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Re-appraisal of the Tocopheroxyl Radical Reaction with β-Carotene: Evidence for Oxidation of Vitamin E by the β-Carotene Radical Cation

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Photobleached β-carotene (Car) is regenerated in hexane on a microsecond timescale in the presence of α-tocopherol (TOH) but not when α-tocopherol is absent, as studied by laser flash photolysis. β-Carotene radical cations ($Car^{\cdot +}$) likewise react with (excess) α-tocopherol: $Car^{+} + TOH \rightarrow Car + TO\cdot + H^{+}$ (second-order rate constant of $k = 1.7 \pm 0.1 \cdot 10^{7}\ M^{-1}\ s^{-1}$ in homogeneous di-*tert*-butylperoxide/benzene at 20°C) rather than α-tocopheroxyl radicals (TO·) reacting with β-carotene. In hexane, hexane radicals formed by pulse radiolysis react considerably faster with β-carotene ($k = 2.1 \pm 0.1 \cdot 10^{9}\ M^{-1}\ s^{-1}$) than with α-tocopherol ($k = 4.9 \pm 0.1 \cdot 10^{6}\ M^{-1}\ s^{-1}$). No evidence was obtained for a slower rate of β-carotene radical cation formation in β-carotene/α-tocopherol mixtures resulting from α-tocopheroxyl radical oxidation of β-carotene. Steady-state radiolysis experiments confirmed that α-tocopherol protects β-carotene from oxidation by hexane radicals. In both solvent systems, β-carotene is regenerated from the radical cation by α-tocopherol rather than α-tocopherol being regenerated by β-carotene from the α-tocopheroxyl radical.

Keywords: β-carotene, α-tocopherol, β-carotene radical cation, α-tocopheroxyl radical, laser flash photolysis, pulse radiolysis

INTRODUCTION

α-Tocopherol (TOH) is the most important lipid-soluble radical scavenging antioxidant in cellular membranes, plasma and lipoprotein.[1,2] Other lipid-soluble antioxidants including the ubiquinones and carotenoids are also considered potent free radical scavengers capable of preventing the chain of lipid peroxidation.[3–6] In both cases, their antioxidant activities may be due to a direct free radical scavenging mechanism or an indirect mechanism which involves re-cycling of α-tocopherol. Free radical scavenging by α-tocopherol involves the formation of the α-tocopheroxyl radical (TO·). Radical interactions with the carotenoids is less understood but generally involves either electron transfer and radical cation ($Car^{\cdot +}$) formation or radical-addition to generate carotenoid adduct-radicals.[7–11] α-Tocopherol and β-carotene exert a synergistic

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effect in inhibiting radical-initiated lipid peroxidation in membranes. α-Tocopherol was found to protect β-carotene from oxidation but the mechanisms involved remain a matter for debate.[12] Carotenoids have recently been shown to reduce the phenoxyl radical to phenol, and it was speculated whether carotenoids had a function in biological systems to recycle phenolic antioxidants by one-electron transfer forming the carotenoid radical cation.[13,14] The regeneration of phenolic antioxidants by carotenoids may also involve ascorbic acid as the final electron donor reacting with the carotenoid radical cation[15] to export potentially damaging free radical oxidants from lipid-rich environments. The relative reactivity of phenoxylic radicals is strongly dependent on the substitution pattern of the phenol. In another recent study, it was thus demonstrated that phenoxyl radicals generated from phenol by peroxidase was able to oxidize β-carotene whereas the phenoxyl radical of 2,2,5,7,8-pentamethyl-6-hydroxychromane (a tocopherol homologue) could not,[16] thereby confirming the lower reactivity of the latter. The delicate balance in the antioxidant hierarchy was nicely demonstrated in the interaction between lycopene and the tocopherol homologues.[17] α-Tocopherol was thus found to reduce the lycopene radical cation whereas lycopene was able to reduce the δ-tocopheroxyl radical. The relative reactivity of the β- and γ-tocopheroxyl radicals was comparable to that of the lycopene radical cation, and a free radical equilibrium was demonstrated between lycopene and β- and γ-tocopherol. It has recently been claimed in a pulse radiolysis study that the α-tocopheroxyl radical could be reduced by most carotenoids including β-carotene but not astaxanthin[18] via reaction (1).

$$\mathrm{TO}\cdot + \mathrm{Car} + \mathrm{H}^+ \rightarrow \mathrm{TOH} + \mathrm{Car}^{\bullet+}. \qquad (1)$$

All the second-order rate constants were close to the diffusion controlled limit ($5.3-26 \cdot 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$).

In light of studies which have provided little evidence supporting reaction (1) in model systems, we carried out experiments combining laser flash photolysis and pulse radiolysis utilising three different solvent systems to establish the validity of reaction (1). No evidence supporting reaction (1) was obtained, in fact, the reverse reaction involving the oxidation of α-tocopherol by the β-carotene radical cation was shown to occur.

