

Review

Generation and Detection of Levuglandins and Isolevuglandins *In Vitro* and *In Vivo*

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Abstract: Levuglandins (LGs) and isolevuglandins (isoLGs), formed by rearrangement of endoperoxide intermediates generated through the cyclooxygenase and free radical induced oxidation of polyunsaturated fatty acids (PUFAs), are extraordinarily reactive, forming covalent adducts incorporating protein lysyl ϵ -amino groups. Because they accumulate, these adducts provide a dosimeter of oxidative injury. This review provides an updated and comprehensive overview of the generation of LG/isoLG *in vitro* and *in vivo* and the detection methods for the adducts of LG/isoLG and biological molecules *in vivo*.

Keywords: levuglandin; polyunsaturated fatty acids; oxidative injury

1. Introduction

Lipids are essential cell membrane components. They incorporate abundant polyunsaturated fatty acids (PUFAs) that are particularly susceptible to oxidative damage. In view of the ready oxidizability of PUFAs and the aerobic environment we live in, it is understandable that lipid oxidation plays a key role in human health. Increasing substantial evidence suggests that lipid oxidation is involved in the

development of many chronic diseases. This has stimulated worldwide efforts to elucidate the mechanisms and pathological consequences of lipid oxidation [1-3].

Levuglandins (LGs) and isolevuglandins (isoLGs)—also referred to as the “isoketals” [4]—are γ -ketoaldehydes that are formed by rearrangement of endoperoxide intermediates generated through the cyclooxygenase and free radical-induced oxidation of arachidonates. The γ -ketoaldehyde functionality of the LGs and isoLGs makes them extraordinarily reactive towards primary amino groups in biomolecules. LGs and isoLGs react with the ϵ -amino groups of lysyl residues in proteins to produce covalent adducts with greater avidity than most other lipid oxidation products, e.g., 4-hydroxynonenal (4-HNE) or malondialdehyde (MDA) [4]. This feature makes covalent LG/isoLG adducts attractive as biomarkers to evaluate oxidative injury in the tissues. LGs/isoLGs initially react with primary amino groups to form in seconds Schiff base adducts which are transformed to pyrrole adducts in minutes. However, these highly alkylated pyrroles are chemically sensitive compounds in the presence of oxygen [5] and are further oxidized in a few hours to stable end products, lactams and hydroxylactams (HLs) [6]. First detected *in vivo* by immunoassays, quantitative analysis of LG/isoLG protein adducts was also achieved by LC-MS after proteolysis to LG/isoLG-lysine derivatives that incorporate the lysine ϵ -amino group into LG/isoLG-derived lactams. Salomon *et al.* reported that isoLGE₂-protein [7], iso[4]LGE₂-protein [8] and iso[7]LGD₂-protein [9] adducts are generated upon oxidation of LDL *in vitro*. Formation of isoLG protein adducts *in vivo* has also been confirmed by a variety of immunological and mass spectrometric methods [10,11]. The levels of isoLG-protein adducts are elevated in diseases associated with oxidative injury [11].

This review will describe levuglandins and isolevuglandins with emphasis on their generation, protein modification and identification of the novel and stable compounds produced during the oxidation of PUFAs which could serve as unique indicators for PUFA-associated oxidative injuries, and provide valuable insights into the pathophysiology of diseases related to oxidative stress.

2. Generation of Levuglandins and Isolevuglandins

Arachidonic acid, a linear twenty carbon methylene interrupted polyunsaturated fatty acid (C₂₀:4 ω 6), is oxidatively transformed by two different enzymatic metabolic pathways catalyzed by cyclooxygenase and lipoxygenase, resulting in a large array of biologically active oxidized 20 carbon fatty acids, collectively called eicosanoids. Besides these enzymatic pathways, growing evidence has also suggested the existence of a free radical-induced metabolic pathway which further greatly expands the family of eicosanoids [9,12-16].

2.1. Enzymatic Pathways

Cyclooxygenase can catalyze a unique transformation, in which two molecules of oxygen are chemoselectively and stereospecifically added to a molecule of AA to produce prostaglandin endoperoxide G₂ (PGG₂). This PGG₂ is a highly reactive molecule which undergoes chemoselective reduction catalyzed by hydroperoxidase, to produce the unstable prostaglandin endoperoxide H₂ (PGH₂, $t_{1/2}$ = 5 min at 37 °C in aqueous solution) [17]. PGH₂ is a pivotal intermediate in regulating a wide variety of cellular activities and undergoes enzymatic and non enzymatic (solvent-induced) rearrangements to provide an array of physiologically active molecules. Lipoxygenases, on the other

