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Real Time Detection of Reactions Between Radicals of Lycopene and Tocopherol Homologues

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Laser flash photolysis of lycopene in homogeneous chloroform solution together with tocopherol homologues results in rapid formation of the lycopene radical cation and slower formation of tocopheroxyl radicals. Time-resolved detection by absorption spectroscopy of decay of the lycopene radical cation, of formation of the tocopheroxyl radicals, and of bleaching of lycopene has shown that α-tocopherol is able to reduce the lycopene radical cation and thereby partially regenerate lycopene on a ms timescale. In contrast, lycopene is able to reduce the δ-tocopheroxyl radical, whereas an equilibrium exists between the lycopene radical cation and β- or γ-tocopherol. The relative stability of these antioxidant radicals is hence: α-tocopheroxyl > lycopene radical cation ~ β-tocopheroxyl ~ γ-tocopheroxyl > δ-tocopheroxyl.

Keywords: Lycopene, tocopherol, laser flash photolysis

INTRODUCTION

Antioxidants, when combined, may show greater antioxidative capacity than what would be expected from their individual activity alone. This synergism may be explained as a consequence of direct interaction between the antioxidants or as a result of different ability of antioxidants to scavenge various reactive oxygen species like peroxyl radicals and singlet oxygen thereby yielding a better overall protection. The interaction between tocopherol and ascorbic acid has been known for some time,[1] and was recently confirmed in cooked minced turkey meat balls in which it was found that α-tocopherol and ascorbyl palmitate acted synergistically in protection against rancidity.[2,3] In lipid bilayers it has been shown that ascorbic acid can reduce α-tocopheroxyl radicals thereby recycling α-tocopherol at the expense of ascorbic acid providing a mechanism for the synergism.[4]

In contrast, a possible interaction between carotenoids and tocopherols is less well-studied. It has been found that β-carotene and α-tocopherol act synergistically in microsomal membranes,[5] however, no detailed study of the interaction has been performed. Recently,[6] it has been found that

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many but not all carotenoids are able to reduce the α-tocopheroxyl radical in hexane solution. We have previously shown that β-carotene is capable of reducing phenoxyl radicals,[7] and we now present results showing how lycopene and tocopherol homologues (see Scheme 1) interact in a homogeneous model system where radicals are generated by laser flash photolysis. Lycopene was selected for these investigations because of the strong current interest in this carotenoid in human nutrition and because of our previous finding of lycopene as may be the most efficient carotenoid scavenger of free radicals.[8,9]

EXPERIMENTAL SECTION

Materials

Lycopene was supplied by Roche A/S (Hvidovre, Denmark) sealed in an ampoule under argon and was used without further purification. Chloroform, HPLC grade, from Lab-Scan (Dublin, Ireland) and α-, β-, γ-, and δ-tocopherol from Merck (Darmstadt, FRG) were used as received. Air-saturated solutions containing $1.0 \cdot 10^{-5}$ M lycopene and $1.0 \cdot 10^{-3}$ M α-, β-, γ-, or δ-tocopherol in chloroform were used for laser flash photolysis experiments the same day as they were prepared.

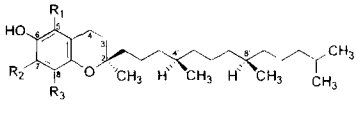
Instrumentation

Laser flash photolysis experiments were carried out with an LKS.50 laser flash photolysis spectrometer from Applied Photophysics Ltd (Leatherhead, UK). The third harmonic at 355 nm of a pulsed Q-switched Nd-YAG laser, Spectron Laser Systems (Rugby, UK), was used for excitation. The intensity of the laser pulse was approximately 50 mJ at 355 nm and the duration of the pulse was around 10 ns. A 1P28 photomultiplier tube from Hamamatsu (Hamamatsu City, Japan) was used to detect transient absorption at wavelengths below 550 nm. Near infrared detection was conducted with an S1336-44BK silicon photodiode from Hamamatsu (Hamamatsu City, Japan). For near infrared measurements, red bandpass filters were used in order to minimize degradation of the carotenoids by the Xe arc lamp used for monitoring, whereas a UV cut-off filter was used for monitoring in the blue-green spectral region. Spectral slit widths were typically 4–5 nm. The samples were excited in 1 cm × 1 cm fluorescence cells from Hellma (Müllheim, Germany).

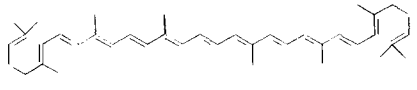
All samples were thermostated at 20.0 ± 0.5°C. Due to degradation of the carotenoids by the laser pulse each sample was subjected to no more than 6 laser pulses.

