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Antioxidants and HNE in redox homeostasis

Wojciech Łuczaj, Agnieszka Gęgotek, Elżbieta Skrzydlewska*

Department of Analytical Chemistry, Medical University of Bialystok, Mickiewicza 2d, 15-222 Bialystok, Poland

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ABSTRACT

Under physiological conditions, cells are in a stable state known as redox homeostasis, which is maintained by the balance between continuous ROS/RNS generation and several mechanisms involved in antioxidant activity. ROS overproduction results in alterations in the redox homeostasis that promote oxidative damage to major components of the cell, including the biomembrane phospholipids. Lipid peroxidation subsequently generates a diverse set of products, including α,β-unsaturated aldehydes. Of these products, 4-hydroxy-2-nonenal (HNE) is the most studied aldehyde on the basis of its involvement in cellular physiology and pathology. This review summarizes the current knowledge in the field of HNE generation, metabolism, and detoxification, as well as its interactions with various cellular macromolecules (protein, phospholipid, and nucleic acid). The formation of HNE-protein adducts enables HNE to participate in multi-step regulation of cellular metabolic pathways that include signaling and transcription of antioxidant enzymes, pro-inflammatory factors, and anti-apoptotic proteins. The most widely described roles for HNE in the signaling pathways are associated with its activation of kinases, as well as transcription factors that are responsible for redox homeostasis (Ref-1, Nrf2, p53, NFκB, and Hsf1). Depending on its level, HNE exerts harmful or protective effects associated with the induction of antioxidant defense mechanisms. These effects make HNE a key player in maintaining redox homeostasis, as well as producing imbalances in this system that participate in aging and the development of pathological conditions.

1. Introduction

A basic feature of cell physiology, specifically respiration and cell signaling, is the generation of reactive oxygen and nitrogen species (ROS/RNS). Cells are in a stable state when the rate of ROS/RNS generation is balanced by the scavenging capacity of various antioxidant compounds. These compounds, which are present at relatively low levels, are able to compete with other oxidizable substrates and thus significantly delay or inhibit the oxidation of the other substrates. On the other hand, the antioxidant defense must permit the ROS-mediated roles in cell signaling and redox regulation that subsequently lead to increased expression of the genes for various antioxidant proteins [1]. The human antioxidant defense includes endogenous antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase, as well as other proteins and low molecular-weight compounds such as glutathione, uric acid, coenzyme Q, and lipoic acid. Moreover, the classical antioxidant system is supported by additional defense systems that remove or repair damaged biomolecules before their accumulation can result in alterations in the cellular metabolism. The interactions between the ROS/RNS and these antioxidants in the redox homeostatic balance produce metabolic responses to endogenous as well as exogenous signals. These signals modulate the appropriate induction of adaptation processes or alternatively, the activation of cell death mechanisms. Therefore, cellular redox homeostasis plays a key role in physiological as well as most pathophysiological processes. Elevated ROS levels that cannot be counteracted by the cellular antioxidant abilities induce redox imbalance that results in uncontrolled oxidative stress. This situation causes oxidative modifications in the

Abbreviations: 15-LOX, 15-lipoxygenase; 4–HNE, 4-hydroxynonenal; AKRs, aldo-keto reductases; ALDHs, aldehyde dehydrogenases; AP-1, activator protein 1; ARE, antioxidant responsive element; Bcl-2, B-cell lymphoma 2; COX-2, cyclooxygenase-2; DHN, 1,4-dihydroxy-2-nonene; DAG, diacylglicerol; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; Gadd45, Growth arrest and DNA-damage-inducible protein; GCL, glutamate cysteine ligase; GSH-Px, glutathione peroxidase; GSSG, glutathione disulfide; GSTs, glutathione *S*-transferases; HIF-1, hypoxia-inducible factor 1; HPETE, 11/15 hydroperoxyeicosatetraenoate; HPODE, 9/13 hydroperoxyoctadecadienoate; Hsf1, Heat shock factor protein 1; IKK, IκB kinase; IL, interleukin; IP3, inositol trisphosphate; IRES, internal ribosomal entry sites; JNK, Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; L4CL, tetralinoleoylcardiolipin; LOOH, lipid hydroperoxides; MAPKs, mitogen-activated protein kinases; Mdm2, Mouse double minute 2 homolog; MRP, multidrug resistance proteins; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PAQR3, Progestin And AdipoQ Receptor Family Member 3; PE, phosphatidylethanolamines; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PPARs, peroxisome proliferator-activated receptors; PS, phosphatidylserines; PUFAs, polyunsaturated fatty acids; Ref-1, Redox effector factor-1; RLIP76, Ral-interacting GTPase-activating protein; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFα, tumor necrosis factor-α; Trx, thioredoxin; TrxR, thioredoxin reductase