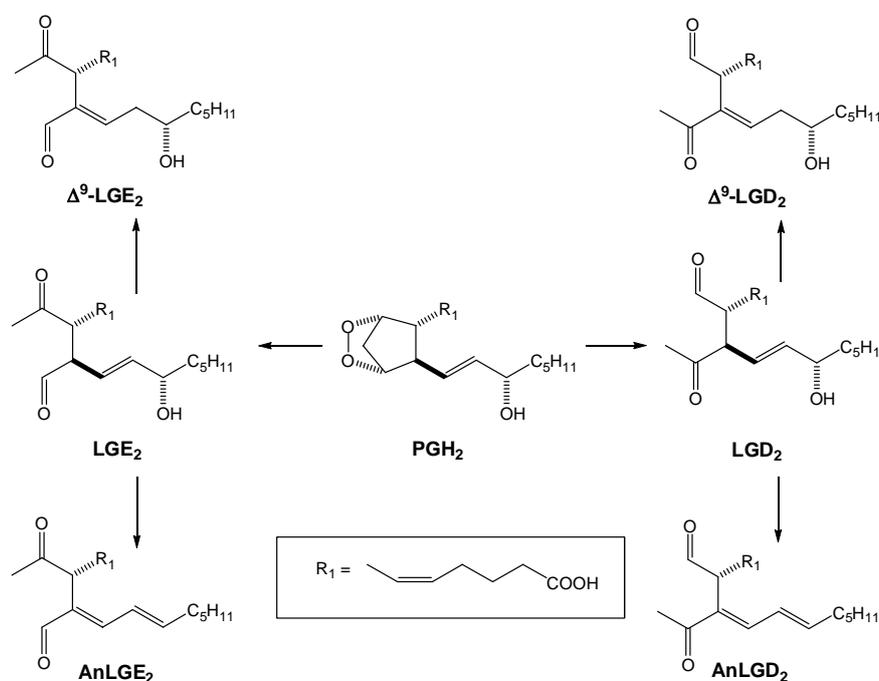
hand, can catalyze the conversion of polyunsaturated fatty acids (PUFAs) into the corresponding fatty acid hydroperoxides by insertion of a molecule of oxygen, for example, soybean lipoxygenase, can generate 13-hydroperoxyoctadecadienoate (13-HPODE) from linoleic acid (LA) and 15-hydroperoxy-eicosatetraenoate (15-HPETE) from AA.

The major products of the enzymatic rearrangement of PGH_2 are prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin and thromboxane A_2 (TXA_2). The solvent-induced nonenzymatic conversion also results in formation I_2 (PGI_2) from PGE_2 and PGD_2 . Reduction of PGH_2 results in prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) [18,19]. Enzyme-induced skeletal rearrangement of the strained bicyclic ring system of PGH_2 results in PGI_2 and TXA_2 . 12-Hydroxyheptadeca-5(*Z*),8(*E*),10(*E*)-trienoic acid (HHT) and malondialdehyde (MDA) are produced as a result of fragmentation of PGH_2 [20,21]. These molecules exert various physiological effects that are often complimentary to each other [22-27].

Salomon [28,29] discovered that a novel alternative rearrangement of PGH_2 also occurs that produces two γ -ketoaldehydes, which were named levuglandin E_2 (LGE_2) and levuglandin D_2 (LGD_2) because they are derivatives of levulinaldehyde with prostanoid side chains [7]. Detailed mechanistic studies revealed that levulinaldehyde was generated as a result of a 1,2-hydride shift during the cleavage of three bonds in a concerted fashion with a polarized transition state during the rearrangements of endoperoxide in aqueous solution [30,31].

Levuglandins are sensitive vinylogous β -hydroxy carbonyl compounds which readily undergo dehydration leading to their anhydro analogs AnLGE_2 and AnLGD_2 as shown in Scheme 1.

Scheme 1. Decomposition of PGH_2 : Generation of levuglandins, anhydrolevuglandins and Δ^9 -levuglandins.



The propensity of LGD_2 to undergo dehydration has precluded its isolation as well as total synthesis [29]. The C10-C11 double bond also migrates to form the more stable conjugated isomers, $\Delta^9\text{-LGE}_2$ and $\Delta^9\text{-LGD}_2$ [30]. Convergent asymmetric total syntheses of LGE_2 and AnLGD_2 were

developed that not only confirmed their structures but also provided adequate amounts of these complex AA metabolites for thorough chemical and biological studies [32,33].

