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Tocopheroxyl Radical

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Abbreviations:
$AscH^-$, ascorbate; $CoQ_{10}H_2$, the reduced form of coenzyme Q; $CoQ_{10}H^{\bullet}$, coenzyme Q radical; ESR, electron spin resonance; FMN, flavin mononucleotede; EDTA, ethylenediaminetetraacetic acid; GO: glucose oxidase; HRP: horseradish perxidase; LH, bisallylic hydrogen; $L^{\bullet}$, lipid radical; $LOO^{\bullet}$, lipid peroxyl radical; LDL: low-density lipoprotein. NRP, nonradical products; $ROO^{\bullet}$, peroxyl radical; PhPH; arbutin (4-hydroxyphenyl-β-D-glucopyranoside); $TO^{\bullet}$, tocopheroxyl radical; α –TOH.

Outline

Abstract

Tocopheroxyl radicals are resonance-stabilized free radicals that are important intermediates in the antioxidation processes of vitamin E. They not only can be generated during the process by which α-tocopherol (vitamin E) scavenges the lipid radicals, but also can be induced by superoxide reacting with oxidation of protonated or deprotonated forms of α-tocopherol. One study even mentioned formation of tocopheroxyl radical by photoionisation. Tocopheroxyl radicals can be recycled by radical-radical reaction, vitamin C and coenzyme Q. They can be directly detected by electron spin resonance (ESR).

Introduction

Tocopheroxyl radicals ($TO^{\bullet}$) are resonance-stabilized free radicals with an unpaired electron donating from α-tocopherol (α-TOH). Usually, they are formed during antioxidant reaction of α-tocopherol [1]. $TO^{\bullet}$ are relative stable free radicals with lifetimes of 12.5s in an average LDL particle [2]. It has been known that α-TOH is biologically and chemically the most active form of vitamin E [3, 11], and the role of α-TOH as an antioxidant is believed to be in scavenging lipid peroxyl radicals which are the chain-carrying species and propagate lipid peroxidation. $TO^{\bullet}$ are generated in this series of reactions. They participate in radical-radical termination reaction that lower the chain-transfer activity and hence the extent of lipid peroxidation [3]. To know the formation, properties and detection of $TO^{\bullet}$ is very essential for us to understand the antioxidant function of vitamin E and treat diseases caused by deficiency of vitamin E.

The chemical structure and physical properties of $TO^{\bullet}$

$TO^{\bullet}$ is the predominant (single) radical in oxidizing LDL because it is thermodynamically the most stable radical [9]. It is a resonance-stabilized and water-insoluble phenoxyl radical with a lipophilic phytyl tail (Fig. 1) [3]. The heterocyclic chromanol ring has an optimised structure for resonance stabilization of the unpaired electron of the α-tocopheroxyl radical (Fig. 2)

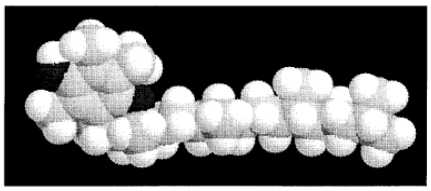


Figure 1. Chemical structure of tocol and tocotrienol and a space-filling model of α-TOH [1].

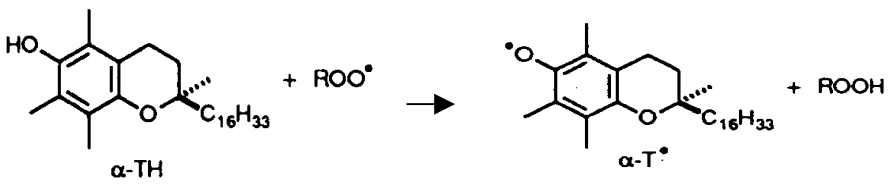
Figure 2. The resonance forms of the α-tocopheroxyl radical [10].

Formation of tocopheroxyl radicals

Tocopheroxyl radicals are mainly generated during the process of which α-tocopherol scavenge the lipid radicals. They also can be induced by superoxide reacting with oxidation of protonated or deprotonated forms of α-tocopheroxyl [4]. One study even mentioned formation of tocopheroxyl radical by photoionation [5]. Here, the formation of $TO^{\bullet}$ will be discussed.

Formed during antioxidant action of vitamin E

The lipid-soluble antioxidant α-TOH (vitamin E) is the principal inhibitor of lipid autoxidation in biological membranes. α-TOH reacts with peroxyl radicals by hydrogen atom transfer to form a hydroperoxide and α-$TO^{\bullet}$, a resonance-stabilized phenoxyl radical that does not readily participate in radical propagation reactions (reaction 1).



$k = 8 \times 10^4\ M^{-1}\ S^{-1}$ (1)

Superoxide induce the formation of $TO^{\bullet}$

Oxidation of protonated (I) or deprotonated (II) forms of α-tocopherol react with superoxide to consequently form α-tocopherol phenoxyl radical (III), α-tocopherol quinone (IV), and α-tocopherol semiquinone radical (V) is shown schematically in Fig. 3.

