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Review

Oxidative changes of lipids monitored by MALDI MS

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ABSTRACT

Oxidation processes of lipids are of paramount interest from many viewpoints. For instance, oxidation processes are highly important under *in vivo* conditions because molecules with regulatory functions are generated by oxidation of lipids or free fatty acids. Additionally, many inflammatory diseases are accompanied by lipid oxidation and, therefore, oxidation products are also useful disease (bio)markers. Thus, there is also considerable interest in methods of (oxidized) lipid analysis.

Nowadays, soft ionization mass spectrometric (MS) methods are regularly used to study oxidative lipid modifications due to their high sensitivities and the extreme mass resolution. Although electrospray ionization (ESI) MS is so far most popular, applications of matrix-assisted laser desorption and ionization (MALDI) MS are increasing. This review aims to summarize the so far available data on MALDI analyses of oxidized lipids. In addition to model systems, special attention will be paid to the monitoring of oxidized lipids under *in vivo* conditions, particularly the oxidation of (human) lipoproteins. It is not the aim of this review to praise MALDI as the "best" method but to provide a critical survey of the advantages and drawbacks of this method.

Contents

Abbreviations: 9-AA, 9-Aminoacridine; amu, Atomar mass unit; AP, Atmospheric pressure; ATT, 6-Aza-2-Thiothymine; BHP, Tert.-butyl-hydroperoxide; CL, Cardiolipin; ClCCA, 4-Chloro-α-cyanocinnamic acid; CHCA, 4-Hydroxy-α-cyanocinnamic acid; DHB, 2,5-Dihydroxybenzoic acid; DNA, Deoxyribonucleic acid; EI, Electron ionization; ESI, Electrospray ionization; ESR, Electron spin resonance; FT, Fourier transform; GC, Gas chromatography; HNE, 4-Hydroxy-2-nonenal; HPLC, High performance liquid chromatography; HSA, Human serum albumine; IgG, Immunoglobulin G; IR, Infrared spectroscopy; LC, Liquid chromatography; LD, Laser desorption; LOX, Lipoxygenase; LP, Lipoprotein; LPC, Lysophosphatidylcholine; LPL, Lysophospholipid; MALDI, Matrix-assisted laser desorption and ionization; MPO, Myeloperoxidase; MS, Mass spectrometry; *m/z*, Mass over charge; NADPH, Nicotinamide adenine dinucleotide phosphate; NMR, Nuclear magnetic resonance; PA, Phosphatidic acid; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; pK, Logarithm of acid dissociation constant; PL, Phospholipid; ppm, Parts per million; PS, Phosphatidylserine; PSD, Post source decay; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; SOD, Superoxide dismutase; SPME, solid phase micro extraction; TAG, Triacylglycerol; TBARS, Thiobarbituric acid reactive substances; TFA, Trifluoroacetic acid; THAP, 2,4,6-Trihydroxyacetophenone; (HP)TLC, (High performance) thin-layer chromatography; TOF, Time-of-flight; UV, Ultraviolet.

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1. Relevance of oxidation processes of lipids

Oxygen constitutes about 20% of the gases of the atmosphere and aerobic organisms have adapted to this highly reactive gas and invented effective protective mechanisms during their evolution (von Sonntag and Schuchmann, 1991). Without oxygen there is no (at least not human) life. However, oxygen must be regarded as a two-faced Janus and exhibits positive as well as negative effects. Humans surely need oxygen to be able to live, but if air is replaced by pure (100%) oxygen, O_2 gets strongly toxic: animals used for experimental purposes die after a few days in a pure oxygen atmosphere as the result of excessive oxidation reactions, which cannot be compensated by the organism. It has also been shown, that the tumor incidence increases if the oxygen partial pressure in the air exceeds only slightly the standard value (Forman and Fisher, 1985).

