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Lipid Hydroperoxide (LOOH) of the Fatty Acid (FA) Nature.

By

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Abbreviations:
CD, conjugated diene
FA, fatty acid
GPx, glutathione peroxidase
LOOH, lipid hydroperoxide
LOX, lipoxygenase
PhGPx, phospholipid hydroperoxide glutathione peroxidase
PUFA, polyunsaturated fatty acid
PV, peroxide value
ROS, reactive oxygen species
UV, ultraviolet

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Abstract:

Lipid oxidation of polyunsaturated fatty acids (PUFAs) leads to the formation of lipid hydroperoxides (LOOHs) as the primary products. LOOHs are one type of reactive oxygen species (ROS) whose biological function is not yet clearly understood. The toxicity of LOOHs in animals has been studied extensively and their elevated levels following instances of cellular injury has been implicated in the disruption of biological membranes, inactivation of enzymes, and damage to proteins and DNA. The increased levels of LOOHs observed in mammalian tissues have also been correlated to the pathogenesis of several disease states such as atherosclerosis and cancer. Based on the toxic implications of LOOH and their probable impact on human health, it is imperative that research into their mechanisms of formation, decomposition and detection be intensely investigated to aid in the etiologic evaluation of disease.

Introduction:

Numerous studies have indicated that uncontrolled oxidation of lipids in biological membranes is a major contributor in several disease states such as atherosclerosis, cancer, and neurodegeneration [9, 12, 14, 15]. Polyunsaturated fatty acids (PUFAs) are predominate target molecules of oxygen radicals in biological membranes. They are characterized by the structural elements, -CH=CH-CH_2-CH=CH-, with one or more double bonds serving as the site for free radical attack.

LOOHs or fatty acid hydroperoxides are the primary products of the oxidation of PUFAs. The elevated levels of LOOHs observed during instances of cellular injury have been correlated to the disruption of biological membranes, inactivation of enzymes, and damage to proteins and DNA [1]. The major biological defense mechanism against LOOHs in mammalian cells is the antioxidant enzymes of the glutathione peroxidase (GPx) family where the phospholipid hydroperoxide glutathione peroxidase (PhGPx) provides the best protection in membranes [10, 11]. These enzymes levels maybe decreased in oxidative stress conditions resulting in further cellular injury or death. [11]. This review paper will discuss the formation, decomposition and detection of lipid hydroperoxides of the fatty acid (FA) lipids.

Lipids and Lipid Oxidation:

Lipids are biological molecules comprising a diverse class of organic compounds that are insoluble in aqueous solutions but are soluble in organic solvents. They are functionally important in biological systems where they serve as structural components of membranes, function as energy reserves, vitamins and hormones, and lipophilic bile acids in lipid solubilization.

As one of the three large classes (i.e., proteins, carbohydrates) of substances found in foods and living cells, lipids are divided into three principal groups that include (FA), triglycerides, and phospholipids. Fatty acids are straight chain organic acids that are of the following types: saturated, monounsaturated and polyunsaturated. PUFAs have two or more *cis* double bonds separated by a single methylene group (-CH=CH-CH_2-CH=CH-).

One striking feature of PUFAs is that they can undergo oxidation in biological systems by a process commonly known as lipid oxidation or lipid peroxidation as it is sometimes called in biological circles [8, 13]. Lipid oxidation is a free radical mechanism that observes a long induction period where the rate is too small to be measured followed by a rapid increase in production as the reaction progresses [5].

The rate of oxidation of PUFA increases with the degree of unsaturation [6], and this increased susceptibility to oxidation is based on the double bonds in the FA that weaken the C-H bond on the allylic carbons enabling easier hydrogen atom ($^{\cdot}$H) removal [16]. The reactivity of PUFA can be best explained by their electronic structure and bonding properties. Unsaturated fatty acids possess pi (π) bonds that are weaker and of lower energy because the π electrons are less firmly held between the two nuclei and are more exposed. The π bond exerts a nucleophilic character to the double bonds of PUFA allowing them to react with electrophilic reagents.

