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Kinetics of Photobleaching of β-Carotene in Chloroform and Formation of Transient Carotenoid Species Absorbing in the Near Infrared

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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Upon laser flash photolysis of β-carotene in chloroform instantaneous bleaching of β-carotene and concomitant formation of near infrared absorbing species are observed. One species, absorbing with maximum at 920 nm, is formed during the laser pulse (10 ns) and is practically gone in one millisecond, the decay showing a bi-exponential behaviour. The second species, absorbing with maximum at 1000 nm, is formed from the species absorbing at 920 nm by first order kinetics with a rate constant of $4.9 \cdot 10^4\ s^{-1}$ at 20°C. This second species decays by second order kinetics and is gone within a few milliseconds. An additional slow bleaching of β-carotene and formation of the species absorbing at 920 nm is observed. This slow bleaching/formation of transient absorption is probably due to processes involving free radicals generated during the instantaneous bleaching. The species absorbing at 920 nm is suggested to be either (i) a free radical adduct formed from β-carotene and chloroform or (ii) β-carotene after abstraction of a hydrogen atom. The species absorbing at 1000 nm is most likely the radical cation. Formation and decay of the near infrared absorbing species and bleaching of β-carotene are independent of whether oxygen is present or absent in the solutions.

Keywords: β-carotene, β-carotene radicals, laser flash photolysis, transient absorption

INTRODUCTION

Carotenoids are known to suppress peroxidation of unsaturated lipids exposed to free radicals, and the carotenoids most stable towards bleaching in peroxidizing systems have been found to be the more effective antioxidants.[1,2] A correlation between the electronic properties of carotenoids and their efficiencies as free radical scavengers has been attempted,[3] but seems to have to await a more detailed understanding of the reaction mechanisms for radical scavenging by carotenoids.

Carotenoids could possibly react as free radical scavengers in a number of ways. One way could be by donation of one of the allylic hydrogens giving rise to a resonance stabilized odd-electron conjugated system. The bond energy of the allylic

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*Corresponding author. Phone: 45 35 28 32 21. Fax: 45 35 28 33 44 hydrogens in retinal[4] (the bond energy of the allylic hydrogens of β-carotene may be expected to be similar) have been calculated to be around 330 kJ/mol, the same as that of phenolic hydrogens which are known to be involved in the free radical scavenging of many chain-breaking antioxidants. Another reaction mechanism of carotenoids could be direct reduction of a free radical yielding the carotenoid cation radical which is also stabilized by resonance. Finally, carotenoids could react with free radicals by addition of the free radical to the π-backbone of the carotenoid giving a (shortened) odd-electron conjugated system. This last mechanism was originally proposed by Burton and Ingold.[5] Of course, carotenoids may react by all of the above mechanisms depending on the conditions and the carotenoid involved. In fact, both the formation of a carotenoid cation radical and an adduct with a free radical in the same experiment have been claimed.[6,7] These species are all expected to absorb in the near infrared spectral region, and time resolved near infrared spectroscopy should prove valuable in further kinetic studies of carotenoids as free radical scavengers.

We present here results of a kinetic study of β-carotene dissolved in chloroform and exposed to light showing direct correlation between bleaching of β-carotene and formation of transient species absorbing in the near infrared. Transient species derived from carotenoids absorbing in the near infrared have been studied before,[6–36] and bleaching of carotenoids subjected to free radicals or light have been examined as well.[6,16,24,35–38] However, a correlation between bleaching in the visible region and formation of transient species absorbing in the near infrared have never been studied in detail before.

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. Chloroform, HPLC grade, from Lab-Scan (Dublin, Ireland) was used as received. A stock solution of 10^{-4} M β-carotene in chloroform was diluted to the required concentration. The solutions were kept in the dark for no longer than a week at 5°C before they were used. The condition of the samples was checked by absorption spectroscopy prior to laser flash photolysis to see if any significant degradation had taken place during storage.

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 was approximately 60 mJ at 355 nm. In a single experiment the second harmonic at 532 nm was used. A 1P28 photomultiplier tube from Hamamatsu (Hamamatsu City, Japan) was used to detect transient absorption at wavelengths below 550 nm. Bleaching was measured at 464 nm (the maximum of β-carotene absorption) at concentrations of β-carotene below $1.0 \cdot 10^{-5}$ 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 either an R928 photomultiplier tube or an S1336-44BK silicon photodiode, both 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 30 laser pulses. After 30 laser pulses no change of the time traces could be discerned though some degradation had taken place as evidenced by absorption spectroscopy.

Time traces were analysed by non-linear least squares fitting using the Levenberg-Marquardt algorithm. Transient absorption spectra were constructed from time traces at 25 individual wavelengths.

RESULTS

Exposure of β-carotene dissolved in chloroform to light was found to induce bleaching of β-carotene. Similar bleaching was not observed when the experiments were repeated in benzene or toluene. In Figure 1 is shown the time trace of β-carotene absorption changes in air-saturated chloroform exposed to a 10 ns laser pulse at 355 nm measured at 464 nm, the maximum of the ground state absorption of β-carotene. A rapid bleaching, i.e. occuring within the time span of the laser pulse (around 10 ns), is observed (Fig. 1A). This laser induced bleaching is not permanent but persists for around 10 μs after which a slower bleaching with a rate constant of the order of 10^{-4} s^{-1} takes place (Fig. 1B). After approximately half a millisecond, partial recovery of the bleaching is observed (Fig. 1B and C).

The time traces of the slow bleaching could be fitted quite well to an exponential decay as may be seen from the insert in Figure 1B. However, the (pseudo) first order decay constant depends upon the concentration of β-carotene as depicted in Figure 2. That is, the slow bleaching is not a first order reaction.

