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Lipid Hydroperoxide (LOOH)

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Abbreviations

ROS, Reactive oxygen species
LOOH, Lipid hydroperoxide
ChOOH, Cholesterol hydroperoxide
LOO•, Lipid peroxyl radical
LOX, Llipoxygenase
COX, Cyclooxygenases
ALA, α-linolenic acid
HPLC-CL, High performance liquid chromatography with chemiluminescent detection

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Abstract

Reactive Oxygen Species (ROS) are generated from the cellular metabolism and by exogenous agents. Lipid-containing structures, such as cell membranes and lipoproteins, are well-known targets of ROS, which can result in lipid peroxidation, a degenerative process that disturbs structure or function of the target system. Lipid hydroperoxides (LOOHs) are prominent non-radical intermediates of lipid peroxidation. Identification of LOOHs is very important in that it can determine whether the primary reaction is mediated by singlet oxygen or oxyradicals. Having increased polarity and long lifetime compared with free radical precursors, LOOHs might be able to migrate from the points of origins to more sensitive sites to cause more pathological changes. LOOHs can also participate in redox reaction, which mediates stress signaling and may evoke a variety of cellular responses from induction of antioxidant enzymes to apoptotic death.

Introduction

ROS can be generated in eukaryotic cells by normal oxidase action and during the course of electron transport in mitochondria or endoplasmic reticulum [1]. In addition to cellular sources, there are numerous environmental sources of ROS, such as being ionizing and non-ionizing radiation [2, 3]. Cell membranes and other organized systems containing unsaturated phospholipids, glycolipids, and cholesterol are the well-known targets of ROS. Oxidation of these lipids leads to lipid peroxidation linking to many disorders, including atherogenesis, ischemia reperfusion injury, and UV-induced carcinogenesis [4]. LOOHs are major non-radical intermediates of lipid peroxidation. LOOHs are more polar than parental lipid, which perturbs the structure and/or function of the membranes or other unsaturated lipid-containing organized system. Understanding the chemical and physical properties of LOOHs as well as metabolism is important in the study of the underlying mechanisms of oxidation reaction, and of oxidative stress signaling pathway.

Formation of LOOHs

Lipid peroxidation may commence with either free radical species, such as oxyl radicals, peroxyl radicals, and hydrogen peroxide, or non-radical species, such as singlet oxygen, ozone, and peroxynitrite. In the former case, the formation of LOOH is illustrated by following equations [5]:

$$LH + HO\bullet \longrightarrow L\bullet + H_2O$$

$$L\bullet + {}^3O_2 \longrightarrow LOO\bullet$$

$$LOO\bullet + LH \longrightarrow LOOH + L\bullet$$

From above equations, we see that free radicals such as hydoxyl radicals triggering chain peroxidation by abstracting an allylic hydrogen from a proximal unsaturated lipid, LH. The resulted carbon-centered lipid radicals (L•) react with $^3O_2$ to generate a lipid peroxyl radical (LOO•), which then reduced to LOOHs. In the latter case, the reaction is rather simple and non-radicals interact directly with unsaturated proximate lipid to generate hydroperoxides. One example is shown following,

$$LH + {}^1O_2 \longrightarrow LOOH$$

where the Δ-state $^1O_2$ can react directly with LH to produce LOOH.

Structure of LOOHs

Membrane phospholipids bearing polyunsaturated *sn*-2 fatty acyl side chains could, in principle, can be a target of ROS. The generated products depend on what kind of species of ROS attack. For example, non-radical species $^1O_2$ attack on 1-palmitoyl-2-linoleoyl-sn-glycerol-3-phophocholine (PLPC) to generate four *sn*-2 positioned hydroperoxides, two non-conjugated (10-OOH, 13-OOH) and two conjugated (9-OOH, 13-OOH); however, free radical species HO· attack PLPC to generate only two conjugated 9-OOH and 13-OOH [6]. Like phospholipids, cholesterol can produce two different products depending on which kinds of ROS species attack. The hydroperoxyl products of cholesterol via ROS attack are shown as following.