Fatty Acid Hydroperoxide Formation:

Fatty acid hydroperoxides are formed through three different mechanisms: autoxidation, photo-oxidation and enzymatic oxidation [13]. These mechanisms can occur separately or simultaneously.

a. Autoxidation:

Autoxidation is defined as a chemical reaction that usually takes place at ambient temperatures involving atmospheric O_2 and organic compounds [5]. It is considered to be a free radical chain reaction that can be divided into three separate processes: initiation, propagation, and termination. These steps have been reviewed by several authors [5, 8, 10, 13] and are listed as follows:

Initiation:	$LH + X^{\cdot} \rightarrow L^{\cdot} + XH \quad (R_i)$	(1)
Propagation:	$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \qquad k_o = 3 \times 10^8\ M^{-1}\ s^{-1}$	(2)
	$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad k_p = 10\text{-}50\ M^{-1}\ s^{-1}$	(3)
Termination:	$LOO^{\cdot} + LOO^{\cdot} \rightarrow \qquad k_t = 10^6 - 10^7\ M^{-1}\ s^{-1}$	(4)
	$LOO^{\cdot} + L^{\cdot} \rightarrow$ (non-radical products) k'_t	(5)
	$L^{\cdot} + L^{\cdot} \rightarrow \qquad k''_t$	(6)

During the initiation step, a lipid radical ($L^{\cdot}$) is formed from an unsaturated fatty acid (LH) (Reaction1) with an initiator X Several processes have been suggested as initiators of autoxidation reactions such as thermal or hemolytic cleavage of an L-H bond, $^{\cdot}H$ abstraction from L-H by an initiator radical [13], and the reaction of FAs with metal ions producing radicals [5, 10]. The rate of initiation, Ri, is considered the overall rate at which chains are started.

Once formed the lipid radical ($L^{\cdot}$) reacts very rapidly with O_2 to form a lipid peroxyl radical ($LOO^{\cdot}$) (Reaction 2) as the first propagation step. The rate constant k_o represents the oxygenation step which is a fast reaction. In the second propagation step the peroxyl radical ($LOO^{\cdot}$) abstracts a $^{\cdot}H$ form the allylic or *bis* allylic position of PUFA (LH) to generate a lipid hydroperoxide (LOOH) and another lipid radical ($L^{\cdot}$) (Reaction 3). This is the kinetically more important step and is also the rate limiting step in the autoxidation process. The rate constant, k_p, is known as the propagation determining constant [16].

Three termination steps (Reactions 4-6) are possible where k_i is the chain termination constant. At normal O_2 partial pressure (100 mm Hg), Reaction 2 occurs so fast that any termination reactions involving $L^{\cdot}$ are not significant. The most important termination reaction is that of $LOO^{\cdot} + LOO^{\cdot}$ (Reaction 4).

A proposed mechanism for the formation of LOOH from PUFA has been developed using oleic acid as a model (Figure 1). In the first step, a $^{\cdot}H$ is abstracted from positions 8 and 11 of oleic acid producing two allylic radicals. The addition of O_2 to the allylic radicals gives rise to four peroxyl radicals, 11-*cis*, 9-*trans*, 8-*cis*, and 10 *trans*. The peroxyl radicals can rearrange to 11-trans and 8-trans peroxyl radicals. Hydroperoxides result from peroxyl radicals undergoing $^{\cdot}H$ abstraction from oleic acid or another unsaturated FA in the vicinity.

