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Free Radical Transients in Photobleaching of Xanthophylls and Carotenes

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Carotenoids in chloroform and carbon tetrachloride photobleach upon nanosecond laser flash photolysis in two steps: instantaneously and in a second-order reaction. The rate constant for second-order reaction (first-order in a solvent derived radical and first-order in (excess) carotenoid) is largest for carotenes ($9.8 \cdot 10^8$ M^{-1} s^{-1} for β-carotene), intermediate for hydroxylated carotenoids, and smallest for carbonyl containing carotenoids ($1.0 \cdot 10^8$ M^{-1} s^{-1} for astaxanthin) in chloroform at 20°C. Near infrared absorbing transients are formed concomitant with photobleaching in chloroform (not detected in carbon tetrachloride). A species formed instantaneously is tentatively identified as either a carotenoid/solvent adduct or an ion-pair. A second species is formed by decay of the instantaneously formed species and is identified as the carotenoid radical cation. This species is formed in a first-order reaction with a rate constant of approx. $5 \cdot 10^4$ s^{-1} and absorbing at longer wavelength than the precursor. The lifetime (second-order decay) of the intermediates appears to be longest for the carotenoids with the longest conjugated system. The results indicate that carotenes are better antioxidants than xanthophylls as the carotenes, at least in the present lipophilic solvents, react faster with free radicals.

INTRODUCTION

Carotenoids have important functions *in vivo* as protectors against light-induced processes such as photosensitizing reactions[1] and against free radical damage during oxidative stress.[1-3] While the photoprotection is relatively well-understood in relation to quenching of excited states of other molecules including singlet oxygen, the free radical scavenging of carotenoids has been more difficult to understand in terms of molecular mechanisms. It has, however, been demonstrated in different investigations that carotenoids react with free radicals in at least two types of reactions: (i) by formation of an adduct between the carotenoid and the free radical and (ii) by electron transfer from the carotenoid to the free radical yielding the carotenoid radical cation.[4-10] An important research goal is now to understand how the structure of different carotenoids influence the reactivity including the balance between the two reaction paths. Thus, in a recent ESR-spin trapping study, β-carotene was found to be prooxidative while other carotenoids showed a net free radical scavenging activity.[11] Upon exposure to light, β-carotene in chloroform has been shown to form two transient species absorb-

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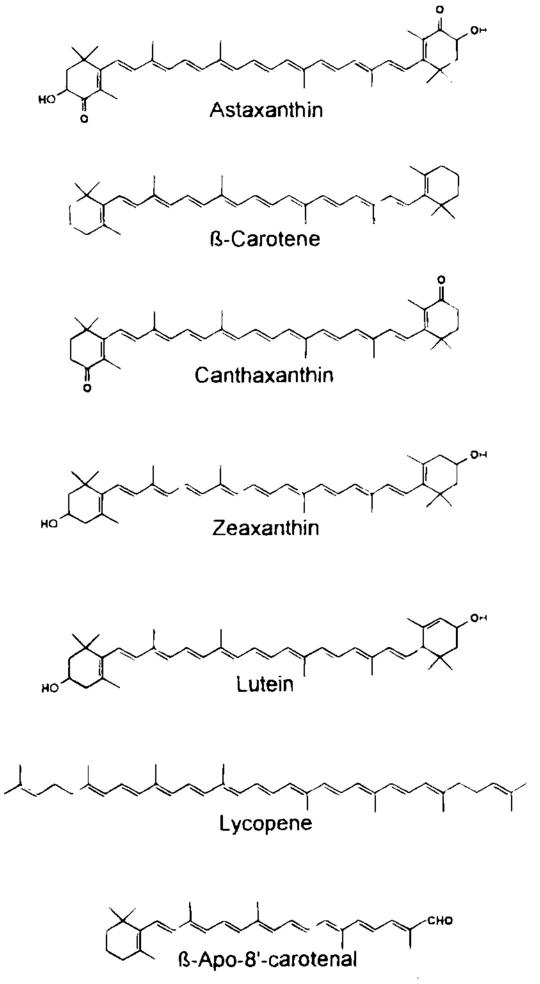
ing in the near infrared spectral region.[9] The two species were tentatively identified as a free radical adduct between β-carotene and chloroform and as the carotenoid radical cation, and the kinetics of their formation and decay was characterised by real-time detection.[9] Since this method provides insight into the reactivity of carotenoids in free radical processes we have initiated investigations of a number of commonly occurring carotenoids (Scheme I) in order to relate structural features to differences in reactivity with the solvent derived free radicals and have also included carbon tetrachloride as solvent.



SCHEME I

EXPERIMENTAL SECTION

Materials

β-Carotene, astaxanthin, canthaxanthin, zeaxanthin, lycopene, lutein and β-apo-8′-carotenal were all supplied by Roche A/S (Hvidovre, Denmark) sealed in ampoules under argon and were used without further purification. Chloroform, HPLC grade, from Lab-Scan (Dublin, Ireland) and carbon tetrachloride, Pronalys AR, from May & Baker Ltd. (Dagenham, England) were used as received. Cyclohexane p.a., toluene p.a., and benzene p.a. were from Merck (Darmstadt, FRG). Stock solutions of 10^{-4} M carotenoid in either chloroform or carbon tetrachloride were diluted to the required concentration. The solutions were kept in the dark for no longer than a week at −23°C before they were used. During this period no discernible degradation of the carotenoid could be observed as checked by absorption spectroscopy (HP 8452 UV-VIS diode-array spectrophotometer, Hewlett Packard, Palo Alto, CA, USA). Oxygen was removed from some of the samples prior to measurements by 3 freeze-pump-thaw cycles.

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 60 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. 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 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 30 laser pulses after which up till 10% degradation was observed (depending on carotenoid and concentration).

Time traces were analysed by non-linear least-squares fitting using the Levenberg-Marquardt algorithm. Transient near infrared absorption spectra were constructed from time traces at every 20 nm, and transient visible absorption spectra from time traces at every 10 nm.