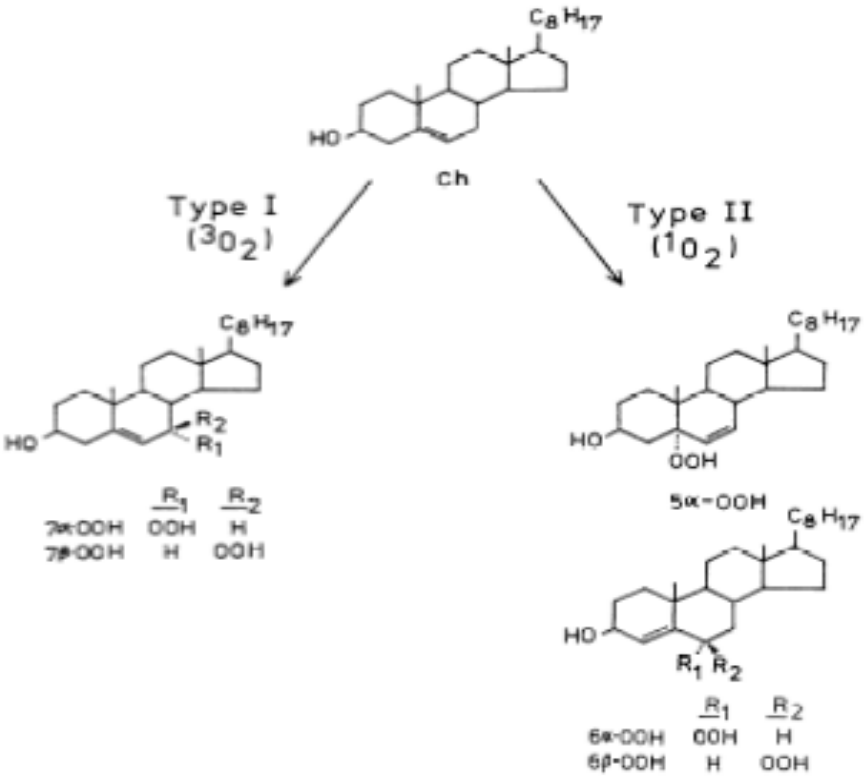
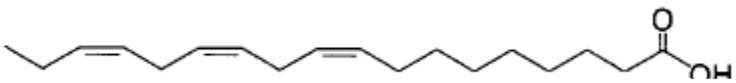


Figure 1. Cholesterol-derived hydroperoxides generated via type I and type II photochemistry [7].

In addition, lipid hydroperoxide can also be in the intermediate products of certain polyunsaturated fatty acid derives such as alpha-linolenic acid (ALA) converted to biological products [8]. These bioconversions responsible are initiated by the oxidative activation of the double bonds, usually in the form of hydroperoxide or endoperoxide intermediate, which result from the action of lipoxygenase (LOXs) and cyclooxygenases (COXs), respectively. And then these intermediates are transformed into flavour and aroma compounds, hormone, etc.



α-Linolenic acid (ALA)

The hydroperoxide products of α-Linolenic acid (ALA):