MATERIALS AND METHODS

Materials

β-Carotene was supplied by Roche A/S (Hvidovre, Denmark) sealed in an ampoule under argon and was used without further purification. Phenol (p.a.) and benzene (p.a.) from Merck (Darmstadt, Germany), hexane (Certified) from Fisher Scientific UK Ltd. (Loughborough, UK) and di-*tert*-butylperoxide from Merck-Schuchardt (Hohenbrunn bei München, Germany) were all used as received. Anhydrous hexane and vitamin E [2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol, α-tocopherol] were obtained from Aldrich (Poole, UK). Nitrous oxide and nitrogen gases were obtained from the British Oxygen Company (Gillingham, UK).

Laser Flash Photolysis

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 (50 mJ per pulse) or the fourth harmonic at 266 nm (80 mJ per pulse) of a pulsed Q-switched Nd-YAG laser, Spectron Laser Systems (Rugby, UK), was used for excitation. A 1P28 photomultiplier tube from Hamamatsu (Hamamatsu City, Japan) was used to detect transient absorption changes in the visible region. Red and near infrared detection was conducted with an S1336-44BK silicon photodiode from Hamamatsu (Hamamatsu City, Japan). For red and near infrared measurements, red bandpass filters were used in order to minimize degradation of β-carotene 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 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.

Solutions contained 10 μM β-carotene and α-tocopherol in the mM range. Two sets of solutions were made; one containing 1.75 M phenol in a mixture of di-*tert*-butylperoxide and benzene (70:30, v/v) with β-carotene and α-tocopherol, and another of β-carotene and α-tocopherol in hexane.

Pulse Radiolysis

The pulse radiolysis facility at the Gray Laboratory Cancer Research Trust has been described previously.[19] In this study 500 ns pulses of 6 MeV electrons were used to deliver doses of *ca.* 9 Gy as determined by thiocyanate dosimetry.[20] Near infrared detection of carotenoid radicals was performed using a tungsten lamp and a Si photodiode. Bleaching of β-carotene and formation of the α-tocopheroxyl radical in the blue-green spectral region was monitored by photomultiplier utilising a Xe lamp. Two solvent systems were used including an N_2O-saturated hexane system and an N_2-saturated *tert*-butanol/water (60:40%) system containing 20 mM β-mercaptoethanol.

Steady-State γ-Radiolysis

Steady-state radiolysis experiments were performed in a ^{60}Co γ-source with nominal activity of 2000 Ci. Solutions were irradiated in gas-tight vials at a rate of 6 Gy min^{-1} as determined by Fricke dosimetry.[21] The degradation of β-carotene was monitored at 450 nm using a Hewlett Packard 8452A Diode Array Spectrophotometer. The radiation chemical yield for the loss of β-carotene, -*G* (β-Carotene)/μmol J^{-1} were calculated from the slopes of the linear plots of β-carotene concentration versus radiation dose in Gy.

RESULTS

Laser Flash Photolysis

Laser flash photolysis at 355 nm of di-*tert*-butylperoxide results in homolytic fission via reaction (2)

$$(CH_3)_3COOC(CH_3) + h\nu \rightarrow 2(CH_3)_3CO\cdot. \quad (2)$$

Quenching of the resultant alkoxyl radical with a large excess of phenol occurs within 10 ns to generate the phenoxyl radical:

$$(CH_3)_3CO\cdot + C_6H_5OH \rightarrow (CH_3)_3COH + C_6H_5O\cdot. \quad (3)$$

The phenoxyl radical then abstracts an electron from β-carotene to give the β-carotene radical cation[13,14] or abstracts a hydrogen atom from α-tocopherol to generate the α-tocopheroxyl radical[22] via reactions (4) and (5) respectively:

$$C_6H_5O\cdot + Car \rightarrow C_6H_5O^- + Car^{\cdot+} \quad (4)$$

$$C_6H_5O\cdot + TOH \rightarrow C_6H_5OH + TO\cdot. \quad (5)$$

The phenoxyl radical reacts with β-carotene[13] and α-tocopherol[22] at comparable rates with $k_4 = 2 \cdot 10^9\ M^{-1}\ s^{-1}$ and $k_5 = 1 \cdot 10^9\ M^{-1}\ s^{-1}$, respectively. In the absence of α-tocopherol, the β-carotene radical cation is rapidly formed (Fig. 1A) and decays bimolecularly on a subsecond time scale[13] according to reaction (6):

