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Editorial Article Open Access

The Need for Commercially Available Defined Oxidized (Phospho)lipids

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Editorial

Phospholipids are essential components of cell membranes and organelles. Furthermore, they are present in body fluids, particularly in the blood, where lipids are made "water-soluble" in the form of lipoproteins to enable their transport in an aqueous environment [1]. The ubiquitous occurrence of lipids led to the development of different analytical "lipidomics" techniques, whereby the majority of these methods are based on mass spectrometry (MS) [2]. Although there was an enormous progress in this field, lipid analysis is still considered to be challenging due to the extreme structural variability of lipids which leads to the appearance of hundreds of different lipid species in a typical biological sample [3]. This is due to (a) the different headgroups such as phosphorylcholine or -ethanolamine, (b) the different fatty acyl residues ranging from saturated residues such as myristic acid (14:0) up to highly unsaturated residues such as docosahexaenoic acid (22:6) and the linkage types (acyl-acyl-, alkyl-acyl-, and alkenyl-acyl) [4]. All these structural aspects cannot be assessed in a single MS experiment using the *m/z* ratios only. Either LC separation prior to MS, sophisticated MS/MS techniques and/or additional methods such as ion mobility spectroscopy is mandatory [5]. It must also be emphasized that quantitative data can only be (if at all) obtained when suitable lipid standards are used: one stable-isotope-labelled (deuterated or ^{13}C-labelled) standard per lipid class is normally needed [6]. These standards are also useful to correct losses of dedicated lipid classes upon the extraction process which is necessary for the enrichment of lipids as well as the removal of salts and other contaminations. Although such isotope-labelled standards are quite expensive, they are nowadays commercially available from many companies. Unfortunately, the situation is much more difficult when oxidized lipids are of interest.

Why are Oxidized Phospholipids of Interest?

From a very general point of view, oxidation plays a major role because the earth's atmosphere contains nearly 20% molecular oxygen [7]. Oxygen is an electronegative, reactive chemical element, which reacts with many biological substrates. It oxidizes, for instance, sulfhydryl groups (the functional group of the amino acid cysteine) into sulfones and, finally, sulfonic acids [7]. Regarding lipids, double bonds in the fatty acyl residues are of particular relevance [8]. They react with oxygen under generation of different oxidation products such as hydroperoxides and peroxides. Chain truncations under generation of aldehydes and carboxylic acids may also occur. These processes cause the rancidity of nutritional fat and vegetable oils and it is assumed that worldwide several megatons of food are destroyed by unwanted lipid oxidation every year [9]. A survey of selected lipid oxidation products is shown in Figure 1 although not all the relevant products are illustrated [10].

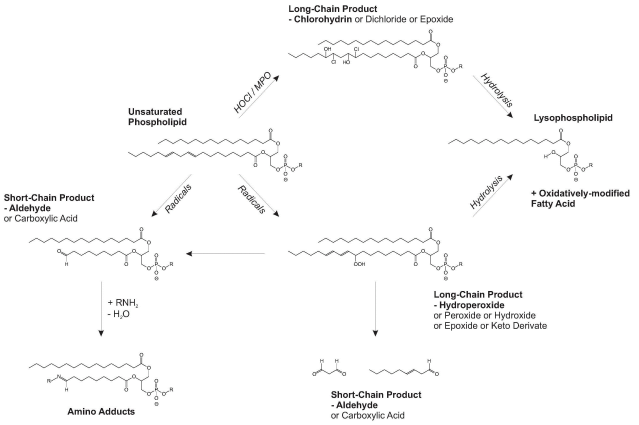

Figure 1: Scheme of potential reaction products from an unsaturated phospholipid (PL) under inflammatory *in vivo* conditions. Please note that "long-chain" products refer to compounds that have a higher mass than the native PL while "short-chain" products have a smaller mass. Although lysophospholipids (LPL) might also be considered as "short-chain" products they are discussed as a distinct lipid class here. This scheme should be regarded as a very crude survey. Not all physiologically relevant compounds are actually listed. Reprinted with permission from Fuchs et al. [8].