RESULTS

The nature of the solvent was found to have strong influence on the photoreactivity of carotenoids. The photodegradation quantum yield of carotenoids in solvents like toluene is rather low, of the order of 10^{-3}–10^{-6} depending on irradiation wavelength.[12] Laser flash photolysis of carotenoids in toluene, benzene, or cyclohexane did not result in any discernible degradation of the carotenoid by a single laser flash. Degradation of the carotenoid was seen after a substantial number of laser pulses (typically less than 5% after more than 1000 laser pulses).[12] On the other hand, laser flash photolysis of the carotenoids in Scheme I in chloroform resulted for each carotenoid in extensive degradation of the carotenoid which could easily be followed by time-resolved absorption spectroscopy, and similar results were obtained in carbon tetrachloride.

1. Carotenoids in Chloroform

Upon laser flash photolysis of carotenoids in chloroform bleaching of the carotenoids in the visible region takes place. Bleaching of the carotenoids takes place in two steps. First there is a very fast, i.e. occurring during the laser pulse (10 ns), bleaching (Fig. 1A) persisting for around 10 μs followed by an additional slower bleaching. This slow bleaching is typically complete within a few milliseconds (Fig. 1B). As Figure 2 shows, the slow bleaching seems to be larger towards the long wavelength side of the absorption band than towards the short wavelength side. Below 420 nm (in the case of lycopene) there is even an increase in absorption (Fig. 2A). This is due to the formation of degradation product(s). That the absorption of the degradation product(s) is only slightly blue-shifted compared to the parent carotenoid indicates that the degradation product(s) consist(s) of conjugated systems shorter than the parent carotenoid by only a few double bonds. The absorption maximum of the degradation product(s) could not be determined due to overlap with the absorption band of the carotenoid. The time traces of the slow bleaching may be fitted to an exponential function indicating a first-order reaction (insert in Fig. 3). However, the (pseudo) first-order rate constant depends upon the concentration of carotenoid as depicted in Figure 3. As shown in Figure 3 the rate of slow bleaching is largely linearly dependent on carotenoid concentration, i.e. first-order in carotenoid, and the second-order rate constants calculated as the slope are given in Table I. From Figure 3 it can also be seen that the carbonyl containing carotenoids (astaxanthin, canthaxanthin, and β-apo-8′-carotenal) are bleached slower than the hydroxylated carotenoids (zeaxanthin and lutein) which again are bleached slower than β-carotene and lycopene.

Concomitant with the bleaching of carotenoid in chloroform is the formation of species absorbing in the near infrared. In Figure 4 is shown the transient absorption spectra of the carotenoids investigated (except β-carotene). In the case of lycopene, lutein, and zeaxanthin it is evident that two species are formed as was also found previously for β-carotene.[9] One species is formed instantaneously

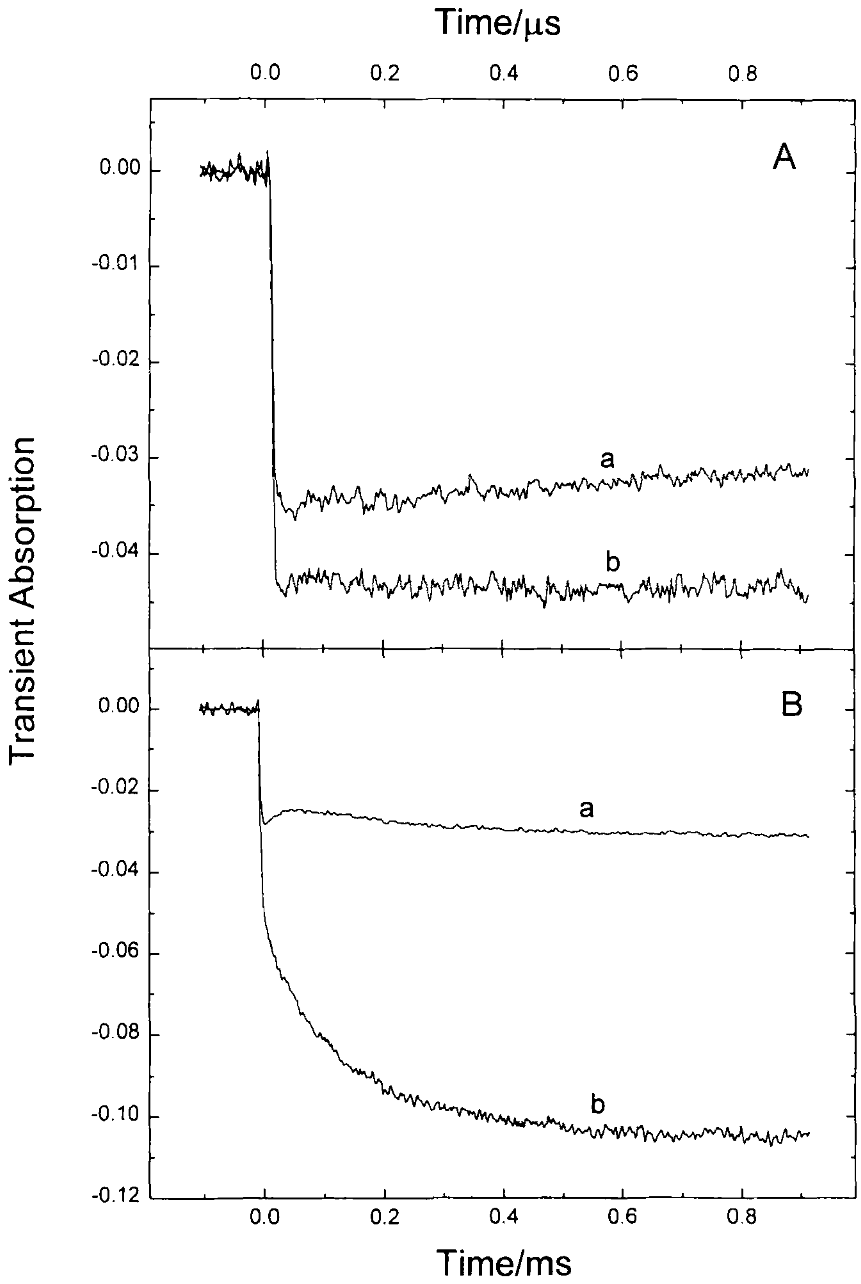


FIGURE 1 Time traces of the bleaching of 8.5 μM lycopene in chloroform at (**a**) 440 and (**b**) 500 nm on two different time scales.