The bleaching of β-carotene is accompanied by formation of transient species absorbing in the near infrared (Fig. 3). It can be seen from Figure 3 that at least two transient species are formed: one, giving rise to an absorption maximum around 920 nm with a secondary maximum in the region 750–800 nm, being formed very fast and another, having an absorbtion maximum around 1000 nm, which appears on a much longer time scale.

The transient absorption spectra of the two species cannot simply be resolved in component spectra because they overlap extensively both spectrally and in time (Fig. 3). Therefore, the formation and decay of the two species were followed not at the maximum of their absorption (920 and 1000 nm, respectively) but in the wings of their absorption bands where the transient absorbtion is still sufficent and the extent of overlap with the absorption of the other species is low. The species absorbing at 920 nm was, hence, monitored at 740 nm and the species absorbing at 1000 nm was monitored at 1060 nm.

The species appearing first in the near infrared, i.e. the one absorbing at 920 nm, is formed instantaneously, i.e. during the laser pulse, and starts to decay immediately (Fig. 4A) with a rate which seems to be independent of the concentration of β-carotene. This rapid decay is followed by a slower decay (Fig. 4B). The overall decay curves fit quite well to a double exponential with two rate constants of the order of 10^5 s^{-1} and 10^3–10^4 s^{-1} (only order of magnitude estimates can be made because the two exponentials decay on a similar time scale making separation of the two decay function difficult, and the non-linear fitting hence unreliable). The rate of decay depends upon the concentration of β-carotene, as shown in Figure 4B, the initial decay being faster at low concentrations. The transient species is completely gone within a millisecond (Fig. 4C).

The species absorbing at 1000 nm is not formed instantaneously, i.e. during the laser pulse, as Figure 5A shows. Fitting an exponential to this time trace yields a first order rate constant of

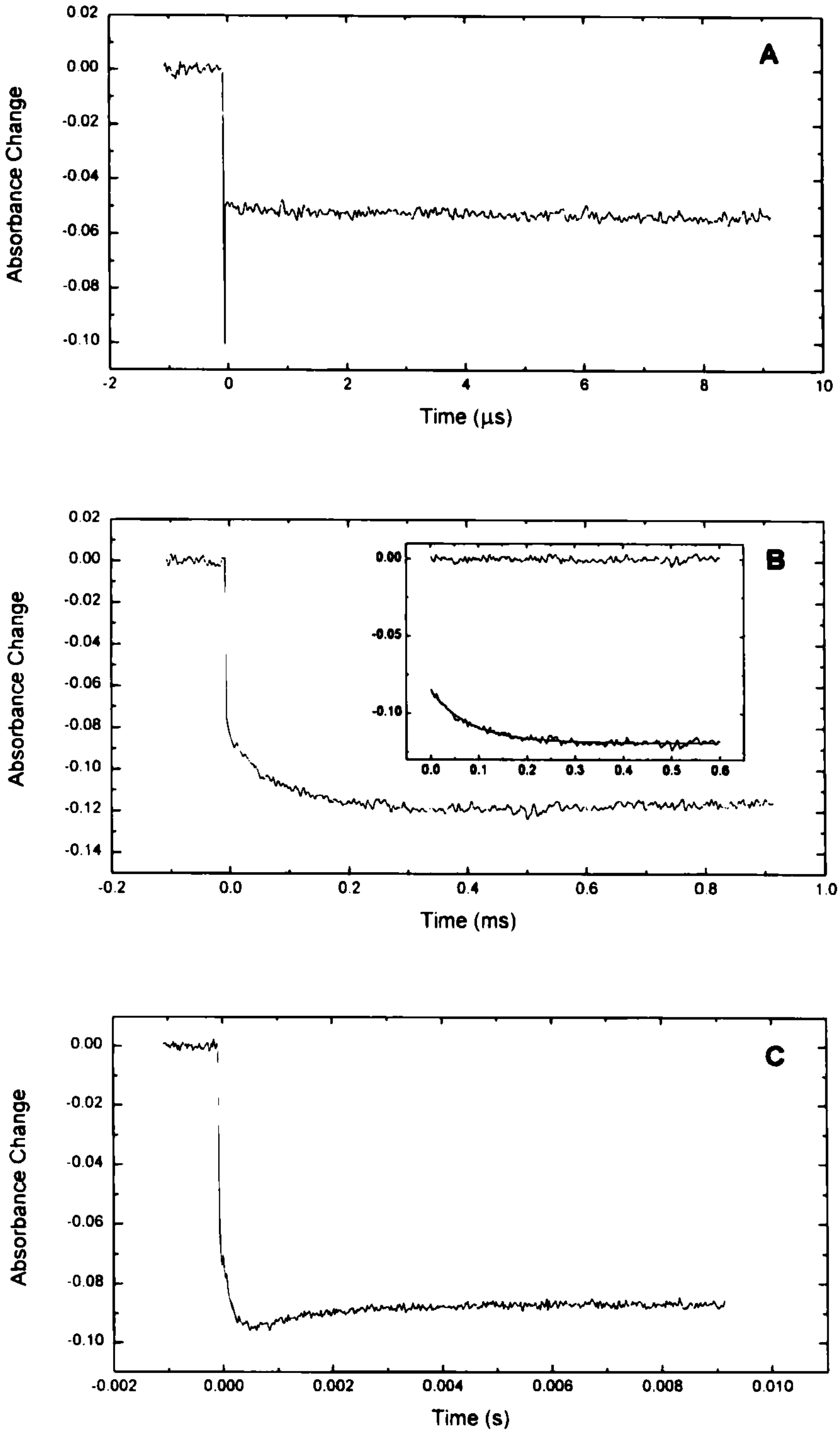

FIGURE 1 Time traces of absorbance changes at 464 nm on three different time scales of a 1.0· 10^{-5} M solution of β-carotene in air-saturated chloroform at 20°C excited at 355 nm with a 10 ns laser pulse. The insert in B shows a fit to a first order expression and the resulting residuals.

