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# Kinetics of Parallel Electron Transfer from β-Carotene to Phenoxyl Radical and Adduct Formation Between Phenoxyl Radical and β-Carotene

ALAN MORTENSEN and LEIF H. SKIBSTED*

*Food Chemistry, Department of Dairy and Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark*

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Phenoxyl radicals generated by laser flash photolysis were found to react with β-carotene with concomitant β-carotene bleaching in two parallel reactions with similar rates: (i) formation of a β-carotene adduct with a (pseudo) first order rate constant of $1\text{–}1.5 \cdot 10^4$ $s^{-1}$ with absorption maximum around 800 nm, and (ii) formation of a β-carotene radical cation with a (pseudo) first order rate constant of $2\text{–}3 \cdot 10^4$ $s^{-1}$ with absorption maximum around 920 nm. Both β-carotene radicals decay on a similar time scale and have virtually disappeared after 100 ms, the β-carotene adduct by a second order process. Oxygen had no effect on β-carotene bleaching or radical formation and decay. The reduction of phenoxyl radicals by β-carotene may prove important for an understanding of how β-carotene acts as an antioxidant.

*Keywords:* β-caroten radicals, phenoxyl radical, laser flash photolysis, transient absorption

## INTRODUCTION

Carotenoids may act as antioxidants (i) by quenching singlet oxygen and (ii) by scavenging free radicals. While the singlet oxygen quenching is well-understood, the radical scavenging has been less investigated. The carotenoid could possible react with a free radical by two different pathways: (i) reduction of the free radical by transfer of an electron or a hydrogen atom from the carotenoid to the free radical or (ii) by addition of the free radical to the conjugated π-electron system of the carotenoid. Pathway (i) would give rise to the carotenoid radical cation or the conjugate base depending on whether only an electron or a hydrogen atom is transferred. Pathway (ii) gives rise to an adduct with the free electron located on the carotenoid moiety as suggested by Burton and Ingold.[1] The species produced by either pathway are fairly stable and may be observed on a millisecond timescale by utilising time-resolved spectroscopy in the near infrared spectral region. Alternatively, the reaction of carotenoids with free radicals may be followed by observing the bleaching taking place in the blue-green spectral region.

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*Corresponding author: Fax: +45 35 28 33 44. E-mail: ls@kvl.dk

Bors and co-workers observed a fast (within a few microseconds) bleaching of crocin, crocetin, and canthaxanthin upon reaction with alkoxyl, peroxyl, or hydroxyl radicals.[2–9] Bleaching of β-carotene upon reaction with thiyl radicals without concomitant formation of near infrared absorbing species has been observed,[10,11] and an adduct between β-carotene and the thiyl radical was believed to be formed. In contrast, $C_2H_5SO_2\cdot$ reacted with β-carotene to form "a composite absorption spectrum" ascribed to both an adduct and the radical cation,[10,11] and $NO_2$ reacted exclusively with β-carotene to form the β-carotene radical cation. In another study, the reaction of β-carotene and lycopene with nitrogen dioxide resulted in transient species detected by two absorption peaks in the near infrared.[12] An absorption maximum at 800 nm was ascribed to an adduct between the carotenoid and nitrogen dioxide, and absorption around 890 nm was ascribed to the carotenoid radical cation.[12]

The reaction of $CCl_3O_2\cdot$ with β-carotene was followed by observing the bleaching and the transient absorption in the near infrared,[13,14] which was ascribed to the radical cation. Reaction took place on a submillisecond timescale. A number of carotenoids have been found to react with $CCl_3O_2\cdot$ by formation of two species absorbing in the near infrared.[15] The species absorbing at the shortest wavelength was believed to be an adduct between $CCl_3O_2\cdot$ and the carotenoid, whereas the longer wavelength peak was assigned to the radical cation. Both species were formed directly by reaction with the trichloromethylperoxyl radical, and furthermore the radical cation was formed by decay of the adduct.[15] Reaction of the carotenoids with $CCl_3\cdot$ only gave rise to one near infrared absorbing species: the radical cation.