2.2. Free Radical Pathways

Generation of stereo and structural isomers of PGs was firstly recognized in a study to quantify the biologically relevant PGs generated enzymatically through the action of COX during AA metabolism [34]. However, the biological significance of this pathway, now called the isoprostane pathway, was ignored for two decades.

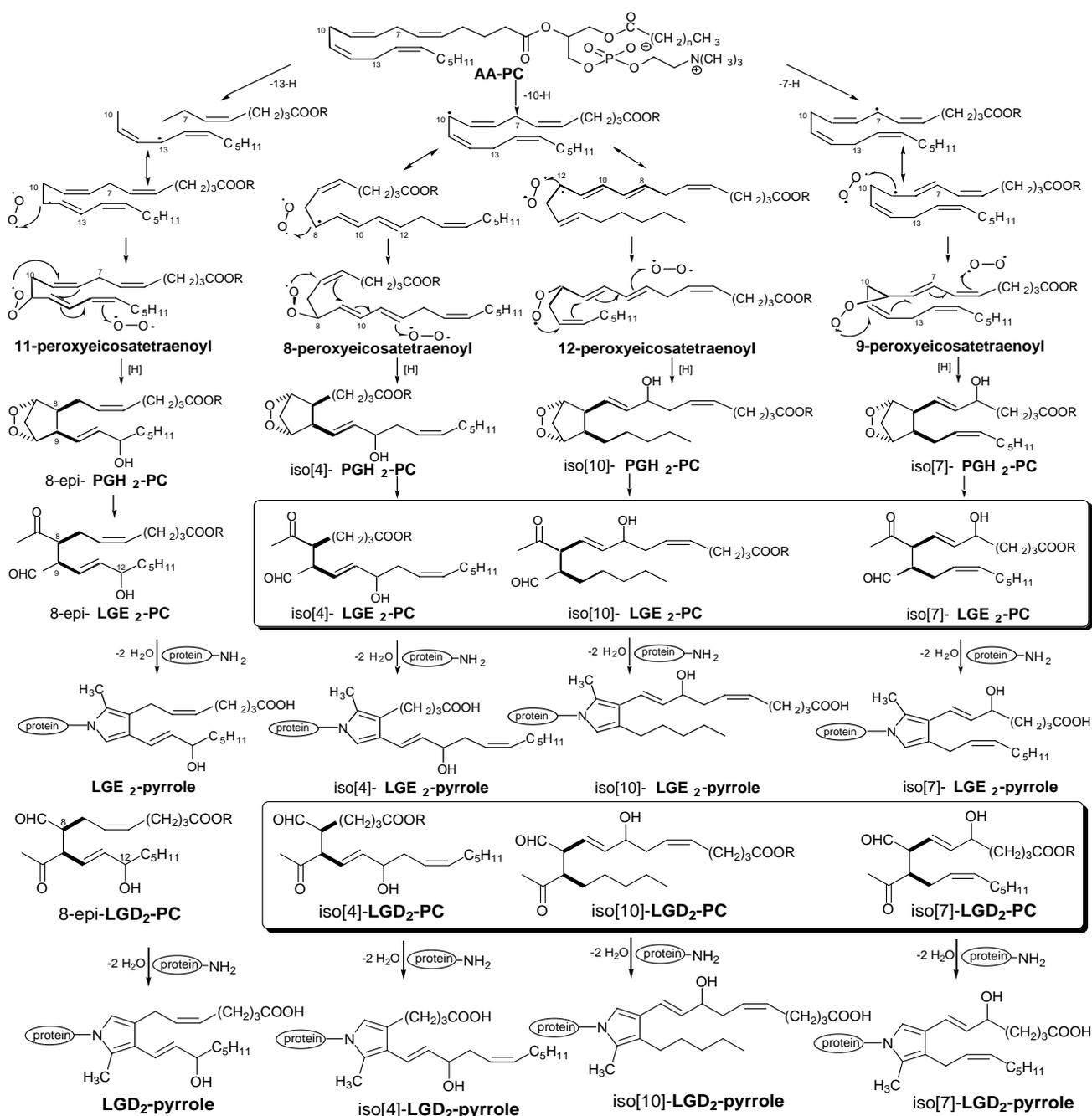
In the early 90s, researchers reported that an *in vivo* non enzymatic generation of PGH₂ isomers occurs in humans resulting in the generation of large amounts of PG isomers upon AA metabolism [12]. The *in vitro* formation of significant amounts of 8-*epi*-PGF_{2α} isomers as well as other isomers of PGs, named isoprostanooids, in aged human plasma, first prompted the rediscovery of this nonenzymatic pathway (isoprostane pathway). Further studies showed the *formation* of these isoprostanooids *in vivo* [13]. The generation of large amounts of 8-*epi*-PGF_{2α} and other isoprostanooids in the plasma of mice treated with free radical initiators like CCl₄ and diquat, further supported a free radical peroxidative mechanism [14]. The fact that the generation of these isoprostanooids could not be suppressed *in vivo*, even after application of cyclooxygenase inhibitors, strongly supports the alternative, free radical pathway [35]. The same researchers also reported that isoprostanes that are PGD₂- and PGE₂-like compounds (D₂/E₂-IsoPs) were found to be generated *in vivo* as phospholipid esters through non-enzymatic rearrangements of stereo and structural isomers of PGH₂ derivatives (isoprostane endoperoxides) that are produced through free radical-induced cyclooxygenation of arachidonyl phosphatidylcholine (AA-PC) [36].