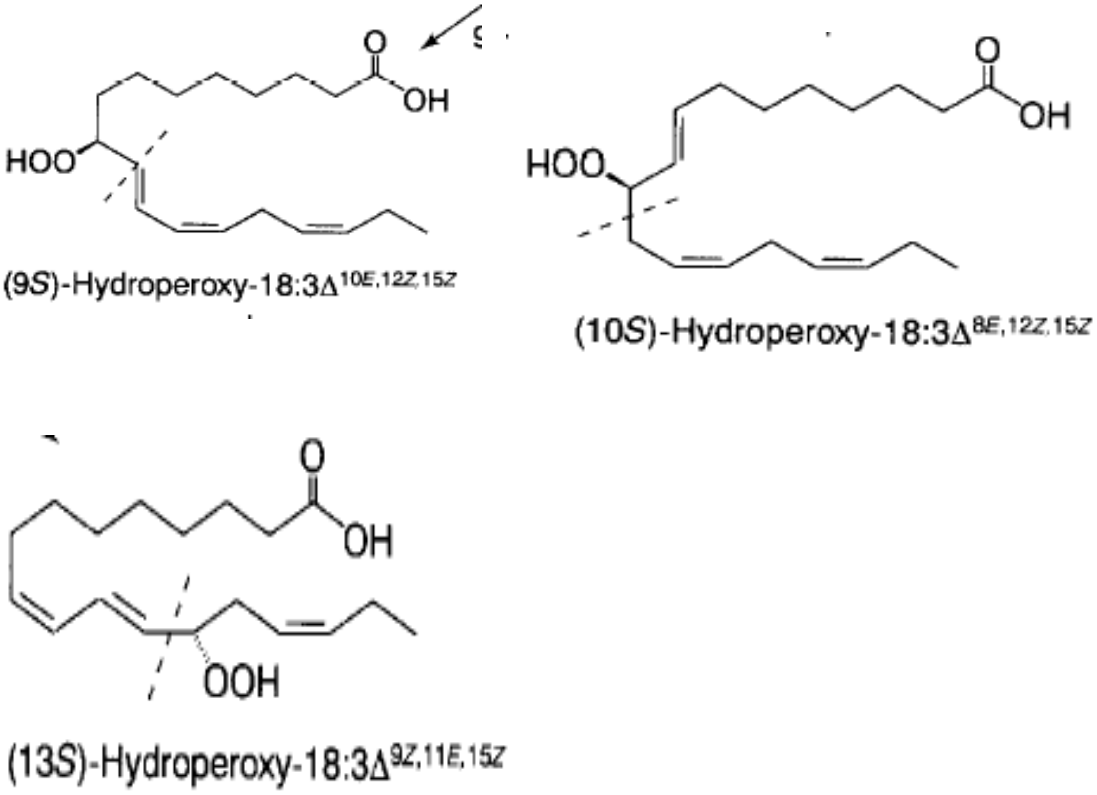


Figure 2. Hydroperoxyl products of α-linolenic acid (ALA)

LOOH metabolism

LOOHs from either cholesterol or phospholipids generated by either free-radical species or non-radical species may either be end-products or reactive intermediate, depending on the reaction system conditions. Accumulated LOOHs tends to perturb

membrane structure/function if systems are lacking reducing agents and/or catalytic (redox-active) iron because LOOHs has greater polarity compared with parental lipids. In the presence of reductants, LOOHs can undergo either one-electron reduction or two-electron reduction (Figure 3) [5]. In the one-electron reduction case, LOOHs are reducted to oxyl radical (LO·) intermediates. LO· can undergo three different changes: (i) direct abstraction of allylic hydrogens and initiation of chain peroxidation; (ii) β-scission with formation of alkyl radicals and aldehydes; (iii) rapid rearrangement and 3O2 addition to give epoxyallylic peroxyl radicals (OLOO·). These products from one-electron reduction of LOOHs are exacerbating lipid peroxidation. In the two-electron reduction case, this is cellular defense process. Three intracellular enzymes have been implicated in detoxification of LOOHs: GSH-peroxidase (GPx1), phospholipid hydroperoxide GSH-peroxidase, and non-seleno GSH-S-transferase (GST). One example is that GPx can convert LOOH to LOH.

