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Non-enzymatic lipid oxidation products in biological systems: Assessment of the metabolites from polyunsaturated fatty acids☆

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ABSTRACT

Metabolites of non-enzymatic lipid peroxidation of polyunsaturated fatty acids notably omega-3 and omega-6 fatty acids have become important biomarkers of lipid products. Especially the arachidonic acid-derived F_2-isoprostanes are the classic *in vivo* biomarker for oxidative stress in biological systems. In recent years other isoprostanes from eicosapentaenoic, docosahexaenoic, adrenic and α-linolenic acids have been evaluated, namely F_3-isoprostanes, F_4-neuroprostanes, F_2-dihomo-isoprostanes and F_1-phytoprostanes, respectively. These have been gaining interest as complementary specific biomarkers in human diseases. Refined extraction methods, robust analysis and elucidation of chemical structures have improved the sensitivity of detection in biological tissues and fluids. Previously the main reliable instrumentation for measurement was gas chromatography–mass spectrometry (GC–MS), but now the use of liquid chromatography–tandem mass spectrometry (LC–MS/MS) and immunological techniques is gaining much attention. In this review, the types of prostanoids generated from non-enzymatic lipid peroxidation of some important omega-3 and omega-6 fatty acids and biological samples that have been determined by GC–MS and LC–MS/MS are discussed.

Abbreviations: AA, arachidonic acid; AD, Alzheimer disease; AdA, adrenic acid; ALA, α-linolenic acid; AMPP, *N*-(4-aminomethylphenyl)pyridinium; APCI, atmospheric pressure chemical ionization; aSAH, aneurysmal subarachnoid hemorrhage; BHT, butylated hydroxytoluene; BSTFA, *N*,*O*-bis(trimethylsilyl)trifuoroacetamide; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; DIPEA, *N*,*N*′-diisopropylethylamine; DMF, dimethylformamide; DTPA, diethylene triamine pentaacetic acid; EBC, exhaled breath condensate; EFSA, European Food Safety Authority; EI, electron ionization; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; ESI, electrospray ionization; GC, gas chromatography; HOTMS, trimethylsilyl hydroxide (trimethysilanol); HPLC, high-pressure liquid chromatography; IAC, immunoaffinity chromatography; IsoPs, isoprostanes; LC, liquid chromatography; *m*/*z*, mass-to-charge ratio; MRM, multiple reaction monitoring; MS, mass spectrometry; NeuroPs, neuroprostanes; NICI, negative-ion chemical ionization; PFB, pentafluorobenzyl; PFB-MO-TMS, pentafluorobenzyl methyloxime trimethylsilyl; PFB-TMS, pentafluorobenzyl trimethylsilyl; PFBBr, pentafluorobenzyl bromide; PGs, prostaglandins; PhytoPs, phytoprostanes; PUFAs, polyunsaturated fatty acids; RIA, radioimmunoassay; ROS, reactive oxygen species; SIL-IS, stable-isotope labeled internal standard; SIM, selected-ion monitoring; SPE, solid-phase extraction; SRM, selected-reaction monitoring; TLC, thin layer chromatography; TMCS, trimethylchlorosilane; TMS, trimethylsilyl; TPP, triphenylphosphine; UPLC, ultra high-pressure liquid chromatography; UV, ultraviolet.

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1. Introduction

Free radicals have been implicated in a number of human diseases such as neurodegenerative, cardiovascular and pulmonary disorders and cancer [1]. Most common free radicals and non-radical species, known as reactive oxygen species (ROS), are able to modify oxidatively lipids, proteins and nucleic acids. Of the lipids in particular the polyunsaturated fatty acids (PUFAs) form a wide variety of oxidized products [2,3].

Among the oxidized lipid products generated, the measurement of isoprostanes (IsoPs) appears to be a promising assay for over two decades due to their specificity and sensitivity for *in vivo* assessment of oxidative stress and lipid peroxidation [4]. The majority of IsoPs are produced *in vivo* by non-enzymatic free-radical-induced peroxidation of PUFAs [5]. These compounds are formed *in situ* on membrane phospholipids and then released in their free form into circulation [6,7]. Depending on the parent PUFAs, different families of IsoPs have been discovered and quantified in several pathological conditions [2]. Among them, F_2-IsoPs are the most represented and extensively studied such that to be the designated "gold" marker by laboratories [8], and to be recently validated by the European Food Safety Authority (EFSA) as biomarkers for oxidative damage in cardiovascular health [9].

Various nomenclature guidelines have been proposed in the literature [10,11]. In this review, the nomenclature reported by Taber and Roberts [10], validated by the International Union of Pure and Applied Chemistry (IUPAC), will be adopted to describe different non-enzymatic oxidized lipid products of arachidonic, adrenic, eicosapentaenoic, docosahexaenoic, and α-linolenic acids. The state-of-the-art analysis of these products, namely F_2-IsoPs, F_3-isoprostanes (F_3-IsoPs), F_4-neuroprostanes (F_4-NeuroPs), F_2-dihomo-isoprostanes (F_2-dihomo-IsoPs) and F_1-phytoprostanes (PhytoPs), in biological samples using GC–MS, GC–MS/MS, LC–MS and LC–MS/MS is reviewed (Fig. 1).

