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Tocopheroxyl Radical

by

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Abbreviations

$Asc^{\bullet-}$ - Ascorbate Radical
$AscH^-$ - Ascorbate
CoQ_{10} – Ubiquinone-10
$CoQ_{10}H^{\bullet-}$ - Ubisemiquinone-10
$CoQ_{10}H_2$ – Ubiquinol-10
EGCG- (-)-Epigallocatechin gallate
EPR – Electron Paramagnetic Resonance
GSH – Glutathione
$HO_2^{\bullet}$ - Hydroperoxyl Radical
LDL – Low Density Lipoprotein
LH – Lipid
$LOO^{\bullet}$ - Lipid Peroxyl Radical
LOOH – Lipid Hydroperoxide
NRP – Nonradical product
$O_2^{\bullet-}$ - Superoxide
TMP – Tochopherol-Mediated peroxidation
$TO^{\bullet}$ - Tocopheroxyl Radical
TOH – Tocopherol

Outline

Abstract

Tocopheroxyl radical is a highly lipophilic compound that is produced as a result of the oxidation of tocopherol. Tocopherol is a potent chain-breaking antioxidant that is important in stopping lipid peroxidation. Two lipid peroxidation schemes involving both tocopherol and its radical propose similar yet very different roles for the antioxidant pair. In "conventional" LDL lipid peroxidation tocopherol acts as an antioxidant, whereas, in tocopherol-mediated peroxidation, tocopherol can be both a pro-oxidant and an antioxidant. The tocopheroxyl radical is able to react with other antioxidants and be recycled back into tocopherol, thus propagating the antioxidant properties of this compound. Two prominent recycling compounds of the tocopheroxyl radical are ascorbate and ubiquinol-10. The reduction of tocopheroxyl radical is important in maintaining antioxidant defenses.

Introduction

Vitamin E was discovered in 1922 by H.M. Evans at Berkeley University [1]. It was not until six decades later, in 1980 that Burton *et al*. recognized its role as a chain-breaking antioxidant [2]. Today, vitamin E is a popular dietary antioxidant. There are eight isoforms of vitamin E; four tocopherol and four tocotrienol structures [3]. The tocopherols (TOH) consist of a phenolic head and a long saturated carbon tail (**Figure 1**). They differ from tocotrienols in that tocotrienol's carbon chain is unsaturated, with three double bonds [3]. The tocopherol isomers give rise to the tocopheroxyl radical ($TO^{\bullet}$) by the oxidation of the hydroxyl group. This radical can be detected by both electron paramagnetic resonance (EPR) [4] and UV spectrometry [5] (**Figure 2)**.