Phenoxyl radicals are formed when phenolic antioxidants react with peroxyl and alkoxyl radicals in biological systems.[16] Other antioxidants like ascorbate may regenerate the phenols as part of a biological defence against peroxidation.[17] The role of carotenoids in such processes is largely unknown, and we have initiated investigations of the reaction of carotenoids with antioxidant radicals and now report results of a kinetic investigation of the reaction of β-carotene with the phenoxyl radical in homogeneous solution.

## 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 cyclohexane (p.a.) from Merck (Darmstadt, Germany) and di-*tert*-butylperoxide from Merck-Schuchardt (Hohenbrunn bei München, Germany) were all used as received. The solutions were made up from stock solutions of $10^{-4}$ M β-carotene in cyclohexane and 2.5 M phenol in di-*tert*-butylperoxide yielding a concentration of β-carotene ranging from 5 to 30 μM and a concentration of phenol of 1.75 M in di-*tert*-butylperoxide/cyclohexane (70/30, v/v). The solutions were stored in the dark at 5°C until they were used. To ensure that no degradation had taken place during storage, sample quality was checked by absorption spectroscopy prior to laser flash photolysis. Oxygen was removed from some of the samples prior to measurements by 3 freeze-pump-thaw cycles.

### Experimental Methods

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 corresponded approximately to 60 mJ at 355 nm. A 1P28 photomultiplier tube from Hamamatsu (Hamamatsu City, Japan) was used to detect transient absorption changes in the visible region. Bleaching was measured at 480 nm (the maximum of β-carotene absorption) at concentrations of β-carotene below 1.0 · $10^{-5}$ M. At higher concentrations, bleaching was measured at a longer wavelength where the absorbance was close to 1. 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. Due to sample degradation each sample was subjected to no more than around 20 laser pulses.

Time traces were analysed by non-linear least squares fitting using the Levenberg-Marquardt algorithm.[18] Transient absorption spectra were constructed from time traces at 18–21 individual wavelengths.

## RESULTS

Upon laser flash photolysis of a solution of β-carotene and phenol in di-*tert*-butylperoxide/cyclohexane using near-UV light for excitation, the initial reaction is a homolytic cleavage of the peroxide bond[19]

$$(CH_3)_3COOC(CH_3)_3 \rightarrow 2(CH_3)_3CO\cdot.$$

The formed alkoxyl radicals react very rapidly (i.e. within 10 ns) with the large excess of phenol present in the solution

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

yielding the phenoxyl radical which was observed by its absorption around 390 nm. The phenoxyl radical then reacts with β-carotene which was observed by the induced bleaching of β-carotene around 400–500 nm. All the alkoxyl radicals react with phenol and not with β-carotene. In the absence of phenol no transient bleaching of β-carotene was observed.

Figure 1 shows the transient absorption at 480 nm induced by a laser pulse in a solution of β-carotene and phenol in di-*tert*-butylperoxide/cyclohexane. At very short times (i.e. below 0.6 μs) a positive transient absorption is observed. This is due to the phenoxyl radical which gives rise to absorption in the entire visible range.[20] This radical is practically gone within a few tenths of a millisecond regardless of the presence of β-carotene as shown by measuring the transient absorption at 385 nm where β-carotene only absorbs weakly (see insert in Fig. 1). The (pseudo) first order rate constant as deduced from the time traces is of the order of $10^3$–$10^4$ $s^{-1}$ increasing with growing concentration of β-carotene. After a few milliseconds (Fig. 1) no further bleaching of β-carotene is observed, and the absorption remains constant at this lower level.

The bleaching of β-carotene in the visible spectral region is followed by concomitant formation of species absorbing in the near infrared. In Figure 2 is shown the near infrared transient absorption spectra following laser flash photolysis of a solution of β-carotene and phenol in di-*tert*-butylperoxide/cyclohexane. Apparently, two species are formed as may be further seen in Figure 3, in which the time traces of absorbance close to the two absorption maxima in the transient near infrared spectra are shown up to a tenth of a second. One species, with an absorption maximum at 920 nm, is formed with a (pseudo) first order rate constant of the order of 2–3 · $10^4$ $s^{-1}$ increasing with increasing concentration of β-carotene. This species reaches its maximum concentration around 200 μs after the laser pulse (Figs. 2 and 3). The other species, absorbing around 800 nm, is formed with a (pseudo) first order rate constant of 1–1.5 · $10^4$ $s^{-1}$ increasing slightly with increasing concentration of β-carotene, and reaches its maximum concentration after approximately 500 μs (Figs. 2 and 3).