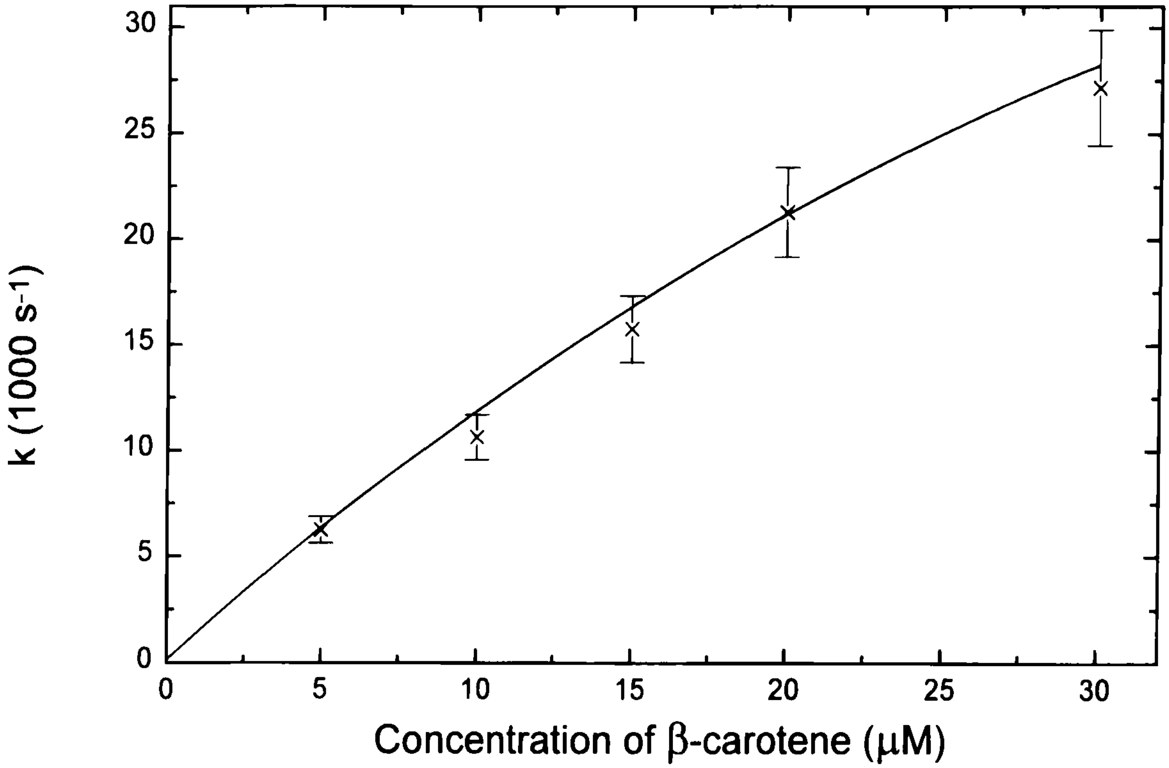

FIGURE 2 Pseudo first order rate constant of the slow bleaching (ms time scale) of β-carotene in chloroform (Fig. 1B) as a function of total concentration of β-carotene. The full line is a guide to the eye.

$6 \cdot 10^4$ s^{-1} at 20°C which increases slightly with increasing concentration of β-carotene. However, the rate constant is only apparently dependent upon concentration of β-carotene. Taking the subsequent decay of the species absorbing at 1000 nm into account (Fig. 5B) yields a first order rate constant of $4.9 \cdot 10^4$ s^{-1} which is independent of concentration of β-carotene. The decay (Fig. 5C), on the other hand, depends upon concentration and fits a second order expression as shown in the inset of Figure 5C. However, a second order rate constant cannot be determined without knowing the concentration of the transient species. Due to the second order decay of this species it persists for a long time. However, within a few milliseconds it is practically gone (Fig. 5C).

The near infrared time traces without oxygen are identical to those where oxygen is present.

DISCUSSION

Time Traces

The initial physical process taking place upon laser flash photolysis at 355 nm of a solution of β-carotene in chloroform (or any other solvent) is excitation of β-carotene. This would, *a priori*, give rise to bleaching. The excited states of β-carotene of interest here is S_1, which cannot be directly populated by absorption due to symmetry restrictions, S_2, the transition to which gives rise to the strong visible absorption, and the level which is responsible for the so-called "cis-band" around 350 nm.[3] The lifetime of these states is very short: around 10 ps (S_1),[39–41] 0.2 ps (S_2),[41–43] and the lifetime of the "cis-level" is probably very short as well. That is, excitation of β-carotene does not by itself give rise to bleaching in the ns regime used