The formation of LGs during nonenzymatic rearrangement of PGH₂ to PGs was discovered by Salomon *et al.* [37]. Based on these observations, they proposed that nonenzymatic *in vivo* generation of LG isomers from endoperoxides could occur during free radical-induced oxidation [7] (Scheme 2). The rearrangement of the 2-lysophosphatidylcholine (PC) ester 8-*epi*-PGH₂-PC delivers 8-*epi*-LGE₂-PC. The 8-*epi*-LGE₂-PC isomer produced in this way is expected to differ in configuration at carbon C8 from that produced by the rearrangement of PGH₂ generated by the cyclooxygenase pathway. It is also expected to be racemic. However, an immunochemical assay (*vide infra*) for protein-bound LG-derived pyrrole would not differentiate between cyclooxygenase-derived or nonenzymatically produced LG isomers as the stereogenic centers at both C8 and C9 in 8-*epi*-LGE₂-PC are destroyed during LGE₂-pyrrole formation.

Furthermore, because hydrogen atom abstraction readily occurs nonregioselectively at any doubly allylic methylene, the free radical pathway can not only produce a stereoisomer mixture of levulinaldehyde derivatives with PG side chains, *i.e.*, isoLGs, but also levulinaldehyde derivatives with non-prostanoid side chains, *i.e.*, iso[n]LGs. Non-regioselective hydrogen atom abstraction from the 7, 10, and 13-positions of an arachidonyl ester produces three regioisomeric pentadienyl radicals (Scheme 2). These then react with molecular oxygen to afford four regioisomeric peroxyeicosa-tetraenoyl radicals that undergo peroxyradical cyclization [38] to deliver four structurally isomeric endoperoxides. Each endoperoxide rearranges to form two LGs or iso[n]LGs, designated as E series if the acetyl substituent is nearer than the formyl substituent to the carboxyl group or as D series if the formyl is nearer than the acetyl to the carboxyl. For iso[n]LGs, the bracketed integers indicate the

length of the carboxylic side chain [7]. For example, hydrogen atom abstraction from the 10-position of AA-PC followed by cyclization of an intermediate 8- radical could lead to *iso*[4]PGH₂-PC and then *iso*[4]LGE₂-PC, where the number in brackets signifies the length of the carboxylic side chain appended to a common 2,3-dioxabicyclo[2.2.1] heptane or levulinialdehyde core.

Scheme 2. Generation of isolevuglandins and isolevuglandin-protein adducts during free radical oxidation of AA and AA-esters.



The terms “isoketal or IsoK” were used [39] as alternatives to the original *iso*LG nomenclature [7] “to distinguish them from levuglandins formed by rearrangement of the cyclooxygenase endoperoxide intermediate, PGH₂”. However, such a distinction is erroneous because the exact same levuglandin molecules, LGE₂ and LGD₂, are generated by both the cyclooxygenase and isoprostane pathways. The difference between the pathways is that stereo and structural isomers are cogenerated with LGE₂ and

LGD₂ in free radical-induced autoxidation of arachidonates. On the other hand, it is important to emphasize the difference between stereoisomers of levuglandins and structural isomers of levuglandins. In the nomenclature, this distinction is reflected in the dichotomy of isoLGs and iso[n]LGs. Thus, for example, LGE₂, one of the stereoisomers designated collectively as isoLGE₂, is a product of both the COX and isoprostane pathways whereas all stereoisomeric iso[4]LGE₂s are only produced through the isoprostane pathway. The alternative names, 15-E2-IsoK and 12-E2-IsoK, respectively, do not convey this fundamental difference.