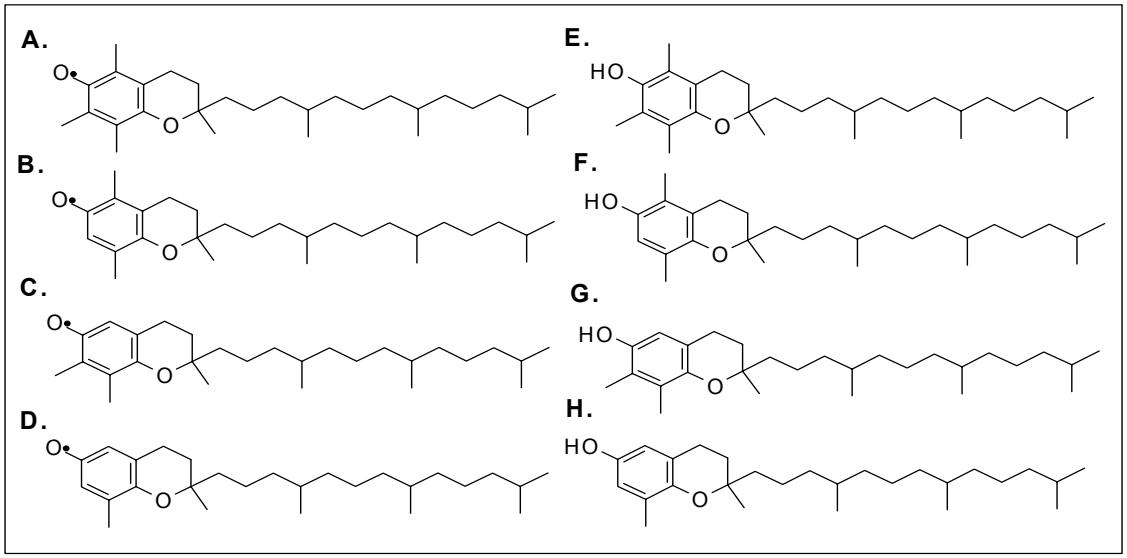

Figure 1. Structure of tocopheroxyl radicals and tocopherols. A. α-$TO^{\bullet}$. B. β-$TO^{\bullet}$. C. γ-$TO^{\bullet}$. D. δ- $TO^{\bullet}$. E. α-TOH. F. β-TOH. G. γ-TOH. H. δ-TOH

Because of their long carbon chain tocopherols and tocotrienols and their radicals are very hydrophobic [6]. This allows them to reside in lipid rich areas, particularly membranes and in LDL, where they act to stop lipid peroxidation. The lipophilic nature of this radical creates

some problems with its accessibility to other antioxidants. This paper will discuss the formation of $TO^{\bullet}$ through the oxidation of TOH and the reduction of the radical back into its antioxidant form.