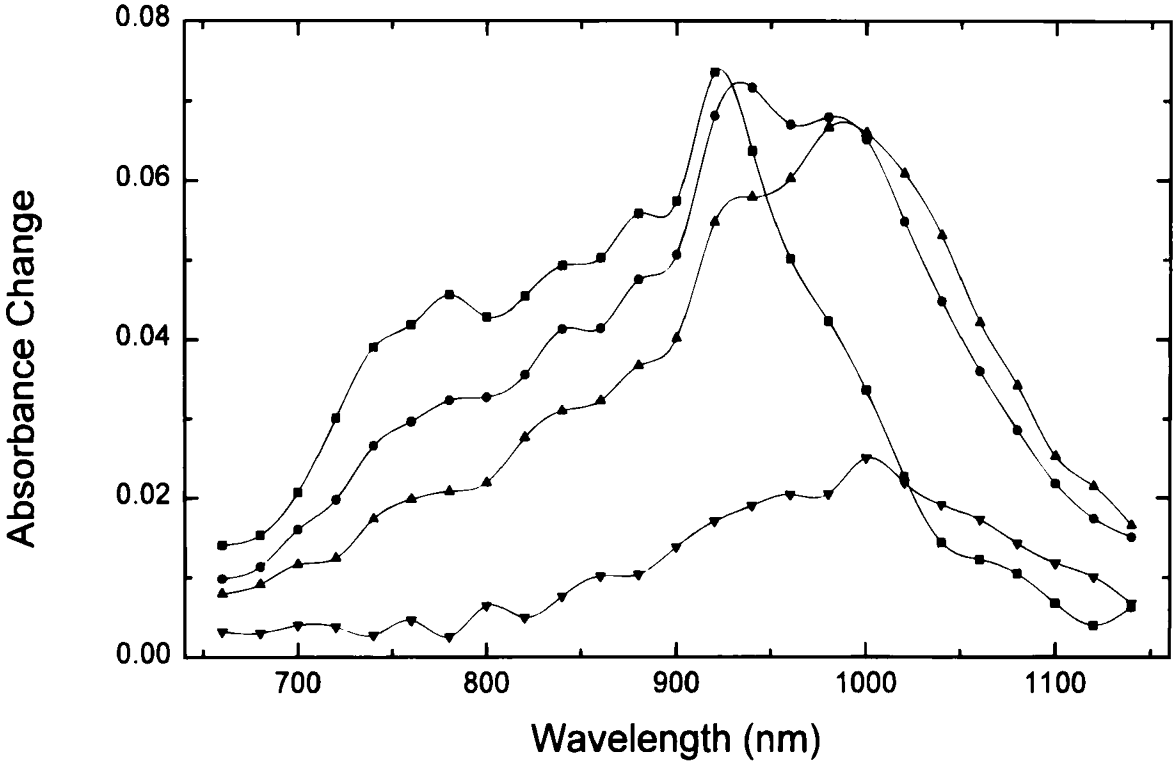

FIGURE 3 Transient absorbtion spectra of 3.0· 10^{-5} M β-carotene in air-saturated chloroform excited at 355 nm. (■) After 4 μs, (●) after 29 μs, (▲) after 89 μs, and (▼) after 1.45 ms. The spectrum at 1.45 ms has been scaled by a factor of 2. The spectra haven been constructed from individual time traces at 25 single wavelengths.

here. The excited state β-carotene must hence be engaged in some sort of unimolecular reaction (e.g. fragmentation) or a bimolecular reaction with the solvent (chloroform) since reaction between β-carotene molecules would not be instantaneous at the concentrations employed here (10^{-5} M). Furthermore, excitation of β-carotene under identical conditions but in other solvents like benzene or toluene was found not to give rise to bleaching. That is, chloroform is actively engaged in the bleaching of β-carotene even though chloroform is not excited under the present conditions. Excitation at 532 nm gave rise to rapid bleaching as well, showing that excitation in the "cis-band" is not a prerequisite for reaction, and that either the S_2 or S_1 state (or both) is involved in the reaction. Possible reaction mechanisms will be discussed later after a discussion of the kinetics.

The product formed instantaneously upon laser excitation of β-carotene in chloroform is the species absorbing at 920 nm (Fig. 3) as evidenced by the time traces in Figure 4. This species then decays with what seems to be a bi-exponential behaviour. This species does not decay back to β-carotene, i.e. the reaction is not reversible, as evidenced by the lack of recurrence of absorption in the visible region (Fig. 1B and C).

The species absorbing at 1000 nm (Fig. 3), which forms slowly ($k = 4.9 \cdot 10^4\ s^{-1}$) after the laser pulse, could be formed by (slow) decay of either β-carotene (Fig. 1B), decay constant of the order of $10^4\ s^{-1}$ (Fig. 2), or by decay of the species absorbing at 920 nm, decay constant of the order of $10^5\ s^{-1}$. However, Figure 5A shows that after 10 μs half of the maximum concentration of the 1000 nm absorbing species has been reached whereas

