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The Fine Print:

Peroxyl Radical And Lipid Peroxidation

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Abbreviation List:

$L^{\bullet}$	lipid alkyl radicals
$LO^{\bullet}$	lipid alkoxyl radicals
$LOO^{\bullet}$	lipid peroxyl radical
LOOH	lipid hydropeoxides
$O_2^{\bullet-}$	superoxide
PUFA	polyunsaturated fatty acids
ROS	reactive oxygen species
TBARS	thiobarbituric acid reactive substances

Outline

Abstract

Lipid is an important component of the membrane surrounding cells and cellular organelles. Polyunsaturated fatty acids can be attacked by O_2 and form lipid peroxyl radicals. The whole process involves several steps: initiation, propagation, and termination. Iron is involved in this process and plays an important role. The possible sources of iron are those iron containing proteins such as ferritin, transferrin and lactoferrin. A lot of methods are available to measure the formation form lipid peroxyl radicals.

Introduction

Polyunsaturated fatty acids are important components of the membrane surrounding cells and cellular organelles. They form a double layer structure of the membrane. They are arranged in a way that the non-polar tail faces towards the inner side of the double layer structure.

Lipid peroxidation has received much attention recently because of its possible contributions to cancer and aging. It is the oxidative deterioration of polyunsaturated fatty acids (PUFA), by which polyunsaturated fatty acids are converted to lipid hydropeoxides (LOOH). Lipid peroxidation is a free radical-related process. Oxidants can react with polyunsaturated fatty acids in cell membranes, to form toxic metabolites. Lipids that contain two or more unsaturated carbon-carbon bonds (C=C) can be attacked by reactive oxygen species (ROS).

Formation of LOO•

Initiation. Initiation of lipid peroxidation occurs when a radical species with significant oxidizing character, such as the hydroxyl radical (•OH), removes an allylic hydrogen from a polyunsaturated fatty acid (PUFA). The removal of the allylic hydrogen from the PUFA forms a lipid radical (L•). An immediate rearrangement occurs, forming a more stable lipid radical, whose dienes are conjugated [1]. In an aerobic environment this radical reacts with oxygen, giving rise to a lipid peroxyl radical (LOO•). Because of its highly reactive properties, hydroxyl radical can react nonselectively with unsaturated fatty acids to form a carbon-centered radical, either by addition to double bonds or by abstraction of an hydrogen atom from the system [2].

$$\text{L-H} + {}^{\bullet}\text{X} \rightarrow \text{L}^{\bullet} + \text{XH}$$

Propagation. Carbon-centered radicals, which are produced from both the initiation and propagation processes, undergo molecular rearrangements. Lipid peroxyl radical abstracts an allylic hydrogen atom from another lipid molecule such as an adjacent PUFA, resulting in a lipid hydroperoxide (LOOH) and a second lipid radical (L•). This second lipid radical can proceed

through the same reactions as the first, generating additional lipid hydroperoxides. The LOO• radicals are able to subtract a hydrogen atom from another lipid molecule such as an adjacent fatty acid. This causes the propagation of the lipid peroxidation. The carbon radical formed can further undergo the oxygen addition reaction to form another peroxyl radical and so the chain reaction of lipid peroxidation continues. The peroxyl radical combines with the hydrogen atom it abstracts, giving a lipid hydroperoxide.

$$LOO^{\bullet} + L\text{-}H \rightarrow LOOH + L^{\bullet}$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

Termination. The termination event can be the result of any reaction with another radical, protein, or compound that acts as a free radical trap, forming a stable end product. The hydroperoxide produced may undergo different reactions to terminate the lipid peroxidation process. They may be reduced to a hydroxy fatty acid or undergo cyclization to produce cyclic endoperoxides [3]. These endoperoxides can start the reactions to form several biologically active prostaglandins, thromboxanes and leukotrienes via the cyclooxygenase and lipoxygenase pathways. Non-enzymatic pathways lead to the formation of compounds such as isoprostanes, aldehydes and alkanes, which can also have concentration-dependent signaling or cytotoxic effects *in vivo* [4]. Formation of these end-products constitute the termination stage of lipid peroxidation. Since PUFAs can have a number of C – H bonds susceptible to free radical attack, several end products can be generated from each PUFA during lipid peroxidation.

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{Non-radical products}$$

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These processes can be illustrated in figure 1 [12].