As molecular oxygen is converted in the human body into more reactive species – commonly termed "reactive oxygen species" (ROS) – nearly all physiologically relevant molecules may be regarded to be "oxidation-sensitive". We will not discuss here the well-known effects of ROS against proteins and DNA but will focus exclusively on oxidation processes of lipids and particularly phospholipids (PL). Although a more comprehensive discussion is beyond the scope of this review, it has to be emphasized, that nowadays many diseases such as atherosclerosis (Yoshida and Kisugi, 2010) or cancer (Ziech et al., 2010). Virtually all inflammatory diseases are associated with oxidation processes.

Due to their abundance and their ubiquitous occurrence, oxidative modifications of lipids are of paramount significance and this important field has been recently comprehensively reviewed (Frühwirth et al., 2007). It is also commonly accepted, that lipid oxidation products lead to the modification of proteins and thus modulate enzymatic activities. Many carbonyl compounds (e.g. aldehydes), that are generated as a consequence of lipid oxidation represent strong electrophiles and react with certain functional groups of proteins, e.g. the side chains of lysine, cysteine and histidine to form predominantly Schiff bases or Michael adducts (Sayre et al., 2006). These irreversible protein modifications make lipid-derived carbonyl compounds even more harmful than the primary ROS.

Nevertheless, the reader should be aware that lipid oxidation is not always an unwanted process. The oxidation of unsaturated fatty acids such as arachidonic acid is of high physiological relevance because molecules with regulatory functions, such as leukotrienes and thromboxanes are generated by this mechanism (Bégin, 1987). Due to the limited available space, we are not able to provide a detailed survey of lipid oxidations. However, we hope that we succeeded in making the importance of qualitative and quantitative analysis of lipid oxidation products evident.

1.1. Technological and commercial aspects

Beside carbohydrates and proteins, lipids – in particular triacylglycerols (TAG) from animal fats and vegetable oils – are the third important component of human nutrition. Lipids provide more energy per gram than proteins or carbohydrates. It is well known, that oils and fats are highly susceptible against oxidation and "rancidity" (unspecific oxidation of lipid-rich food) is an enormous problem in food sciences (Sun et al., 2011). Huge food amounts are destroyed each day by these processes although food is normally supplemented with antioxidants (vitamins) that (at least partially) help to overcome this problem. Food destruction is a particular problem in poor countries where cooling of food is often not possible. In order to prevent such unwanted reactions, methods for the determination of the contents of oxidized lipids within the food are urgently required.

1.2. Important oxidants

Although a lot of different oxidants are used in laboratory chemistry, we will discuss here exclusively compounds that are of relevance in biological systems. Therefore, oxidants such as organic per acids, $K_2Cr_2O_7$ or $KMnO_4$ will not be discussed in this review. Nevertheless, the reader should note that these chemicals are even nowadays very important if the position of a double bond within a certain fatty acyl residue of a lipid is of interest (Fuchs and Schiller, 2009a). Oxidants discussed in this review comprise primarily atmospheric oxygen (O_2), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and hydroxyl radicals ($HO^•$).

1.2.1. Atmospheric oxygen

Oxygen is the most abundant chemical element on earth due to its abundance in the atmosphere, water and metal oxides. Beside fluorine, oxygen exhibits the highest level of electronegativity and is, thus, among the most reactive elements. Nevertheless, common triplet oxygen ($^3O_2$) is still not reactive enough to induce significant oxidative damages to lipids. However, oxygen is the *in vivo* starting material of all further, more reactive species. A coarse survey of the generation of different ROS as well as some selected reactive nitrogen species (RNS) is shown in Fig. 1. Please note that we will focus here on oxidative processes mediated by neutrophilic granulocytes that are of particular relevance under inflammatory conditions. Therefore, some enzymes typical of granulocytes such as myeloperoxidase are also mentioned in Fig. 1.

1.2.2. Superoxide and hydrogen peroxide

There are enzymes available ("NADPH oxidases") that are capable of transferring an electron from NADPH to molecular oxygen