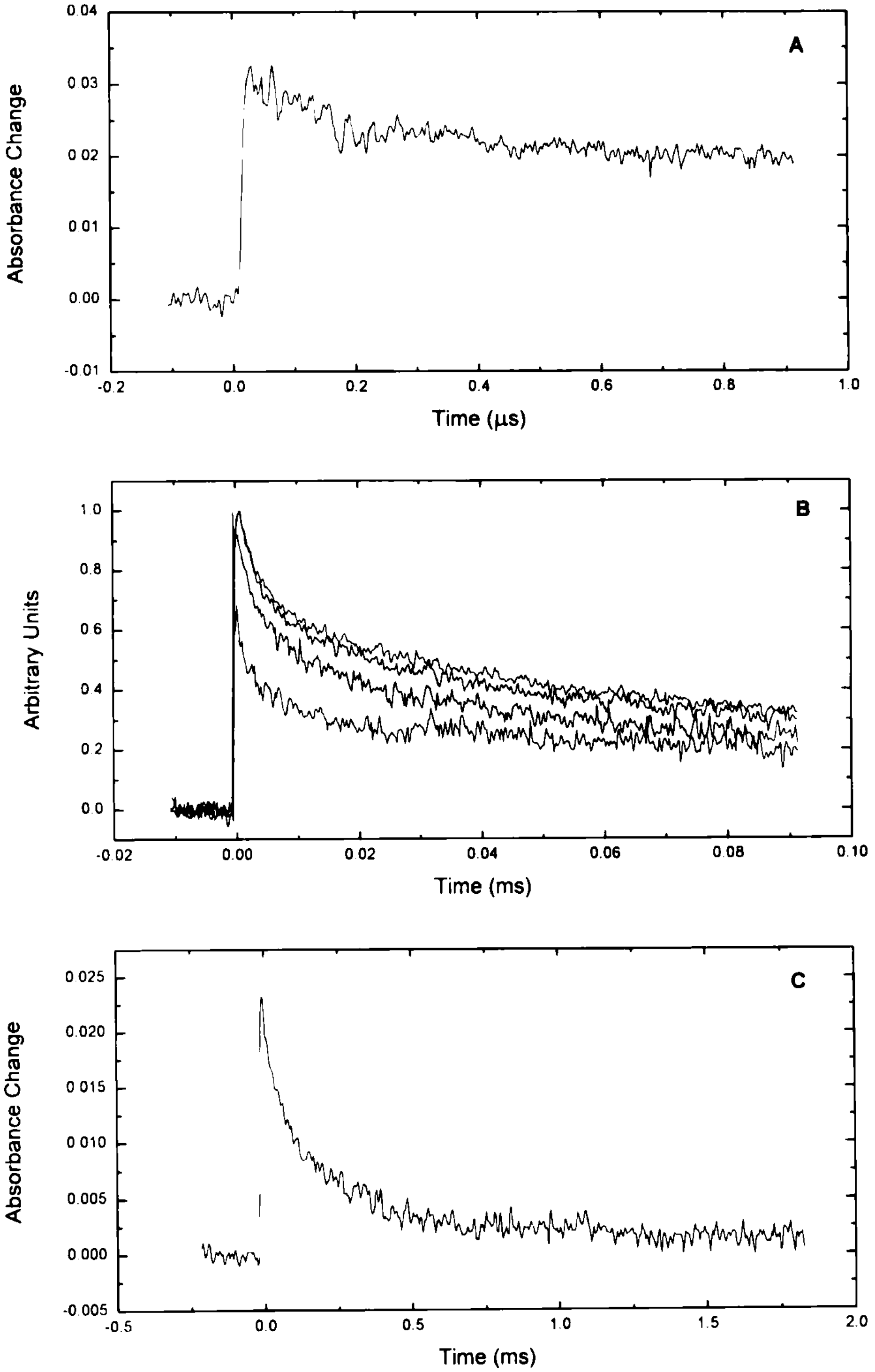

FIGURE 4 Time traces on three different time scales of absorbance changes at 740 nm of a β-carotene solution in chloroform at 20°C excited at 355 nm with a 10 ns pulse. A and C: β-carotene concentration $2.0 \cdot 10^{-5}$ M. B: β-carotene concentration (top to bottom) $2.0 \cdot 10^{-5}$ M, $1.5 \cdot 10^{-5}$ M, $1.0 \cdot 10^{-5}$ M, and $0.5 \cdot 10^{-5}$ M. In B, the time traces have been normalised.

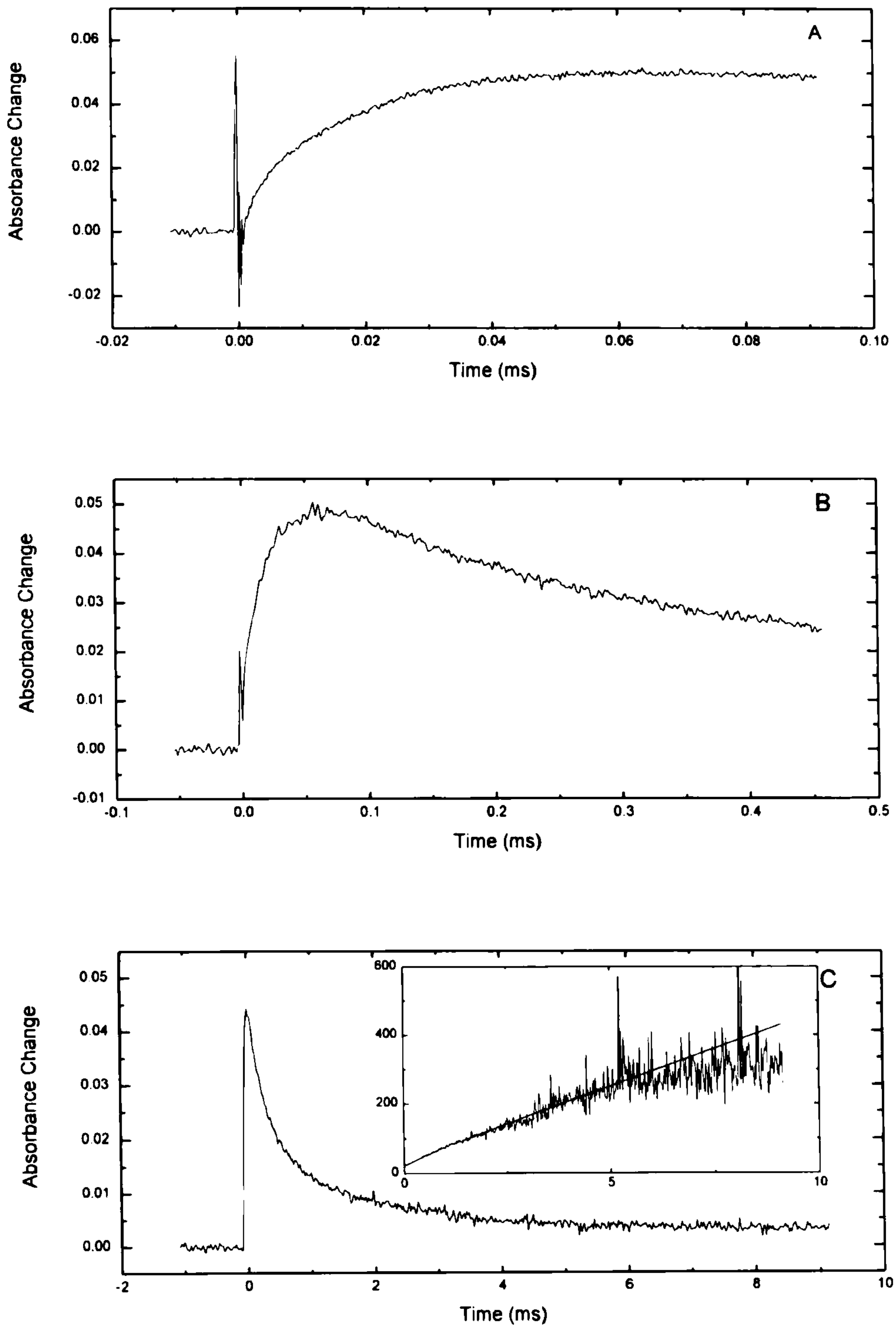

FIGURE 5 Time traces on three different time scales of absorbance changes at 1060 nm of a 2.0· 10^{-5} M solution of β-carotene in air-saturated chloroform at 20°C excited at 355 nm with a 10 ns pulse. The insert in C shows a second order plot for decay of the transient absorption (reciprocal absorption as a function of time).

