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Review Article

Protein lipoxidation: Detection strategies and challenges

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ABSTRACT

Enzymatic and non-enzymatic lipid metabolism can give rise to reactive species that may covalently modify cellular or plasma proteins through a process known as lipoxidation. Under basal conditions, protein lipoxidation can contribute to normal cell homeostasis and participate in signaling or adaptive mechanisms, as exemplified by lipoxidation of Ras proteins or of the cytoskeletal protein vimentin, both of which behave as sensors of electrophilic species. Nevertheless, increased lipoxidation under pathological conditions may lead to deleterious effects on protein structure or aggregation. This can result in impaired degradation and accumulation of abnormally folded proteins contributing to pathophysiology, as may occur in neurodegenerative diseases. Identification of the protein targets of lipoxidation and its functional consequences under pathophysiological situations can unveil the modification patterns associated with the various outcomes, as well as preventive strategies or potential therapeutic targets. Given the wide structural variability of lipid moieties involved in lipoxidation, highly sensitive and specific methods for its detection are required. Derivatization of reactive carbonyl species is instrumental in the detection of adducts retaining carbonyl groups. In addition, use of tagged derivatives of electrophilic lipids enables enrichment of lipoxidized proteins or peptides. Ultimate confirmation of lipoxidation requires high resolution mass spectrometry approaches to unequivocally identify the adduct and the targeted residue. Moreover, rigorous validation of the targets identified and assessment of the functional consequences of these modifications are essential. Here we present an update on methods to approach the complex field of lipoxidation along with validation strategies and functional assays illustrated with well-studied lipoxidation targets.

Contents

Abbreviations: ACR, acrolein; AGE, advanced glycation end product(s); ALE, advanced lipoxidation end product(s); CID, collisional induced dissociation; CHH, 7-(diethylamino)coumarin-3-carbohydrazide; cyPG, cyclopentenone prostaglandin(s); DNPH, 2,4-dinitrophenylhydrazine; ESI, electrospray ionization; GO, glyoxal; 4-HHE, 4-hydroxyhexanal; HNE, 4-hydroxynonenal; HEL, Nε-hexanoyl-lysine; MALDI, matrix assisted laser desorption/ionization; MDA, malondialdehyde; MS, mass spectrometry; NLS, neutral loss scans; ONE, 4-oxononenal; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; RCS, reactive carbonyl species.

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1. Introduction

Redox balance is emerging as an important physiological and pathophysiological regulator of cellular behavior and outcome. The functions and activities of a growing number of proteins have been found to be altered by the oxidation state of the protein, especially at redox-sensitive cysteines. Proteins can also be modified by covalent reactions with oxidized sugars, often referred to as advanced glycation end products (AGE), or with oxidized products of lipids (advanced lipoxidation end products, or ALE) [1]. These reactions can occur mainly on the nucleophilic residues cysteine, histidine, arginine and lysine, although reactions with glutamine and asparagine have also been reported; this leads to the formation of a wide variety of adducts, as described previously [1–4]. While, originally, such modifications were considered solely as detrimental to protein function, more recently there have been some reports of protein lipoxidation increasing the activity or altering the nature of the activity of specific proteins, and therefore lipoxidation is starting to be considered alongside cysteine thiol-sulfenate-disulfide switches as an additional mechanism of protein regulation [3,5,6]. The aim of this article is to provide a succinct update on the importance of lipoxidation *in vivo* and progress in the methods employed for its study.