In comparison to air oxidation, the situation gets even more complex considering oxidation reactions under *in vivo* conditions. Even though life of higher animals is not possible without oxygen, an excess of oxygen is harmful and is known to promote, for instance, the development of cancer [7]. Oxidation unequivocally also plays an important role in inflammation: the invasion of organisms such as bacteria into the human body leads to an activation of the immune system. Inflammatory cells such as macrophages and granulocytes appear at the site of inflammation. Besides the release of bactericidal proteases, these cells are also capable of generating reactive oxygen species (ROS) being more reactive than common molecular oxygen. This is schematically illustrated in Figure 2: Oxygen (O_2) is first converted into a superoxide anion radical ($O_2^{\bullet-}$), which dismutase either spontaneously or particularly in the presence of the enzyme superoxide dismutase into hydrogen peroxide (H_2O_2), which is the educt for other more reactive compounds such as hypochlorous acid, singlet oxygen and peroxynitrite [11]. Additionally, H_2O_2 may be converted into hydroxyl radicals ($HO^{\bullet}$) by "Fenton Chemistry", i.e. in

the presence of redox-active transition metals like Fe^{2+} or Cu^{+}. $HO^{\bullet}$ radicals are the strongest oxidizing species known so far. Although inflammation is a natural and beneficial process, excessive inflammation is harmful to the organism and may lead to the destruction of tissues such as the cartilage layer of joints [12]. It is now commonly accepted that well-known diseases like cancer, arthritis, atherosclerosis and many others are accompanied by inflammation and the excessive production of ROS [13].

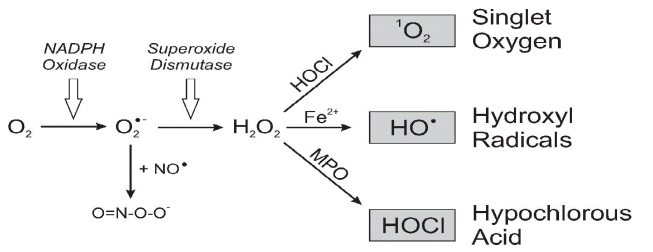

Figure 2: Generation of selected ROS under *in vivo* (particularly inflammatory) conditions. Please note that this is a very simplified summary of potential chemical reactions whereby the most relevant reactive species are set into shaded boxes. Reactive nitrogen species (RNS) and peroxynitrite are not at all considered in this scheme. The enzyme myeloperoxidase is abbreviated by MPO.

Although proteins are more susceptible to oxidation due to their sulfhydryl and amino groups, lipids are also interesting ROS targets because of their high concentration in biological systems. This finally leads to the products shown in Figure 1.

Therefore, it is not surprising that the mass spectrometric identification and quantification of oxidized lipids plays an increasingly important role. However, analysis of oxidized lipids is more challenging compared to "normal" lipids for the following reasons:

1. Usually oxidized lipids are only present in much smaller amounts than the normal bulk lipids such as phosphatidylcholines. Therefore, LC separation and/or mass spectrometers with significant dynamic ranges are needed.

2. Some oxidation products (such as hydroperoxides, cf. Figure 1) are transient products and decay into other products [14]. This particularly applies for *in vivo* conditions where reactions with other biomolecules may further decrease the lifetime of such products. For instance, selected lipid oxidation products, such as aldehydes, possess reactive functional groups that may react with amino groups of abundant proteins (cf. Figure 1).

3. Quantitative lipidomics studies require the addition of suitable standards [6]. Unfortunately, the availability of oxidized lipid standards is so far extremely limited.

The last point is, in our opinion, the most crucial one: to the authors best knowledge only a handful of standards is available so far, namely 1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine(POVPC) 1-palmitoyl-2- (9'-oxo-nonanoyl) -sn-glycero-3- phosphocholine, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine, 1-hexadecyl-2-azelaoyl-sn-glycero-3-phosphocholine and 1- palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (cf. Figure 3) are available from the AVANTI Polar Lipids company. Even if this is a very limited compound list, these standards are extremely useful products because they are chemically pure.