the slow bleaching of β-carotene has not commenced yet at this point (Fig. 1A). On the other hand, the species absorbing at 920 nm starts to decay immediately after its formation (Fig. 4A) and decays with a first order time constant (of the order of 10^5 s^{-1}) equal to, within experimental error, the first order time constant ($4.9 \cdot 10^4$ s^{-1}) of formation of the species absorbing at 1000 nm. That is, the first order formation of the species absorbing at 1000 nm correlates with the first order decay of the species absorbing at 920 nm.

Apparently, the slow bleaching of β-carotene (Fig. 1B) does not lead to the formation of any new species absorbing in the visible or near infrared region. However, the bi-exponential behaviour of the decay of the species absorbing at 920 nm (Fig. 4B) indicates that at longer times another process influences the decay. This other process could be additional formation of the species absorbing at 920 nm, beyond that induced directly be the laser pulse, and thereby yielding an apparently slower decay rate for this species. This idea is corroborated by the observations that the decay of the species absorbing at 920 nm is faster at low concentrations of β-carotene (Fig. 4B), and that the slow bleaching of β-carotene is slower at low concentrations of β-carotene (Fig. 2). The (first order) decay rate of the species absorbing at 920 nm is expected to be independent of concentration of β-carotene. However, due to additional formation of this species, the apparent decay rate becomes dependent on concentration of β-carotene. Furthermore, because the bleaching is faster at high concentrations of β-carotene (Fig. 2), the formation of the species absorbing at 920 nm is also faster at high concentrations, and the apparent decay rate (Fig. 4B) is, therefore, slower at high concentrations. That the rate of formation of the species absorbing at 1000 nm is independent of concentration of β-carotene reflects the fact that this species is mainly being formed (Fig. 5A), from the species absorbing at 920 nm, before the additional bleaching of β-carotene takes place (Fig. 1B).

Near Infrared Absorbing Species

Species derived from carotenoids absorbing in the near infrared spectral region may be formed in a variety of ways. Reaction of β-carotene with electron acceptors (Lewis acids) like I_2, $SbCl_3$, HNO_3, Br_2, ICl, O_2^-, NO_2, AsF_5, and SO_3, and free radicals like $CCl_3O_2\cdot$ and $C_2H_5SO_2\cdot$[6–17,35,36] have all been found to produce species absorbing in the near infrared. The exact nature of these species is not known with certainty, but they have been described as adducts,[6,7,14,15] as the cation radical, β-carotene$^{+\cdot}$,[7,9,19] including dimers and trimers of the radical cation and one or two β-carotene molecules,[17] the dication (β-carotene^{2+}),[16] and as a charge transfer complex.[11,13,20] Acids also induce the formation of species absorbing in the near infrared,[21–23] as do photolysis of Photosystem II membranes.[10,24–27] In the latter case, the near infrared transient absorption has been ascribed to the carotenoid cation radical. Both the β-carotene radical cation and radical anion (β-carotene$^{+\cdot}$ and β-carotene$^{-\cdot}$) absorb in the near infrared spectral region. The radical anion absorbs at a shorter wavelength than the corresponding radical cation. The anion radical has been formed by pulse radiolysis[14,27–30] of solutions of carotenoids. Formation of the cation radical has been by way of pulse radiolysis,[27–32] electrolysis,[33,34] sensibilized laser flash photolysis,[18] and reaction with $CCl_3O_2\cdot$[7,35,36] or $C_2H_5SO_2\cdot$[6] made by pulse radiolysis of solutions containing CCl_4 and $C_2H_5SO_2Cl$, respectively. The species being described as adducts between carotenoids and electron acceptors show peak absorption in the region 700–950 nm[7,14,15] depending on the nature of the adduct and the solvent. The β-carotene radical cation usually has its strongest absorption in the range 880–1050 nm[7,9,15,24–36] showing a hypsochromic shift with increasing solvent polarity. This strong transient absorption is accompanied by a much weaker absorption around 1400 nm[32,34] due to a lower lying state. The dication of β-carotene absorbs in the same spectral region

(1000 nm) as the β-carotene radical cation.[16,34] The extinction coefficient of the β-carotene radical cation has been shown[27] to be of the order of 10^5 M^{-1} cm^{-1}, the same order of magnitude as the extinction coefficient of β-carotene in the visible region. The extinction coefficient of the dication has been estimated to be of the same order of magnitude[16] in a solid film but an order of magnitude smaller in solution.[34] However, these are only estimates due to the possible interference of other absorbing species like the β-carotene radical cation.

Comparing Figures 1, 4, and 5 shows that β-carotene and the two near infrared absorbing species all have extinction coefficients of the same order of magnitude. Again, only order of magnitude estimates can be made due to spectral overlap, and lacking information of the actual concentration due to decay.

