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DANGER: LIPID ALKOXYL RADICALS
Detrimental Biochemical Implications

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Abbreviations:
^{t}BuOOH, *tert*-butyl hydroperoxide; DMPO, 5,5-dimethyl-pyrroline-1-dioxide, EPR, electron paramagnetic resonance; 4-HNE, 4-hydroxy-2-nonenol; LH, lipid; LO$^{\bullet}$, alkoxyl radical; LOO$^{\bullet}$, peroxyl radical; LOOH, lipid hydroperoxide; LPO, lipid peroxidation; POBN, α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone; PUFA, polyunsaturated fatty acid,

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Abstract

The oxidative deterioration of lipids, also known as lipid peroxidation, contributes to the development and progression of many diseases such as inflammation, ischemia/reperfusion, atherosclerosis, and cancer. In biological systems, polyunsaturated fatty acids that possess two or more *bis*-allyic carbon-carbon double bonds are often the target of electron extraction and initiate lipid peroxidation. The propagation of the lipid peroxidation cycle produces alkoxyl radicals non-enzymatically *via* a Fenton reaction, a one-electron reduction, or the combination between two peroxyl radicals. Alkoxyl radicals are highly oxidizing (high $E^{o'}$ value) consequently the direct and indirect reaction of the alkoxyl radical with surrounding organic material often has dire biochemical implications. For example, alkoxyl radicals can react with DNA bases to form heptanone-ethano-adducts and failure to repair these DNA lesions can lead to mutations and apoptosis [1]. Due to the importance of these implications many techniques have been developed to study and measure the alkoxyl radical.

Introduction

The reaction between molecular oxygen and lipids (LH) and the subsequent formation of lipid hydroperoxides (LOOH) is commonly referred to as lipid peroxidation (LPO) [2]. Polyunsaturated fatty acids (PUFAs), fatty acids, such as docoshexenoic acid, that contain two or more *bis*-allylic double bonds, are highly susceptible to oxidative deterioration. LPO has been shown to participate in many diseases including inflammation, ischemia/reperfusion, vascular disease [3,4,5,6], Alzheimer's disease, and aging [7]. It also plays a crucial role in the mechanisms of various treatments such as photodynamic therapy of cancer, therapeutic hyperthermia, and chemotherapy of cancer. The mechanism of these treatments is due to the radicals produced during the initiation and propagation phases of LPO cycle.

Both peroxyl ($LOO^{\bullet}$) and alkoxyl ($LO^{\bullet}$) radicals are highly reactive making them dangerous to cell. The lipid alkoxyl radical can attack DNA or other surrounding organic molecules inducing irreparable damage to cells [8]. The aim of this work is to illustrate how alkoxyl radicals are formed, highlight their bio-chemical implications, and discuss how alkoxyl radicals can be detected.

Formation of Alkoxyl Radicals

In biological systems, Alkoxy radicals can be formed either enzymatically or non-enzymatically. The enzymatic triggers of peroxidation are cycloxygenases, oxidases, lipoxygenases, peroxidases, and NADPH-cyt P_{450} reductases.

The non-enzymatic formation of an alkoxyl radical can occur three ways: a LOOH-derived Fenton reaction, a reductive cleavage, or combination of two peroxyl radicals. The LOOH-derived Fenton reaction is the most likely mechanism to form an alkoxyl radical and occurs when a Fe^{2+} reduces a lipid hydroperoxide forming Fe^{3+} and $LO^{\bullet}$. This reaction, detailed in **Figure 1** [9]**,** not only produces $LO^{\bullet}$ but also OH^{-}. Iron (II) is used as the initiator of LPO in this model however it should be noted that other substances are capable of extracting an electron

