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Lipid Alkoxyl Radicals

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chl, chlorophyll; DMPO, 5, 5-dimethyl-pyrroline-1-oxide; EMPO, 5-ethoxycarbonyl-5-methyl-1-pyrroline N-oxide; EPR, electron-paramagnetic-resonance; ESR, electron-spin-resonance; HPLC, high performace liquid chromatography; HSO_3^-, bisulfite; $L^\bullet$, lipid alkyl radical; LH, linoleic acid; $LnO^\bullet$, α-linolenic (LN, 18:3 n-3) radical; $LO^\bullet$, lipid alkoxyl radical; LOOH, linoleic acid hydrogenperoxide; NDGA, nordihydroguaiaretic acid; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species.

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Abstract

During the process of lipid peroxidation, lipid alkoxyl radical is an intermediate product and participates in propagation of lipid peroxidation. The organic Fenton reaction is the major way to generate lipid alkoxyl radicals in biological systems. It can also be produced in experimental conditions using metal or non-metal induced reductive cleavage or radiolysis and photolysis induced reductive cleavage. Lipid alkoxyl radical is very reactive and harmful to cells. It undergoes cyclization and β-scission to generate aldehyde and allylic radicals that are another intermediate radical in lipid peroxidation. It can also react with antioxidants and many biomolecules and usually end up with aldhyde, ketone, and secondary alcohol. Alkoxyl radicals can be detected by many biological methods, among which ESR/EPR with spin traps is the most popular.

Introduction

Almost all bio-molecule can be attacked by free radicals; but lipids, are the most susceptible. Lipids are present in cell membrane, lipoprotein. The attacking free radical reacts with the doubly allylic methylene groups in polyunsaturated fatty acids (PUFA) of lipids [1]. This oxidative destruction of PUFA is known as lipid peroxidation. Lipid alkoxyl radicals are generated from reduction of PUFA by some metal complexes and metal proteins. They are very reactive and damaging oxygen-centered lipid radicals; some evidence supports that PUFA akoxyl radicals exist as carbon-centered epoxyallylic radicals [9]. It was found that alkoxyl radicals are important intermediate products that break down to fatty acid aldehydes and to alkyl radicals in the process of lipid peroxidation. $LO^{\bullet}$ has a very short half-life: about a microsecond [2], so it is thought to be a minor propagating species in lipid peroxidation [3]. They are unstable with increasing solvent polarity [1].

Generation of lipid alkoxyl radicals

PUFAs create oxidative stress in biological systems as they undergo lipid peroxidation, forming free radicals such as peroxyl and alkoxyl radicals. Under experimental conditions, lipid alkoxyl radicals can be generated in three ways from the respective hydro- or dialkylperoxides [7].

Generation of lipid alkoxyl radicals under biological systems.

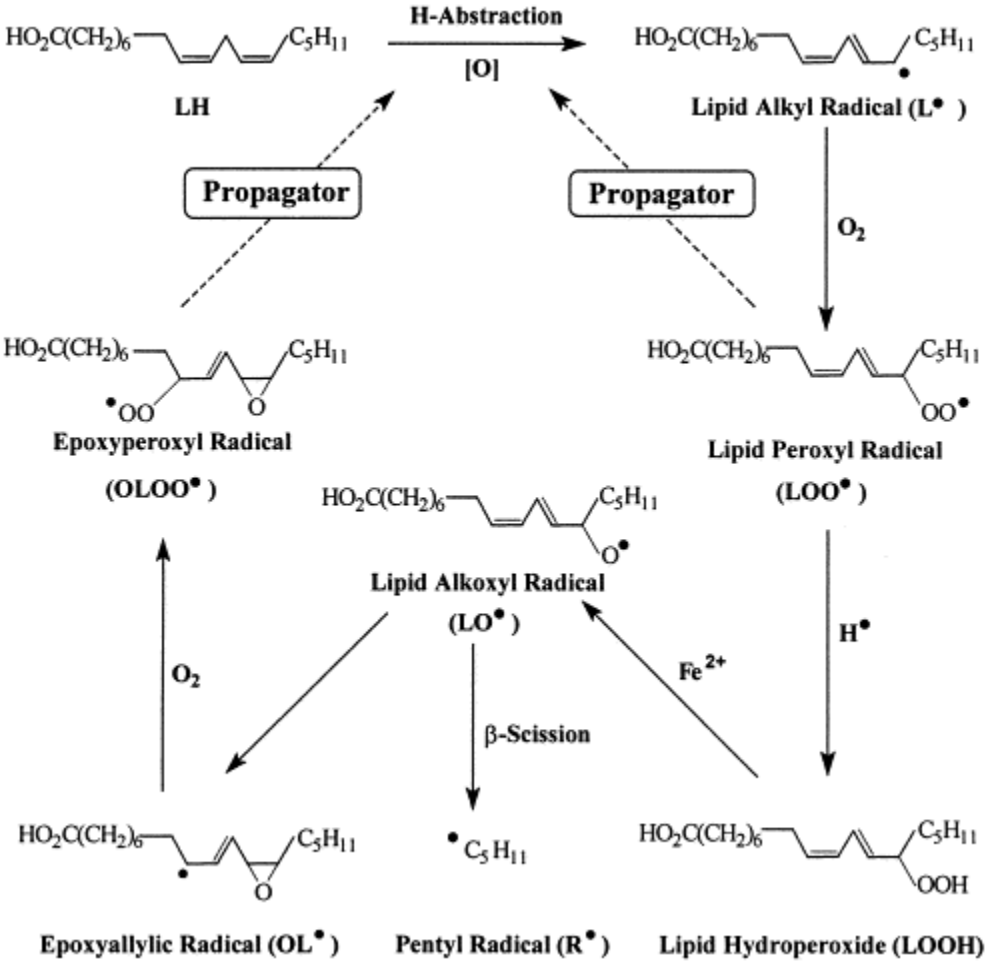
1. Organic Fenton Reaction.

