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An overview of the chemistry and biology of reactive aldehydes

Kristofer S. Fritz, Dennis R. Petersen *

Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver Anschutz Medical Center, 12850 E. Montview Blvd, Campus Box C-238, Aurora, CO 80045, USA

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ABSTRACT

The nonenzymatic free radical generation of reactive aldehydes is known to contribute to diseases of sustained oxidative stress including rheumatoid arthritis, atherosclerosis, neurodegeneration, and a number of liver diseases. At the same time, the accumulation of lipid electrophiles has been demonstrated to play a role in cell signaling events through modification of proteins critical for cellular homeostasis. Given the broad scope of reactivity profiles and the ability to modify numerous proteomic and genomic processes, new emphasis is being placed on a systems-based analysis of the consequences of electrophilic adduction. This review focuses on the generation and chemical reactivity of lipid-derived aldehydes with a special focus on the homeostatic responses to electrophilic stress.

Introduction

Unsaturated fatty acids are abundant constituents sequestered in *sn*-1 and *sn*-2 esterfied forms in glycerol phospholipids within cellular membranes. In addition, unsaturated free fatty acids are abundant within various cellular compartments. The fact that lipids constitute a major portion of the plasma and mitochondrial and endoplasmic membranes establishes the presence of massive concentrations of unsaturated fatty acids within membranous structures. Likewise, the concentration of unsaturated fatty acids, in the free form or bound to specific transport proteins, in cells is noteworthy. It is also well recognized that the polyunsaturated fatty acids are bioactive mediators of diverse pathways involved in cellular homeostasis or, in some cases, interact with cellular macromolecules resulting in cell death. These cellular responses may be a consequence of the vulnerability of unsaturated fatty acids to diverse oxidation reactions or radical reactions both of which result in formation of electrophilic lipid products. Certain of the oxidation reactions involving unsaturated fatty acids are enzymatically mediated by families of non-heme-containing metallo-enzymes including the lipoxygenases (LOX), cyclooxygenases (COX), and cytochrome P450. Because these reactions are integral for an organisms response to a range of stimuli, they are generally well controlled and generate a spectrum of bioactive products which are ligands for highly specific, receptor-mediated responses including vasodilation, vasoconstriction, and proinflammatory or anti-inflammatory cascades (for informative comprehensive reviews of the production and actions of enzymatically generated bioactive lipids see the entire volume of *Chem. Rev*. **111**:2011) [1].

The electrophilic lipid products generated by free radical-mediated lipid oxidation are different in many respects than those generated by enzyme-mediated oxidation. First, free radical-mediated production of electrophilic products of polyunsaturated fatty acids proceeds by autocatalysis and is, as a result, not well regulated. Thus, free radical-mediated lipid peroxidation is more commonly associated with diseases of sustained oxidative stress including rheumatoid arthritis, atherosclerosis, neurodegeneration, and a number of liver diseases. Further, the overproduction of electrophilic lipids resulting from free radical-mediated peroxidation of polyunsaturated fatty acids has been demonstrated to initiate cell death through modification of proteins critical for cellular homeostasis. A series of recent reviews highlight the advances in identification of lipid substrates, mechanisms of lipid peroxidation, and protein targets of electrophilic lipid modification [2,3]. At the same time, there is emerging data demonstrating that, like the lipid products produced by the LOX, COX, and P450-mediated oxidation of unsaturated fatty acids, the electrophilic products of radical-generated lipid peroxidation also initiate responses that are cytoprotective and thus integral for cellular homeostasis and survival. This review will provide an overview of free radical-mediated lipid peroxidation involved in cellular injury followed by a more comprehensive examination of recent studies describing the potential of electrophilic products of lipid peroxidation to modulate signaling pathways involved in antioxidant responses. The final section of this perspective will assess the potential of antioxidant therapy to enhance or abrogate antioxidant responses initiated by electrophilic lipid signaling.

Generation of reactive aldehydes

The generation of oxygen-derived free radicals and oxidants is a consequence of cellular metabolism and bioenergetic processes

* Corresponding author. Fax: +1 303 724 7266.
E-mail address: dennis.petersen@ucdenver.edu (D.R. Petersen).

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throughout the animal kingdom [4]. It is evident that a host of biochemical mechanisms mediate persistent free radical generation and damage. An insightful review by Hermann Esterbauer details the integration of oxidative processes in human physiology [5]. In it, he states that the adult human with a daily energy 0 requirement of 10,000 kJ consumes approximately 660 g of oxygen per day, with roughly 90–95% of that oxygen converted to harmless water through mitochondrial respiration. The remaining 5–10% of that oxygen undergoes univalent and divalent reduction, yielding reactive oxygen species, such as superoxide radicals. When taken in the context of the average human life span, the human body consumes a massive 17,000 kg of oxygen which results in the concomitant production of 800–1700 kg of oxygen radicals. These figures provide a fascinating perspective on the detoxifying mechanisms aerobic organisms have integrated into biology and the critical role they play in diffusing oxygen radicals and maintaining life.