RESULTS

Photolysis of carotenoids in chloroform or carbon tetrachloride results in degradation of the carotenoid. Application of short light pulses and time-resolved detection (laser flash photolysis) has shown that photodegradation of the carotenoid is complex proceeding via two near infrared absorbing transient species.[8,9] In a pre-



	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3



Lycopene

SCHEME I Structure of tocopherols and lycopene.

vious study,[9] lycopene has been shown to react faster with solvent-derived radicals than many other carotenoids. In the presence of tocopherols, lycopene, which on the basis of these previous studies was found to be the most efficient free radical scavenger, was found to photodegrade differently indicating significant interaction with tocopherol.

Photodegradation of Lycopene in Chloroform

One intermediate observed in the near infrared is formed instantaneously, i.e. within 10 ns, upon laser flash photolysis of lycopene in chloroform. Another intermediate, absorbing at a longer wavelength, is formed in a first-order reaction by degradation of the initially formed intermediate. Excited states may be quenched by electron transfer, and it has been suggested that carotenoids react in this way with carbon tetrachloride.[10] The slowly formed intermediate is identified as the carotenoid radical cation[8,9] whereas identification of the initially formed intermediate is less certain. Adducts between carotenoids and free radicals absorb at a shorter wavelength than the corresponding radical cation[11] and the following set of reactions may be envisaged[8,9]

$$\text{Lycopene}^{*} + CHCl_3 \rightarrow [\text{Lycopene} - CHCl_2]\cdot + Cl\cdot \quad (1)$$

$$[\text{Lycopene} - CHCl_2]\cdot \rightarrow \text{Lycopene}^{+\bullet} + CHCl_2^{-}. \quad (2)$$

The excited state lycopene reacts with chloroform to yield an adduct and a chlorine atom. The adduct goes on to decay (by first-order kinetics) to the radical cation. However, this set of reactions imply that a (relatively) strong covalent bond between lycopene and chloroform is broken. Furthermore, reaction (1) does not comply with the general notion that excited states are quenched by electron transfer. However, if the slowly formed species is the radical cation, the initially formed species must be a precursor of this. The following set of reactions may explain the observations[9]

$$\text{Lycopene}^{*} + CHCl_3 \rightarrow [\text{Lycopene}^{+\bullet} \cdots CHCl_3^{-\bullet}] \quad (3)$$

$$[\text{Lycopene}^{+\bullet} \cdots CHCl_3^{-\bullet}] \rightarrow \text{Lycopene}^{+\bullet} + CHCl_3^{-\bullet}. \quad (4)$$

Excited state lycopene reacts with chloroform to yield an ion-pair which then decays to the radical cation. The radical cation decays by second-order kinetics.[8,9] The solvent-derived radical formed in reaction (4) causes a slow bleaching of the carotenoid

$$CHCl_3^{-\bullet} \rightarrow CHCl_2\cdot + Cl^{-} \quad (5)$$

$$\text{Lycopene} + CHCl_2\cdot \rightarrow [\text{Lycopene}^{+\bullet} \cdots CHCl_2^{-}]\cdot \quad (6)$$

The instantaneously formed intermediate thus decays bi-exponentially: first-order decay (reaction (4)) and pseudo first-order formation (reaction (6)).[8,9]

Photolysis of Lycopene and Tocopherols

If tocopherols are capable of reducing the lycopene radical cation

$$\text{Lycopene}^{+\bullet} + \text{TocOH} \rightarrow \text{Lycopene} + \text{TocO}\cdot + H^{+} \quad (7)$$

one would expect that the lycopene radical cation would decay faster than it does in the absence of tocopherols. Figure 1 shows that the lycopene radical cation does decay faster in the presence of α-tocopherol. In the presence of β- or γ-tocopherol, the lycopene radical cation decays faster at short times but at longer times, the decay is slower than in the absence of these tocopherol homologues (Fig. 1). δ-Tocopherol, perhaps surprisingly, increases the lifetime of the lycopene radical cation significantly (Fig. 1).

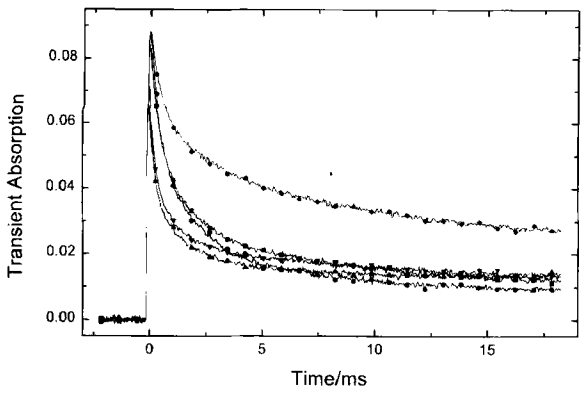


FIGURE 1 Time traces of near infrared absorption of the lycopene radical cation at 1060 nm following laser flash photolysis at 355 nm of $1.0 \cdot 10^{-5}$ M lycopene and 1.0 mM α-tocopherol (●), β-tocopherol (▲), γ-tocopherol (▼), δ-tocopherol (♦) or without tocopherol (■).