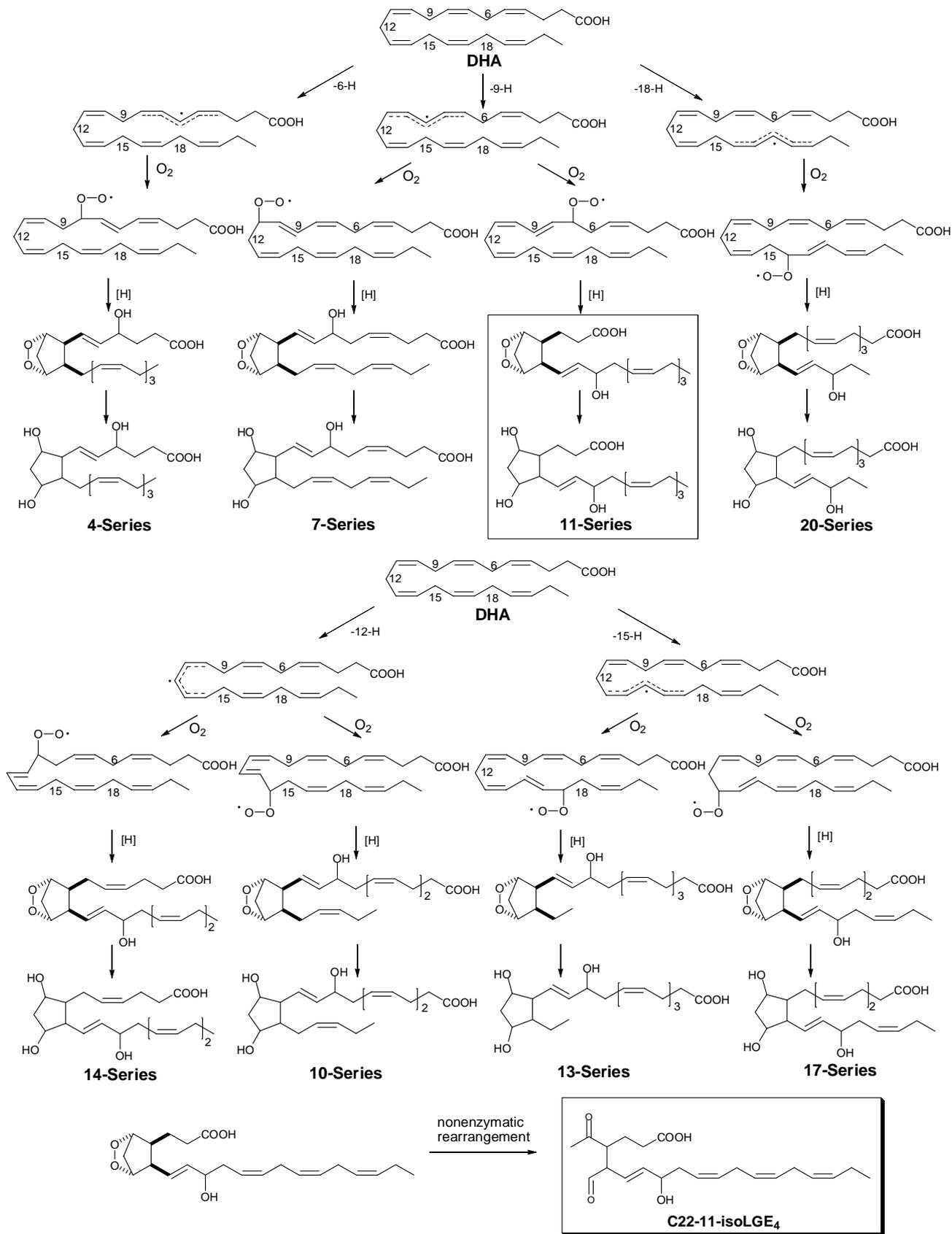
Studies reveal that myeloperoxidase (MPO) serves as an enzymatic catalyst for initiation of lipid peroxidation and lipoprotein oxidation *in vivo* [40]. MPO is an abundant heme protein secreted by phagocytes in response to stimulation [41]. MPO [42] and its distinct products [HOCl-damaged proteins [43] and 3-chlorotyrosine [44] are enriched in human atherosclerotic aortic intima and LDL recovered from atheroma. MPO uses H₂O₂ together with low molecular weight cosubstrates like chloride [45], tyrosine [46], and nitrite (NO₂⁻) [47,48] to generate a variety of reactive oxidants and diffusible radical species [49,50]. With the MPO-H₂O₂-NO₂⁻ catalytic system, Salomon group confirmed the *in vitro* generation of isoLGs, isoLGE₂, iso[4]LGE₂ and iso[7]LGE₂, during the free radical-induced peroxidation of AA-PC. These facts further confirmed the feasibility of the “isolevuglandin pathway” in nonenzymatic AA metabolism.