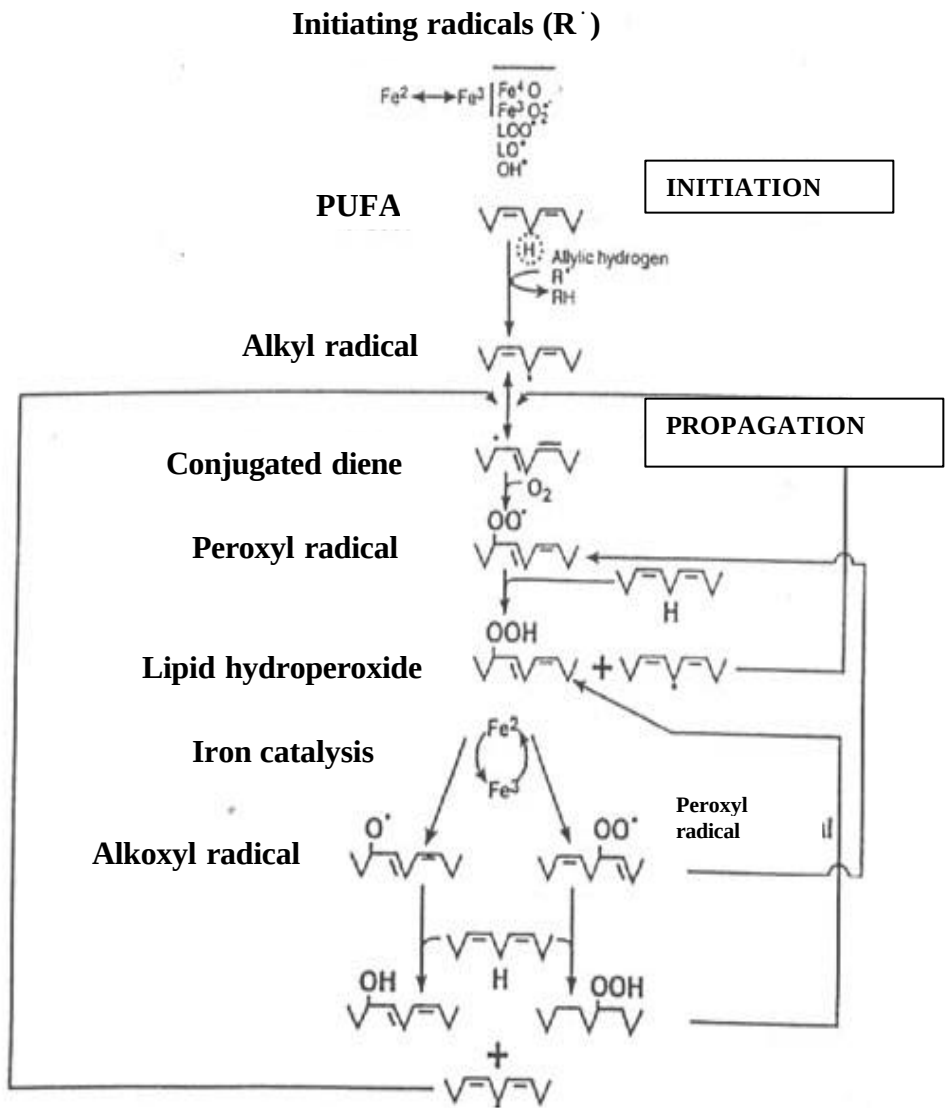

Figure 1. Chemistry of the initiation and propagation phases of lipid peroxidation. [12]

Involvement of iron LH composing biological membranes can be oxidized to LOOH as a consequence of cell disruption. The concentration of preexisting LOOH strongly influences the formation of new LOOH [5]. When the preexisting LOOH concentration is low, new LOOH will be produced predominantly by reactive species that have enough energy to break an allylic

C-H bond of LH. Hydrogen abstraction and lipid alkyl radicals ($L^{\bullet}$) formation are involved in this process. When the preexisting LOOH are abundant, new LOOH will be produced predominantly by an iron-catalyzed decomposition of LH to highly reactive lipid alkoxyl radicals ($LO^{\bullet}$), which then form $L^{\bullet}$ radicals by hydrogen abstraction from LH. In both cases, Fe(II) is required.

The reaction could be:

$$LOOH + Fe(II) \rightarrow LO^{\bullet} + Fe(III)$$

Possible sources of Fe(II) are those iron-binding proteins such as ferritin, transferrin and lactoferrin [6]. Because these proteins have higher affinity for Fe(III) than for Fe(II), they bind Fe(III) rather than Fe(II). Upon encountering with suitable reductants, they release iron in form of Fe(II) [7]. These suitable reductants may be superoxide ($O_2^{\bullet -}$) [8] and ascorbate [9].