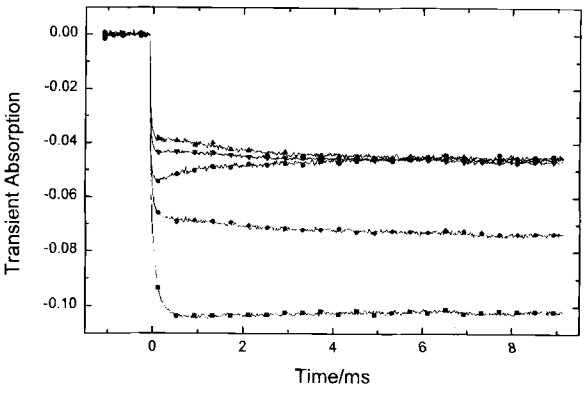


FIGURE 2 Time traces of bleaching of $1.0 \cdot 10^{-5}$ M lycopene at 530 nm following laser flash photolysis at 355 nm. With 1.0 mM (●), β-tocopherol (▲), γ-tocopherol (▼), δ-tocopherol (♦) or without tocopherol (■).

The slow bleaching of lycopene is complete within a millisecond after which the absorbance stays at this lower level apart from a slight increase due to diffusion (Fig. 2). In the presence of α-tocopherol, lycopene is partially reformed according to reaction (7) as evidenced by a slight increase in absorption until around 5 ms after the laser pulse. On the other hand, when β-, γ-, or δ-tocopherol is present, a slow and slight bleaching beyond that induced by the radicals derived from chloroform (reaction (4)) takes place on a rather long time scale (Fig. 2).

α-Tocopheroxyl radicals are characterised by their absorption around 420–430 nm and their long lifetime on the order of milliseconds.[4] In Figure 3 is shown the changes in absorbance at 420 nm following laser flash photolysis. Lycopene absorbs in this region too which explains the instantaneous bleaching observed. The positive transient absorption is due to formation of the tocopheroxyl radicals.

DISCUSSION

As may be seen from Figures 1, 2, and 3, α-tocopherol behaves qualitatively different in interaction with lycopene radicals formed by laser flash photolysis in chloroform compared to β- and γ-tocopherol which, on the other hand, behave in the same manner. δ-Tocopherol neither behaves like α- nor β- and γ-tocopherol. These observations provide the background for a comparison of the free radical scavenging efficiency of lycopene with the efficiency of the tocopherol homologues.

α-Tocopherol

Figure 1 shows that α-tocopherol reduces the lifetime of the lycopene radical cation by reacting with it. Figure 2 shows that lycopene is partially being reformed by reaction between the lycopene radical cation and α-tocopherol as evidenced by the increase in absorption between 0.2 and 5 ms. The lycopene radical cation and α-tocopherol thus reacts according to reaction (7) which may also be seen by the increasing concentration of the α-tocopheroxyl radical at long times (Fig. 3). However, Figure 1 shows that the lifetime of the lycopene radical cation only decreases slightly in the presence of α-tocopherol, and Figure 2 shows that lycopene is only being reformed to a small extent. Reaction (7) is thus only of minor importance compared to the decay pathway in the absence of α-tocopherol

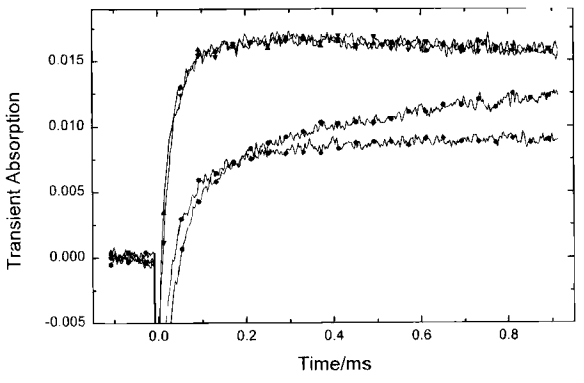


FIGURE 3 Times traces of bleaching of $1.0 \cdot 10^{-5}$ M lycopene and formation of tocopheroxyl radicals at 420 nm following laser flash photolysis at 355 nm. With 1.0 mM α-tocopherol (●), β-tocopherol (▲), γ-tocopherol (▼) or δ-tocopherol (♦).

$$2\,\text{Lycopene}^{+\cdot} \rightarrow \text{non-radical products}, \quad (8)$$

even though α-tocopherol (1.0 mM) is at a much higher concentration than the lycopene radical cation, as only a small fraction of the 10^{-5} M lycopene is present at any time as radicals.