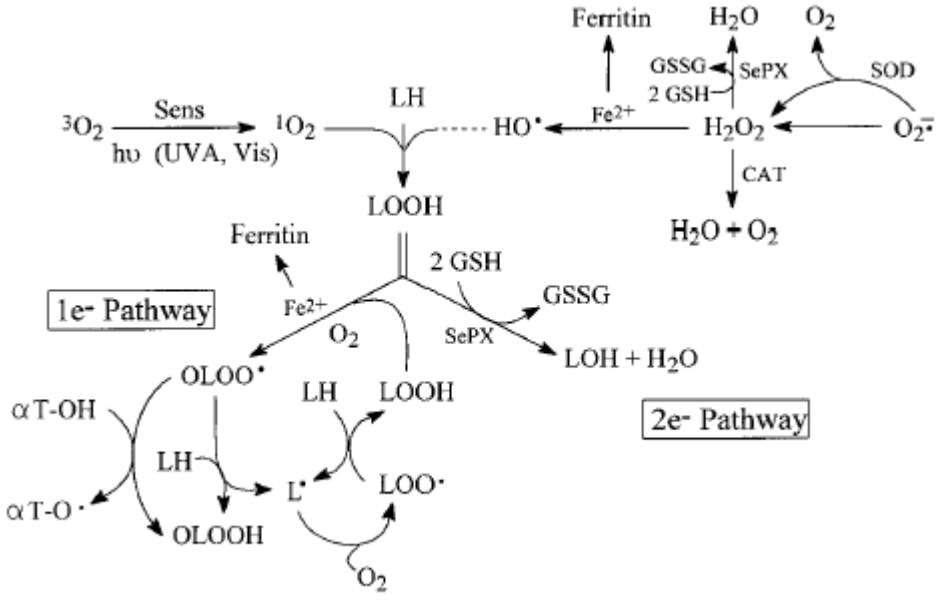


Figure 3. Diagram of important routes of lipid hydroperoxide formation and metabolism.

Detection of LOOH

Variety of techniques can be used to measure LOOHs, ranging from relatively simple "bulk" methods such as thiobarbituric acid assay to sophisticated HPLC-based approaches with extremely high sensitivity and specificity. Chemiluminescence detection HPLC-CL and mercury cathode electrochemical detection HPLC-EC (Hg) have been developed specifically for LOOH separation and detection [9, 10, 11].

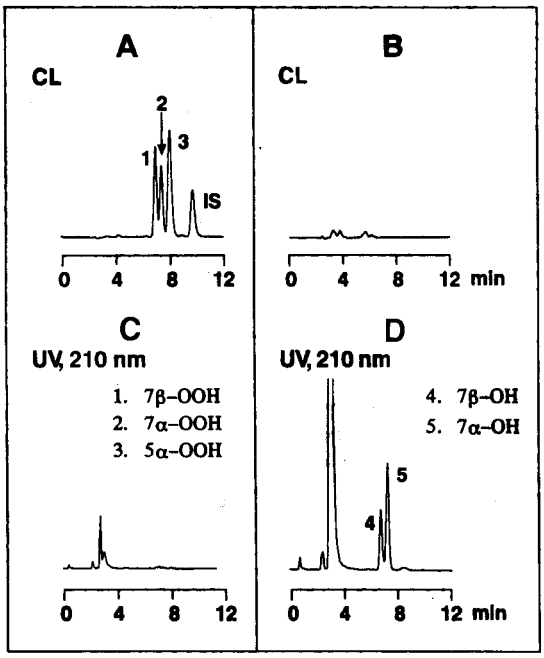


Figure 4. Chromatographic separation of cholesterol hydroperoxides (ChOOH) and corresponding diols in standard mixtures. (A, C) A 10-µl sample containing known ChOOH in methanol. (B, D) A 10-µl sample containing known hydroxycholesterol in methanol. CL, chemiluminescent detection; UV, ultraviolet detection.

For the purpose of illustration, HPLC-CL is discussed here to show how HPLC-CL was used to separate cholesterol hydroperosides (ChOOH) as well as determine CHOOHs [10]. In this process, cholesterol was irradiated with a halogen lamp at 10°C

with oxygen for 8h to generate the hydroperoxide products of cholesterol. Then ChOOHs were separated and determined by reverse-phase HPLC with post-column chemiluminescent (CL) detection.

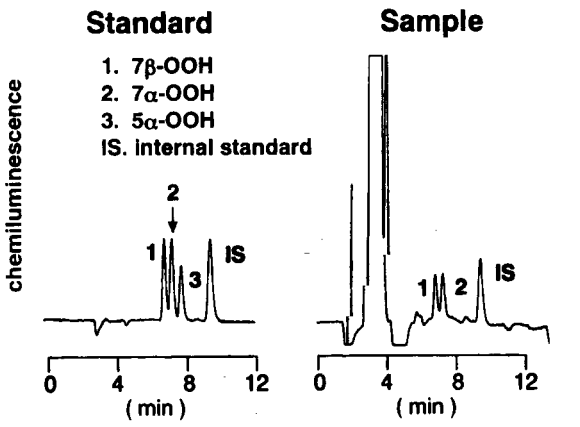


Figure 5. Chromatograms from high-performance liquid chromatography with chemiluminescent detection for the erythrocyte sample from a healthy volunteer, a mixture of standard Ch-OOH, and the IS.