(Fig. 5Aa) and starts to decay right after it is formed. The decay seems to follow neither simple first- nor second-order kinetics. The other species, absorbing at a longer wavelength than the species formed instantaneously, is formed slowly (Fig. 5Ab) by first-order kinetics. The rate constants estimated may be found in Table I. This species decays by second-order kinetics but since the molar absorbance is not known for the transient, the time traces cannot be converted to rate constants. In the

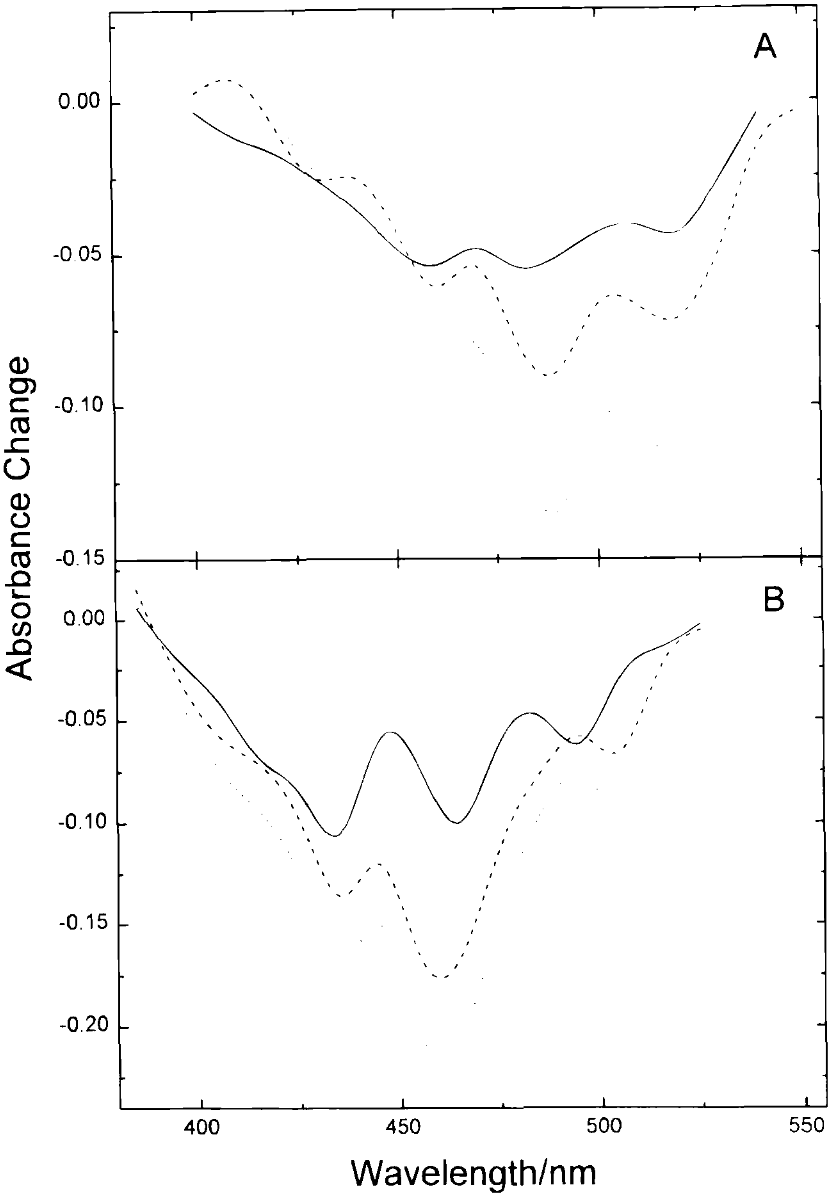


FIGURE 2 Transient absorption difference spectra of (A) 8.5 μM lycopene in chloroform and (B) 10 μM β-carotene in carbon tetrachloride at 40 ns (full), 40 μs (dashed) and 890 μs (dotted) after the laser pulse.

case of the carbonyl containing carotenoids astaxanthin, canthaxanthin, and β-apo-8′-carotenal it is less evident that two species are formed (Fig. 4). However, scrutinizing the transient spectra shows that the absorption maximum shifts slightly towards longer wavelengths as a function of time (Fig. 4), and from the time traces (Fig. 5C) it is clear that the kinetics appear different at the red and blue side of the absorption band. The absorption maxima of the two near infrared absorbing species for the six carotenoids together with β-carotene are given in Table I. Due to the extensive overlap of the two near infrared absorbing species in the case of the carbonyl containing carotenoids, the absorp-

