation leads to the formation of truncated phospholipids that still contain a long hydrophobic fatty acid in position *sn*−1 but have replaced the natural fatty acid in position *sn*−2 by a shorter tail with a hydrophilic terminal functional group. The ω-ends of the latter may consist of a variety of functional groups including aldehyde and carboxylic acid residues. Typical truncated phospholipids have been isolated from biological sources (*e.g.* oxidized lipoproteins, atherosclerotic plaques) and can meanwhile be prepared by chemical synthesis. The most widely investigated compounds are oxidation products of 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (PAPC) and 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC). PAPC generates 1-palmitoyl-2-valeroyl-phosphatidylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-phosphatidylcholine, whereas PLPC is a source of 1-palmitoyl-2-oxononanoyl-phosphatidylcholine (PoxnoPC) and 1-palmitoyl-2-azelaoyl-phosphatidylcholine (PazePC). The minor structural differences between the aldehydo (POVPC and PoxnoPC) and the respective homologous (PGPC and PazePC) glycerophospholipids lead to significant changes in biophysical and biochemical properties. *In silico* studies [1] on the molecular dynamics of oxidized PazePC and PoxnoPC have revealed that the fragmented acyl chains are expelled from the hydrophobic regions of the bilayer. Whereas the carboxyl residue of the former lipid protrudes into the aqueous phase, the less polar aldehyde function resides in the lipid–water interface of the bilayer (monolayer) [2–4]. As a consequence, the respective structural motifs can interact with both the molecules in the same supramolecular assembly as well as with (cell, lipoprotein) surfaces from outside [5]. The lipid conformations seem to be very important for the bioactivities of the respective compounds, since the biological effects of truncated phospholipids depend to a much larger extent on the fragmented acyl chains than on their polar head groups [6]. The carboxylate phospholipids can only physically interact with the biomolecules in their immediate vicinity. In contrast, phospholipid aldehydes can undergo chemical reactions with free amino groups of proteins and aminophospholipids (phosphatidylethanolamine and serine) (Fig. 1) by Schiff base formation [7]. Substantial evidence is already available from experiments *in vitro* and *in vivo* showing that protein modification by aldehydolipids can lead to a plethora of functional and biophysical consequences including association with membranes, aggregation, gain and loss of function [7,8]. 4-Hydroxynonenal is currently the most profoundly studied lipid oxidation product in this respect [9]. Less information is available about phospholipid aldehydes [7]. Kinnunen and colleagues provided evidence that modification of

Abbreviations: MD, molecular dynamics; SCH, Schiff base lipid; POVPC, 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; QM, quantum mechanics; SI, supplementary information

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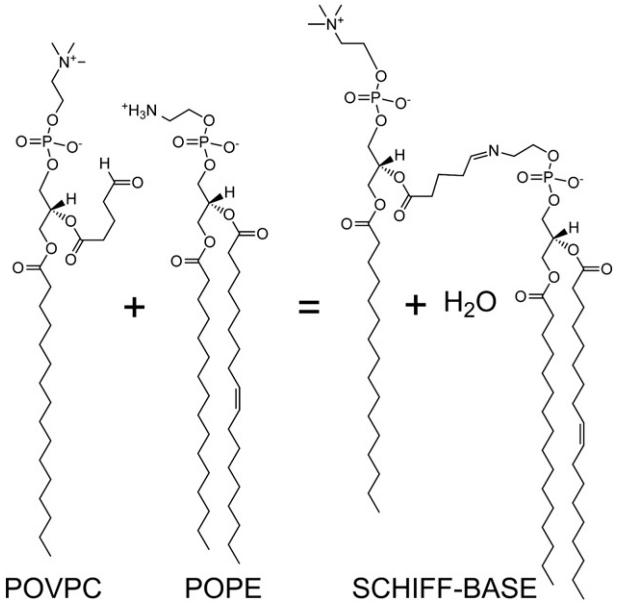

Fig. 1. The Schiff base condensation reaction between a POPE lipid and an oxidized POVPC lipid.

enzymes and antimicrobial peptides improves association with artificial membranes [10]. In a recent study, primary protein targets of a fluorescent POVPC have been identified in cultured RAW macrophages [11]. In order to verify their functional roles in the cells, these polypeptides are currently subject to biophysical (spatial lipid–protein proximity) and biochemical (gain and loss of function) studies in live cells.

