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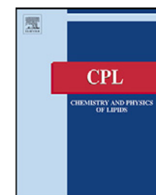

Closely related oxidized phospholipids differentially modulate the physicochemical properties of lipid particles

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ABSTRACT

Oxidation of glycerophospholipids results in the formation of large variety of oxidized phospholipid products that differs significantly in their chemical compositions and molecular structures. Biological activities of these oxidized products also differ considerably. Here we report the comparisons of the physicochemical properties of non-oxidized phospholipid particle containing two closely related tOx-PLs: 1-palmitoyl-2-(5-keto-6-octendioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC) and 1-palmitoyl-2-(9-keto-10-dodecendioyl)-*sn*-glycero-3-phosphocholine (KDdiA-PC). DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) was used as a model membrane non-oxidized phospholipid. Physicochemical properties of the lipid particles were characterized by using fluorescence spectroscopy, native polyacrylamide gel and agarose gel electrophoresis. Our result shows that the presence of closely related tOx-PLs, which differ only in the chemical composition of the oxidized fatty acyl chains at the *sn*-2 position, exerts considerably different effect on the physicochemical properties of non-oxidized phospholipid particles containing them.

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1. Introduction

In vivo oxidation of glycerophospholipids leads to addition of oxygen atom(s) to the unsaturated fatty acyl chain resulting in the generation of different type of oxidized products (Bochkov et al., 2010; Catalá, 2010). These oxidized products are involved in the variety of pathological and physiological functions (Catalá, 2009; Deigner and Hermetter, 2008; Fruhwirth et al., 2007; Fu and Birukov, 2009). Lipoprotein particles and cellular membranes are enriched in polyunsaturated fatty acids (PUFAs) containing phospholipids and PUFAs are considered as the main source for oxidized phospholipid generation. In mammalian phospholipids, saturated fatty acyl chain of either 16 or 18 carbons are present at the *sn*-1 position while the *sn*-2 position usually contains long carbon chain PUFAs (Fruhwirth et al., 2007; Fu and Birukov, 2009). Various oxidized products generated during phospholipid oxidation can be broadly classified into following categories: oxygenated phospholipids (Oxy-PLs), truncated oxidized phospholipids (tOx-PLs) and other low molecular weight oxidized species. In Oxy-PLs, the number of carbons in the oxidized fatty acyl chain is similar to that of the parent non-oxidized phospholipid molecules. Examples of Oxy-PLs includes 1-palmitoyl-2-(5,6-epoxyisoprostaneE2)-*sn*-glycero-3-phsphocholine (PEIPC), 1-palmitoyl-2-(5,6-epoxycyclopentenone)-*sn*-glycero-3-phsphocholine (PECPC) and isoketal-containing oxidized phospholipids (IsoketalPC). These Oxy-PLs are derived from the oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) and contains oxidized fatty acyl chain of 20 carbons at the *sn*-2 position. In tOx-PLs, the chain length of the oxidized fatty acid is shorter in length than the parent non-oxidized phospholipid molecules (Catalá, 2009; Deigner and Hermetter, 2008; Fruhwirth et al., 2007). Lipids which comes under these category includes 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), 1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (POVPC), KOdiA-PC, and 5-hydroxy-8-oxo-6-octenedioic acid ester of 2-lysophosphocholine (HOdiA-PC) (Catalá, 2009; Deigner and Hermetter, 2008; Fruhwirth et al., 2007). These lipids are also derived from the oxidation of PAPC and compared to PAPC these lipids contains truncated (shorter than 20 carbons) oxidized fatty acyl chain at the *sn*-2 position. Other products of phospholipid oxidation include low molecular weight oxidized species. Under this category comes 4-hydroxy-2-nonenal (4-HNE) and 4-hydroxy-2-hexenal (HHE), the main aldehydes formed during the peroxidation and fragmentation of $n-6$ and $n-3$ PUFAs, respectively, esterified at the *sn*-2 position of phospholipid

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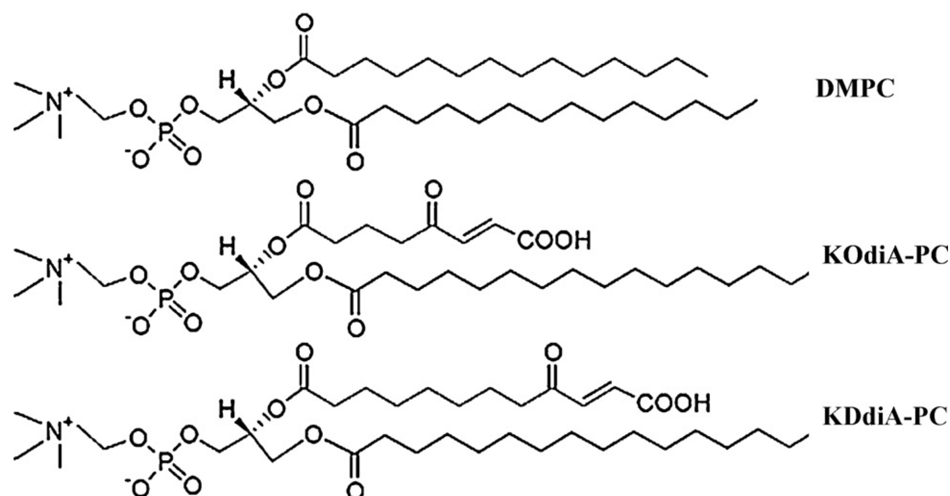

Fig. 1. The structures of the lipids used in the present study. DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; KOdiA-PC, 1-palmitoyl-2-(5-keto-6-octendioyl)-*sn*-glycero-3-phosphocholine; KDdiA-PC, 1-palmitoyl-2-(9-keto-10-dodecendioyl)-*sn*-glycero-3-phosphocholine.

molecules (Catalá, 2009). Thus the generation of different types of oxidized phospholipid products depends on the length of carbon chain and number and position of double bonds in the acyl chain of the phospholipid molecules.