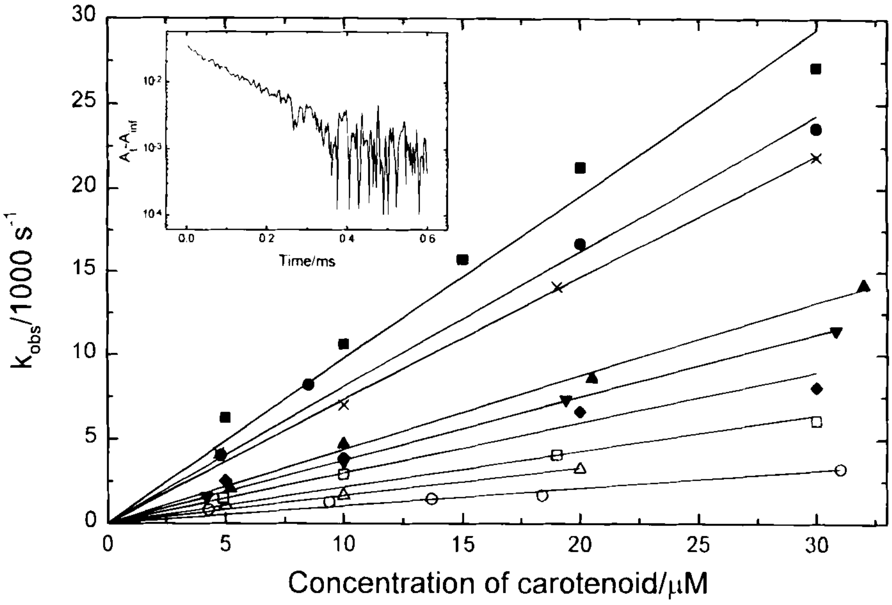


FIGURE 3 Pseudo first order rate constant of the slow bleaching of the carotenoids as a function of concentration of carotenoid. ■: β-carotene in chloroform, ●: lycopene in chloroform, X: β-carotene in carbon tetrachloride, ▲: zeaxanthin in chloroform, ▼: lutein in chloroform, ◆: canthaxanthin in carbon tetrachloride, □: β-apo-8′-carotenal in chloroform, △ canthaxanthin in chloroform, and O: astaxanthin in chloroform. The full lines are least-squares linear fits to the data. Insert: First-order plot of absorbance change at 524 nm for the slow bleaching of 8.5 µM lycopene in chloroform.

tion maxima are somewhat uncertain, and in the case of astaxanthin it could not be determined for the transient species absorbing at the longer wavelength.

2. Carotenoids in Carbon Tetrachloride

Carotenoids dissolved in carbon tetrachloride and exposed to laser flash photolysis show the same trends in the visible region as do carotenoids dissolved in chloroform, i.e. instantaneous bleaching followed by a slower pseudo first-order bleaching (Fig. 1). The rate of slow bleaching is similar to that observed in chloroform solutions (Fig. 3). However, only a very weak transient absorption (Fig. 5D) is observed in the near infrared region even though the carotenoids are degraded to a significant extent (Fig. 2B).

3. Carotenoids in Deaerated Solutions

When deaerated solutions of carotenoids in carbon tetrachloride are exposed to the laser pulse, bleaching of the carotenoid is still observed. However, in the NIR spectral region weak transients appear and disappear (Fig. 6A). These transients extend from the red end of the spectrum into the NIR and are not observed in deaerated carbon tetrachloride without carotenoid. The weak transients were also observed in deaerated chloroform solutions, however, only in regions where the two strongly absorbing transient species absorb weakly (Fig. 6B), i.e. in the wings of the absorption bands. From Figure 6 it would seem that the weak transients look the same in chloroform and carbon tetrachloride solutions. The first weak transient appears after 10 µs, the second appears around 100 µs, and a third 150 µs after the laser pulse (Fig. 6). The weak transients were not observed in the same solutions after air had been introduced into the sample. Apart from these weak transients, the formation and decay characteristics of the two strongly absorbing transient species are not affected by the presence or absence of oxygen (Fig. 6).

TABLE I

Carotenoid	Rate constant of slow bleaching ($M^{-1}\ s^{-1}$)	Rate constant of formation of the radical cation (s^{-1})	Absorption maximum of adduct (nm)	Absorption maximum of radical cation (nm)	Absorption maximum of carotenoid (nm)
Zeaxanthin($CHCl_3$)	$4.4 \cdot 10^8$	$5.7 \pm 0.2 \cdot 10^4$	905	980	463
Lutein($CHCl_3$)	$3.7 \cdot 10^8$	$7.8 \pm 3.3 \cdot 10^4$	880	940	456
Lycopene($CHCl_3$)	$8.1 \cdot 10^8$	$5.4 \pm 0.9 \cdot 10^4$	960	1040	485
β-Carotene[a]($CHCl_3$)	$9.8 \cdot 10^8$	$4.9 \pm 0.1 \cdot 10^4$	920	1000	464
β-Carotene(CCl_4)	$7.3 \cdot 10^8$	—	—	—	464
Astaxanthin($CHCl_3$)	$1.0 \cdot 10^8$	—	855	—	490
Canthaxanthin($CHCl_3$)	$1.6 \cdot 10^8$	—	860	900	485
Canthaxanthin (CCl_4)	$3.0 \cdot 10^8$	—	—	—	480
β-Apo-8′-carotenal($CHCl_3$)	$2.1 \cdot 10^8$	—	835	855	476

[a] From Ref. 9.