The endogenous generation of reactive aldehydes has been studied for decades and is known to contribute to numerous disease pathologies by altering proteomic, genomic, cell signaling, and metabolic processes [6,7]. Reactive aldehydes compose a class of highly reactive organic chemical compounds obtained by oxidation of primary alcohols, characterized by the common group R-CHO consisting of a carbonyl center bonded to hydrogen and an R group [8]. These compounds arise predominantly as a consequence of oxidative stress within the cellular microenvironment, where prooxidant forces overcome natural antioxidant capacities. Numerous studies demonstrate that at low levels, these compounds contribute to regulating cell proliferation among many other processes; however, a delicate balance exists between basal levels of these aldehydes and cytotoxic concentrations [5,6,9,10].

The production of reactive aldehydes occurs through a number of pathways, including enzymatic and nonenzymatic processes. One of the most prominent sources for the generation of reactive aldehydes is through nonenzymatic free radical mechanisms [11]. Specifically, a profusion of reactive nitrogen species (RNS) and reactive oxygen species (ROS), including peroxynitrite, superoxide radicals, and hydroxyl radicals, provides an overabundance of initiating chemicals. A major source of these aldehydes is the autoxidation of polyunsaturated fatty acids (PUFA), including arachidonic and linoleic acid. Lipid peroxidation occurs in three major phases, comprised of an initiation event, chain propagation, and termination (Fig. 1) [3]. Typical initiating chemical species are hydroxyl and superoxide radicals. Both are present under normal physiologic conditions and are produced at much higher concentrations in situations of oxidative stress. Initiation occurs through the abstraction of a bis-allylic hydrogen from a lipid chain (LH) to yield a lipid radical (L̇). Propagation proceeds when oxygen is added to the carbon-centered radical, where L̇ is rapidly converted to an oxygen-centered peroxyl radical (LOȮ). The LOȮ reacts with another LH to generate L̇ and an unstable lipid hydroperoxide (LOOH), which in turn yields new peroxyl and alkoxyl radicals, degrading further to secondary products through β-scission and hock cleavage, among others. A single initiation reaction is postulated to result in 200 to 400 propagation cycles, rapidly amplifying free radical damage under highly oxidizing PUFA-rich environments [12]. Chain-breaking events are known to occur, as α-tocopherol (vitamin E) radicals form while converting LOȮ to LOOH [13]. Termination of the propagation cycle occurs when two free radical species combine to yield nonradical species. The degradation of peroxyl and alkoxyl radicals into secondary products is thought to generate well over a hundred different reactive species, each with a wide range of reactivity, size, and specificity [5,6]. This overabundance of chemical species greatly increases the complexity of studies attempting to characterize lipid peroxidation-derived electrophilic damage. The more prominent products 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), and acrolein (ACR) have been examined under various disease models through in vitro assays, immunohistochemical staining, and analytical HPLC methods [7,14,15].

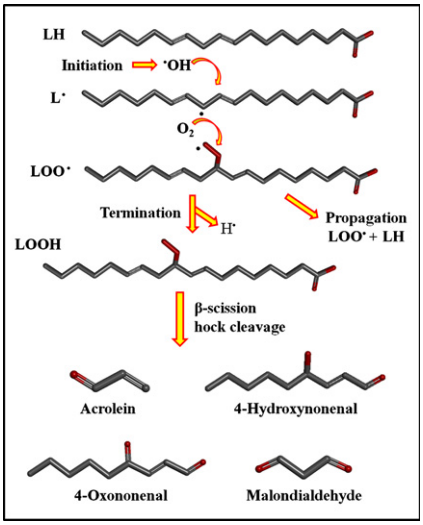

Fig. 1. The generation of reactive aldehydes through the initiation, propagation, and termination stages of lipid peroxidation. For simplicity, Fig. 1 shows the generation of reactive aldehydes from a C18 fatty acid; however, in cells, the fatty acids are largely esterified within phospholipids. Furthermore, reactive aldehydes can also occur in esterified form, which may also form adducts with proteins and other macromolecules, although this is a less well-studied area.

Cellular location is an important consideration in determining the extent of free radical-mediated damage resulting in the generation of reactive aldehyde species. As illustrated in Fig. 2, lipid-rich membranes provide an optimal environment for producing a high abundance of these cytotoxic compounds. Microsomal and mitochondrial intramembrane concentrations of these lipid peroxidation-derived electrophilic compounds have been estimated to accumulate to as high as 10 mM in vivo [9,16]. The regulatory function and cytotoxicity of these aldehydes hinges on abundance, reactivity, and longevity. Longevity varies greatly, with compounds such as 4-oxononenal (4-ONE) and 4-HNE displaying half-lives of roughly 1 s and 2 min, respectively [9,17]. Persisting on a scale of minutes, some aldehydes preferentially retain the ability to transiently modify distant proteins, membranes, and DNA. These factors may contribute to a localized bias for molecular adduction by reactive aldehydes, particularly those with short half-lives, as nucleophiles located within the vicinity of lipid peroxidation remain more susceptible to higher concentrations of electrophiles. However, long-lived species may have a more pronounced global impact by altering a broader array of proteomic and genomic targets due to their transient nature.

Chemical mechanisms

The chemical specificity of reactive aldehydes is impacted through direct and indirect forces. Direct factors include characteristics of the electrophile and nucleophile species, such as pK_a, chemical potential, and electrophilic index. Indirect forces include solvent pH and adjacent peptide sequence, which can play an important role through steric hindrance and charge stabilization. Both factors play an integrated role in determining the specificity for any given set of electrophile–nucleophile pairs. To better understand the stability and reactivity of these adducts, a number