Assuming that the literature assignments are correct, the species absorbing at 920 and 1000 nm in our experiments is either the β-carotene radical cation, the dication or an adduct. None of them is the β-carotene radical anion. This latter species absorbs below 900 nm[14,27–30] and besides it is unlikely that this species should be oxidized to one of the oxidized β-carotene species mentioned above, which should then absorb at 1000 nm, as this would be a two-electron process. The species being described as adducts of β-carotene with electron acceptors usually absorb below 900 nm[7,13,15] except when the adduct is between β-carotene and superoxide, in which case the absorption is around 950 nm.[14] Hill *et al.*[7] found that an adduct was not formed between a carbon-centered radical ($CCl_3\cdot$) and β-carotene but only between the oxygen-centered radical $CCl_3O_2\cdot$ and β-carotene. On the other hand, they found that the β-carotene radical cation was formed irrespective of whether oxygen was present or absent from the solution. That is, in the presence of oxygen both an adduct and the cation radical are formed. The adduct absorbed at a shorter wavelength than the cation radical, which was formed both by reaction with a free radical and by decay of the adduct. In another study[6] it was observed that thiyl radicals, RS·, did not give rise to any near infrared absorbing species though bleaching of β-carotene did take place, and the formation of an adduct was postulated. On the other hand, reaction with $RSO_2\cdot$ generated both an adduct and the β-carotene cation radical.[6] In our experiments, the formation of the two near infrared absorbing species is independent of whether oxygen is present or not. This together with the usual absorption range of the adducts indicates that the species absorbing at 920 nm is not an adduct of β-carotene with a(n) (oxygen-centered) radical. Besides, the instantaneous formation of the species absorbing at 920 nm (Fig. 4A) shows that this species does not involve oxygen in any form because if oxygen was involved, a diffusion controlled (i.e. "slow") reaction, and hence formation of this species, would be observed.

Another species which, possibly, could absorb in the near infrared is the neutral β-carotene radical (β-carotene·), i.e. the conjugate base of the β-carotene cation radical. To the best of our knowledge this species has not been observed or, at least, has not been assigned to any observations.

At least, it seems plausible that the two species absorbing in the near infrared are indeed free radicals of some sort since the absorption is expected to be at a longer wavelength than absorption of the parent molecule because transitions between electronic energy levels of low energy which in β-carotene are occupied, in the free radicals are only half-filled. Another possibility is charge-transfer complexes. Possible reaction mechanisms, and hence species, will be discussed below.

Possible Reactions

The very rapid bleaching of β-carotene upon laser flash photolysis (Fig. 1A) excludes free radicals as being responsible for the bleaching as free radicals would react slowly with β-carotene due to diffusion. The very first stage in bleaching of β-carotene is excitation of β-carotene. The excited state β-carotene then reacts rapidly (during the lifetime of the excited states, i.e. within 10 ps) with chloroform. Two types of reactions fast enough, i.e. complete within 10 ns, the time resolution of the spectrometer may, be envisaged: 1) electron transfer from excited β-carotene molecules to chloroform and 2) formation of a complex between excited state β-carotene and chloroform.

In a previous study[44] it was found that laser flash photolysis of β-carotene in CCl_4 gave rise to a species which showed an ESR signal. The following reaction was proposed

$$\beta\text{-carotene} + CCl_4 \rightarrow \beta\text{-carotene}^{+\bullet} + CCl_4^{-}\bullet \qquad (1)$$

but the transient absorption was not monitored by near infrared absorption. If it is assumed that the β-carotene radical cation is the species giving rise to the absorption at 920 nm in our experiments, a number of decay paths giving rise to the species absorbing at 1000 nm may be envisaged

$$\beta\text{-carotene}^{+\bullet} + CCl_4 \rightarrow \beta\text{-carotene}^{2+} + CCl_4^{-}\bullet \qquad (2)$$

or alternatively

$$2\beta\text{-carotene}^{+\bullet} \leftrightarrows \beta\text{-carotene}^{2+} + \beta\text{-carotene}. \qquad (3)$$

$CCl_4^{-}\bullet$ degrades to $CCl_3\bullet$ and Cl^- in a few microseconds.[44] Reactions (1) and (2) may apply to our system if CCl_4 is substituted for $CHCl_3$ in the reactions above.

Reaction (3) is known to take place.[22,34] However, it is not compatible with the present observations. Firstly, it would imply an increasing decay rate with increasing concentration of β-carotene$^{+\bullet}$ but as Figure 4B shows, the opposite is observed. Secondly, the rate of formation of β-carotene^{2+}, the species absorbing at 1000 nm (Fig. 5A), should depend on the concentration of β-carotene$^{+\bullet}$ but this rate has been found to be first order, and not second order as implied by reaction (3). Reaction (2) could explain the observations. The free radicals formed from $CHCl_3$ could then give rise to the slow bleaching of β-carotene (Fig. 1B) and the additional formation of β-carotene$^{+\bullet}$ (Fig. 4B) as explained above.

Finally, the β-carotene radical cation could react with β-carotene to give dimers

$$\beta\text{-carotene}^{+\bullet} + \beta\text{-carotene} \rightarrow (\beta\text{-carotene})_2^{+\bullet}, \qquad (4)$$

the existence of which have been claimed.[17] This would explain the slow bleaching but, as in the case of reaction (3), it is inconsistent with the decreasing rate of decay of the species absorbing at 920 nm with increasing β-carotene concentration.

The β-carotene radical cation is found to absorb at 1000 nm in CH_2Cl_2[33,34] and is thus not likely to absorb at 920 nm i chloroform. Besides, the extinction coefficient of the dication is an order of magnitude lower that that of the radical cation,[34] though an extinction coefficient of the same order of magnitude have been claimed,[16] and Figure 3 shows that the extinction coefficient of the two species is of the same order. Therefore, the species absorbing at 920 nm is most likely not the β-carotene radical cation.