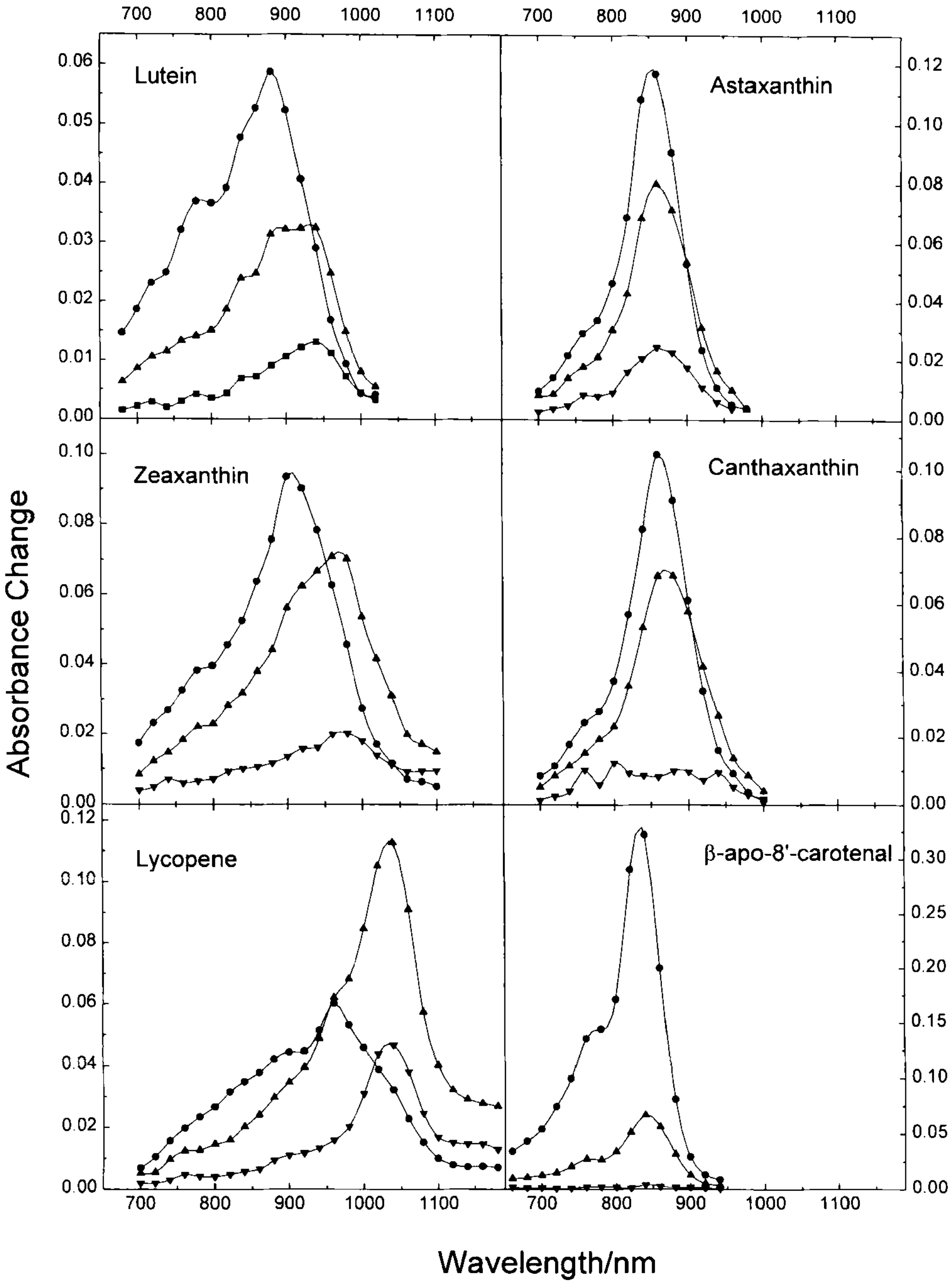


FIGURE 4 Transient absorption spectra of 31 μM lutein, 32 μM zeaxanthin, 30 μM lycopene, 31 μM astaxanthin, 30 μM canthaxanthin, and 30 μM β-apo-8'-carotenal in chloroform at (●) 4, (▲) 89, and (▼) 890 μs after the laser pulse. The corresponding β-carotene absorption spectra have absorption maxima at 780 and 920 nm after 4 μs and at 980 nm after 89 μs.[9]

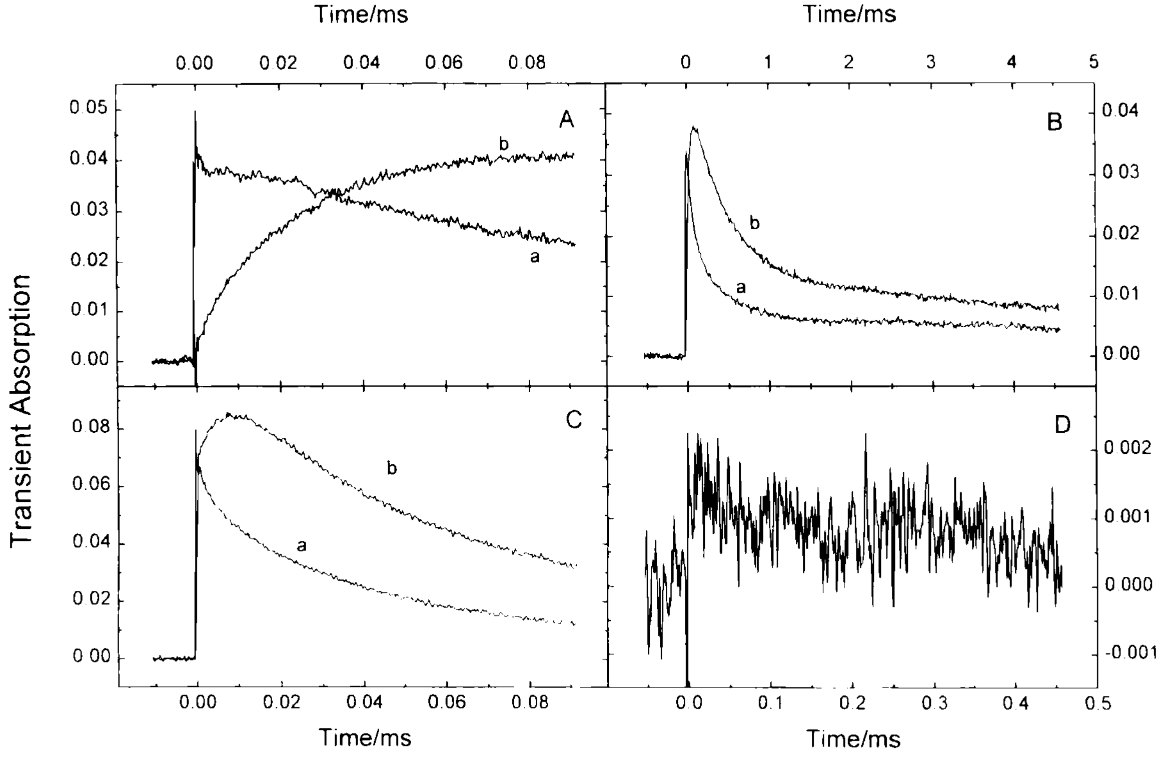


FIGURE 5 Time traces of near infrared absorption of carotenoid transient species in chloroform (**A**, **B**, and **C**) or carbon tetrachloride (**D**). A and B: 30 μM lycopene at (a) 860 and (b) 1100 nm. C: 30 μM β-apo-8′-carotenal at (a) 700 and (b) 880 nm. D: 30 μM β-carotene at 700 nm.