The time trace of the decay of the species absorbing at 920 nm could neither be fitted to a

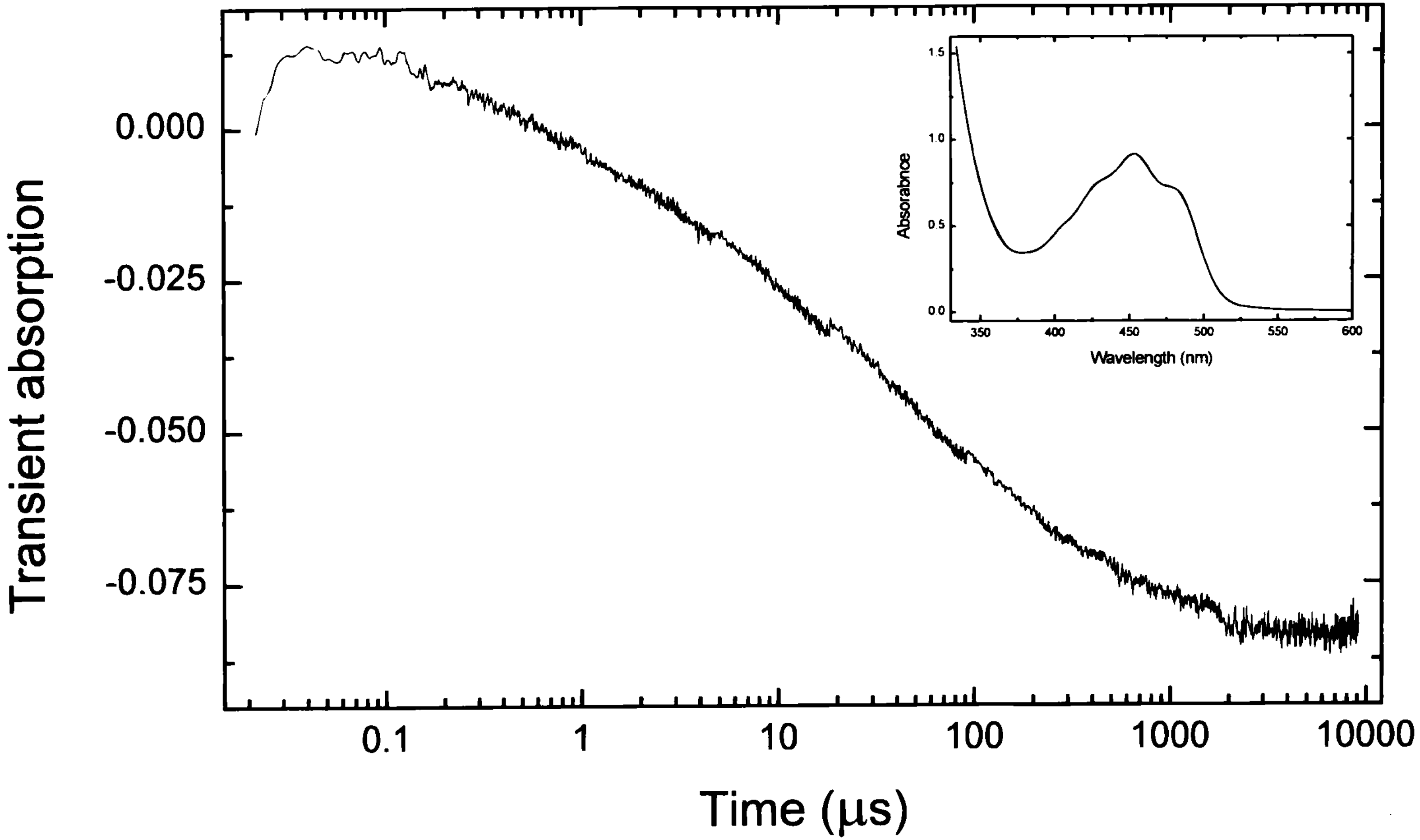

FIGURE 1 Time trace of absorbance changes at 480 nm of $1.0 \cdot 10^{-5}$ M β-carotene and 1.75 M phenol in air-saturated di-*tert*-butylperoxide/cyclohexane (70/30, v/v) at 20°C excited at 355 nm with a 10 ns laser pulse. Insert: Absorption spectrum of the reaction