Under biological conditions, the generation of lipid alkoxyl radicals is dependent on hydroperoxide precursors. This is the most common route to form alkoxyl radicals. The peroxidation starts from hydrogen abstraction from the lipid. It is believed to occur by intramolecular and intermolecular hydrogen abstraction. LOOH is generated following addition of oxygen and hydrogen. The lipid hydroperoxides (LOOH) can promote a Fenton reaction to

generate lipid alkoxyl radicals (scheme 1) [4]. The reductive role of metal ions depends on the ion type, pH, solvent, and other factors [5].

$$Fe^{2+} + LOOH + H^{+} \rightarrow Fe^{3+} + LO^{\bullet} + H_2O \quad \text{(Reaction 1)}$$

$$LOOH + Me^{n+} \xrightarrow{\text{reductive cleavage}} LO^{\bullet} + OH^{-} + Me^{(n+1)} \quad \text{(Reaction 2)}$$



Adapted from [3].

Scheme 1. An overview of the formation and propagation of lipid-derived radicals during lipid peroxidation in biological systems. All lipid-derived radicals participate or propagate the lipid peroxidation. The lipid alkoxyl radicals are generated from lipid hydroperxide in the presence of

ferrous iron and undergo β-Scission or cyclization to propagate lipid peroxidation. They do not play a major role in the whole processes because of its short lifetime.

2. Combination of two peroxyl radicals.

Re-combination of two peroxyl radicals is a minor way to generate lipid alkoxyl radicals in biological systems. It occurs only in heavily peroxidized material. The rate constant is $3x10^8$ $M^{-1}S^{-1}$ [7].

$$\mathbf{2\ LOO^{\bullet} \xrightarrow[K= 3x10^8\ S^{-1}]{\text{radical decay}} 2\ LO^{\bullet} + {}^1O_2} \qquad \text{(Reaction 3)}$$

Generation of lipid alkoxyl radicals under experimental conditions.

1. Homolytic cleavage of LOOH by non-metals.

Non-metals such as bisulfite, sulfite can reduce hydroperoxide to generate lipid alkoxyl radicals [6].

$$\mathbf{HSO_3^- + LOOH \rightarrow LO^{\bullet} + HSO_3^- + OH^-} \qquad \text{(Reaction 4)}$$

2. Metal-induced reductive cleavage.

Lipid alkoxyl radical can be generated during the formation of epoxides from linoleic acid peroxidation by the cysteine • $FeCl_3$ catalyst [8].

3. Reductive cleavage by hydrated electrons (e_{aq}^-) produced by pulsed radiolysis or UV lights [7].

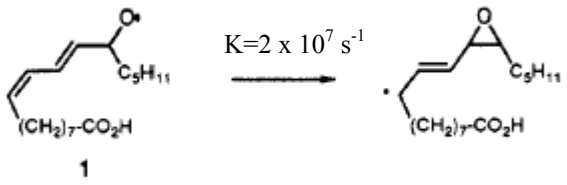
$$\mathbf{LOOH + e_{aq}^- \longrightarrow LO^{\bullet} + OH^-} \qquad \text{(Reaction 5)}$$

Reactions of Lipid alkoxyl radicals

Lipid alkoxyl radicals are very reactive and short-lived. They can react in multiple ways to propagate lipid peroxidation and terminate their functions in biological systems.