DISCUSSION

1. Formation and Decay of Transient Species in Chloroform Solutions

The two near infrared absorbing transient species formed upon laser flash photolysis of carotenoids in chloroform solutions have previously for β-carotene been tentatively identified as an adduct between the carotenoid and chloroform (the species formed instantaneously) and as the carotenoid radical cation (the species formed slowly).[9]

The instantaneous formation of the adduct correlates with the instantaneous bleaching of the carotenoid (Fig. 1A). The adduct starts to decay right after it is formed. At the same time, the absorbance starts to increase on the blue side of the absorption band of the carotenoid (Fig. 1A), and the radical cation appears (Fig. 4). The increase in absorbance on the short wavelength side of the absorption band is due to the formation of products from degradation of the adduct. The slow bleaching (Fig. 1B) follows first-order kinetics with an observed first-order rate constant proportional to the carotenoid concentration (Fig. 3). Each laser flash results only in bleaching of a minor fraction of carotenoid (Fig. 2), and the slow bleaching reaction observed is a reaction between a radical species formed during the instantaneous bleaching and the nonbleached carotenoid. Photoexcitation of carotenoids in carbon tetrachloride or chloroform has previously been found to initiate free radical processes.[9,13] The second-order rate constant of bleaching (Table I) is for β-carotene larger in chloroform than in carbon tetrachloride as expected on the basis of differences in viscosity (0.969 cp for CCl_4 and 0.58 cp for $CHCl_3$)[14] while the opposite is observed for canthaxanthin.

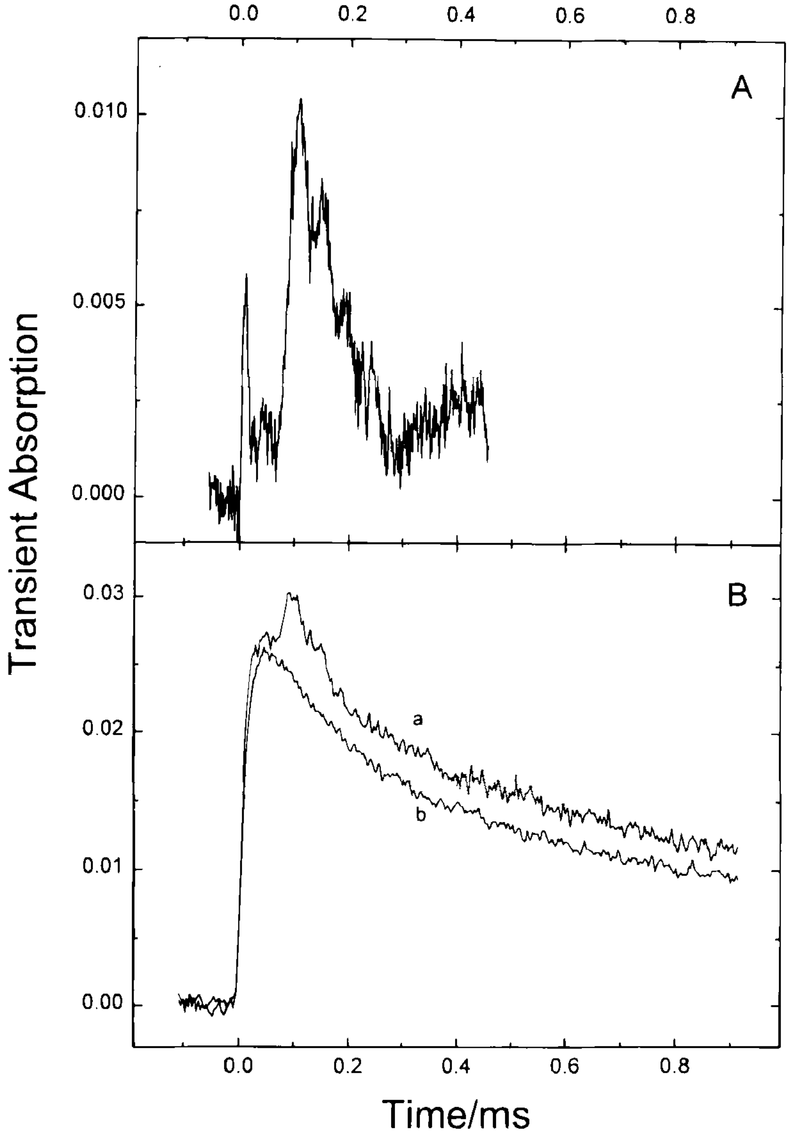


FIGURE 6 Time traces of 30 μM canthaxanthin at (A) 900 nm in deaerated carbon tetrachloride and at (B) 940 nm in deaerated (a) and air-saturated (b) chloroform.

The formation of the radical cation, however, does not correlate with the slow bleaching of the carotenoids. Comparing Figure 3 and Table I for each of the carotenoids shows that the rate of slow bleaching is less than the rate of formation of the radical cation though the observed rate constants are of the same order of magnitude, i.e. 10^4 s^{-1}. Besides, the formation of the radical cation *is independent* of concentration of carotenoid whereas the slow bleaching (Figure 3) is not, and the slow bleaching does not commence until 10 μs after the laser pulse at which point the concentration of the radical cation has already reached a significant level (Fig. 5A). On the other hand, the formation of the radical cation correlates with the decay of the adduct, as the radical cation is formed in a first-order reaction and the decay of the adduct seems to happen by two first-order processes. The biexponential decay of the adduct may be explained as follows. The adduct decays by first-order kinetics. However, at longer times an additional formation of the adduct takes place which correlates with the slow bleaching of the carotenoid (Fig. 1B). The exponential decay of the adduct and the slow (pseudo) first-order additional formation of this species leads to the biexponential decay characteristics (two first-order processes). This kinetic scheme also explains why the decay of the adduct is faster at low concentrations of carotenoid. As Figure 3 shows, the bleaching of carotenoids is slower at low concentrations. The slow formation of the adduct is therefore also slower at low concentrations, and the overall decay hence faster. The time traces of decay of the adduct cannot reliably be fitted to a biexponential decay function because decay of the adduct and the additional formation of this species takes place on a similar time scale (on the order of $10^4\ s^{-1}$, Fig. 3 and Table I). Moreover, the adduct also decays to degradation products yielding an even more complex decay scheme.