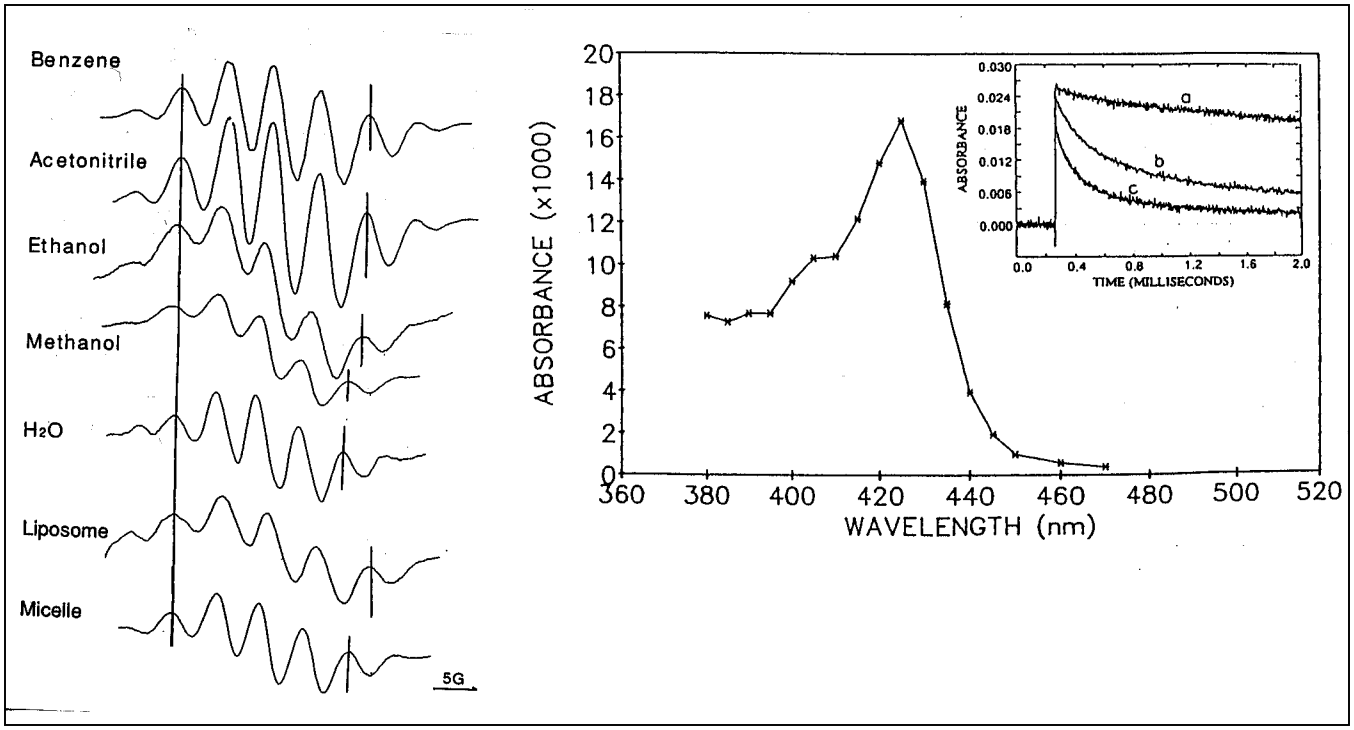

Figure 2. Detection of $TO^{\bullet}$. A. EPR spectra of $TO^{\bullet}$ in several different solutions [4]. UV spectrum of $TO^{\bullet}$. Inset. Decay of $TO^{\bullet}$ at 425 nm at varying pH (a. pH 6.6; b. pH 4.2; c. pH 3.2) [5].

Oxidation of Tocopherol to Form Tocopheroxyl Radical

Phenols are typically chain-breaking antioxidants [7]. Tocopherol is a chain breaking antioxidant that is able to stop lipid peroxidation. In doing so, its hydroxyl group of the TOH head is oxidized to become $TO^{\bullet}$ [1]. If not stopped, lipid peroxidation in membranes can reach 1 to 5 nmol mg^{-1} protein min^{-1} [1]. TOH is present in the membrane at a ratio of 1 to 2000 phospholipids [1,8]. Even at this small concentration (less than 0.05 to 0.1 nmol mg^{-1} protein [1]), TOH is able to protect the membrane against lipid peroxidation.

All forms of vitamin E are able to stop lipid peroxidation in this manner. However, there is a hierarchy to the efficiency of the isomers. α-TOH is the best chain-breaking antioxidant of

the isomers with a rate constant of 2.4 x 10^6 M^{-1} s^{-1}, followed by β-TOH (1.7 x 10^6 M^{-1} s^{-1}), γ-TOH (1.6 x 10^6 M^{-1} s^{-1}), and finally δ-TOH (6.5 x 10^5 M^{-1} s^{-1}) [6]. α-TOH also is the most abundant isomer of vitamin E found in mammalian membranes [8,9].

"Conventional" LDL Lipid Peroxidation

In "conventional" lipid peroxidation, TOH acts only as an antioxidant to stop lipid peroxidation (**Figure 3**). The radical reaction is started with an initiation event in which a hydroperoxyl causes the oxidation of a polyunsaturated fatty acid. This reacts with oxygen to forms $LOO^{\bullet}$. This is carried out in the propagation step where an uncertain number of peroxidation cycles take place. In the inhibition (or termination) step, TOH donates a hydrogen atom to $LOO^{\bullet}$ and is oxidized to form $TO^{\bullet}$, thus stopping the chain of peroxidation [1,9,10,11].

$$LOO^{\bullet} + TOH \rightarrow LOOH + TO^{\bullet}$$

When $TO^{\bullet}$ is recycled back in to TOH, TOH can act to stop another propagation of lipid peroxidation. However, TO• may react with LOO• to form a nonradical product (NRP) [9,10].