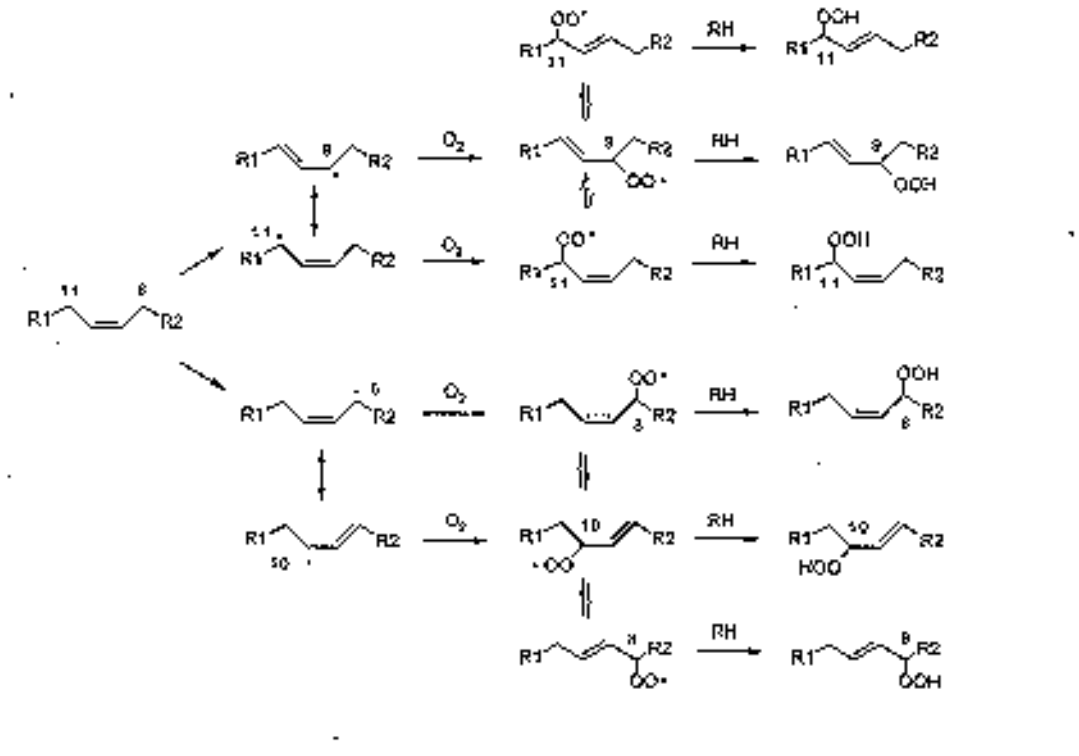

Figure 1. Formation of oleic acid hydroperoxides (Adapted from 13)

b. Photo-oxidation

Exposure to light accelerates the formation of hydroperoxides from PUFA. This acceleration could be due to photochemical or photosensitized oxidations [3, 8]. In photochemical oxidation, or direct oxidation, free radicals are generated from PUFA during exposure to ultraviolet (UV) irradiation (Reaction 7). This reaction is of little significance since light absorptions at wavelengths less than 220 nm are unable to reach lipids unless they are left unprotected in direct sunlight or exposed to fluorescent light.

$$LH \xrightarrow{hv} L^{\cdot} + H^{\cdot} \qquad (7)$$

Photosensitized oxidation occurs in the presence of photosensitizers (i.e. chlorophyll, hameproteins, riboflavin and synthetic dyes) and visible light [8]. Sensitizers have two excited states, the singlet (^{1}sens) and the triplet (^{3}sens). The ^{3}sens sensitizer has a longer half-life and is able to initiate photo-oxidations. There are two types of processes that govern the actions of the ^{1}sens and ^{3}sens sensitizers and they are divided into Type 1 and Type 2 reactions. In Type 1 reactions the sensitizer in the triplet state (^{3}Sens) reacts with FA substrate (LH) by hydrogen atom or electron transfer to form radicals (intermediates), which then react with O_2 to form hydroperoxides (Scheme 1).

$$^3\text{Sens} + \text{LH} \xrightarrow{hv} (\text{intermediate}) + O_2$$
$$\downarrow$$
$$\text{hydroperoxides} + \text{Sens} \qquad \text{(Scheme 1)}$$

In the Type 2 reaction, the triplet sensitizer (^{3}Sens) reacts with O_2 by energy transfer to generate singlet oxygen ($^1O_2$) which further reacts with PUFA (LH) to produce hydroperoxides (Scheme 2).

$$^3\text{Sens} + O_2 \xrightarrow{hv} (\text{intermediate}) + {}^1\text{Sens} \rightarrow {}^1\text{O2}$$
$$\downarrow \text{LH}$$
$$\text{hydroperoxides} \qquad \text{(Scheme 2)}$$

c. Enzymatic Oxidation

Lipoxygenases (LOX) are found widely in plants, fungi and animals. They catalyzed the controlled peroxidation of PUFA to give hydroperoxides [4]. LOXs contain one iron per molecule of enzyme. The ferric form (LOX-Fe^{+3}) of the enzyme is responsible for oxidizing the PUFA (RH) to form a pentadienyl radical (R·). The R· reacts with O_2 to form ROO· which abstracts a ·H to form ROOH (Figure 2).

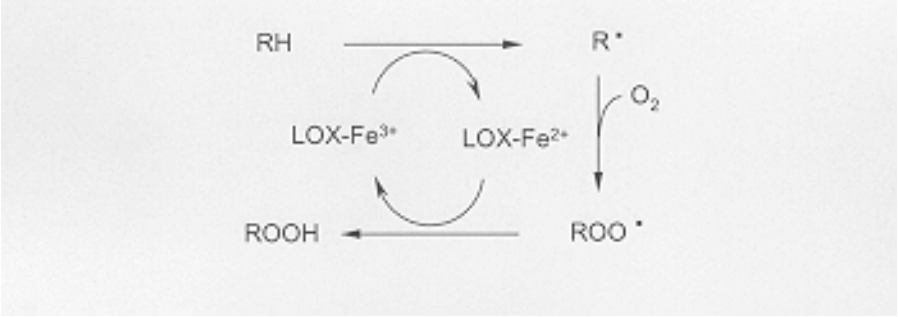

Figure 2. Lipoxygenase action. (Adapted from 4).