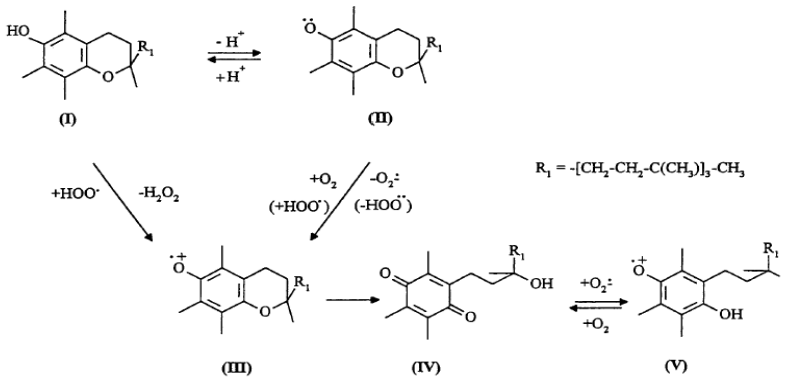


Figure 3. A scheme illustrating superoxide-induced formation of $TO^{\bullet}$ [4].

Formation of tocopheroxyl radical by photoionation.

The studies of the kinetics of oxidation of α-tocopherol and the reduction of the tocopheroxyl radical have shown that the tocopheroxyl radical ($TO^{\cdot}$) was formed by photoionation [5]. Time-resolved resonance Raman spectroscopy indicated that the tocopheroxyl radical cation ($TOH^{+\bullet}$), with a pKa of –1.4, was the predominant species in strongly acidic solution.

$$TOH + h\nu \rightarrow TOH^{+\bullet} \rightarrow TO^{\bullet} + H^{+} \quad \lambda = 800 \text{ nm} \qquad (2)$$

Repair-Recycling of tocopheroxyl radical

The mechanism of repair-recycling of tocopheroxyl radical is complex. It involves radical-radical reaction, the function of coenzymes and vitamin C. Details will be described as follows.

Radical-radical reaction

The classical action of α-TOH is that of a chainbreaking antioxidant that reflects its ability to react rapidly with the chain-carrying lipid peroxyl radical ($LOO^{\bullet}$). Alternatively, α-TOH can directly scavenge the radical that initiates peroxidation. In both cases, the relatively nonreactive α-tocopheroxyl radical (α-$TO^{\bullet}$) is formed. This radical is free to diffuse in homogeneous

solutions and rapidly reacts with another available radical to yield nonradical products (NRP) (Fig. 4) [3], resulting in termination if lipid peroxidation and consumption of α-TOH. Each molecules of α-TOH can destroy redicals and thereby terminate two potential chain reactions.

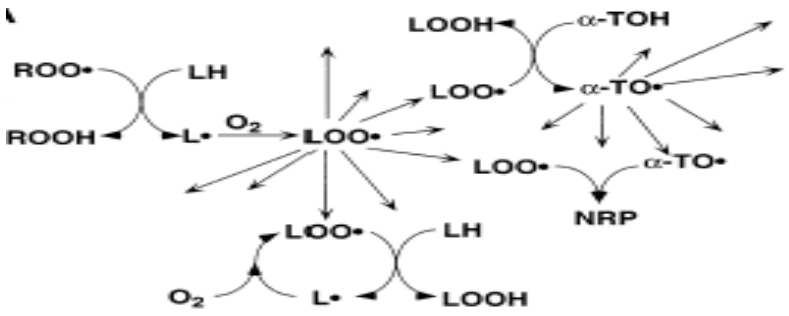


Figure 4. Repair-recycling of tocopheroxyl radical by radical-radical reaction.
$L^{\bullet} + O^2 \rightarrow LOO^{\bullet}$ $k = 3 \times 10^8$ M^{-1} S^{-1}, $LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH$ k = 10-50 M^{-1} S^{-1}, $LO_2^{\bullet} + TOH \rightarrow LOOH + TO^{\bullet}$ $k = 2.5 \times 10^6 M^{-1}S^{-1}$.

Repaired by Ubiquinone-10 ($CoQ_{10}H_2$; the reduced form of coenzyme Q)

Vitamin E acts as the primary donor of hydrogen to a peroxyl radical, and the phenoxyl radical of α-tocopherol (α-$TO^{\bullet}$) thus formed is reduced by coenzyme Q [4].

$$\alpha\text{-}TO^{\bullet} + CoQ_{10}H_2 \rightarrow \alpha\text{-}TOH + CoQ_{10}H^{\bullet} \quad (3)$$

$$\alpha\text{-}TO^{\bullet} + CoQ_{10}H^{\bullet} \rightarrow \alpha\text{-}TOH + CoQ_{10} \quad (4)$$

$K_3 \approx 3 \times 10^6$ $M^{-1}S^{-1}$. $K_4 = 3.7 \times 10^5$ $M^{-1}S^{-1}$ and 2.2×10^5 $M^{-1}S^{-1}$ at 25°C in benzene and ethanol, respectively. Whether the fully reduced form of coenzyme Q, ubiquinol-10 ($CoQ_{10}H_2$), and/or its semireduced form, ubisemiquinone-10 ($CoQ_{10}H^{\bullet-}$), participates in reducing the α-tocopherol phenoxyl radical is currently unknown [3].