$$2Car^{\cdot+} \rightarrow Products(s). \quad (6)$$

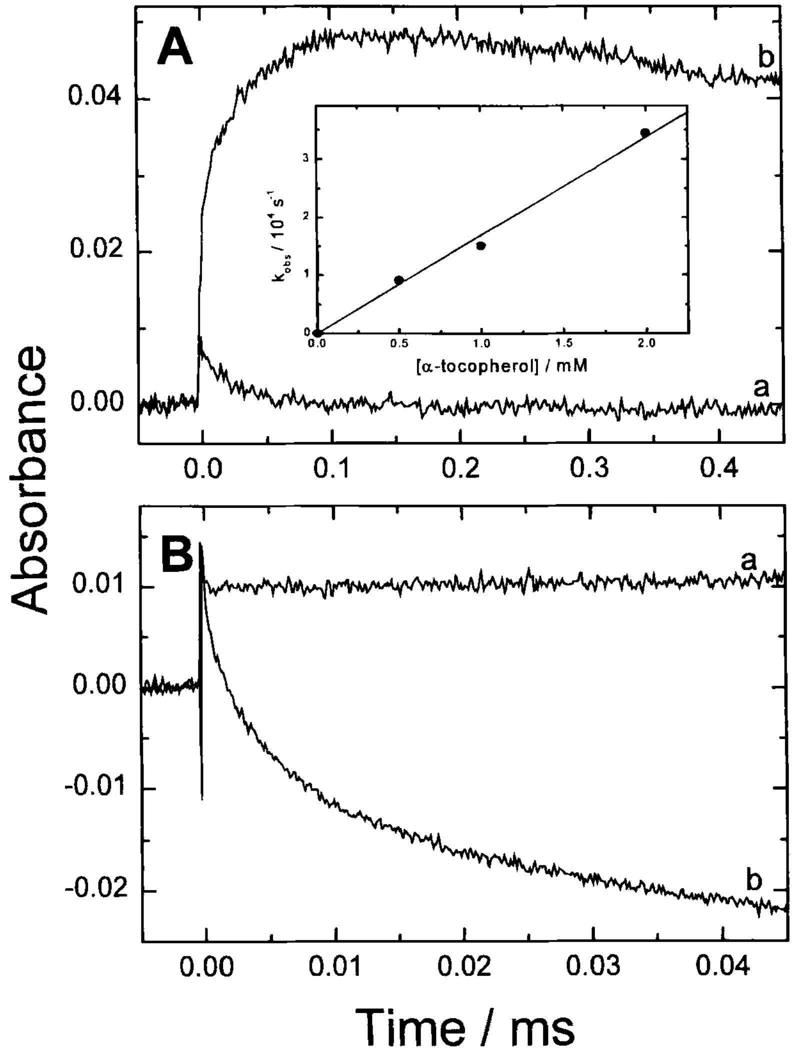

FIGURE 1 Time traces of transient absorption following laser flash photolysis (8 ns) at 355 nm of (a) 10 μM β-carotene, 1 mM α-tocopherol and 1.75 M phenol and (b) 10 μM β-carotene and 1.75M phenol in di-*tert*-butylperoxide/benzene (7:3, v/v) at **(A)** 920 nm (β-carotene radical cation absorption) and **(B)** 480 nm (β-carotene absorption). The insert in (A) shows the observed pseudo first-order rate constant of reaction (7) as a function of α-tocopherol concentration.

In the presence of a large excess of α-tocopherol (100-fold), most phenoxyl radicals are scavenged via reaction (5) and only a small amount of β-carotene radical cation is formed via reaction (4) (Fig. 1A). However, the lifetime of the β-carotene radical cation is much shorter in the presence of α-tocopherol, and disappears completely in less than 100 μs. This indicates that the β-carotene radical cation is scavenged by α-tocopherol to generate the α-tocopheroxyl radical via reaction (7):

$$\mathrm{Car}^{\bullet +} + \mathrm{TOH} \rightarrow \mathrm{Car} + \mathrm{TO}\bullet + \mathrm{H}^+. \qquad (7)$$

The decay was exponential and first-order in α-tocopherol concentration. By varying the concentration of α-tocopherol, the rate constant of reaction (7) was determined to be $k_7 = 1.7 \pm 0.1 \cdot 10^7\ M^{-1}\ s^{-1}$ from the linear slope of the observed rate constant *versus* α-tocopherol concentration in Fig. 1A. Kinetic traces recorded in the 400–500 nm spectral region (where both the β-carotene ground-state absorption and the α-tocopheroxyl radical absorption occur) confirmed reaction (7). In the absence of α-tocopherol, a positive transient is observed at short times at 480 nm (Fig. 1B) due to the formation of the phenoxyl radical (reaction (3)), and bleaching of β-carotene via reaction (4) is observed on a longer time scale. In the presence of α-tocopherol, no bleaching of β-carotene is observed but only formation of the α-tocopheroxyl radical which has a very long lifetime in this system.[22] This is consistent with the kinetic observations of the β-carotene radical cation (Fig. 1A) and is entirely consistent with reaction (7). The complete inhibition of β-carotene degradation by the phenoxyl radical in the presence of α-tocopherol indicates that the resultant α-tocopheroxyl radicals generated in reaction (5) do not react with β-carotene via reaction (1) as has been suggested.[18]

The α-tocopheroxyl radical may also be generated directly by photolysis at 266 nm in hexane

$$TOH + h\nu \rightarrow TO\cdot + H\cdot. \qquad (8)$$

The hydrogen atom is rapidly ($k = 1.5 \cdot 10^8\ M^{-1}\ s^{-1}$)[23] scavenged by hexane

$$H\cdot + C_6H_{14} \rightarrow H_2 + C_6H_{13}\cdot. \qquad (9)$$

The α-tocopheroxyl radical generated by photolysis has a rather short lifetime (Fig. 2A) compared to the lifetime in di-*tert*-butylperoxide/benzene (Fig. 1B). The lifetime, however, is greatly increased in the presence of β-carotene (Fig. 2A). The short lifetime may be explained by the reaction between the α-tocopheroxyl radical and the hexane radical:

$$TO\cdot + C_6H_{13}\cdot \rightarrow \text{product(s)}. \qquad (10)$$

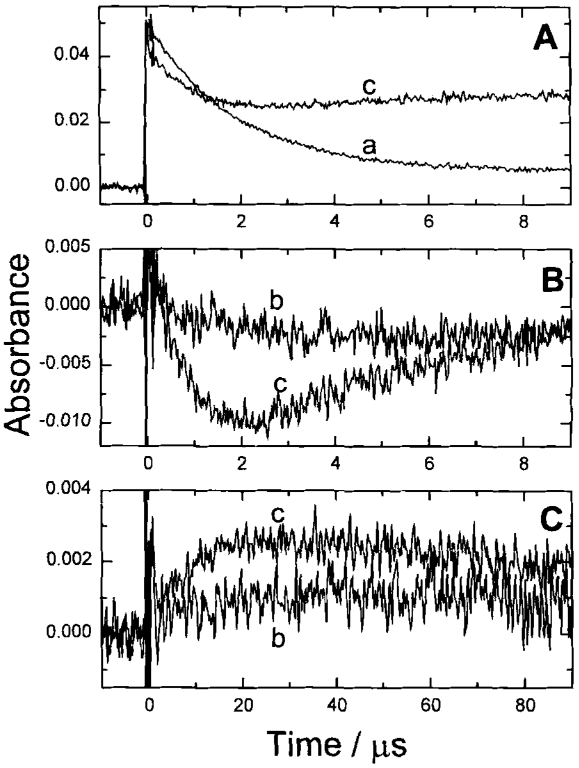

FIGURE 2 Time traces of transient absorption following laser flash photolysis (8 ns) at 266 nm of **(a)** 1 mM α-tocopherol, **(b)** 10 μM β-carotene and **(c)** 10 μM β-carotene and 1 mM α-tocopheroxyl radical absorption), (B) 450 nm (β-carotene absorption) and (C) 1040 nm (β-carotene radical cation absorption).

In the presence of β-carotene the lifetime of the α-tocopheroxyl radical is increased because the hexane radical is scavenged by β-carotene in reaction (11):

$$Car + C_6H_{13}\cdot \rightarrow Car^{\cdot+} + C_6H_{13}^{-}. \qquad (11)$$

The increased lifetime of the α-tocopheroxyl radical in the presence of β-carotene indicates that β-carotene effectively competes with the α-tocopheroxyl radical for the hexane radical. Despite the much higher concentration of α-tocopherol than of the α-tocopheroxyl radical and β-carotene, α-tocopherol does not seem to compete effectively for the hexane radical

$$TOH + C_6H_{13}\cdot \rightarrow TO\cdot + C_6H_{14}. \qquad (12)$$

If it did, a much longer lifetime of the α-tocopheroxyl radical were to be expected, and besides the instantaneous formation of α-tocopheroxyl radical due to photolysis, a slower formation due to reaction 12 would be expected. Neither is observed (Fig. 2A). The decay kinetics of the α-tocopheroxyl radical does not change in the presence of oxygen, indicating that either the hexane radicals react faster with the α-tocopheroxyl radical than with oxygen or the hexylperoxyl radicals formed by reaction between hexane radicals and oxygen react just as fast with the α-tocopheroxyl radical as the hexane radical does. β-Carotene is bleached to a small extent by the laser pulse at 266 nm (Fig. 2B). In the presence of α-tocopherol the extent of bleaching is higher due to reaction (11). However, β-carotene is only regenerated in the presence of α-tocopherol (via reaction (7)) but not in the absence of α-tocopherol (Fig. 2B). Fig. 2C shows that the β-carotene radical cation is generated to a larger extent in the presence of α-tocopherol, in accordance with reaction (11). These results provide further support for reaction (7) but once again no evidence was obtained for the reverse reaction (1).

Pulse Radiolysis

A previous pulse radiolysis study of α-tocopherol/carotenoid mixtures in hexane claimed to provide evidence for the oxidation of different carotenoids by the α-tocopheroxyl radical via reaction (1).[18] In light of our laser photolysis experiments which provided evidence for reaction (7) rather than reaction (1), further pulse radiolysis experiments were performed in N_2O-saturated hexane. Hexane radicals generated by radiolysis react with both β-carotene and α-tocopherol to generate the β-carotene radical cation and the α-tocopheroxyl radical, respectively. The relative reactivity of hexane radicals toward the two antioxidants is significantly different. Pulse radiolysis of α-tocopherol in hexane generated transient absorption with a maximum at 420 nm (Fig. 3A) attributable to the α-tocopheroxyl radical.[18,24] The formation of the α-tocopheroxyl radical was exponential and first-order in (excess) α-tocopherol concentration (0.1–8 mM). Plotting the observed first-order rate constant against α-tocopherol concentration yielded the second-order rate constant $k = 4.9 \pm 0.1 \cdot 10^6\ M^{-1}\ s^{-1}$ (insert Fig. 3A). The lifetime of the α-tocopheroxyl radical generated by pulse radiolysis is much longer than the lifetime when generated by photolysis (compare Fig. 3A with Fig. 2A) probably because of reaction (10) in the latter. The formation of the β-carotene radical cation at 999 nm[18,25] was also exponential and first-order in β-carotene concentration (10–80 μM) as shown in Fig. 3B but occurs significantly faster at a rate close to the diffusion controlled limit $k = 2.1 \pm 0.1 \cdot 10^9\ M^{-1}\ s^{-1}$. In marked contrast to the α-tocopheroxyl radical, the β-carotene radical cation is relatively short-lived in hexane and decays bi-molecularly to products via reaction (6) in less than 200 μs at the same dose per pulse *ca.* 3 Gy. Lower doses per pulse could not be used since the radiation chemical yield of the radical cation in hexane is very low (a fairly large dose per pulse of 3 Gy resulted in radical cation absorption as low as *ca.* 1 milli-absorbance unit at 999 nm).