Information about the (supra)molecular properties of Schiff bases formed between phospholipid aldehydes and aminophospholipids is still scarce for two reasons. First of all, such compounds have not been isolated yet from biological sources. They are labile and require stabilization before isolation, especially if the natural lipid content is low. Secondly, defined biophysical studies require chemically defined lipids. Efficient methods are still not available for synthesis, and thus, we are developing procedures for the preparation of such compounds. Therefore, *in silico* studies are for the moment the only way to get an idea of how such follow-up products of phospholipid oxidation might behave. If an aminophospholipid containing two hydrophobic acyl chains reacts with an oxidized phospholipid, an amphipathic compound with a large hydrophobic moiety and a less polar head group will be formed. This may lead to significant changes in membrane organization and changes in bimolecular (lipid–lipid and lipid–protein) interactions. Thus, it was the aim of this study to understand the behavior of such adducts in a phosphatidylcholine bilayer as well as the consequences of adduct formation on the supramolecular properties of the bilayer itself. Here we report on a molecular dynamics (MD) study on a model Schiff base generated from POPE and POVPC. We found that substantial amounts of this unusual lipid can be incorporated in a membrane. However, lipid packing and membrane thickness are significantly altered. These data are relevant not only to effects of oxidized phospholipids on membrane organization but also to functions of membrane proteins that critically depend on these parameters.

2. Materials and methods

Simulations of the Schiff-base lipid (SCH) in POPC bilayers were carried out with SCH:POPC ratios of 1:16, 1:8, 1:4 and 1:1. Below, the force-field derivation for the Schiff-base is described, followed by the simulation protocols.

2.1. Force field parameters

The modified Berger force field [12], with parameters adapted from http://moose.bio.ucalgary.ca/ was used for POPC and POPE. Water was represented by a single point charge (SPC) water model [13]. The parameters around the C═N bond were obtained using standard protocols on the parent compound shown in Fig. 2. In short, the structure was geometry optimized using Gaussian 03 [14] at the B3LYP/6-311++G** level of theory. The electrostatic potentials were sampled for each molecule at a number of points at the HF/6-31G* level of theory and the atomic partial charges were calculated using an electrostatic potential (ESP) fit. The optimized structures had their non-polar hydrogen atoms combined to the attached carbon atoms to create the united atom structures. The nitrogen atom was not protonated. The final partial charges are shown in Fig. 2. Atoms were assigned atom types from the Berger force field whenever possible.

2.2. System construction

The pure POPC bilayer contained 64 lipids in each leaflet. The bilayer had been pre-equilibrated and simulated for approximately 100 ns. The initial rectangular box sizes were 64.07 Å × 64.07 Å × 88.82 Å. Equal numbers of POPC lipids were replaced with SCHs from each leaflet. The initial conformation of the SCH molecules was such that the palmitoyl tail of the POVPC moiety pointed into the aqueous phase. The bilayer simulations will be referred to as BIL8, BIL16 and BIL32, with the trailing number corresponding to the number of SCHs in the bilayer. At higher concentrations of SCH (BIL32), the POVPC palmitoyl tails tended to aggregate in the aqueous phase, trapping the system in an intermediate state where SCHs aggregated in the lipid bilayer, and the POVPC palmitoyl tails could not insert into the lipid bilayer. For BIL32, therefore, simulations were implemented where the terminal methyl groups of the POVPC palmitoyl tails were pulled into the bilayer with a harmonic force for 50 ns. The pulling force constant was 500 kJ mol^{-1} nm^{-2}. This pulled the POVPC palmitoyl into the lipid bilayer. The forces were removed after 50 ns, and the system was simulated for at least another 1 μs. This simulation is referred to as PULL32.

The SCH carries one negative charge, and K^+ ions instead of Na^+ ions were used as counterions to avoid force-field related artifacts or bilayer condensation [15].

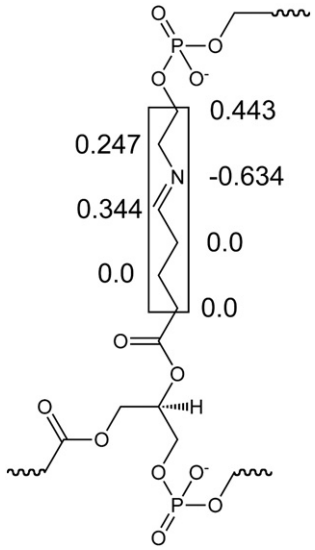

Fig. 2. The parent molecule used to develop force field parameters for the C═N bond, shown in the box. The final partial charges on united atoms are shown. The partial charges were adjusted slightly to sum up to reasonable charge groups. The nitrogen atom was not protonated.