2.3. Free Radical Oxidation of Docosahexaenoic Acid (DHA)

Docosahexaenoic acid (C₂₂:6 ω 3) (DHA), a member of ω -3 PUFAs, is an essential requirement for the function and development of the human brain and retina [51]. While the content of DHA in human plasma LDL is low, the DHA content in brain and retina ranges from 20%–60% of total fatty acid content and is present esterified in phospholipids [52]. Although the exact role of DHA is not clear, deficiency of DHA is associated with the abnormalities in brain function [53]. Similar to other PUFAs, DHA is readily oxidized *in vitro* because of the presence of six homoallylic double bonds. Considering of the structural similarity between AA and DHA, analogue isoprostanes could be generated upon the free radical-induced peroxidation of DHA. Enhanced lipid oxidation has been reported as a consequence of oxidative injuries or decrease in antioxidant capacity in human brain and retina as measured using the simple thiobarbituric acid (TBA) test [54,55]. The free radical-induced peroxidation of DHA might play an important role in the pathogenesis of neurodegenerative diseases, e.g., Alzheimer's disease, Parkinson's disease, *etc.* [56-58].

Despite the complications involved, identification of DHA peroxidation products have been carried out by several research groups [59,60]. The quantification of such peroxidation products might provide a unique marker of oxidative injury in human brain and retina, and also a better understanding of their biological roles. In analogy to the free radical-induced mechanism that produces F₂-isoprostanes from arachidonic acid, F₄-C₂₂-isoprostanes, 22-carbon analogues of PGF_{2 α} , were generated during the *in vitro* nonenzymatic peroxidation of DHA [59]. The mechanism of the formation of F₄-C₂₂-isoprostanes (Scheme 3) involves generation of DHA radicals at doubly allylic methylene positions, addition of molecular oxygen, followed by the formation of bicyclic endoperoxide intermediates from the resulting peroxy radicals. The bicyclic endoperoxide intermediates are then reduced to form F₄-C₂₂-isoprostanes. It is known from previous studies [61] that such bicyclic endoperoxides can also readily undergo another kind of rearrangement to produce levuglandin-like products.

Scheme 3. F₄-C22-isoprostane and C22-isolevuglandin (11-isoLGE₄) generation from nonenzymatic peroxidation of docosahexaenoic acid (DHA).



3. Detection of Biological Adducts of Isolevuglandins

3.1. Immunological Detection of Protein Adducts of LGs and IsoLGs

Previous studies by Salomon *et al.* had shown that the γ -ketoaldehyde functional array in LGE₂ has an extraordinary proclivity towards rapid covalent adduction with proteins. For examples, human serum albumin binds 10 equivalents of LGE₂ within 1 min [62]. Consistent with their earlier result, Brame confirmed the rapid consumption of LGE₂ ($t_{1/2} = 20$ s) upon exposure to bovine serum albumin using mass spectrometry [63]. LGE₂ reacts with ϵ -lysyl amino groups of proteins to form Schiff base adducts [64]. Pyrrole derivatives are then generated upon rapid cyclization and dehydration [65]. Further oxidation delivers lactam and hydroxylactam stable end products [63]. Pyrrole and Schiff-base derivatives of phospholipids are generated by analogous reactions with LGE₂ stereoisomers with phosphatidyl ethanolamine [66].

Because LGs are rapidly sequestered by covalent adduction with proteins, Salomon *et al.* developed assays to detect their presence *in vivo* using antibodies that would recognize their protein adducts [67]. Existence of LGE₂-protein adducts *in vivo* was first detected in human cerebral vasculature using polyclonal rabbit antibodies [10]. However, they noticed in these studies that they could not distinguish between a cyclooxygenase formation or a possible alternative free radical-induced autoxidative biogenesis for these LGE₂-protein adducts *in vivo*. Thus, Salomon predicted that epitopes that would be recognized by LGE₂-protein adduct antibodies could be generated by free radical-induced autoxidation of arachidonyl phospholipids [68]. Experimental evidence supporting this hypothesis was first provided by the demonstration that LGE₂-protein adducts are generated upon free radical-induced autoxidation of low-density lipoprotein *in vitro* [7]. Thus, antibodies against LGE₂-protein adducts were useful tools for detecting products of the isoprostane pathway for *in vitro* systems that do not contain cyclooxygenase activity. The discovery of isolevuglandins (also referred to as isoketals) exploited rabbit polyclonal antibodies against LGE₂-protein adducts to detect the production of LGE₂ through free radical-induced oxidation of LDL *in vitro* [7]. Further evidence supporting the conclusion that these immunoreactive protein modifications were derived from LGE₂ stereoisomers (isoLGs) was then secured by mass spectroscopic analysis (*vide infra*) of the modified lysine excised from LDL protein through exhaustive proteolysis [63].