Removal of lipid peroxyl radicals

Cells have a number of ways to protect against the constant threat of the radicals produced in lipid peroxidation. There are enzymatic species that act to minimize the detrimental effect of radicals. These enzymes include superoxide dismutase, glutathione peroxidases, and catalase [10]. Also, there are numerous removal and repair enzymes, such as phospholipase A2, are available to repair the damaged molecules [11]. Cells are also known to utilize many non-enzymatic antioxidant compounds which react directly against oxidizing agents. Two of the major antioxidant compounds that have received considerable attention are vitamin E (alpha-tocopherol) and vitamin C (ascorbic acid) [12].

Alpha-tocopherol, a fat-soluble vitamin, is considered to be the major membrane-bound antioxidant used by the cell. On the other hand, ascorbic acid is regarded as the major aqueous-phase antioxidant. Recent evidence suggests that alpha-tocopherol and ascorbic acid function together in the cyclic-type of reaction seen in figure 1 [13]. During this process, alpha-tocopherol is converted to a radical by donating a labile hydrogen to a lipid peroxyl radical. The oxidized alpha- tocopherol radical is energetically stable and has low reactivity with other molecules within the membrane. Oxidized alpha-tocopherol can then be re-reduced to its original form by ascorbic acid. This regeneration of reduced alpha-tocopherol presumably occurs at the surface of the membrane where ascorbic acid and alpha-tocopherol can meet. Along with acting as a reducing agent for alpha-tocopherol, ascorbic acid is also considered a preventative antioxidant because of its ability to scavenge for reactive radicals [13].

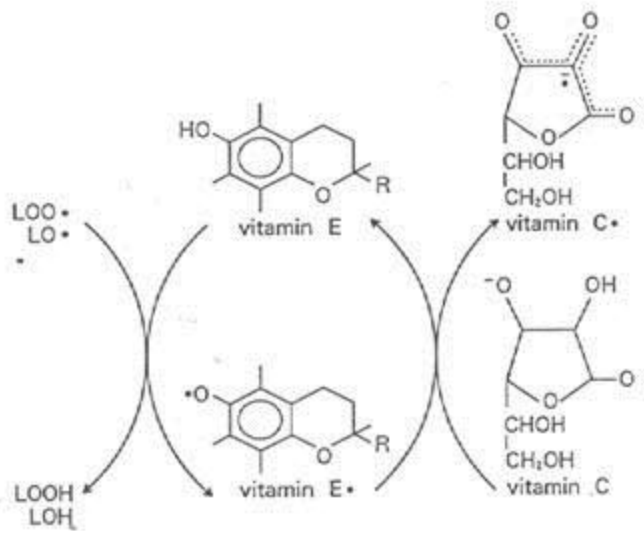

Figure 2. Chain reaction of vitamin E and vitamin C with lipid peroxyl radical [13].

Measurement

There is a range of methods available for measurement of markers of lipid peroxidation, whether by measuring lipid hydroperoxides, or degradation products. However, a number of problems need to be resolved before we feel confident in these measurements. These problems include variability of the standards used. And small differences in the technique used can have a huge effect on the result achieved.

Evidence for damage to lipids *in vivo* is obtained by measurement of peroxides or isoprostanes in blood and urine. The major problem which needs to be considered is to distinguish peroxides formed as a consequence of in vivo oxidative stress and those ingested from dietary sources. The levels of peroxide in cells and tissues present a balance between peroxide formation and peroxide metabolism or decomposition. That is, they exist in a steady state.

A number of methods are available to measurement levels of lipid hydroperoxides. It can be measured by HPLC following dissolving the hydroperoxide into a polar solvent, which can separate between less polar triacylglycerol and cholesterol hydroperoxides and the more polar free fatty acids and phospholipid hydroperoxides [14].

Until recently, the thiobarbituric acid reactive substances (TBARS) test was probably the most utilized assay for measuring lipid peroxidation. The process of the TBARS test is relatively simple. TBA plus acid are added to the material under study, perhaps with added metal ions, chelating agents or other reagents. Then the mixture is heated to 100 degrees Celcius. Then the formation of a pink color is measured at 523 nm. The simplicity to perform the test conceals its essential complexity. The apparent "TBA reactivity" varies with the exact concentration of acid,

type of acid and period of heating used in the TBA assay [15]. The values obtained depend on such factors as the iron salt concentration. And to get the changes in the absolute values for the "TBA reactivity", the same assay has to be employed in the same way each time.

Summary

Peroxidation of lipids causes damages to cell membrane because lipids are the main component of the cell membrane. Its initiation occurs when a radical species attacks and removes an allylic hydrogen from an unsaturated fatty acid, resulting in a radical chain reaction. Once lipid peroxidation begins, iron may participate in driving the process. The possible sources of iron in body are those iron-containing proteins. There are a number of methods available to measure the markers of lipid peroxidation, either by measuring the formation of products, or the degradation of reactants. The most utilized method is TBARS test.

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