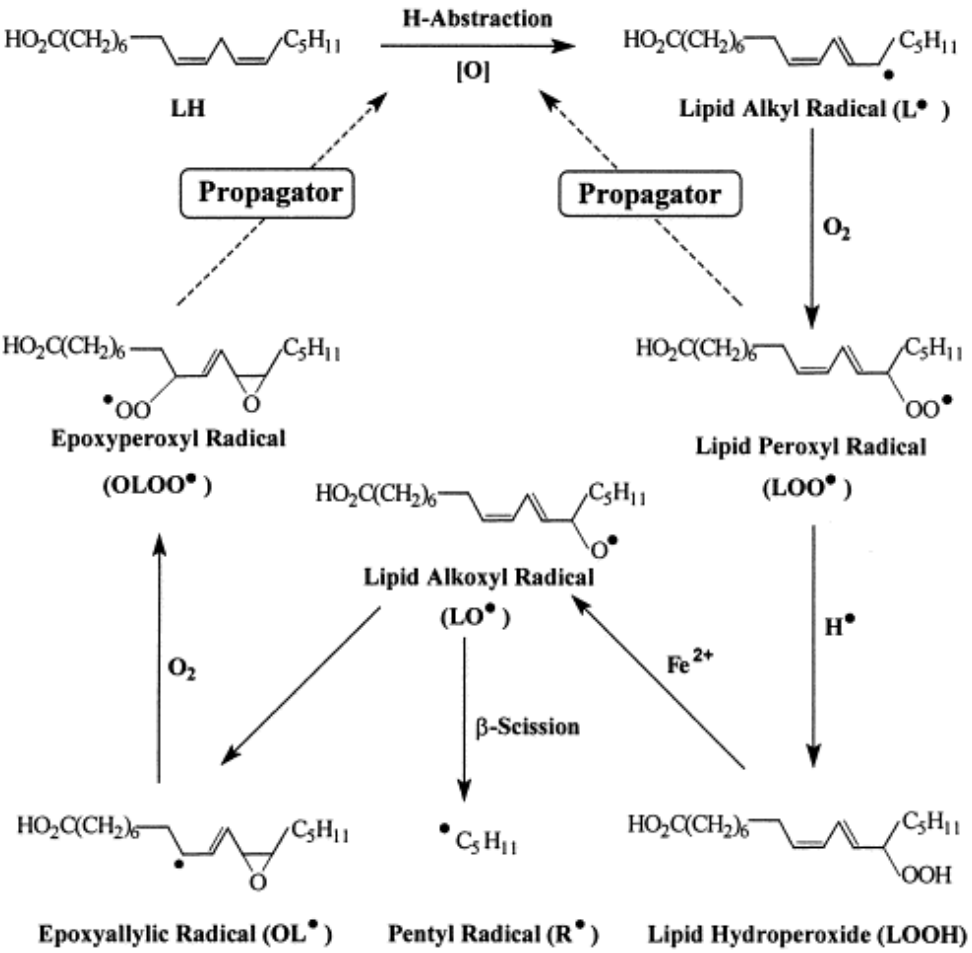

Figure 1. An overview of the chemistry of the formation of lipid-derived radicals ($L_d^{\bullet}$) produced during lipid peroxidation in the presence of ferrous iron. This scheme shows some of the radical species formed in the peroxidation of linoleic acid. Three different propagating species are shown. It is currently thought that $OLOO^{\bullet}$ may be the major propagating species in this type of system. The species $LO^{\bullet}$ is thought to be a very minor propagating species because of its very short lifetime. It is estimated that the rate constant of cyclization of $LO^{\bullet}$ would be ~$2 \times 10\ s^{-1}$ while the rate constant for β-scission would be ~$1 \times 10\ s^{-1}$. (This figure and text

from the lipid. This includes but is not limited to ionizing radiation, copper, hydroxyl radicals, and other lipid derived radicals.

The formation of an alkoxyl radical *via* a one electron reduction requires the presence of a transition metal. However it is believed that this decomposition of LOOH, as shown in **Reaction 1**, is thought to be a minor reaction.