* Corresponding author.
E-mail address: elzbieta.skrzydlewska@umb.edu.pl (E. Skrzydlewska).

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structure and function of cellular components and consequently leads to cell, tissue and organ injury. Thus, redox imbalance plays a significant role in development of numerous disease conditions, which demonstrates the biological relevance of redox regulation.

2. Lipid peroxidation

Among the cellular components, the most sensitive to oxidative modifications are the membrane phospholipids. Specifically, these lipids exhibit elevated susceptibility to oxidation by various endogenous ROS/RNS as well as exogenous inducers such as some transition metal ions, high energy irradiation and xenobiotics that are capable of initiating lipid peroxidation in the biomembranes.

Lipid peroxidation encompasses various mechanisms that can be classified as free radical-mediated oxidation, free radical-independent non-enzymatic oxidation and enzymatic oxidation [2]. The main pathway resulted from oxidative stress is free radical peroxidation proceeding by chain reactions in the biomembranes. These reactions are initiated by various types of active species and are involved in development of pathologies.

The solubility of molecular oxygen and therefore the ROS/RNS generation in the biomembranes is high. Thus, the membrane phospholipids, which containing high levels of polyunsaturated fatty acids (PUFAs) including arachidonic, linoleic, linolenic, docosahexaenoic, and eicosapentaenoic acids, are extremely sensitive to attack by ROS/RNS and other electrophiles [3]. Moreover, after reacting with the free radicals, the PUFAs themselves become reactive free radicals and are capable of propagating lipid peroxidation chain reactions [4].

The most powerful initiators of lipid peroxidation are the hydroxyl and hydroperoxyl radicals. Hydroxyl radicals are generated from hydrogen peroxide during cellular oxygen metabolism via the Fenton and Haber–Weiss reactions, in the presence of free iron or copper ions, and from peroxynitrite or as a result of high energy irradiation [5]. Superoxide and nitric oxide, which are generated by the activities of NADPH oxidase, xanthine oxidase, and nitric oxide synthase, are not able to initiate lipid peroxidation, but they react to give peroxynitrite, which may initiate chain reactions [3]. However, the protonated form of superoxide, the hydroperoxyl radical, is a sufficiently strong oxidant that it can initiate oxidation of PUFAs and causes the transformation of hydrogen peroxide to the hydroxyl radical [6].

2.1. HNE generation

As a result of the ROS/RNS reactions with PUFAs, hydrogen is abstracted from the alpha carbon with the insertion of oxygen, which results in the generation of lipid peroxyl radicals that are subsequently oxidized to lipid hydroperoxides (Fig. 1). As the result of LOOH (lipid hydroperoxides) decomposition, diverse products are generated. Among these, the α,β-unsaturated aldehydes deserve special attention, particularly 4-hydroxy-2-nonenal (4-hydroxy-2,3-trans-nonenal, HNE), which is a major product of the decomposition of hydroperoxides of ω-6 fatty acids such as linoleic and arachidonic [7].