2. Generation of lipid oxidation products

2.1. Metabolites of arachidonic acid

The biosynthesis of the majority of the IsoPs involves a free radical-induced process of arachidonic acid (AA) peroxidation initiated by the presence of radical species mainly centered on the oxygen such as the hydroxyl radical. Generation of prostaglandins (PGs) from free AA is cyclooxygenase dependent, whereas IsoPs made in phospholipid membranes are cyclooxygenase independent. IsoPs are released in their free forms by the platelet-activating factor acetylhydrolase and possibly by other phospholipases [12,13]. It was recently found that they circulate predominantly in high-density lipoproteins (HDL) [14] and then subsequently are metabolized and excreted in urine. It was reported that a significant proportion of F_2-IsoPs in urine are conjugated as glucuronides [15]. Each series of F_2-IsoPs contains eight possible isomers. As each regioisomer includes its racemic counterpart, a total of 64 different F_2-IsoPs can be generated; however, it is unknown and not investigated whether these numerous F_2-IsoPs generated would have similar properties. Among the F_2-IsoPs regioisomers, most human studies have focused on the 15-F_2-IsoP series, in particular 15-F_{2t}-IsoP, which is also known as 8-*iso*-$PGF_{2\alpha}$ and often used as an index for F_2-IsoPs. Nevertheless, Li and coworkers [16] discovered that 5-F_{2t}-IsoPs are present in urine at higher concentration than 15-F_{2t}-IsoPs.

The fundamental difference in the biosynthesis between PGs and IsoPs displays the specificity in the stereostructure of the metabolites. The non-specific initial radical hydrogen atom abstraction at one of the three possible bisallylic positions (7, 10 and 13) in AA is the first difference between the IsoPs synthesis and the PGs synthesis, where H-abstraction is regiospecific and occurs only at the 13 position. The subsequent pentadienyl radicals formed in the IsoPs synthesis react with molecular oxygen generating six different pentadienyl peroxyl radicals. Only four of them can produce the four different series of IsoPs compounds *via* a subsequent irreversible radical cascade (double 5-exo trig cyclization), followed by a final oxygenation leading to G_2-IsoPs. Consecutive hydroperoxide and endoperoxide reductions generate the specific F_2-IsoPs. The endoperoxide reduction can also be perturbed and following a Kornblum–DeLaMare rearrangement can give E- and D-IsoPs. Dehydration of membrane-bound E_2- and D_2-IsoPs is facile under physiological conditions [17] and produces cyclopentenone A_2- and J_2-IsoPs, respectively [18]. The particular *cis*-orientation of the side chains in IsoPs in contrast to *trans*-orientation in PGs reflects another crucial specificity, linked to the localization of the radical process. For IsoPs biosynthesis, the membrane is the place of action, and therefore conventional chemistry rules apply (lower transition state energy during the double 5-exo-trig cyclization) to generate the 1,2-*cis*-orientations of the side chains compared to enzymatically driven three-dimensional *trans*-orientation of the side chains obtained with PGs synthesis. Furthermore, two stereochemistries – the all-*syn* (represented as subscript "c"; see 5-F_{2c}-IsoP) and *syn*-anti-*syn* stereochemistry (represented as subscript "t"; see 15-F_{2t}-IsoP) – are present in IsoPs synthesis, and they are well explained by the Beckwing-Houk model of the lower transition states possible during a radical cyclization process; chair- and boat-like transition states are shown in Scheme 1.

The number of theoretical regioisomers further complicates the complexity of IsoPs biosynthesis; the four F_2-IsoPs regioisomers having each 8 diastereoisomers could generate 64 racemic compounds. It was shown that the 5- and 15-series IsoPs are the most abundant *in vivo*, likely due to the fact that the 8- and 12-series IsoPs are more readily oxidized further [19–22].

2.2. Metabolites of adrenic acid

Adrenic acid (AdA) is the elongated form of arachidonic acid (AA). Being a two-carbon analog of AA, it will also provide four series of dihomo-isoprostanes (dihomo-IsoPs), the 7- and 17-series being the major metabolites [23]. Similarly to IsoPs, 64 racemic F_2-dihomo-IsoPs can theoretically be found *in vivo*, and so far only the F-series was investigated in biological and analytical studies [23–25].

2.3. Metabolites of eicosapentaenoic acid

Eicosapentaenoic acid (EPA) was also found to provide 6 series of F_3-IsoPs, the 5- and 18-isomers being the most abundant *in vivo* [26–28]. Theoretically, 96 racemic isomers of F_3-IsoPs can be observed and quantified as a total *in vivo*. F_3-IsoPs are not the most studied isoprostanoids, probably because EPA is less abundant than AA in human tissues. However, Rokach and coworkers [27,29] have shown that 5-F_3-IsoP can be quantified in urine and may represent a β-oxidized metabolite of 7-F_4-NeuroP from DHA [27,29,30].

2.4. Metabolites of docosahexaenoic acid

The peroxidation of docosahexaenoic acid (DHA) follows a similar process as described for IsoPs. A total of 8 possible regioisomers identified as 4-, 7-, 10-, 11-, 13-, 14-, 17- and 20-series NeuroPs, and a total of 128 theoretical compounds could be generated. Among these 4- and 20-series represent the two most abundantly found NeuroP isomers *in vivo* [31].

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