Reaction 1 $\mathrm{LOOH + Fe^{2+} + 1e^{-} \longrightarrow LO^{\bullet} + OH^{-} + Fe^{3+}} \quad k = 3.2 \times 10^{2}\ \mathrm{M^{-1}\ s^{-1}}$

Biologically, both the single electron and the transition metal have many possible sources. One possible electron donor has recently been shown to be ascorbate (Vitamin C) [10]. Iron (II) is once again shown in this reaction however copper is also another possible source.

Another possible mechanism in the formation of the alkoxyl radical is the reaction between two $LOO^{\bullet}$ radicals. However, it should be noted that in order for this reaction, as shown in **Reaction 2**, to occur a highly peroxidizing environment is required [11].

Reaction 2 $$2LOO^{\bullet} \xrightarrow{\text{radical decay}} 2LO^{\bullet} + {}^{1}O_2$$

The rate constant (k) for this reaction is 3 x 10^8 M^{-1} s^{-1}.

Biochemical Implications

Alkoxyl radicals have an $E^{o'}$ value of 1600 mV at pH 7 which makes them good oxidizing agents compared to $ROO^{\bullet}$ which has an $E^{o'}$ of 1000 mV [12]. **Table 1** compares the electron potential of the alkoxyl radical to the hydroxyl radical, peroxyl radical, vitamin E, and iron (III). The alkoxyl radical has the ability to oxidize means that the radical can abstract a $H^{\bullet}$ from other molecules (an important step in the propagation of LPO) or undergo a rapid molecular rearrangement to other radical species [13]. **Reaction 3** illustrates the reaction between an alkoxyl radical and a lipid resulting in an alkoxide and a lipid radical.

Table 1.

Couple	$E^{o'}$ / mV
$HO^{\bullet}$, H^+ / H_2O	2310
$RO^{\bullet}$, H^+ / ROH	1600
$ROO^{\bullet}$, H^+ / ROOH	1000
$TO^{\bullet}$, H^+ / TOH (Vitamin E)	500
Fe(III) / Fe(II) (aqueous)	110

This table is adapted from Buettner 1993.

Reaction 3 $$RO^{\bullet} + LH \longrightarrow ROH + L^{\bullet} \qquad k = 1.1 \text{ x } 10^7 \text{ } M^{-1} \text{ } s^{-1}$$

Reaction 4 $$LOOH + RO^{\bullet} \longrightarrow LOO^{\bullet} + ROH$$

Reaction 5 $$LO^{\bullet} \longrightarrow OL^{\bullet} \qquad k = 2 \text{ x } 10^7 \text{ } M^{-1} \text{ } s^{-1}$$

The reaction depicted in **Reaction 4** illustrates the possibility of an alkoxy radical reacting with a lipid hydroperoxide forming a peroxyl radical as well as an alkoxyl radical. The formation of $L^{\bullet}$ and $LOO^{\bullet}$ can continue to propagate lipid peroxidation. **Reaction 5**, also shown in Figure 1**,** shows the reconfiguration of the oxygen-centered alkoxyl radical to a carbon centered radical. The rate constant of this cyclization reaction is 2 x 10^7 s^{-1} while the β-scission, shown in Figure 1 is approximately 1 x 10^6 s^{-1} [9]. This means that 95% of the alkoxyl product is converted to the epoxyallylic radical ($OL^{\bullet}$) making it the major product.

Lee *et al.* (2001) have demonstrated that when the $LO^{\bullet}$ is attached to a PUFA then an intramolecular radical propagation reaction readily occurs creating α,β-unsaturated aldehyde genotoxins, such as 4-hydroxy-2-nonenal (4-HNE) [10]. The Michael addition of nitrite ($-NH_2$) to a double bond or a Schiff base formation with a –CHO group can cause DNA adducts, protein adducts, and phospholipids adducts [13,14].