Determination of the rate constant of formation of the radical cation is influenced by the overlapping contribution of the adduct and as Table I shows, the uncertainty is larger when the separation between the absorption maxima of the adduct and the radical cation is small. In the case of the carbonyl containing carotenoids this rate constant could not be determined due to the extensive overlap. Among the four carotenoids for which the rate constant of formation could be determined there does not seem to be any significant difference. The radical cation decays by second-order kinetics, and a determination can therefore not be made of the rate constant without knowing the concentration. However, Figure 4 shows that there are differences in the lifetimes of the different carotenoid radical cations. The lycopene radical cation has the longest lifetime and the β-apo-8′-carotenal radical cation has the shortest lifetime. This is not surprising considering that the conjugated system is longest in lycopene and shortest in β-apo-8′-carotenal. This is also reflected in the position of the absorption band (Table I), the absorption band of the lycopene radical cation being at a lower energy than the absorption band of the β-apo-8′-carotenal radical cation. The double bonds in the rings do not contribute to a large extent to the conjugated system, and it is therefore more or less of the same length in the four other carotenoids, and as Figure 4 shows there is no significant difference in the lifetime of the radical cations of these four carotenoids.

As Figures 4 and 5 show, the absorption of the radical cation of the carbonyl containing carotenoids is rather weak compared to the absorption of the adduct in contrast to the other carotenoids. This could either be due to a lower extinction coefficient of the radical cation compared to the adduct, or could be due to smaller amounts of the radical cation being formed upon decay of the adduct. Considering that the extinction coefficients of the adducts of carbonyl containing carotenoids seem to be of the same order of magnitude as that of the other carotenoids, the last possibility seems to be more likely.

Among the four carotenoids without a carbonyl group there exist a correlation between the maximum of the carotenoids' absorption and that of the adduct and radical cation (Table I): the lower the energy of the second excited state (S_2, which gives rise to absorption in the visible region) of the carotenoid, the lower the energy of the second excited state (D_2, which gives rise to absorption in the NIR region)[15] of the adduct and the radical cation. However, this correlation does not hold true if the carbonyl containing carotenoids are considered. Whereas a carbonyl group has the effect of lowering the energy of the S_2 state of the carotenoid, it has the opposite effect on the D_2 state of the adduct and the radical cation (Table I).

2. Reactions of Transients

Neither depletion of the ground state population of carotenoid nor formation of triplet carotenoid can explain the instantaneous bleaching observed. The lifetime of the singlet excited states of carotenoids is only a few picoseconds,[16–20] and can hence not be observed on the time scale of the present experiments, and the intersystem crossing yields are too low (on the order of 10^{-3}).[12] Besides, reaction between carotenoid and solvent takes place in the excited singlet state manifold.[13]

First of all, the evidence seems to be in favor of the transient species absorbing at the longer wavelength is the radical cation.[9] The species formed instantaneously, however, has not been identified unambigously. The excited state carotenoid could conceivably react with the solvent (chloroform) in a number of ways. It has been suggested that excited state carotenoids[13] and 1,4-diphenylbutadiene[21] react with the solvent by electron transfer

$$\text{carotenoid} + \text{CHCl}_3 \rightarrow \text{carotenoid}^{+\bullet} + \text{CHCl}_3^{-}\cdot. \quad (1)$$

There are now two possibilities. Firstly, the transient species formed slowly is not the radical cation but a species derived from the radical cation, for instance the neutral radical

$$\text{carotenoid}^{+\bullet} \rightarrow \text{carotenoid}\cdot + \text{H}^{+}. \quad (2)$$

To the best of our knowledge, the neutral radical has never been observed, or at least has never been assigned to any transient absorptions. Secondly, the species formed slowly is the radical cation, and the species formed instantaneously is not. Reaction (1) may be modified as follows

$$\text{carotenoid}+\text{CHCl}_3 \rightarrow [\text{carotenoid}^{+\bullet}\cdots\text{CHCl}_3^{-}\cdot] \quad (3)$$

in which an ion-pair is formed. This ion-pair may then be the species formed instantaneously giving rise to NIR absorption at the shorter wavelength. This ion-pair may be expected to decay by first-order kinetics

$$[\text{carotenoid}^{+\bullet}\cdots\text{CHCl}_3^{-}\cdot] \rightarrow \text{carotenoid}^{+\bullet} + \text{CHCl}_3^{-}\cdot \quad (4)$$

yielding the radical cation, the species believed to be the one absorbing at the longer wavelength. The formed anion radical $\text{CHCl}^{-}_3\cdot$ decays accordingly

$$\text{CHCl}_3^{-}\cdot \rightarrow \text{CHCl}_2\cdot + \text{Cl}^{-}. \quad (5)$$

The radical formed in this reaction could then go on to react with more carotenoid

$$\text{carotenoid} + \text{CHCl}_2\cdot \rightarrow [\text{carotenoid}^{+\bullet}\cdots\text{CHCl}_2^{-}] \quad (6)$$

giving rise to additional bleaching and formation of a species similar to the product of reaction (3) and absorbing at the shorter wavelength.