It is important to emphasize that distinguishing LGE₂ production through the two entirely different biochemical pathways, COX and isoprostane, is a redoubtable challenge. The LGE₂-protein adduct immunoreactivity detected in human cerebral vasculature was cited as an example of the localization of “isoK adducts” [69]. However, antibodies against LGE₂-protein adducts cannot distinguish the operation of a free radical-promoted isoLG (isoK) biogenesis from a COX mediated biosynthesis through the levuglandin pathway *in vivo* unless the COX activity has been inhibited.

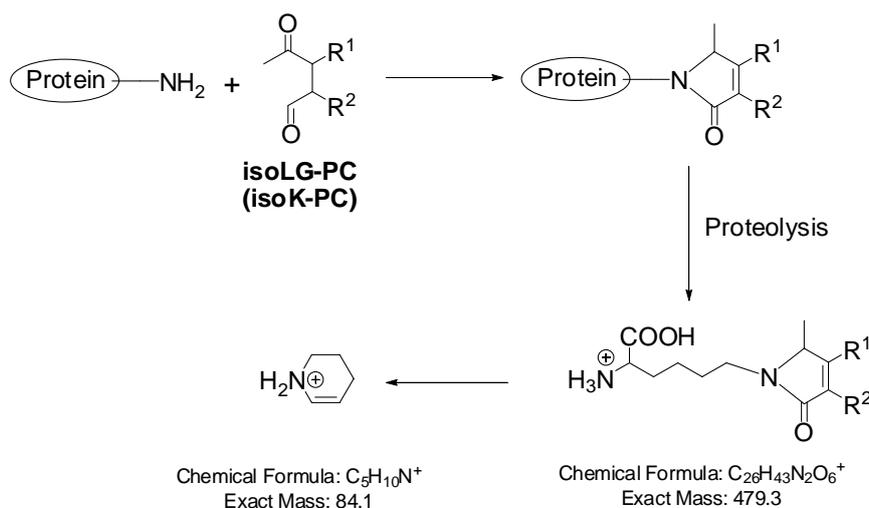
Structural isomers of LGE₂, designated collectively as iso[n]LGE₂s, are unique products of the free radical pathway (Scheme 2). Therefore, to provide a valuable tool for the unambiguous assessment isoLG production *in vivo*, antibodies against iso[4]LGE₂-protein adducts [8] were raised and used to demonstrate the presence of isolevuglandins in human blood [11]. Antibodies raised against iso[7]LGE₂-protein adducts further confirmed the operation of the isolevuglandin pathway *in vivo* [9]. The mean levels of the iso[4]LG and iso[7]LG adducts, as well as those detected with antibodies raised

against LGE₂-protein adducts [10], are elevated in plasma from individuals with atherosclerosis compared to individuals with no cardiovascular disease.

3.2. Mass Spectrometric Detection of Protein Adducts of LGs and IsoLGs

Mass spectrometry is increasingly being employed to identify the structures of biomolecules from *in vivo* samples, especially of proteins (proteomics), lipids (lipidomics) and metabolites (metabolomics). This is mostly owing to the discovery of soft ionization techniques, such as electrospray ionization (ESI) [70] and matrix-assisted laser desorption ionization (MALDI) [71,72] over 20 years ago. Also, the design of new more robust instruments and user-friendly software that allows multiple data processing and analysis have made mass spectrometry an accessible technique for the study of biomolecules in general. Briefly, organic mass spectrometry is an analytical technique which allows measuring the molecular weight and relative abundances of an analyte. In addition, generally by using tandem mass spectrometry, structural information of molecules can also be obtained. The most common ionization methods used in the analysis of phospholipids are electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). These soft ionization methods produce almost no fragmentation, and allow the ionization of non-volatile and thermolabile samples. Usually, this is an advantage, especially in the analysis of mixtures, but only information about the molecular weight of the compounds can be obtained. However, it is possible to induce the fragmentation of the sample ions, commonly by using collision induced dissociation (CID). In this technique called tandem mass spectrometry, MS/MS, fragmentation of a selected ion by collision with an inert gas is induced and the product ions are analyzed. High sensitivity analysis, usually in the fmol range, of complex sample mixtures and the capacity of coupling with separation techniques, namely liquid chromatography (LC) are other advantages of this technique. It is beyond the scope of this review to give a comprehensive outline of the mass spectrometry fundamentals. Encompassing this emergent trend, mass spectrometry is becoming increasingly important in phospholipid oxidation research. One of the main reasons for this, other than the increasing spread of mass spectrometers, is that interpreting mass spectra of oxidized phospholipids is usually straightforward [73,74].