Detection of Alkoxyl Radicals

Pulse radiolysis or flash photolysis has been used to study the kinetics of alkoxyl radicals in both polar and non-polar environments. Alkoxyl radicals derived from *tert*-butyl hydroxide (^{t}BuOOH) in aqueous solutions is a simple model system used to measure $LO^{\bullet}$ in a polar environment [8]. Alkoxyl radicals have a low molar absorption making direct spectroscopical analysis difficult; however $LO^{\bullet}$ can be measured via their reaction with other oxidizable substrates. This is an indirect method that measures either the loss of the substrate or the formation of the product.

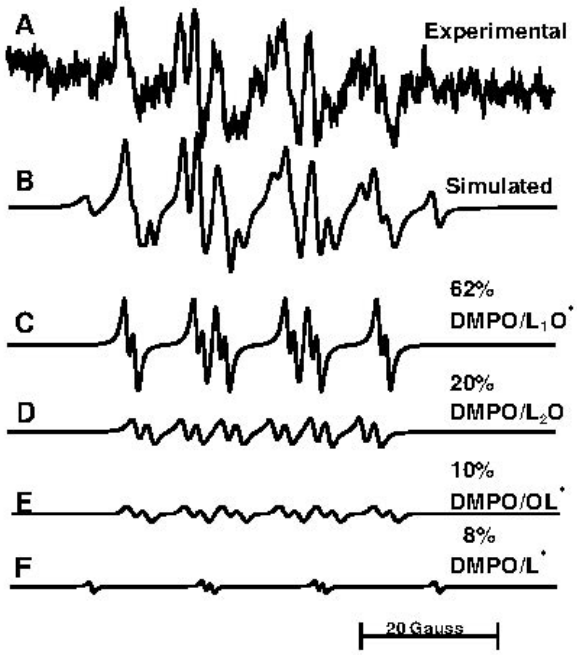

Figure 2. EPR spectrum of DMPO/lipid radical adduct derived from the Fe^{2+}-mediated oxidation of DHA in aerobic conditions. This figure shows both experimental and simulated including the contribution of different radicals, including the $LO^{\bullet}$ in part C. (Figure is modified from Venkataraman *et al*. (2004))

Alkoxyl radicals can be measured using electron paramagnetic resonance (EPR). An example of an EPR alkoxyl radical signal is shown in **Figure 2**. EPR has been used to directly measure radicals produced from both linoleate and arachidonate, unfortunately this is not necessarily possible in cells. Consequently, a spin trap is required. α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone (POBN) is a good spin trap for carbon centered radicals while 5,5-dimethyl-pyrroline-1-dioxide (DMPO) is a good spin trap for oxygen centered radicals. The combination

of these two spin traps can provide information about the radicals, including $LO^{\bullet}$, produced through LPO [15].

Since the $LO^{\bullet}$ easily reacts proteins or other PUFAs it is also necessary to detect these genotoxic products. Both Lee *et al.* (2001) and Sowell *et al.* (2004) have developed methods using liquid chromatography and mass spectrometry for such a purpose both *in vitro* and *in vivo*, respectively. The *in vivo* showed the human plasma from 11 healthy subjects had a concentration of 1.30±0.74 μM (mean ± standard deviation) of the Vitamin C HNE conjugate which is derived from the alkoxyl radical [16].

Discussions and Conclusions

The progression and development of many diseases has been linked to the oxidation of lipids and it is LPO that produced alkoxyl radicals. Alkoxyl radicals have been shown to a component of the genotoxic effect related to LPO, both directly through the $LO^{\bullet}$ reacting with proteins, DNA, or other lipids and indirectly through toxins such as HNE. Due to its reactivity and its propensity of inducing irreparable damage, $LO^{\bullet}$ has become a topic of intense study. The advent of flash photolysis, EPR, liquid chromatography, and mass spectrometry has provided researchers the opportunity to elute the role of $LO^{\bullet}$ in diseases such as atherosclerosis and Alzheimer's. Perhaps these past works and current studies will also improve treatments, such as photodynamic therapy, where LPO is a mechanism of inducing cell death.

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