Another possible mechanism would be formation of an exciplex which then decays fast

$$[\text{carotenoid}\cdots\text{CHCl}_3]^{*} \rightarrow [\text{carotenoid}\cdots\text{CHCl}_2]\cdot + \text{Cl}\cdot \quad (7)$$

to the adduct [β-carotene· · ·CHCl_2]· which then is the species giving rise to absorption at the shorter wavelength. This mechanism is in accordance with previous assignments[4,5] of adducts between carotenoids and free radicals. The adduct could then decay (again by first-order kinetics)

$$[\text{carotenoid}\cdots\text{CHCl}_2]\cdot \rightarrow \text{carotenoid}^{+\bullet} + \text{CHCl}_2^{-} \quad (8)$$

yielding the radical cation. The chlorine atom formed in (7) could then go on to react with carotenoid

$$\text{carotenoid} + \text{Cl}\cdot \rightarrow [\text{carotenoid}\cdots\text{Cl}]\cdot \quad (9)$$

yielding another adduct. This adduct may be expected to absorb at the same wavelength as the adduct formed in (7) because the chromophore is the same (an odd-electron conjugated system). Reaction (9) hence explains the slow bleaching and additional formation of the transient species absorbing at the shorter wavelength.

The radical cation decays by second-order kinetics. One possible reaction could be by dismutation

$$2\,\text{carotenoid}^{+\cdot} \leftrightharpoons \text{carotenoid}^{2+} + \text{carotenoid}. \quad (10)$$

The reaction in (10) is known to take place,[15] however, the dication absorbs at 700–800 nm,[15] and Figure 4 shows that no species absorbing in this region is being formed. Besides, (10) implies that the carotenoid is being reformed but as Figure 2 shows, the products from degradation of the radical cation absorb at a shorter wavelength than the carotenoid.

The sequence of events may hence be depicted as in Figure 7. Excited state carotenoid reacts with chloroform, possibly *via* the formation of an exciplex, to yield either an adduct or an ion-pair. This instantaneously formed species decays by first-order kinetics to degradation products and more importantly to the radical cation. The radical cation then decays by second-order kinetics to degradation products. This scheme assumes that the species formed slowly is the radical cation. If this is not the case, reactions like (1) and (2) could be a possibility.

3. Carotenoids in Carbon Tetrachloride

Carotenoids in carbon tetrachloride are bleached in the same way as in chloroform, i.e. instantaneously and by a slower reaction largely first-order in carotenoid (Fig. 3), and the bleaching, and hence degradation, of the carotenoids is of the same order of magnitude in both solvents. Perhaps surprisingly, only very weak near infrared transient absorption is observed. There are now two possibilities: either the near infrared absorbing transients are not formed in carbon tetrachloride, or they are formed but are difficult to detect by the present technique. If the latter is true, it could be due to a very much lower extinction coefficient of these transient species in carbon tetrachloride than in chloroform, hence leaving the transient species almost undetectable by absorption spectroscopy. However, the extinction coefficient in carbon tetrachloride would have to be very much lower (2 orders of magnitude) than in chloroform to account for the missing intensity. We do not consider such large variations to be true for rather similar solvents. Another possibility would be that the lifetime of these species is much shorter in carbon tetrachloride than in chloroform. The rationale behind this is that chloroform as a polar solvent may better stabilize the formed radical cation than carbon tetrachloride yielding a longer lifetime in chloroform. This would imply a difference of many orders of magnitude between the lifetimes in the two solvents. Jeevarajan *et al.*[13] found that the yield of carotenoid radical cation upon photolysis was very low in carbon tetrachloride: the ESR signal was very weak even in solutions containing 0.2 mM carotenoid (an order of magnitude higher than in our experiments). This could on the other hand imply that carotenoids in carbon tetrachloride degrade by a so far unknown mechanism different from that in chloroform and without formation of the near infrared absorbing transient species.

4. Carotenoid Transients in Deaerated Solutions

The weakly absorbing transients formed upon laser flash photolysis of carotenoids in deaerated

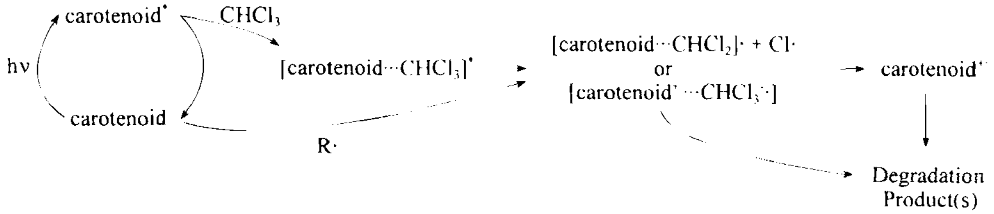


FIGURE 7 Possible reactions upon laser flash photolysis of carotenoids in chloroform.

chloroform and carbon tetrachloride solutions (Fig. 6) seem to be due to the same phenomenon in both solvents considering the similarity of the transients. The appearance of these weak transients could indicate some kind of oscillating phenomenon, perhaps changes in refractive index due to local heating of the sample upon laser flash photolysis. The transients are completely absent in air-saturated solutions and in deaerated solutions after air has been admitted to the sample suggesting that they are due to species quenched by oxygen.

CONCLUSIONS

The second-order reaction between carotenoids and free radicals in chloroform and carbon tetrachloride is fastest for the carotenes β-carotene and lycopene, and among the xanthophylls the carbonyl containing carotenoids (canthaxanthin, astaxanthin, and β-apo-8′-carotenal) react slower with the free radicals than the hydroxylated carotenoids (lutein and zeaxanthin). Similar differences in photophysics between the carbonyl containing carotenoids and other carotenoids have been observed before.[12,22] Degradation of the carotenoids involves near infrared absorbing intermediates in the case of chloroform whereas the same transients are not observed in carbon tetrachloride. This could either be due to a shorter lifetime of these transients in carbon tetrachloride or the fact that they are not formed. The near infrared absorbing intermediate absorbing at the shorter wavelength is either an ion-pair or an adduct, and the other intermediate is the radical cation. The lifetime of these intermediates is longest for lycopene and shortest for β-apo-8′-carotenal as expected considering the length of the conjugated systems.

The present results indicate that carotenes are better antioxidants than the more polar xanthophylls in lipophilic systems.

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