Biological effects of LOOH

The balance of LOOH between one-electron reduction and two-electron turnover in a given cells determine the biological consequence of LOOH. Serious peroxidative damage can be averted if two-electron detoxification of strategic primary LOOHs overtakes one-electron toxicity enhancement. On the other hand, if LOOHs accumulate at a high rate and iron-catalyzed reduction takes hold, then detoxification is overwhelmed and potentially lethal chain peroxidation may be unleashed. The extent of peroxidative injury in stressed cell may determine whether it ultimately survival or succumbs to apoptosis, or necrotic death (Figure 6).

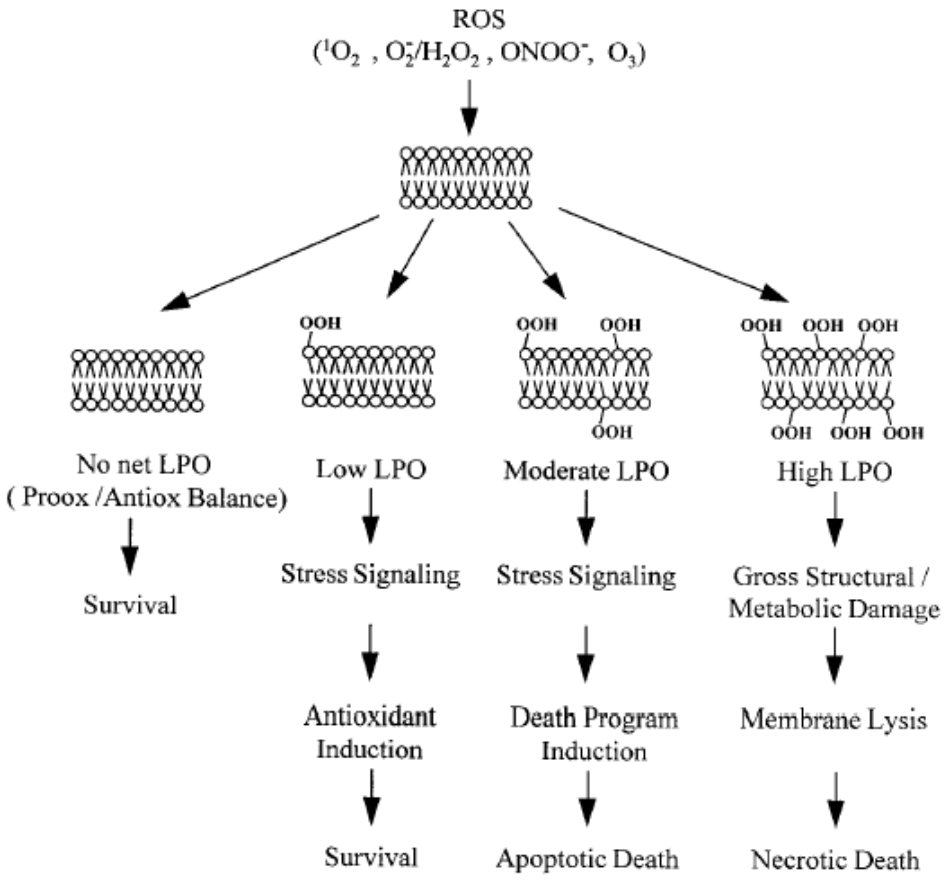


Figure 6. Scheme showing possible levels of lipid peroxidation (LPO) involvement in ROS-induced signal transduction [5].

Summary

Lipid hydroperoxides, the intermediates of peroxidative reactions, are typically much longer-lived than free radical precursors or products. This makes LOOHs potentially more dangerous. Furthermore, LOOH toxicity and effector action could conceivably be manifested far beyond the site of LOOH origin because LOOH can migrate from one membrane to another. And many factors can affect the fate of LOOH, such as the damage expansion of one-electron reaction on one hand and detoxifying reaction of two-electron reaction on the other hand. In addition, LOOH may be involved

in the stress signaling pathway, which may determine whether a cell survives or succumbs to an oxidative damage. In order to understand these rather complex areas, it is very important to know the physiological and biochemical process of LOOHs.

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