2. Types of oxidized lipids that generate adducts

Phospholipid peroxidation occurs following radical attack, usually on polyunsaturated fatty acyl chains, and generates many different products including full-chain length oxidized fatty acids or phospholipids, chain-shortened oxidized phospholipids and small fragmentation products from the chain scission reactions. These reactions are now quite well understood and have been described in detail in several recent reviews [7–9], showing that the structure of the parent lipid and the site of radical damage determine the products. There are also enzymatic pathways for producing oxidized fatty acids and phospholipids, starting with cytochrome P450 enzymes, lipoxygenases and cyclooxygenases; products of the latter are further metabolized by a variety of prostaglandin synthases [10]. Many of the products generated by both enzymatic and non-enzymatic pathways are reactive and electrophilic owing to the presence of carbonyl groups (aldehydes or ketones) or α, β-unsaturated moieties, and can be categorized into five principal groups: alkanals (and hydroxyalkanals), 2-alkenals, 4-hydroxy–2-alkenals, keto-alkenals, and alkanedial (dialdehydes) [3]. The most reactive and commonly studied are malondialdehyde (MDA), acrolein (ACR), 4-hydroxyhexanal (4-HHE) and 4-hydroxynonenal (HNE), which also reflects the fact that these products are produced at higher levels than many other products [7] (please see Fig. 1 for the structures of some electrophilic lipids involved in protein lipoxidation). In addition, compounds with more complex structures, such as oxidized phospholipids, arachidonic acid metabolites and nitrated fatty acids are emerging as important lipid mediators in pathophysiological situations, in some cases associated with the onset and/or the resolution of inflammation. The type of adducts formed depends on the reactivity of the oxidized lipid species. Compounds containing aldehydes or ketones can react with amines (e.g. on lysine) to form Schiff base adducts by loss of water, whereas those containing an α, β-unsaturated moiety form Michael adducts by a nucleophilic addition reaction of the protein sidechain at the β-carbon. Furthermore, some electrophilic lipids have been described to contain epoxide moieties, which also react with nucleophiles giving rise to different structures. It is interesting to note that some bi-functional lipid oxidation products, such as dialdehydes or hydroxyalkenals, do react with proteins and still present free carbonyls, which can be exploited in some detection procedures, as discussed below. Nevertheless, in many cases, the carbonyl group is involved in the reaction and is not available for detection. In addition, bifunctional electrophilic lipids can induce protein cross-linking, as has been shown for HNE, isoketals and cyclopentenone prostaglandins (cyPG) with dienone structure, and this may have important consequences on protein fate [11–13].

3. Pathophysiological relevance of lipoxidation adducts

Evidence for occurrence of lipoxidation products *in vivo* has expanded greatly in the last 10 years, as more sensitive and specific methodology has been developed, and now there are many examples of lipoxidized proteins in both healthy and diseased tissues. Much of the work has focused on HNE, but there are also many examples of adducts formed by other short chain electrophilic products, whereas studies of lipoxidation by long chain and esterified products are rarer.

As discussed below, generation of reactive species is increased in pathological conditions, and, in parallel, levels of protein lipoxidation increase in several diseases, favoring progress in the detection of adducts and identification of the modified proteins. A

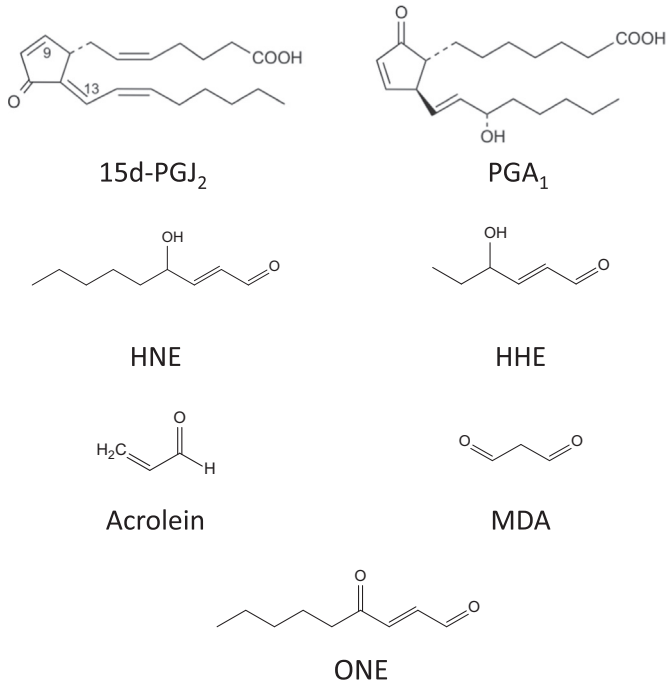

Fig. 1. Structure of some of the electrophilic lipids involved in protein lipoxidation.