It has been previously reported[18] that the rate of β-carotene radical cation formation in a 10 μM β-carotene/100 μM α-tocopherol mixture is much slower than with 10 μM β-carotene alone. This slower formation of the radical cation has been ascribed to reaction (1) where electron transfer occurs from the carotenoid to the α-tocopheroxyl radical.[18] We obtained no evidence for a slower rate of radical cation formation in the presence of 100 μM α-tocopherol. Indeed, Fig. 4B clearly shows that the observed rate of formation of the β-carotene radical cation increased with increasing concentrations of α-tocopherol (0.1–33 mM) at constant dose per pulse (*ca.* 2.9 Gy) and constant β-carotene concentration (20 μM). The increase in rate was also accompanied by an increase in β-carotene radical cation absorption at 999 nm (Fig. 4A). In order to ascertain whether this was due to reaction (1),

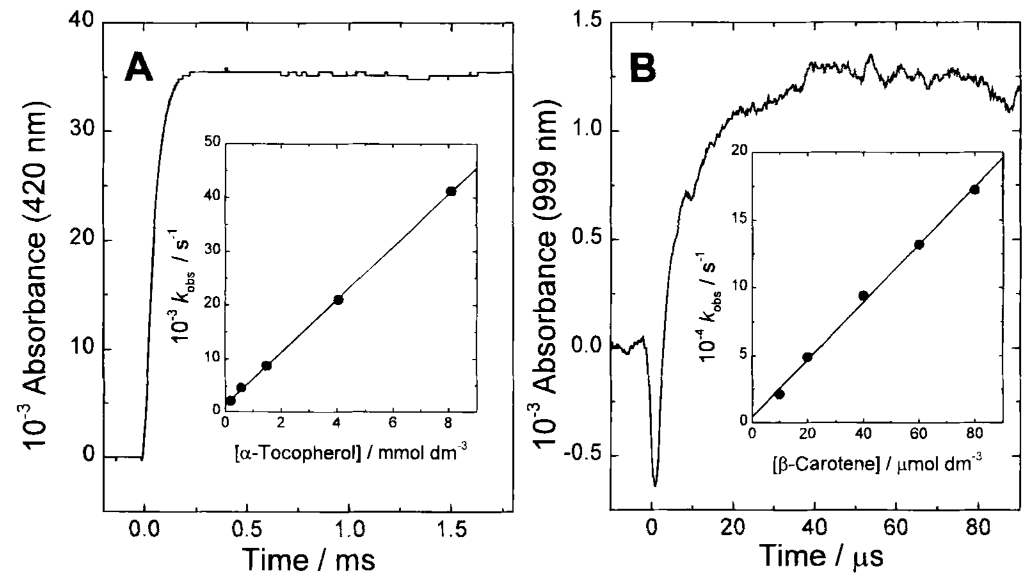

FIGURE 3 **(A)** Typical transient absorption of the α-tocopheroxyl radical generated by pulse radiolysis (9 Gy) of N_2O-saturated hexane containing 4.1 mM α-tocopherol. The insert in (A) shows the observed pseudo first-order rate constant for α-tocopheroxyl radical formation as a function of α-tocopherol concentration. Panel **(B)** shows a typical transient absorption of the β-carotene radical cation generated by pulse radiolysis (2.9 Gy) of N_2O-saturated hexane containing 20 μM β-carotene. The insert in panel (B) shows the observed pseudo first-order rate constant for β-carotene radical cation formation as a function of β-carotene concentration.