LC-ESI/MS/MS analysis of the lysyl lactam adduct of LGE₂ shows a parent ion at m/z 479.3 and a daughter ion at m/z 84.1 that arises from the lysyl moiety [63]. All isoLG-derived lysyl lactams can deliver parent and daughter ions with these same exact masses (Scheme 4). LC-ESI/MS/MS analysis of the amino acids obtained by exhaustive proteolysis of isoLG-apoB adducts, generated upon oxidation of LDL *in vitro*, indicated a complex mixture of products exhibiting the m/z 479.3–84.1 transition as expected for a family of structurally isomeric isoLG-derived lysyl lactams. As mentioned above, this experiment confirmed their earlier finding, based on immunological evidence, that isolevuglandins are generated through free radical-induced oxidation of LDL *in vitro* [7]. The LC-MS technique was also applied to the quantitative analysis of lysyl lactams obtained by exhaustive proteolysis of plasma proteins [75]. The lactam derivatives of isoLGE₂ prepared from [³H₂, ¹³C₆]-lysine were used as an internal standard. This analytical method provides a convenient measure of total LG and isoLG protein adducts, but does not distinguish COX products, *i.e.*, levuglandins, from isolevuglandins (isoketals).

Scheme 4. MS/MS fragmentation of isolevuglandin-derived lysyl lactams.

The Schiff base adducts generated initially in the reaction of LGE2 with proteins can be stabilized by reduction with sodium borohydride. Subsequent exhaustive proteolysis gives reduced adducts of lysine that exhibit a dehydrated parent ion at m/z 467.3 [64]. MS/MS analysis of this ion produces a daughter ion with m/z 321.2 that probably is a protonated tetrahydrofuryl ion generated by intramolecular nucleophilic displacement of lysine.

All isoLG-derived reduced Schiff base adducts of lysine can give parent and daughter ions with these same exact masses. Recently, the LC-ESI/MS/MS technique was applied to the quantitative analysis of reduced Schiff base adducts obtained by exhaustive proteolysis of liver proteins from rats treated with CCl_4 , a model of oxidant injury to liver [75]. Schiff base adduct levels prior to base treatment were barely detectable. Apparently, more than 97% of the Schiff base adducts were incorporated in phospholipid esters. Because only free AA is a substrate for COX, these phospholipid Schiff base adducts are unique products of free radical-induced cyclooxygenation. The provenance of Schiff base adducts that are not incorporated in phospholipid esters must be determined independently because they can also be generated through the COX pathway [76].

A remarkable dichotomy between the Schiff base and lactam adducts was observed in these experiments. While the isoLG-derived Schiff base derivatives of proteins were present in the form of phospholipid esters, the lactam derivatives were present as free acids [75]. IsoLG-Schiff base adducts are intermediates that are converted into pyrroles and then lactams. Three possible alternative explanations for the preferential incorporation of Schiff base adducts into phospholipid esters are: (1) Schiff base esters are far superior substrates for esterases than lactam esters; (2) Cyclization of Schiff bases to give pyrroles is strongly disfavored by the membrane environment to which phospholipids are anchored and (3) Oxidation of pyrroles to give lactams is strongly disfavored by the membrane environment. In other words, the last two alternatives mean that: (1) Pyrrole formation from a Schiff base or (2) Pyrrole oxidation occurs much more readily for the free acid adducts than for the phospholipid adducts. It seems likely that investigation of this dichotomy will provide valuable insights into the unique environment present at the membrane/water interface.

Except for detection of protein adducts, Salomon *et al.* [77,78] report the detection *in vivo* and quantitative analysis of LG/isoLG adducts that incorporate the primary amino group of

phosphatidylethanolamines (PEs) into LG/isoLG-derived hydroxylactams (HLs) using HPLC-MS/MS. In pilot clinical studies, the levels of isoLG-PE-HL in plasma from Age-related Macular Degeneration (AMD) patient samples are elevated compared with those in plasma from control samples. And the levels of isoLG-PE-HL are significantly elevated in murine model of alcoholic liver disease.

4. Conclusions

In summary, the generation of levuglandin/isolevuglandin upon free radical-induced peroxidation of polyunsaturated fatty acid plays important roles in the pathology of diseases related to oxidative stress. The discoveries of adducts of LG/isoLG and biological molecules will provide clues about the biomarkers in the field of oxidative injuries.

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Sample Availability: Samples of the compounds are available from the authors.

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