Chemico-Biological Interactions 239 (2015) 87–99

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Angiotensin II modification by decomposition products of linoleic acid-derived lipid hydroperoxide

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ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 27 May 2015
Accepted 20 June 2015
Available online 22 June 2015

Keywords:
Lipid peroxidation
Linoleic acid hydroperoxide
Reactive aldehydes
Peptide modification
Angiotensin II
Mass spectrometry

ABSTRACT

Polyunsaturated fatty acids are highly susceptible to oxidation induced by reactive oxygen species and enzymes, leading to the formation of lipid hydroperoxides. The linoleic acid (LA)-derived hydroperoxide, 13-hydroperoxyoctadecadienoic acid (HPODE) undergoes homolytic decomposition to reactive aldehydes, 4-oxo-2(*E*)-nonenal (ONE), 4-hydroxy-2(*E*)-nonenal, *trans*-4,5-epoxy-2(*E*)-decenal (EDE), and 4-hydroperoxy-2(*E*)-nonenal (HPNE), which can covalently modify peptides and proteins. ONE and HNE have been shown to react with angiotensin (Ang) II (DRVYIHPF) and modify the N-terminus, Arg^2, and His^6. ONE-derived pyruvamide-Ang II (Ang P) alters the biological activities of Ang II considerably. The present study revealed that EDE and HPNE preferentially modified the N-terminus and His^6 of Ang II. In addition to the N-substituted pyrrole of [N-C_4H_2]-Ang II and Michael addition products of [His^6(EDE)]-Ang II, hydrated forms were detected as major products, suggesting considerable involvement of the vicinal dihydrodiol (formed by epoxide hydration) in EDE-derived protein modification in vivo. Substantial amounts of [N-(EDE – H_2O)]-Ang II isomers were also formed and their synthetic pathway might involve the tautomerization of a carbinolamine intermediate, followed by intramolecular cyclization and dehydration. The main HPNE-derived products were [His^6(HPNE)]-Ang II and [N-(HPNE – H_2O)]-Ang II. However, ONE, HNE, and malondialdehyde-derived modifications were dominant, because HPNE is a precursor of these aldehydes. A mixture of 13-HPODE and [$^{13}C_{18}$]-13-HPODE (1:1) was then used to determine the major modifications derived from LA peroxidation. The characteristic doublet (1:1) observed in the mass spectrum and the mass difference of the $[M + H]^+$ doublet aided the identification of Ang P (N-terminal α-ketoamide), [N-ONE]-Ang II (4-ketoamide), [Arg^2(ONE – H_2O)]-Ang II, [His^6(HNE)]-Ang II (Michael addition product), [N-C_4H_2]-Ang II (EDE-derived N-substituted pyrrole), [His^6(HPNE)]-Ang II, [N-(9,12-dioxo-10(*E*)-dodecenoic acid)]-Ang II, and [His^6(9-hydroxy-12-oxo-10(*E*)-decenoic acid)]-Ang II as the predominant LA-derived modifications. These modifications could represent the majority of lipid-derived modifications to peptides and proteins in biological systems.

1. Introduction

Modifications of protein are known to be associated with various human diseases through the modulation of protein activity, function, and macromolecular interactions [1]. Recently, the protein modifications by lipid hydroperoxide-derived reactive aldehydes have gained more attention because of their possible involvement in the etiology and/or progress of degenerative diseases such as diabetes, cardiovascular diseases, and Alzheimer's disease [2,3]. Although these biological effects have been mainly attributed to the capacity of reactive aldehydes to react with the nucleophilic sites of proteins [4], the overall biological process of oxidative protein modifications is complicated and remains largely undefined. Lipid-derived aldehyde derivatives are formed during