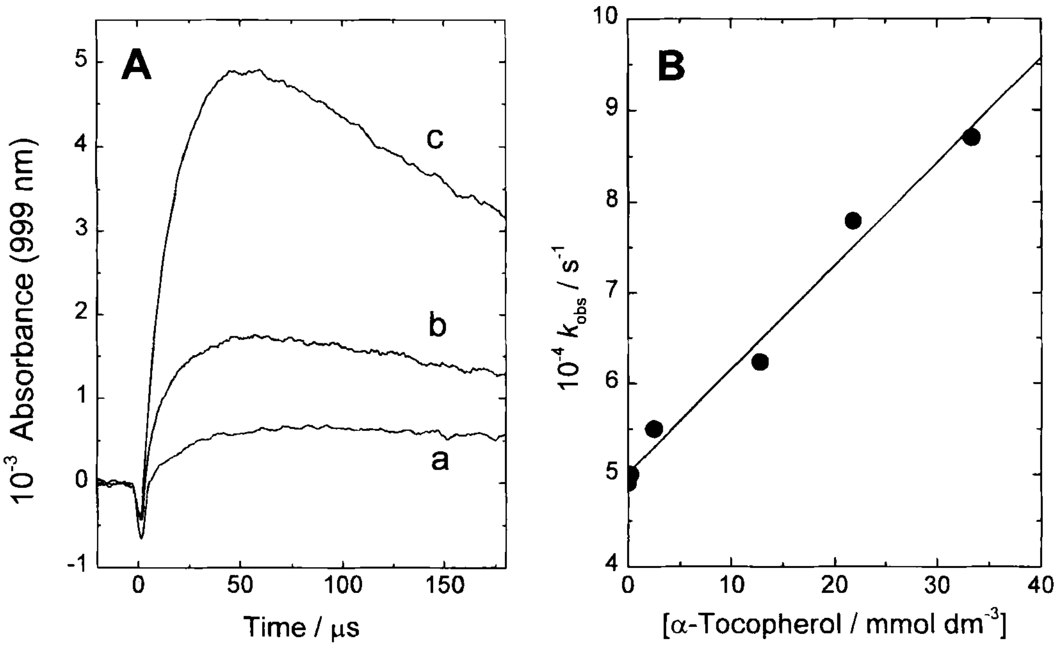

FIGURE 4 In panel A, pulse radiolysis (2.9 Gy) of 20 μM β-carotene in N_2O-saturated hexane generates the β-carotene radical cation **(a)**. The β-carotene radical cation absorption increases on the introduction of **(b)** 200 μM and **(c)** 33 mM α-tocopherol. Panel B shows the increase in the observed pseudo first-order rate constant for β-carotene radical cation formation as a function of α-tocopherol concentration at constant dose (2.9 Gy) and β-carotene concentration (20 μM).

further experiments were performed in *tert*-butanol/water mixtures containing 20 mM β-mercaptoethanol and various β-carotene and α-tocopherol concentrations. The thiyl radicals (RS·) generated in this system react exclusively with β-carotene to generate adduct-radicals [RS-Car]· in reaction (13), and unlike many other radical species thiyl radicals are unique in that they do not produce the β-carotene radical cation.[7,19] Thiyl radicals also abstract a hydrogen atom from α-tocopherol to generate the α-tocopheroxyl radical according to reaction (14).

$$RS\cdot + Car \rightarrow [RS-Car]\cdot \qquad (13)$$

$$RS\cdot + TOH \rightarrow RSH + TO\cdot \qquad (14)$$

Thus, any β-carotene radical cation formation in this system could only be attributable to reaction (1). Pulse radiolysis of excess α-tocopherol (~ 10 mM) in the presence of 20 μM β-carotene did not generate any radical cation absorption despite the fact that the α-tocopheroxyl radical is stable for ms after the electron pulse.

In the hexane system the decay of the β-carotene radical cation changed from pure second-order kinetics (Fig. 5, transient a) to first-order in the presence of excess α-tocopherol (Fig. 5, transient b). The mixed-order kinetics associated with β-carotene

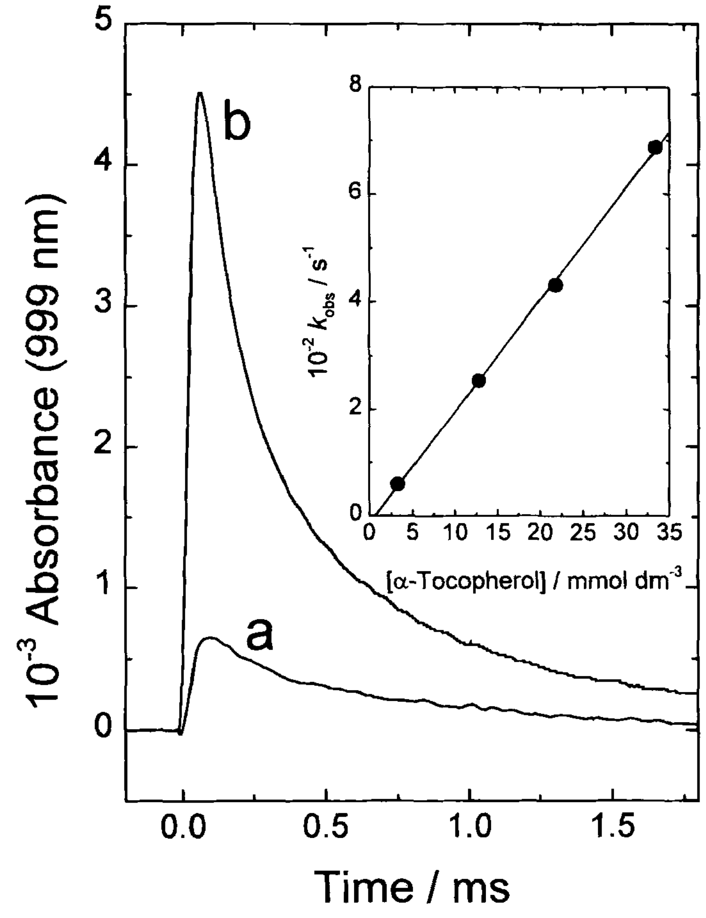

FIGURE 5 The decay of the β-carotene radical cation in the absence of α-tocopherol is second-order **(a)** and exhibits mixed-order kinetics in the presence of 33 mM α-tocopherol **(b)**. The insert shows the increase in the observed pseudo first-order rate constant for β-carotene radical cation decay with an increase in α-tocopherol concentration at constant dose (2.9 Gy) and β-carotene concentration (20 μM).