The first mechanism that was proposed for the formation of HNE involved the generation of hydroperoxyperoxides (9/13 hydroperoxyoctadecadienoate (HPODE) and 11/15 hydroperoxyeicosatetraenoate (HPETE)) as derivatives of linoleic and arachidonic acid [8,9]. HNE was obtained from these intermediates as a result of Hock cleavage (in the presence of Fe^{2+}) in the mechanism proposed by Pryor and as β-scission in another mechanism [10,11]. Two alternative nonenzymatic mechanisms of HNE generation were reported later. One of these involves cyclization of peroxyl radicals to form a dioxetane that can rearrange resulting in chain cleavage, and the second, similar to that for hydroperoxide decomposition, involves Hock rearrangement and Hock cleavage [12,13]. It should also be noted that HPODE and HPETE were considered to be intermediates that commonly resulted from free radical mechanisms in which hydrogen was abstracted at bis-allylic sites and molecular oxygen was added by an enzymatic process that involved the 15-lipoxygenases (15-LOX). In this process, 13-HPODE is produced by the oxidation of linoleic acid by 15-LOX-1, whereas 15-HPETE is generated during oxidation of arachidonic acid by 15-LOX-2 [13,14]. Thus, HNE may be an end-product of free radical-mediated as well as enzymatic oxidation of the ω-6 PUFAs. Moreover, cyclooxygenase-2 (COX-2) was also suggested to be involved in the HNE formation triggered by Escherichia faecalis, a human intestinal commensal [15]. More recently, a novel mechanism of HNE formation, specific for linoleic acid, was demonstrated to occur, which represents an alternative for the bis-allylic hydrogen abstraction. This mechanism involves oxidation of a mitochondrial cardiolipin, tetralinoleoylcardiolipin (L4CL). On the basis of its four linoleic acid chains, this molecule is very prone to free radical oxidation and results in cross-chain peroxyl radical addition and decomposition [16].

The level of HNE generated during lipid peroxidation primarily depends on the availability of endogenous PUFAs as well as exogenously provided with the food. This statement refers especially to ω-6 PUFAs, which have been shown to be major precursors of HNE [7]. It makes the content of ω-6 PUFAs versus ω-3 PUFAs in the food is crucial in this matter. Therefore the ratio of ω-6/ω-3 fatty acids, increased in Western diet during the past few decades up to 17:1, is very important factor pivotal for oxidative stress intensification. Thus delivering of ω-6 PUFAs with the diet results in an increase of HNE level what leads to development of many diseases including rheumatoid arthritis, inflammatory bowel disease, nonalcoholic fatty liver disease, obesity, cardiovascular diseases, neurodegenerative diseases and cancer [17]. Increased content of ω-3 PUFA in the diet is strictly associated with prevention, thus the decrease of the ratio of ω-6/ω-3 fatty acids significantly reduces the risk of chronic diseases. However, recent studies showed possible dual implications of PUFAs intake. It has been shown that ketogenic diet high in linoleic and arachidonic acids enhanced oxidative stress with significant increase of 4-HNE-modified proteins level [18]. Another study indicated for upregulation of ω-1 hydroxylation of 4-hydroxynonenoate leading to decrease of the HNE level as consequence of ketogenic diet [19]. The correlation between high content of ω-6 PUFAs in diet and enhanced oxidative stress and HNE pathological action remains unquestionable.

2.2. Antioxidants versus lipid peroxidation

Lipid peroxidation is regulated by antioxidants including proteins and low molecular compounds that participate in biomembrane protection. The most relevant anti-peroxidative antioxidant is GSH-Px, which catalyzes the continuous reduction of lipid peroxides at the expense of reduced glutathione. This reaction depends on the presence of selenium. Most isoforms of glutathione peroxidase contain a selenocysteine residue and have a Lys-92 residue at their active sites. The lysine can easily form a Schiff base with HNE that is reversible in an aqueous environment [20]. Formation of this adducts leads to inhibition of the glutathione peroxidase activity, which has been observed in aging and during oxidative stress in neurons [21]. Moreover, an increased HNE level is accompanied by a decrease in the glutathione peroxidase activity in various pathologies such as cancer, hypertension, and Lyme disease [22–24]. However, an elevated activity of this antioxidant is observed in skin cells after UV irradiation [25].

GSH-Px activity also depends on the level of its co-substrate, GSH, which is oxidized in stress conditions and can react with HNE [26]. The oxidized form of GSH, glutathione disulfide (GSSG), can be reduced by glutathione reductase using NADPH as an electron donor. However, HNE decreases the glutathione reductase activity [27]. The reaction of GSH with HNE, which is catalyzed by the glutathione*S*-transferases (GSTs), leads to the formation of GS-HNE adducts that cause decreases in the cellular GSH level and GSH-Px activity [28]. This situation promotes lipid peroxidation and the production of signaling molecules [29–31]. HNE regulates the GSH level by stimulating γ-glutamyl