As mentioned above, excited state β-carotene might also react with chloroform to form a complex. This reaction may be very fast due to the close proximity of chloroform and β-carotene in the solution. The reaction may be written as follows:

$$\beta\text{-carotene}^* + CHCl_3 \rightarrow [\beta\text{-carotene} \ldots CHCl_3]^*. \qquad (5)$$

The product formed in reaction (5) is not a free radical and is hence not expected to absorb in the near infrared unless a charge transfer effect is involved. However, the complex may degrade rapidly, i.e. during the laser pulse, giving rise to a free radical absorbing at 920 nm. One such degradation pathway could be a reaction similar to reaction (1), the product formed in reaction (5) being a short-lived intermediate in reaction (6)

$$\beta\text{-carotene} + \mathrm{CHCl_3} \rightarrow \beta\text{-carotene}^{+\bullet} + \mathrm{CHCl_3^{-}}\bullet \qquad (6)$$

As discussed above, β-carotene$^{+\bullet}$ is not likely to be identical to the species absorbing at 920 nm. Another possible mechanism of degradation of the complex in (5) could be by decomposition into two neutral free radicals. This seems a very reasonable explanation because the slow bleaching of β-carotene (Fig. 1B) is probably due to a reaction with a free radical. The rate constant (Fig. 2) depends upon concentration indicating that it is not a unimolecular process but involves at least one other reactant. It seems most likely that one of the carbon-chlorine bonds should cleave as this is weaker than the carbon-hydrogen bond in chloroform

$$[\beta\text{-carotene} \ldots \mathrm{CHCl_3}]^* \rightarrow [\beta\text{-carotene} \ldots \mathrm{CHCl_2}]\bullet + \mathrm{Cl}\bullet. \qquad (7)$$

The free radical adduct formed in (7) could give rise to the absorption at 920 nm. The adduct could then decay to the β-carotene radical cation

$$[\beta\text{-carotene} \ldots \mathrm{CHCl_2}]\bullet \rightarrow \beta\text{-carotene}^{+\bullet} + \mathrm{CHCl_2^{-}} \qquad (8)$$

which absorbs at 1000 nm. The chlorine atom formed in (7) may then react with β-carotene and induce the slow bleaching (Fig. 1B) and additional formation of the species absorbing at 920 nm (Fig. 4B), or it could react with chloroform by abstraction of hydrogen to produce $\mathrm{CCl_3}\cdot$ which then could react with β-carotene. A variety of adducts may thus be formed. However, their absorption characteristics are probably very similar because the chromophore (the odd-electron conjugated system) will be the same. This sequence of events explains the occurence of the two species absorbing in the near infrared.

Another possible route could be the following

$$\beta\text{-carotene}^* + \mathrm{CHCl_3} \rightarrow \beta\text{-carotene}\bullet + \mathrm{CHCl_2}\bullet + \mathrm{HCl} \qquad (9)$$

where hydrogen is transferred from β-carotene to chloroform yielding β-carotene· which could be the species absorbing at 920 nm. The β-carotene cation radical could then be formed by proton transfer from chloroform

$$\beta\text{-carotene}\bullet + \mathrm{CHCl_3} \rightarrow \beta\text{-carotene}^{+\bullet} + \mathrm{CCl_3^{-}}. \qquad (10)$$

The free radical formed in (9), $\mathrm{CHCl_2}\cdot$, could then add to β-carotene giving rise to the slow bleaching (Fig. 1B) and the additional formation of the species absorbing at 920 nm (Fig. 4B), and could hence explain the observed time traces.

The fate of the species absorbing at 1000 nm is not known. It decays by second order kinetics (Fig. 5C), and one possible mechanism could be dismutation yielding β-carotene and a two-electron oxidized species (reaction (3)). However, β-carotene is not reformed to a significant extent (Fig. 1B and C), and another decay path is therefore followed. Possibly, β-carotene is not being reformed at all, the small recurrence of absorption being due to degradation products which absorb in the near UV but have a small extinction coefficient in the blue spectral region.

CONCLUSIONS

The overall possible reaction pathways may thus be depicted as in Figure 6. Excited β-carotene reacts in some way instantaneously with chloroform to yield the species absorbing at 920 nm, which could be a free radical adduct (β-carotene . . . $\mathrm{CHCl_2}\cdot$), or following hydrogen abstraction the radical β-carotene·, according to reactions (5) and (7) or (9), respectively. Besides that, excited β-carotene relaxes to the electronic ground state. After approximately 10 µs ground state β-carotene (β-carotene relaxes to the ground state within picoseconds) reacts with some species (Fig. 1B) probably formed (as a by-product) during the instantaneous formation of the species absorbing at 920 nm yielding more of the species

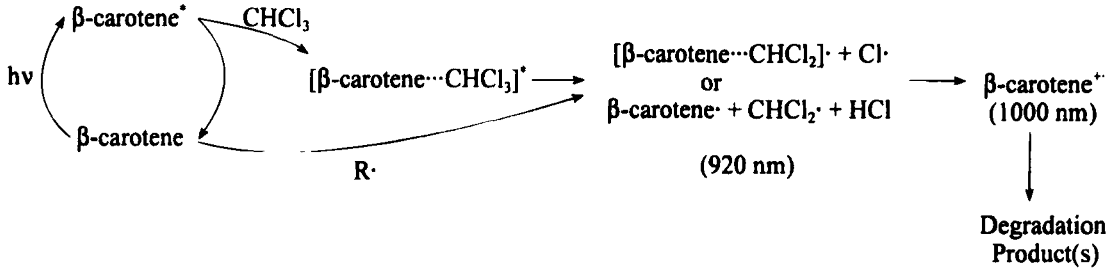

FIGURE 6 Possible reactions involved in the bleaching of β-carotene.

absorbing at 920 nm. The species absorbing at 920 nm immediately starts to decay (first order) to another species absorbing at 1000 nm, which is probably the cation radical as shown in reactions (8) and (10). The decay seems to become slower due to additional formation of the species absorbing at 920 nm (Fig. 5B). The species absorbing at 1000 nm decays by second order kinetics.

We thank one of the reviewers for making us aware of a paper published while this paper was being reviewed.[45] This paper corroborates some of our results even though the two systems being studied are not identical. Everett *et al*366[45] also observed a second order decay of the β-carotene radical cation. However, in their system the β-carotene radical adduct absorb in the same region as β-carotene itself whereas the radical adduct formed in our system absorb in the near infrared spectral region.

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