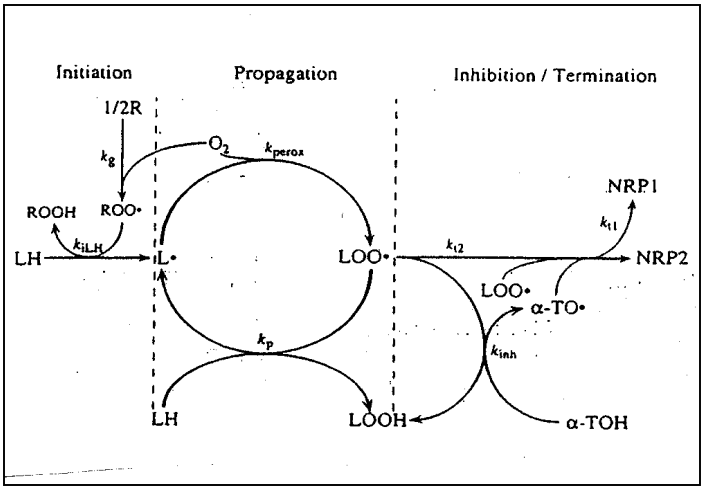

Figure 3. "Conventional" LDL lipid peroxidation. The radical then oxidizes LH to begin the cycle of lipid peroxidation. This step cycles as a part of propagation to create an indefinite amount of $LOO^{\bullet}$. Inhibition or termination occurs when TOH reduces $LOO^{\bullet}$ to LOOH, forming $TO^{\bullet}$. Alternatively, $LOO^{\bullet}$ may react with itself or $TO^{\bullet}$ to form a nonradical product [9].

Tocopherol-Mediated Peroxidation

In tocopherol-mediated peroxidation, as proposed by Stocker *et al*. [9-12], TOH can act as either a pro-oxidant or an antioxidant (**Figure 4**). In this case TOH transfers a peroxidation

reaction from the aqueous phase into the lipid phase, shown in the following equation [11].

$$ROO^{\bullet} + TOH \rightarrow ROOH + TO^{\bullet}$$

The radical reaction is now facilitated within the lipid environment *via* the oxidation of LH by $TO^{\bullet}$ [12].

$$TO^{\bullet} + LH \xrightarrow{k_{TMP}} \xrightarrow{O_2} TOH + LOO^{\bullet}$$

The rate constant for this reaction (k_{TMP}) is approximately 0.1 M^{-1} s^{-1} [12]. Although this reaction is very slow, $TO^{\bullet}$ must wait for several minutes before being recycle back to TOH by another antioxidant. This provides ample time for TMP to occur [11,12]. Lipid peroxidation is thus initiated by $TO^{\bullet}$ and is then terminated by TOH. This creates a cycle of tocopherol-mediated peroxidation [7,9-11].

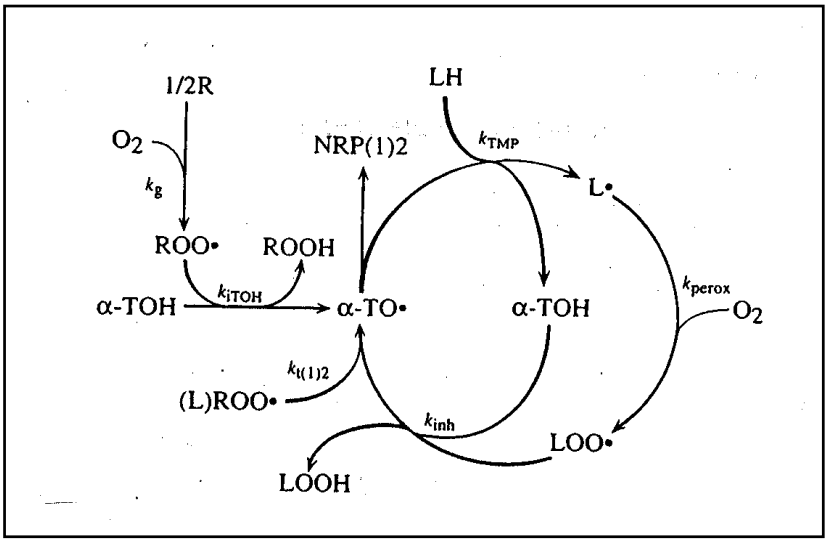

Figure 4. Tocopherol-mediated peroxidation cycle. Hydroperoxyl radicals oxidize TOH to $TO^{\bullet}$. Tocopheroxyl radical then acts as a pro-oxidant, oxidizing LH, which forms $L^{\bullet}$. The addition of oxygen to makes $LOO^{\bullet}$ which reacts with TOH to reform $TO^{\bullet}$ and restart the cycle of peroxidation [9].