radical cation decay was resolved into the contributing first-order component due to reaction (7) and the second-order component of the competing reaction (6). The slope of the linear plot of observed first-order rate constant versus α-tocopherol concentration gave the rate constant $k_7 = 2.0 \pm 0.1 \cdot 10^4$ M^{-1} s^{-1} which is approximately three orders of magnitude slower than that determined by laser photolysis in the di-*tert*-butylperoxide/benzene system. Though we have no ready explanation for this large difference in rate constant between this system and the phenol/di-*tert*-butylperoxide/benzene system, the difference could be due to differences in reaction medium.

Further evidence supporting these observations was derived from simple steady-state radiolysis experiments of β-carotene/α-tocopherol mixtures in N_2O-saturated hexane. Under identical experimental conditions, the radiation chemical yields for the loss of the antioxidants are -*G*(β-Carotene) = 0.15 μmol J^{-1} and -*G*(α-tocopherol) = 0.21 μmol J^{-1}, respectively. Pulse radiolysis and laser photolysis experiments have demonstrated the stability of the α-tocopheroxyl radical in hexane. If oxidation of β-carotene by the α-tocopheroxyl radical via reaction (1) was taking place in this system, then the irradiation of β-carotene in the presence of excess α-tocopherol should result in a *ca.* 29% increase in β-carotene degradation. Figure 6 shows a plot of -*G*(β-Carotene) /μmol J^{-1} *versus* α-tocopherol concentration (0–500 μM) which clearly demonstrates that excess α-tocopherol (500 μM) completely suppresses hexane radical-induced degradation of β-carotene (80 μM). If reaction (1) could occur in this system, then the complete scavenging of hexane radicals by α-tocopherol should not have offered any protection. The reactivity of hexane radicals toward β-carotene is greater than with α-tocopherol by over three orders of magnitude (see Fig. 3). As shown in Figure 6 when [α-tocopherol] < [β-carotene], the hexane radicals are primarily scavenged by β-carotene but α-tocopherol still offers protection. A low dose rate of 6.2 Gy min^{-1} was used to minimise radical-radical reactions, mainly the bimolecular decay of the β-carotene radical cation via reaction (6). The mechanism of protection may therefore be due to the scavenging of the β-carotene radical cation by α-tocopherol and regeneration of β-carotene via reaction (7).

DISCUSSION

In the recent paper by Böhm and colleagues[18] a slower formation of the β-carotene radical cation in the presence of 100 μM α-tocopherol than in the absence of α-tocopherol was observed upon pulse radiolysis in hexane. This slower formation was ascribed to reaction between the α-tocopheroxyl radical and β-carotene. The rate of this reaction was found to be close to diffusion controlled for a number of carotenoids (in the case of zeaxanthin even higher than diffusion controlled). The only exception was astaxanthin where evidence was obtained for the reverse reaction (7) where the carotenoid radical cation oxidised α-tocopherol to the α-tocopheroxyl radical. Our laser flash photolysis and radiolysis experiments clearly demonstrate that reaction (7) appears to be the rule rather than the exception (at least in the case of β-carotene).

Pulse radiolysis of β-carotene/α-tocopherol mixtures in hexane is an unnecessarily complicated system for the study of the interaction of these two antioxidants. The increase in β-carotene radical cation absorption in the presence of increasing concentrations of α-tocopherol may possibly be due to another α-tocopheroxyl radical species such as the α-tocopherol radical cation ($TOH^{\bullet+}$) which may generate the β-carotene radical cation following electron transfer from β-carotene

$$\mathrm{TOH}^{\bullet+} + \mathrm{Car} \rightarrow \mathrm{Car}^{\bullet+} + \mathrm{TOH}. \qquad (15)$$

Reaction (15) may also explain the observed increase in the rate of β-carotene radical cation formation in the presence of α-tocopherol. Generally,