Abbreviations: AA, L-ascorbic acid; Ang, angiotensin; Ang P, pyruvamide-Ang II; APM, aspartame; ARA, arachidonic acid; Arg, arginine; CID, collision-induced dissociation; COX, cyclooxygenase; Cys, cysteine; DODE, 9,12-dioxo-10(*E*)-dodecenoic acid; EA, 4,5-epoxy-2(*E*)-alkenal; EDE, *trans*-4,5-epoxy-2(*E*)-decenal; ESI, electrospray ionization; HNE, 4-hydroxy-2(*E*)-nonenal; HODD, 9-hydroxy-12-oxo-10(*E*)-decenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HPNE, 4-hydroperoxy-2(*E*)-nonenal; His, histidine; HPODE, hydroperoxyoctadecadienoic acid; LA, linoleic acid; LC, liquid chromatography; LOX, lipoxygenase; Lys, lysine; $[M + H]^+$, protonated molecule; MDA, malondialdehyde; MS, mass spectrometry; MS/MS, tandem mass spectrometry; ONE, 4-oxo-2(*E*)-nonenal; PB, phosphate buffer; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; *t*Boc, N^{α}-*tert*-butoxycarbonyl; TFA, trifluoroacetic acid; TMP, 1,1,3,3-tetramethoxypropane; t_R, retention time.

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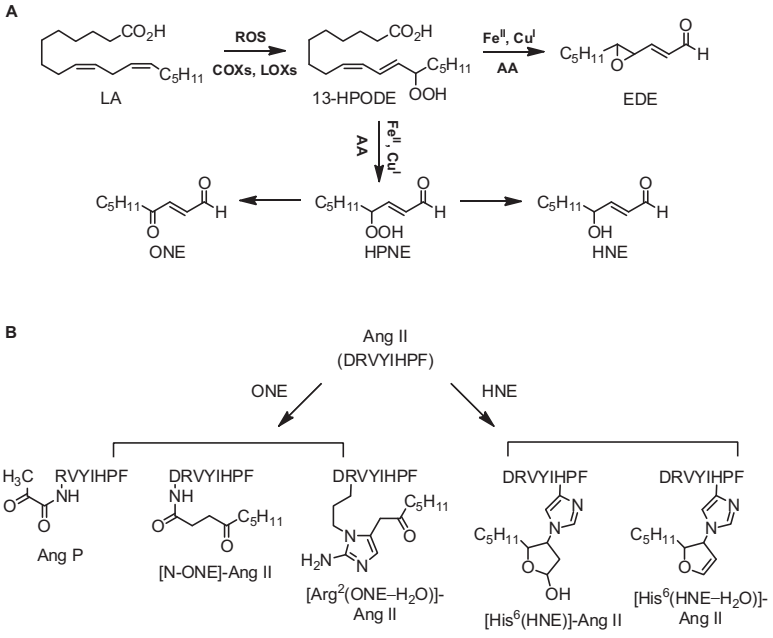
http://dx.doi.org/10.1016/j.cbi.2015.06.029
0009-2797/

the propagation stage of lipid peroxidation. Degradation of polyunsaturated fatty acid (PUFA) hydroperoxides resulted in the generation of a wide variety of breakdown products, which include alkanals, 2-alkenals, hydroxy-alkenals, keto-alkenals, and alkanedials [3]. Therefore, it is important to understand the structure of carbonyl chain, the nature of linkage, and the site of adduction in order to better clarify the biological effect of protein modification and use the modified protein as biomarkers of oxidative damage in vivo.