solution. The strong absorption at 300 nm is mainly due to di-*tert*-butylperoxide.

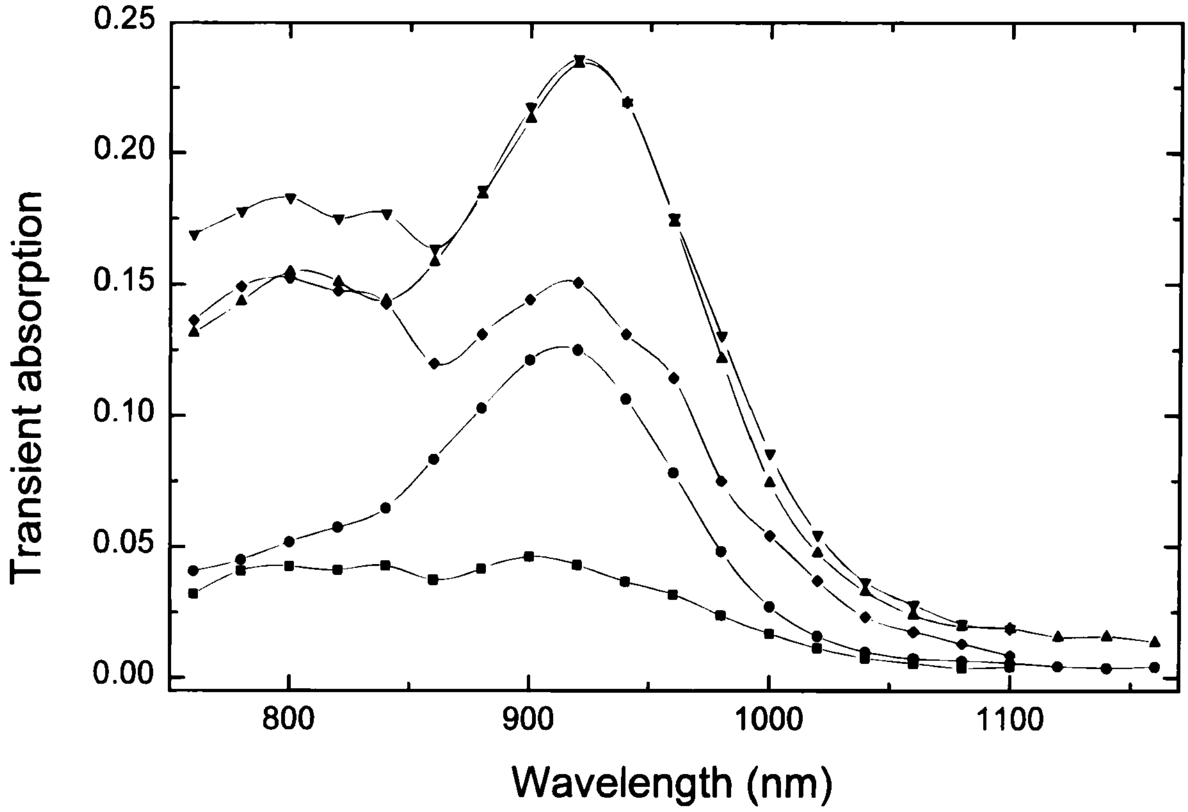

FIGURE 2 Transient absorbtion spectra of 3.0 · $10^{-5}$ M β-carotene and 1.75 M phenol in air-saturated di-*tert*-butylperoxide/cyclohexane (70/30, v/v) at 20°C excited at 355 nm. (●) 9 μs, (▲) 89 μs, (▼) 290 μs, (◆) 1.9 ms, and (■) 19 ms after a 10 ns laser pulse.