Physicochemical properties of the phospholipid particles can be characterized by various methods. Due to the immediate response of fluorescence parameters of fluorescent probe to the properties of its microenvironment in the lipid particles, fluorescence spectroscopy has been routinely used to characterize the physicochemical properties of lipid particles (Reichardt, 1994). Various fluorescent probes are commercially available that possess unique chemical properties which enables them to reside in a particular location in the lipid particle and report the information of the microenvironment from that region (Reichardt, 1994). Similarly, native polyacrylamide gel electrophoresis (native PAGE) and agarose gel electrophoresis are powerful electrophoretic techniques that can be used to determine not only the homogeneity of the lipid and lipoprotein particles but also to characterize the size and surface properties of the lipid aggregates (Pérusse et al., 2001; Litman, 1973; Sparks and Phillips, 1992; Sparks et al., 1992; Pande et al., 2010).

Various reports indicate that the biological activities of even closely related tOx-PLs differs considerably (Fruhwirth et al., 2007; Fu and Birukov, 2009; Johnstone et al., 2009). However, whether these closely related tOx-PLs also exert different effect on the physicochemical properties of non-oxidized phospholipid particles containing them has not been studied in detail. In this study we report the comparison of the physicochemical properties of particles made up of model membrane lipid DMPC and containing closely related tOx-PLs. KOdiA-PC and KDdiA-PC, two tOx-PLs used in this study, are the oxidation products of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC) and 1-palmitoyl-2-arachidonolyl-*sn*-glycero-3-phosphocholine (PAPC) containing PL, respectively (Deigner and Hermetter, 2008). These tOx-PLs plays important role in many pathologies and increased levels of these two tOx-PLs have been reported in the lipoprotein particles and in the atherosclerotic lesions (Bochkov et al., 2010). These two tOx-PLs carries same polar functional group at the end of oxidized *sn*-2 fatty acyl chain (γ-oxygenated-α,β-unsaturated carboxyl group) but differ only in the chain length of *sn*-2 fatty acyl chain: KOdiA-PC and KDdiA-PC contains 8 and 12 carbons chain, respectively (Fig. 1). In this report we have compared the physicochemical properties of lipid particle containing these tOx-PLs. Our results show that because of the difference in the chemical compositions of the oxidized fatty acyl chain esterified at the *sn*-2 position, these two closely related tOx-PLs exert significantly different effect on the physicochemical properties of non-oxidized phospholipid particles containing them and the effect is dependent on the concentration of particular tOx-PL present.

2. Materials and methods

2.1. Materials

KOdiA-PC and KDdiA-PC were purchased from Cayman Chemical (Ann Arbor, MI). Agarose, chloroform, EDTA, ethanol, sodium chloride, DMPC, 1,6-diphenylhexatriene (DPH) and 9-diethylamino-5H-benzo[α]phenoxazine-5-one (Nile Red) were purchased from Sigma–Aldrich, Bangalore, India. *N*-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (F-DHPE) and 4-(2-(6-(dioctylamino)-(2-naphthalenyl)-(ethenyl)-1-(3-sulfopropyl)-pyridinium inner salt (di-8-ANEPPS) were obtained from Molecular Probes (Invitrogen, Bangalore, India). NBD-DMPG was purchased from Avanti Polar Lipids (Alabaster, AL). Precasted tris–glycine gel (4–20%), tris–glycine native running buffer, tris–glycine native sample buffer and NativeMark unstained protein standards were purchased from Invitrogen (Bangalore, India). All other reagents used were of analytical grade. Buffer used was prepared in double distilled deionized water.

2.2. Phospholipid particles preparation

Stock solutions of the fluorescent dyes were made in the following solvents: Nile Red in acetone, F-DHPE and di-8-ANEPPS in ethanol and DPH in dimethyl formamide. Appropriate amount of lipid stock solutions in chloroform were mixed to obtain desired lipid molar ratio. The solvent was then removed under nitrogen followed by desiccation for ~3 h under high vacuum. The dry lipid film was then hydrated by adding appropriate amount of aqueous buffer containing 20 mM HEPES, 150 mM NaCl, 1 mM EDTA (pH 7.4) and vortexing the tubes at room temperature to form multilamellar vesicles (MLVs). Appropriate amount of fluorescent dye was then added from the respective stocks to attain the dye/lipid molar ratio of 1/200. NBD-DMPG (0.1 mol%) labeled phospholipid particles, used in the electrophoresis, were prepared by adding an

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