δ-Tocopherol

Apparently, the lifetime of the lycopene radical cation is significantly increased in the presence of δ-tocopherol (Fig. 1). This is not due to an increased stability, and hence longer lifetime, of the lycopene radical cation. The apparent longer lifetime of the lycopene radical cation must rather be ascribed to additional (slow) formation of the lycopene radical cation

$$\text{Lycopene} + \delta\text{-TocO}\cdot \rightarrow \text{Lycopene}^{+\cdot} + \delta\text{-TocO}^{-}. \quad (9)$$

The δ-tocopheroxyl radical is formed by reaction with the radical formed from chloroform in reaction (5). Figure 2 also shows that bleaching of lycopene in the presence of δ-tocopherol takes place long after bleaching has stopped in the absence of tocopherol. The δ-tocopheroxyl radicals thus act as a pool of ("stable") radicals that react slowly with lycopene after the reaction with the more reactive solvent-derived radicals has stopped.

β- and γ-Tocopherol

The reactions of β- and γ-tocopherol with lycopene are identical (Fig. 1, 2, and 3) which is not surprising considering the similarity of their structures. At short times, i.e. high concentration of lycopene radical cation, β- and γ-tocopherol reduces the lifetime of the lycopene radical cation (Fig. 1) according to reaction (7) resulting in a smaller degree of bleaching (Fig. 2) than what is observed with the other tocopherols. However, at longer times, the concentration of the lycopene radical cation decreases more slowly in the presence of β- or γ-tocopherol (Fig. 1) than in the absence of these tocopherols. As in the case of δ-tocopherol, this must be ascribed to reaction (9). Thus, an equilibrium exists

$$\text{Lycopene}^{+\cdot} + \beta/\gamma\text{-TocOH} \leftrightarrows \text{Lycopene} + \beta/\gamma\text{-TocO}\cdot + \text{H}^{+} \quad (10)$$

which at short times (high concentration of lycopene radical cation) predominantly runs forward but at longer times (low concentration of lycopene radical cation) shifts to the left. This can further be seen from Figure 2, which shows that lycopene undergoes an additional slow bleaching in the presence of β- or γ-tocopherol in marked contrast to what is seen for α-tocopherol.

Comparison of tocopherols

β- and γ-tocopherol seem to react faster than the other tocopherols with both the solvent-derived radicals, as seen from the more rapid formation of the tocopheroxyl radicals (Fig. 3), and with the lycopene radical cation (Fig. 1). This higher reaction rate compared to α-tocopherol could be due to less steric hindrance at the phenolic group as both β- and γ-tocopherol only have one ortho methyl group in contrast to two for α-tocopherol.

The reaction rate for formation of antioxidant radicals is not necessarily correlated with the stability of the free radicals formed. From the present results it is possible to make a hierarchy of stability of one-electron oxidized lycopene and tocopherols. Since α-tocopherol is able to reduce the lycopene radical cation (reaction (7)), the α-tocopheroxyl radical must be more stable than the lycopene radical cation. The equilibrium (reaction (10)) between the lycopene radical cation and β- or γ-tocopherol shows that the lycopene radical cation and the β- and γ-tocopheroxyl radicals have similar stability. The fact that lycopene can reduce the δ-tocopheroxyl radical (reaction (9)) shows that the δ-tocopheroxyl radical is less stable than the lycopene radical cation. The ordering of free radical stability: α-tocopheroxyl > lycopene radical cation ~ β-tocopheroxyl ~ γ-tocopheroxyl > δ-tocopheroxyl, is for the homologue tocopherols in agreement with what is found for their efficiency as antioxidants in model food systems[2,12]

CONCLUSIONS

Even though the reaction between the lycopene radical cation and α-tocopherol (reaction (7)) only accounts for a minor amount of the degradation of the lycopene radical cation compared to the bimolecular self-reaction (reaction (8)), it may be of importance in biological systems where the concentration of the lycopene radical cation is less than in these experiments.

We have previously[9] shown that lycopene is among the most efficient carotenoid free radical scavengers, because the lycopene radical cation is more stable than many other carotenoid radical cations. β- and γ-tocopherol, and perhaps even δ-tocopherol, may hence be able to reduce other carotenoid radical cations due to the lower stability of these carotenoid radicals compared to the lycopene cation.

Acknowledgment

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