In more detail, reactive oxygen species (ROS) are generated continuously by a variety of enzymatic and nonenzymatic processes in living cells. ROS can interact with numerous cellular components, including DNA, protein, and lipids [5–7]. The multiple methylene groups positioned between double bonds make PUFAs extremely sensitive to oxidation, which results in the formation of lipid hydroperoxides. For linoleic acid (LA), ROS result in the formation of two 13-hydroperoxyoctadecadienoic acid (HPODE) isomers, and two 9-HPODE isomers, each of which exists as a mixture of R- and S-enantiomers. Enzymes such as cyclooxygenases (COXs) and lipoxygenases (LOXs) can also oxidize PUFAs [8,9]. In contrast to ROS-mediated oxidation, COX- and LOX-mediated conversion of PUFAs to lipid hydroperoxides is highly stereoselective. For example, COX-2 converts LA to 9(*R*)-HPODE and 13(*S*)-HPODE [10]. LA is also a substrate for both 15-LOX-1 and 15-LOX-2, which exclusively produce 13(*S*)-HPODE [11,12]. In our previous studies [13–16], 4-oxo-2(*E*)-nonenal (ONE), 4-hydroxy-2(*E*)-nonenal (HNE), *trans*-4,5-epoxy-2(*E*)-decenal (EDE), and 4-hydroperoxy-2 (*E*)-nonenal (HPNE) were identified as the major decomposition products from the incubation of 13(*S*)-HPODE in the presence of L-ascorbic acid (AA), Fe^{II}, or Cu^{I} (Scheme 1A). The same bifunctional electrophiles also arise from 15(*S*)-hydroperoxyeicosatetraenoic acid (HPETE), an arachidonic acid (ARA)-derived hydroperoxide [17]. These bifunctional electrophiles were derived from the ω terminus of 13(*S*)-HPODE or 15(S)-HPETE. It has been proposed that EDE arises from α-cleavage of an alkoxy radical [18]. A Hock rearrangement of a *bis*-hydroperoxide intermediate leads to the formation of HPNE, which undergoes reduction to HNE or 2-electron oxidation to ONE [13–15,19].

ONE and HNE have been extensively studied because of their abundance, toxic effects, and reactivity towards DNA and proteins [10,20,21]. Upon reaction with proteins, ONE and HNE form Michael addition products with nucleophilic amino acids, cysteine (Cys), histidine (His), and lysine (Lys) [21]. ONE can also modify arginine (Arg) [22,23] and Lys [24] through the formation of Schiff base intermediates to produce a substituted imidazole and a stable 4-ketoamide (an apparent ONE-Lys Michael addition product), respectively. In our recent studies with bioactive octapeptide angiotensin (Ang) II and other Ang peptides [25,26], ONE was shown to mediate the conversion of the N-terminal amino acid to α-ketoamide (Scheme 1B). In addition, unusual modifications by ONE or HNE can be derived from specific amino acid sequences and conformations of peptides or proteins. For example, ONE recognizes the specific amino acid motifs of His^{75}, alanine[76], and Lys^{77} in bovine histone H4 and forms a novel cyclic structure that incorporates the imidazole ring of His and a newly formed pyrrole derived from Lys [27]. HNE produces dehydrated Michael addition products upon reaction with His^{6} in Ang II because the specific conformation of Ang II facilitates the dehydration of Michael addition products (Scheme 1B) [23]. EDE reacts with Lys producing N-substituted pyrroles and N-substituted 2-hydroxyalkyl pyrroles through cyclization of the Schiff base to a 5-membered ring [28,29]. EDE can also form Michael addition products with His [30]. Although HPNE is a direct precursor of ONE and HNE, it is reported that it can modify Lys to generate adducts of unusual structure through a mechanism involving hydroperoxy group-mediated intramolecular oxidation [31].

As a continuation of our previous studies on ONE- and HNE-derived modifications to Ang II [23,25], we carried out further investigations on Ang II modification by EDE and HPNE. Liquid chromatography (LC)/electrospray ionization (ESI)-mass spectrometry (MS) was used to identify the modified peptides, and model reactions were performed using aspartame (APM), N^{α}-*tert*-butoxycarbonyl (*t*Boc)-His or malondialdehyde (MDA) to confirm the modifications observed in Ang II reactions. Subsequently, a mixture of 13-HPODE and [$^{13}C_{18}$]-13-HPODE



Scheme 1. (A) Decomposition of LA-derived lipid hydroperoxide (13-HPODE) to highly reactive aldehydes EDE, HPNE, ONE, and HNE. (B) Identified Ang II modifications derived from ONE and HNE.