Decomposition of Lipid Hydroperoxides:

Hydroperoxides are relatively stable under favorable conditions such as low temperature, dilute solution, in the presence of antioxidants and in the absence of catalyst. Under normal circumstances, this is usually not the case and LOOHs become susceptible to chemical changes based on their environment. LOOH can decompose through homolytic free radical reactions for instance where the formation of either a peroxyl (Reaction 8) or alkoxyl radical (Reaction 9) is formed.

$$LOOH \rightarrow LOO\cdot + H \quad (8)$$
$$LOOH \rightarrow LO\cdot + \cdot OH \quad (9)$$

The formation of LOO· is a reversible reaction whereas the LO· is irreversible and will lead to the formation of decomposition products.

Detection Methods for Lipid Hydroperoxides:

Most of the detection and measurement techniques for assessing lipid oxidation are centered on the determination of the secondary end products and do not concentrate on the primary end products. There are however some methods that will allow LOOHs to be detected and may serve as more suitable indicators when elucidating mechanisms of cellular injury.

a. Peroxide Value (PV):

Hydroperoxides are the main products of lipid oxidation. They can be measured based on their abilities to liberate iodine (I_2) from potassium iodide (KI). The theory behind this method is that the oxidized sample is dissolved in a chloroform acetic acid mixture and KI is added as a reducing agent. The liberated I_2 is titrated with sodium thiosulfate ($Na_2S_2O_3$) to a starch endpoint (Scheme 3) [2].

$$2KI + 2CH_3CO\text{-}OH \rightarrow 2HI + 2CH_3CO\text{-}OK$$
$$LOOH + 2HI \rightarrow LOH + H_2O + I_2$$
$$I_2 + Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \qquad \text{(Scheme 3)}$$

The PV value is expressed as milliequivalents of I_2 per kilogram (MEQ/kg) of lipid or as millimole of hydroperoxide per kg lipid (mmol/kg). PV as (MEQ/kg) = 2 x (mmol/kg). The method has a tendency to generate data that is not reproducible due to interferences from O_2 in the air and light exposure. The results from a study in large part are dependent upon the experience and training of the end user. Under astringent conditions, it could be a reliable method.

b. Conjugated Dienes:

The oxidation of PUFAs results in conjugated diene (CD) hydroperoxide structures with a double bond-single bond-double bond arrangement. The conjugated double bond system is more stable than the isolated double bond based on the bond dissociation energies of 126kJ/mol

versus 111 kJ/mol. The CD products absorb UV light in the wavelength range of 230-235 nm and can be determined spectrophotometrically by their maximum absorbance at 234 nm. An extinction coefficient of 24000 M^{-1} cm^{-1} makes this a sensitive way of quantifying conjugated hydroperoxides. In principle, a weighed sample is diluted in alcohol, (methanol for esters and isooctane for triacyglycerols) [7]. The CD value of lipids is quantified by their UV absorbance at 234 nm and is expressed as umol hydroperoxides/g sample. This method is sensitive and is most applicable during the early stages of lipid oxidation where there is little or no decomposition.

Conclusion:

Research has shown that lipid hydroperoxide formation from the oxidation of unsaturated fatty acids produces deleterious effects on the cellular level. Uncontrolled lipid peroxidation has been implicated in the pathogenesis of several disease states such as cancer [12], atherosclerosis [9], Alzheimer's [14] and the aging process [15]. Studies into the lipid hydroperoxide formation have been extensive over the years with well defined kinetics [16] of the autoxidation reactions of PUFAs.

The product formation involving lipid oxidation can be very complex and is not limited to LOOHs making the evaluation of damage assessment and the mechanisms difficult. The key to understanding the mechanisms of injury related to the toxicity of LOOHs will be strongly related to the detection and measurement techniques currently available and still under investigation. There are no stand alone methods for assessing damages caused by lipid peroxidation, therefore; several approaches should be undertaken to aid in the etiologic evaluation of disease.

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