1. Rearrangement of PUFA alkoxyl radicals.

Because of the unsaturation of PUFA hydroperoxide, PUFA alkoxyl radicals tend to rearrange into epoxyallylic radicals, even it is possible to abstract hydrogen [3, 5]. Epoxyallylic radicals will be further oxidized to epoxyperoxyl radical that propagate lipid peroxidation (Sheme 1) [3]. The rate constant of cyclization of lipid alkoxyl radical is approximately ~ 2 x 10^7 s^{-1} [3].

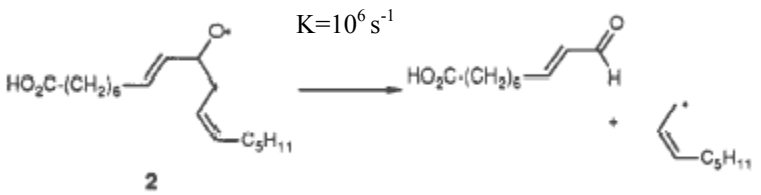


1

(Reaction 7)

2. β-Scission of lipid alkoxyl radicals.

β-Scission of lipid alkoxyl radicals results in carbon-carbon chain cleavage and generates low molecular weight compounds (scheme 1) [3, 5]. Aldehyde and allylic radical are the products [9]. Pentane is the terminal compound to end these propagating reactions. The rate constant for β-scission is ~ 1x 10^6 s^{-1} [3].



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(Reaction 6)

The extensive cyclization and β-scission of lipid alkoxyl radical are driven by production of an allylic carbon-centered radical. Some alkoxyl radicals undergo cyclization or β-scission to produce allylic radicals (reaction 5 and 6), while some can undergo both β-scission and cyclization to produce allylic radicals [9].

3. Ketone termination of lipid alkoxyl readical.

Ether-linked products are formed by either combination or intermolecular addition of alkoxyl radicals and lipid alkyl radicals [5].

$$\mathbf{LO^{\bullet} + L^{\bullet} \longrightarrow LOL} \qquad \text{(Reaction 8)}$$

4. Reaction with chlorophyll.

The alkoxyl radical would react with chl and leading to its destruction. The first step is a one-electron oxidation of chl to chl^+ which subsequently forms irreversible oxidation products [6].

$$\mathbf{LO^{\bullet} + chl + H^{+} \longrightarrow LOH^{\bullet} + chl^{+}} \qquad \text{(Reaction 9)}$$

$$\mathbf{LO^{\bullet} \longrightarrow Decomposition\ products} \qquad \text{(Reaction 10)}$$

5. Reaction with antioxidants.

Because PUFA alkoxyl radicals are very reactive oxidizing radicals, some known phenolic antioxidants NDGA, proplgallate, Trolox and some isoflavonoids, and some phenolic compounds can effectively scavange the PUFA alkoxyl radicals. The intermolecular reactivities of PUFA alkoxyl radicals have been established using crocin assays [7]. Table 1 lists the relative rate constants of photolyticaly generated PUFA alkoxyl radicals with phenols and phenolic antioxidants.

Table 1 The relative rate constants of photolyticaly generated PUFA alkoxyl radicals with phenols and phenolic antioxidants. (Adapted from [7]).

Phenols:	9-LO•	13-LO•	13-LnO•
Trolox c	0.43	0.32	0.33
3,4-dihydroxytoluene	0.061	0.055	0.083
3,4-dihydroxycinnamic acid	0.075	0.072	0.063
NDGA	0.20	0.31	0.20
2' ,4'-dihydroxyacetophenone	0.012	0.010	0.009
2' ,5'-dihydroxyacetophenona	0.070	0.063	0.065
3',4'-dihydroxyacetophenone	0.070	0.085	0.085
propylgallate	0.23	0.167	0.20
Flavonoids:			
Hesperetin	0.037	0.032	0.017
Texasin	0.16	0.15	0.17

Table 1: The alkoxyl radicals are very reactive and can be scavenged by various antioxidants including phenols and flavonoids. The listed antioxidants are some examples that react with lipid alkoxyl radicals.

As a member of ROS, LO• attacks many bimolecules. It can be scavenged by DNA molecules and generate DNA radicals to cause DNA damage [11]. Damage to PUFA decreases membrane fluidity. The intermolecular reactivities of PUFA alkoxyl radicals have been established using crocin assays. The relative rate constants for a number of biomolecules are listed below [7].

Table 2. Relative rate constants of photolytically generated PUFA alloxyl radials with nucleic acid derivatives.

Substrate	9-LO•	13- LO•	9- LnO•	13- LnO•
Nucleic acid bases:				
adenine	0.072	0.057	0.067	0.052
cytosine	0.016	0.032	0.027	0.035
thymine	0.019	0.017	0.020	0.021
uracil	0.062	0.066	0.021	0.036
Nucleosides				
adenosine	0.076	0.061	0.060	0.055
cytidine	0.073	0.030	0.027	0.035
gouanosine	0.006	0.069	0.055	0.066
thymidine,	0.036	0.023	0.623	0.015

Table 2: Some biomolecules are effective scavengers of PUFA alkoxyl radicals, nucleic acid bases and nucleosides are listed as examples of biomolecules that are attracted by lipid alkoxyl radicals [7].