first order nor a second order expression. The time trace (at 780 nm, Fig. 3) of the species absorbing at 800 nm, on the other hand, seemed to fit a second order expression reasonably well (see insert in Fig. 3). This could indicate for instance disproportionation yielding β-carotene and a two-electron oxidized species. However, β-carotene is not being reformed as Figure 1 shows. The degradation products of β-carotene oxidation by alkyl, alkoxyl, and peroxyl free radicals have recently been studied.[21] The products formed by reaction with phenoxyl radicals, however, still remain unidentified. Both species decay on a similar time scale (Fig. 3).

Neither bleaching of β-carotene nor formation of the two near infrared absorbing species were influenced by whether oxygen was present in the solutions or not.

## DISCUSSION

The formation of two near infrared absorbing species in the present system with a phenoxyl radical reacting with β-carotene is in accordance with the findings of a previous study on the reaction of carotenoids with the trichloromethylperoxyl radical in aqueous solutions,[15] in which two near infrared absorbing species also were detected. If the assignment of Hill *et al.*[15] of the two transient species is correct, we may assign the species absorbing at 920 nm in our experiments to the β-carotene radical cation and the species absorbing at 800 nm to an adduct between the phenoxyl radical and β-carotene.

On the other hand, Everett *et al.*[11] found that the adduct between β-carotene and thiyl or sulfonyl radicals absorbed in the same region as β-