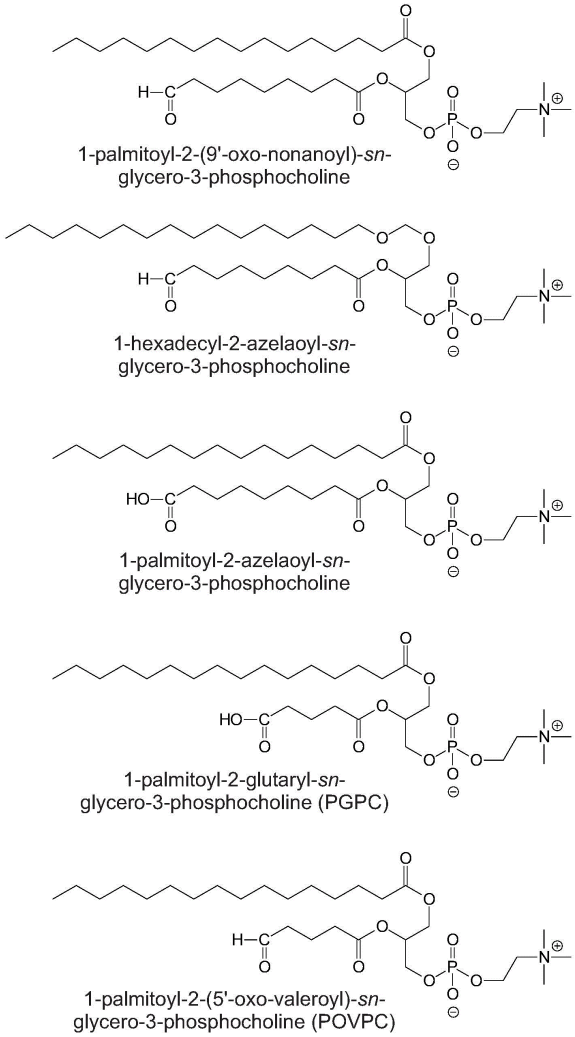

Figure 3: Survey of the so far (to our best knowledge) commercially available, purified lipid oxidation products.

Compounds available from other companies, such as oxidized PAPC (oxPAPC), are as far as we know usually mixtures which is also correctly announced by the corresponding companies. This maybe one reason why different results are obtained by different research groups using identical compounds from the same companies. Many scientists working in this field are biologists and do often neither have the knowledge or the instrumental resources to evaluate the compositions of such complex lipid mixtures. So, there are often contradictory reports about the effects of oxidized lipids on different cell lines.

We conclude with two requests: first, we would like to encourage all suppliers of oxidized lipids to emphasize problems related to the

stability of their products. Second, all companies are advised to expand their portfolio of oxidized lipids because this is a really hot topic in the bio and health sciences. In particular, compounds labelled with stable isotopes such as ^{2}H or ^{13}C would be urgently required in order to perform reliable mass spectrometric studies of oxidized lipids in biological systems.

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References

1. Mu H, Porsgaard T (2005) The metabolism of structured triacylglycerols. Progress in Lipid Research 44: 430-448.
2. Kasuga K, Suga T, Mano N (2015) Bioanalytical insights into mediator lipidomics. J Pharm Biomed Anal 113: 151-162.
3. Han X, Gross RW (2005) Shotgun lipidomics: Electrospray ionization mass spectrometric analysis and quantitation of the cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev 24: 367-412.
4. Fuchs B, Süss R, Schiller J (2010) An update of MALDI-TOF mass spectrometry in lipid research. Prog Lipid Res 49: 450-475.
5. Harkewicz R, Dennis EA (2011) Applications of mass spectrometry to lipids and membranes. Annu Rev Biochem 80: 301-325.
6. Wang M, Wang C, Han X (2016) Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry - What, how and why? Mass Spectrom Rev, in press 9999: 1-22.
7. von Sonntag C, Schuchmann HP (1991) The elucidation of peroxyl radical reactions in aqueous solution with the help of radiation-chemical methods. Angew Chem Int Ed 30: 1229-1253.
8. Fuchs B, Bresler K, Schiller J (2011) Oxidative changes of lipids monitored by MALDI MS. Chem Phys Lipids 164: 782-795.
9. Sun YE, Wang WD, Chen HW, Li C (2011) Autoxidation of unsaturated lipids in food emulsion. Crit Rev Food Sci Nutr 51: 453-466.
10. Domingues MR, Reis A, Domingues P (2008) Mass spectrometry analysis of oxidized phospholipids. Chem Phys Lipids 156: 1-12.
11. Schiller J, Fuchs B, Arnhold J, Arnold K (2003) Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. Curr Med Chem 10: 2123-2145.
12. Fuchs B, Schiller J (2014) Glycosaminoglycan degradation by selected reactive oxygen species. Antioxid Redox Signal 21: 1044-1062.
13. Valko M, Jomova K, Rhodes CJ, Kuča K, Musílek K (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Arch Toxicol 90: 1-37.
14. Catalá A (2009) Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys Lipids 157: 1-11.