Dection of lipid alkoxyl radicals

When an atom or molecule with an unpaired electron is placed in a magnetic field, the spin of the unpaired electron can align either in the same direction or in the opposite direction as the field. Alkoxyl radicals have unpaired electron, so the common methods to detect them are electron-paramagnetic-resonance (EPR) or electron-spin-resonance (ESR) spectroscopy that measures the absorption of microwave radiation by an unpaired electron when it is placed in a strong magnetic field. Due to the short half-life of alkoxyl radicals, the ESR/EPR with DMPO/DEPMPO/EMPO spin trapping is usually used [12].

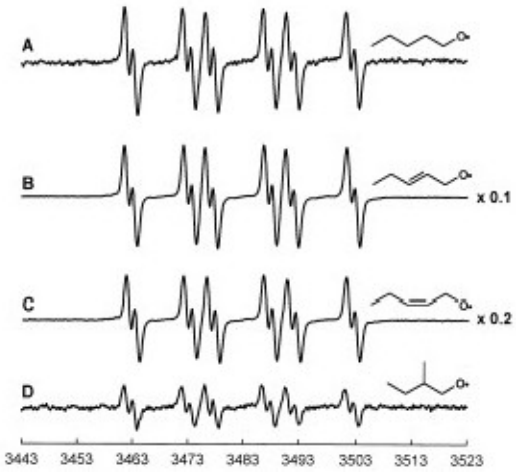

Fig.1. ESR spectrum of DMPO adducts with various primary alkoxyl radicals as indicated.

Adapted from [13].

Many other methods can detect alkoxyl radicals too, such as HPLC to detect lipid hydroperoxide and lipid alkoxyl radical, some chemical reactions. Each has its disadvantages and advantages. The most applied method is ESR.

Summary

Lipid alkoxyl radicals can be generated in both biological systems and experimental conditions. They mainly participate in lipid peroxidation process and undergo cyclization and β-scission to propagate lipid peroxidation. They may react with chlorophyll, antioxidants, biomolecules etc and cause damages. ESR and EPR with spin trap are the most used methods to detect lipid alkoxyl radicals.

References:

1. Erben-Russ M, Michel C, Bors W, and Saran M. (1987) Absolute rate constants of alkoxyl radical reactions in aqueous solution. *Am Chem Soc*. **91:** 2362-2365.

2. Armstrong D, Sohal RS, Cutler RG, Slater TF. *Free radicals in molecular biology, aging, and disease.* **Vol. 27** Raven press.

3. Qian SY, Wang HP, Schafer FQ, Buettner GR. (2000) EPR detection of lipid-derived free radicals from PUFA, LDL, and cell oxidations, *Free Radic Biol Med*. **29:** 568-579.

4. Stark G. (1991) The effect of ionizing radiation on lipid membranes. Biocimica et Biophysica Acta. **1071**: 103-122.

5. Gargner HW. (1989) Oxygen radical chemistry of polyunsaturated fatty acids. *Free Radic Biol Med* **7:**65-86.

6. Peiser GD and Yang SF. (1978) Chlorophyll destruction in the presence of bisulfite and linoleic acid hydroperoxide. *Phytochemistry*. **17**: 79-84.

7. Bors W, Erben-Russ M, Michel C, Saran M. (1990*) Free radicals, lipoproteins, and membrane lipids*. Plenum Press, Newyork.

8. Gardner, HW; Jursinic PA. Degradation of linoleic acid hydroperoxides by a cysteine. $FeCl_3$ catalyst as a model for similar biochemical reactions. I. Study of oxygen requirement, catalyst and effect of pH. *Bioch Biophy Acta*, **665:** 100-112, 1981.

9. Wilcox AL, Marnett LJ. (1993) Polyunsaturated fatty acid alkoxyl radicals exist as carbon-centered epoxyallylic radicals: A key step in hydroperoxide-amplified lipid peroxidation. *Chem. Res. Toxicol*, **6:** 413 – 416.

11. Sun Y. (1990) Free radicals, antioxidant enzymes, and carcinogenesis, *Free Radic Biol Med,* **8**: 583-599.

12. Stolze K, Udilova N, Nohl H. (2000) Lipids radicals: properties and detection by spin trapping. *Acta Biochimica Polonica.* **47**: 923-930.

13. Dikalov SI, Mason RP. **(**2001) Spin trapping of polyunsaturated fatty acid-derived peroxyl radicals: reassignment to alkoxyl radical adducts**.** *Free Radic Biol and Med*. **30**:187-197.