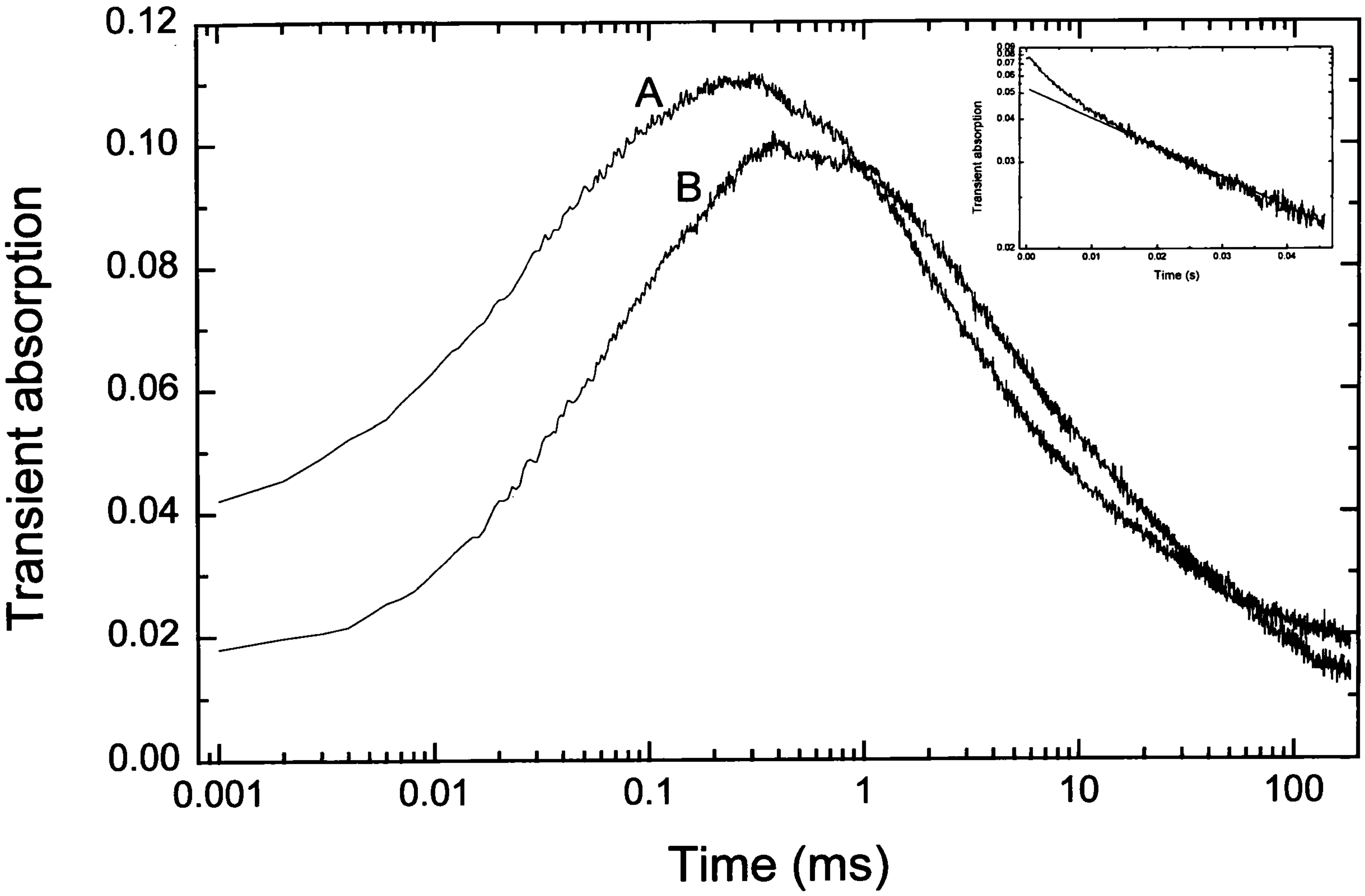

FIGURE 3 Time traces of absorbance changes at 950 nm (A) and 780 nm (B) of 1.4 · $10^{-5}$ M β-carotene and 1.75 M phenol in air-saturated di-*tert*-butylperoxide/cyclohexane (70/30, v/v) at 20°C excited at 355 nm with a 10 ns laser pulse. The insert shows a second order plot for decay of the transient absorption at 780 nm (reciprocal absorption as a function of time). Deviation from second order behaviour at early stages is due to spectral interference from the radical cation and to the continuing formation (up till a few milliseconds, Fig. 1) of the adduct.

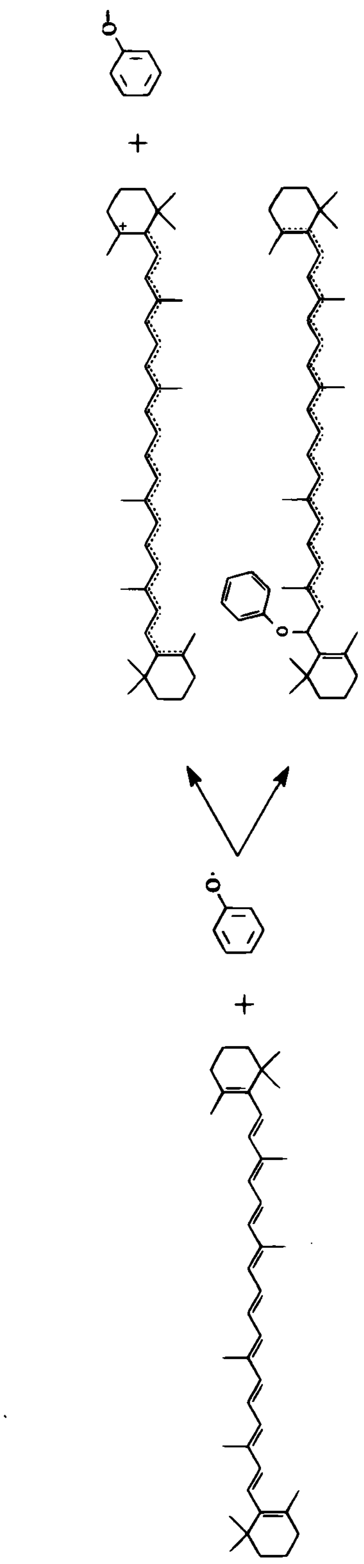

FIGURE 4 Competitive reaction of phenoxyl radical with β-carotene to yield phenoxyl adduct of β-carotene (the phenoxyl radical may in principle add to the β-carotene conjugated π-electron system in various positions) and β-carotene radical cation.