Reduction of Tocopheroxyl Radical

The tocopheroxyl radical can be recycled back into tocopherol by various antioxidants. Many antioxidants are able reduce to $TO^{\bullet}$, such as ascorbate [10,12-15,19] ubiquinol-10 [11,16,17], GSH [1,8,12], EGCG [18], cartenoids [19], BHT [10], and even TOH [2]. Ascorbate and ubiquinol-10 are described in more detail below.

Ascorbate

Ascorbate ($AscH^-$) goes through a one-electron reduction of $TO^\bullet$ to regenerate TOH [5,19].

$$\alpha\text{- } TO^\bullet + AscH^- \rightarrow \alpha\text{-TOH} + Asc^{\bullet-}$$

In solution (water/isopropanol/acetone), the rate constant for the reduction of $TO^\bullet$ by ascorbate is 1.55×10^6 M^{-1} s^{-1} [14]. In a phosphotidylcholine liposome the rate constant increases to 2×10^5 M^{-1} s^{-1} [12,14,15]. Because $AscH^-$ is hydrophilic, it is not able to be in the membrane component as is the highly hydrophobic $TO^\bullet$. The radical center of $TO^\bullet$ is at the lipid-aqueous interphase of the membrane [9]. Here, the $AscH^-$ is able to react with the unpaired electron of $TO^\bullet$. This reduction moves the radical center from the hydrophobic membrane to the aqueous phase it so the radical can no longer react with LH [12]. Because $Asc^{\bullet-}$ is resonance-stabilized, it is relatively unreactive and is unlikely to propagate more free radical reactions [7].

Ubiquinol-10

Ubiquinone (Coenzyme Q, CoQ_{10}) is an electron carrier in the electron transport chain. Because of its position in the mitochondrial membrane, ubiquinol-10 (fully reduced ubiquinone) is able to react with $TO^\bullet$ and reduce it back to TOH [16].

$$TO^\bullet + CoQ_{10}H_2 \rightarrow TOH + CoQ_{10}H^{\bullet-}$$

The second order rate constant for this reaction was found to be 3.74×10^5 M^{-1} s^{-1} in benzene and 2.15×10^5 M^{-1} s^{-1} in ethanol [16]. While TOH can be oxidized by protonated superoxide ($k = 2 \times 10^5$ M^{-1} s^{-1}), superoxide also acts as an ally by helping to reduce $TO^\bullet$ [16]. It does so by a one-electron reduction reaction of CoQ10 to form ubisemiquinone-10 radical [17].

$$CoQ_{10} + O_2^{\bullet-} \rightarrow CoQ_{10}H^{\bullet-} + O_2$$

Two ubisemiquinone-10 radicals react to form ubiquinol-10, which then can reduce $TO^{\bullet}$ as shown above [17].

$$2CoQ_{10}H^{\bullet -} + 2H^{+} \rightarrow CoQ_{10}H_2 + CoQ_{10}$$

Vitamin E and Disease

When vitamin E was first discovered it was known to be essential for reproduction [2, 20]. Since then it biological implications have gained great importance. In arterial walls TOH stop the oxidation of LDL, which heads off atherosclerosis by preventing the recruitment of macrophages to ingest the LDL and for the foam cells of an atherosclerotic lesion [9,10]. TOH also prevents oxidation of polyunsaturated fatty acids in synaptosomes, which may play a key preventative role in Parkinson's disease [21]. Finally, TOH antioxidant properties help protect against the oxidative stresses that are a factor in carcinogenesis [21]. As discussed earlier, the properties of tocopheroxyl radical are an important part of vitamin E cycling that allows tocopherol to continuously do its job.

Conclusion

Tocopheroxyl radical is an important factor in free radical chemistry on lipid peroxidation. According to "conventional" LDL peroxidation TOH acts only as an antioxidant and the only role for $TO^{\bullet}$ is to be reduced back into TOH [1,9-11]. However, tocopherol-mediated peroxidation, a more recent model, suggests that TOH is a pro-oxidant and an antioxidant in lipid peroxidation, and $TO^{\bullet}$ help propagate the radical reactions [9-12]. This model increases the emphasis on the importance of ascorbate and ubiquinol-10 in the antioxidant system.

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