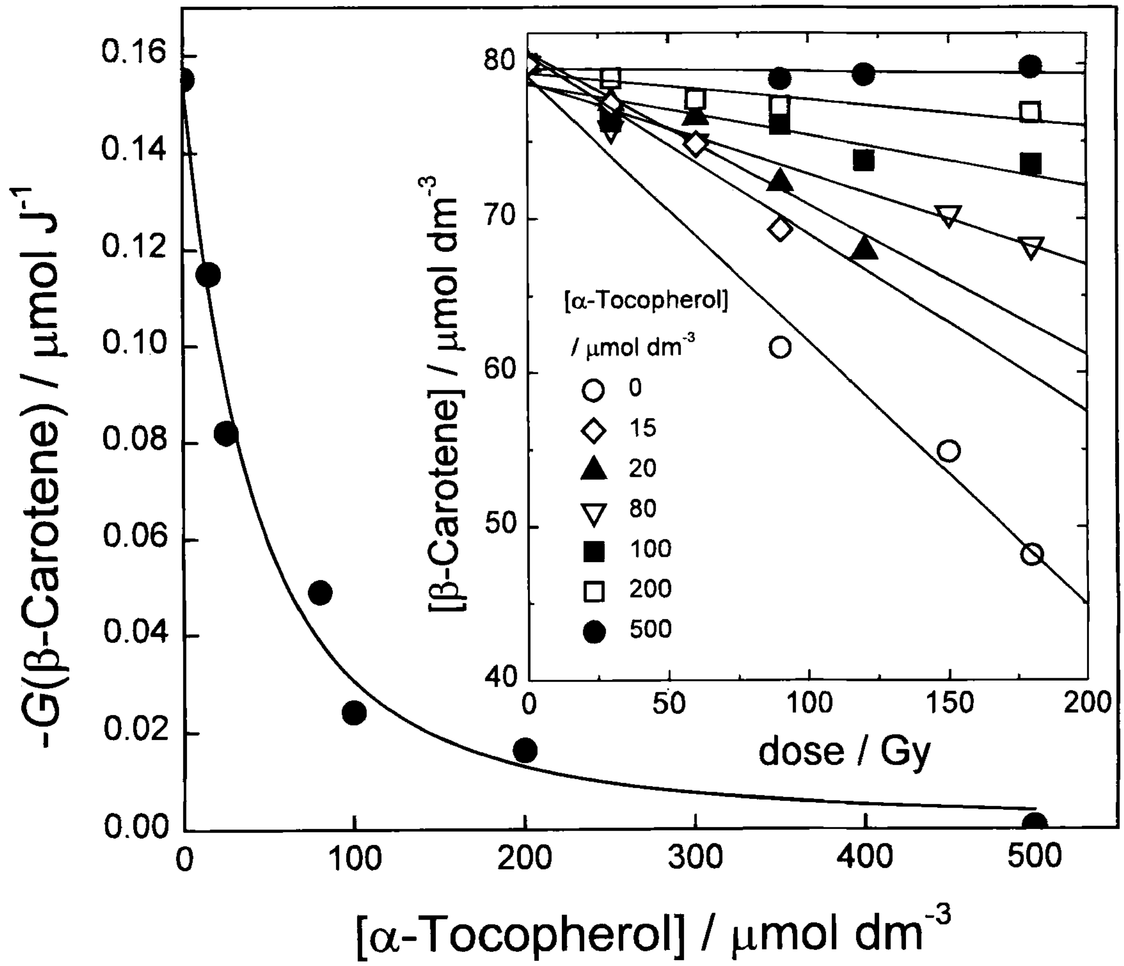

FIGURE 6 Variation of the radiation chemical yield of β-carotene degradation, -G(β-Carotene)/μmol J^{-1} during the steady-state γ-radiolysis of 80 μM β-carotene in N_2O-saturated hexane with increasing α-tocopherol concentration. Excess α-tocopherol completely inhibits β-carotene degradation. The insert shows the corresponding degradation of the β-carotene as a function of radiation dose in the presence of various concentrations of α-tocopherol.

phenoxyl radical cations have pK_a values of *ca.* 5.[26] The α-tocopherol radical cation would be expected to deprotonate very rapidly at neutral pH in aqueous systems but is very stable in acidic acetonitrile where the rate of deprotonation to the α-tocopheroxyl radical is considerably slower.[27] In the present study no spectral evidence for a tocopherol radical cation was obtained in hexane so the importance of this species in the hexane system remains a matter for debate.

Generating the α-tocopheroxyl radical by laser flash photolysis in the presence of β-carotene helped to circumvent competition kinetics associated between reactions (6) and (7) in the hexane system. Despite the fact that the rate constant $k_7 = 1.7 \cdot 10^7\ M^{-1}\ s^{-1}$ has been determined in homogeneous solution it is sufficiently fast to at least implicate reaction (7) in carotenoid/α-tocopherol interactions within heterogeneous lipophilic environments particularly cell membranes and lipoprotein. Based on this investigation we have found no evidence that β-carotene can recycle the α-tocopheroxyl radical back to α-tocopherol. However, our results do indicate that α-tocopherol recycles β-carotene by scavenging the β-carotene radical cation.

CONCLUSIONS

Previous studies of the interaction between α-tocopherol and carotenoids have indicated that the α-tocopheroxyl radical cannot be reduced by carotenoids but that the carotenoid radical cation may be reduced by α-tocopherol.[12,16,17] The present results have clearly demonstrated that this is indeed the case, and a rate constant $k_7 = 1.7 \pm 0.1 \cdot 10^7\ \mathrm{M^{-1}\ s^{-1}}$ for this reaction has been determined. Our results are consistent with the hypothesis that α-tocopherol protects β-carotene during membrane-associated oxidative stress and not *vice versa*.[18]

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Note added in proof

Recent findings by Val-gimigli *et al.*[28] corroborate our findings, i.e. that β-carotene cannot reduce the α-tocopheroxyl radical.

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