carotene itself. Furthermore, Everett *et al.*[11] found that only one near infrared absorbing species (the radical cation) was formed upon reaction of β-carotene with $NO_2$, whereas Böhm *et al.*[12] observed two near infrared absorbing species (an adduct and the radical cation). Obviously, there is some inconsistency here. As far as the difference in absorption between adducts of β-carotene and thiyl or sulfonyl radicals (absorbing in the blue-green spectral region[11]) and between β-carotene and trichloromethylperoxyl radicals (absorbing in the near infrared[15]), it may be speculated that this is due to the nature of the attacking radicals and the formed adducts. If the sulfur containing radicals add to β-carotene at the center of the molecule, the length of the odd-electron conjugated π-electron system (the chromophore) is shorter than if it added to the end of the β-carotene molecule, and the resultant absorption would be at a shorter wavelength. As far as the reaction with peroxyl radicals is concerned, product studies have shown[21] that they primarily add to β-carotene at the end of the molecule yielding a long odd-electron π-electron system.

The rate constant deduced from observing either the bleaching of β-carotene or the formation of the products should be the same in a parallel reaction (β-carotene → adduct, and β-carotene → β-carotene$^{+\cdot}$) and equal the sum of the two individual rate constants.[22] However, as mentioned previously the observed rate constant from bleaching of β-carotene is lower than that deduced from observing the formation of the products. This is due to interference from the phenoxyl radical which absorbs in the same spectral region. The fact that the observed rate of formation of the radical cation is higher than that of the adduct (though they should be the same, *vide supra*) is either due to differences in decay rate of the cation radical and the adduct or is due to decay of the adduct to the cation radical (adduct → β-carotene$^{+\cdot}$) resulting in two different pathways of formation of the cation radical and hence a higher observed rate constant. In the study of Hill *et al.*[15] it was observed that both the radical cation and the adduct were formed by direct reaction with the trichloromethylperoxyl radical. Besides this direct formation, the radical cation was also formed by decay of the adduct in accordance with the present findings. Previously, we reported the decay of an adduct between β-carotene and chloroform to yield the β-carotene cation radical.[23]

Hill *et al.*[15] observed a (pseudo) first order rate constant of formation of the adduct and the radical cation of $2 \cdot 10^5\ s^{-1}$ at a concentration of β-carotene of $10^{-4}$ M. This yields a second order rate constant of $2 \cdot 10^9\ M^{-1}\ s^{-1}$, a value close to the value of the second order rate constant we obtain at a concentration of β-carotene of $10^{-5}$ M ((pseudo) first order rate constant of the order of $10^4\ s^{-1}$). In another study[13], a second order rate constant for the reaction of the trichloromethylperoxyl radical with β-carotene of $1.5 \cdot 10^9\ M^{-1}\ s^{-1}$ in t-butanol/water was deduced from the bleaching of β-carotene.

A very recent study by Liebler and McClure[21] showed that alkyl, alkoxyl, and peroxyl free radicals react with β-carotene both by addition and by hydrogen atom abstraction, the equivivalent of a one-electron transfer. β-Carotene seems hence to be able to scavenge a vide variety of free radicals, and scavenging takes place by two different pathways.

## CONCLUSIONS

The present results show that β-carotene reacts as a scavenger of phenoxyl radicals in two ways (as summarized in Fig. 4): (i) by formation of an adduct with the free radical and (ii) by donation of an electron to the free radical. Both the adduct and the radical cation are formed by direct reaction with the phenoxyl radical and then decay to unidentified degradation products. Besides this, the radical cation may be formed by decay of the adduct. The fact that the time trace of decay of the radical cation could not be fitted to neither a first nor second order expression indicates that the kinetics of formation of this species is complex, possible due to two different pathways of formation. If it is assumed that the extinction coefficients of the radical cation and the adduct are similar, then Figure 2 shows that both reaction pathways depicted in Figure 4 are equally important in the reaction of β-carotene with free radicals. Phenoxyl radicals are important intermediates in biological defence against peroxidation. The present studies show that β-carotene may play a role in the regeneration of one-electron oxidized phenolic antioxidants like tocopherols and flavonoids, which may be important for an understanding of β-carotene's role as an antioxidant.

## *Acknowledgements*

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