Recycling of tocopheroxyl radical with vitamin C

α-TOH in LDL can be regenerated from its radical, α-$TO^{\bullet}$, by ascorbate (vitamin C) [2].

$$\alpha\text{-}TO^{\bullet} + AscH^- \rightarrow TOH + Asc^{\bullet -} \qquad K = 2 \times 10^5\ M^{-1}S^{-1} \quad (5)$$

Detection of tocopheroxyl radical

Oxidation of tocopherols can produce persistent tocopheroxyl free radicals, which can be observed with electron spin resonance (ESR) [6]. ESR analysis of the perturbation of free radical signals by paramagnetic ions of perturbation of NMR signals of fatty acyl chain residues are tools that can be used for studying the location of the tocopheroxyl radical in the membrane lipid bilayer. The oxidation methods have been proved effective in both chemical and biological systems. Here, two different methods will be discussed as following.

Peroxidases linked to enzymatic hydrogen peroxide generators.

For studies of natural membranes, an enzyme system for generating phenoxyl radicals has been shown to specifically generate the tocopheroxyl radical. The system consists of horseradish peroxidase coupled to a hydrogen peroxide-generating system:

$$\text{Glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2 \quad (5)$$

$$H_2O_2 + 2\text{PhOH} \rightarrow 2H_2O + \text{PhO}^{\bullet} \quad (6)$$

$$\text{PhO}^{\bullet} + \alpha\text{-TOH} \rightarrow \text{PhOH} + \alpha\text{-TO}^{\bullet} \quad (7)$$

Treatment of a variety of tocopherol-containing liposomes with this system has been shown to yield the ESR spectrum of the tocopheroxyl radical (Fig. 5).

Peroxidases linked to photochemical peroxide sources

An excellent photosensitizer is flavin mononucleotede (FMN), which absorbs electrons from a variety of organic molecules. A hybrid photochemical-enzymatic system for generating

phenoxyl radical, consisting of FMN, EDTA, and horseradish peroxidase yields tocopheroxyl radicals in liposomes (Fig. 6).

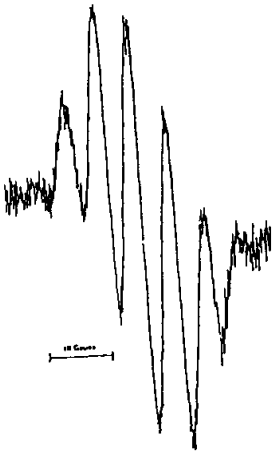


Fig. 7. ESR spectrum of the d-α-tocopheroxyl. In a typical liposome preparation, consisting of dioleoylphosphatidylcholine at 100 mg/mL and d-α-to-copherol at 230 μM, an oxidation system consisting of 5 mM glucose, 0.4 U/mL GO, 100 μM arbutin, and 0.2 U/mL HRP produced ESR signals of tocopheroxyl [6].

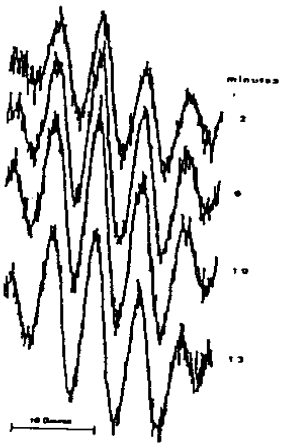


Fig. 8. ESR spectra of tocopheroxyl radical in liposomes using enzymatic oxidation. The reaction mixture contained 100mg/mL dioleoylphosphatidylcholine, 200 μM *d-α*-tocopherol, 10 μM FMN, 1 mM EDTA, 100 U/mL superoxide dismutase, 0.2 U/mL horseradish peroxidase, and 100 μM arbutin; irradiation was at 450mn, using an interference filter with a 50 nm bandwidth [6].

Summary

Tocopheroxyl radicals ($TO^{\bullet}$) are free radicals with an unpaired electron donating from α-tocoperol (α-TOH). They are mainly formed during antioxidant reaction of α-tocoperol. Superoxide and photoionisation also can induce the formation of tocopheroxyl radicals. They are stable, water-insoluble radicals and can be recycled and repaired by radical-radical reaction, reaction with $CoQ_{10}H_2$ and Vitamin C. Finally, tocopheroxyl radicals can be directly detected by electron spin resonance (ESR).

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