Importance of Carotenoid Structure in Radical-Scavenging Reactions

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The rate of (parallel) reaction between carotenoids and phenoxyl radical to yield (i) carotenoid/phenoxyl adducts and (ii) carotenoid radical cations (in effect regenerating the phenol by reduction) has been found to increase for an increasing number of (coplanar) conjugated double bonds in the carotenoid and to decrease in the presence of hydroxy and especially keto groups. The reactions to yield the carotenoid radical have half-lives of tenths of milliseconds, and when studied by transient visible and near-infrared absorption spectroscopy using nanosecond laser flash photolysis for radical generation, the relative rates in di-*tert*-butyl peroxide/benzene (7/3, v/v) at 20 °C were lycopene (1.66), β-carotene (1), zeaxanthin (0.79), lutein (0.70), and echinenone (0.68), while canthaxanthin and β-apo-8′-carotenal hardly reacted and astaxanthin not at all. When formed, the carotenoid radicals are rather stable (second-order decay on a millisecond time scale). The lower the energy of the near-infrared transition in the radical cation, the more effective is the carotenoid as phenoxyl scavenger, and lycopene is concluded to be the more efficient antioxidant.

Keywords: *β-Carotene; lycopene; lutein; zeaxanthin; echinenone; β-apo-8′-carotenal; canthaxanthin; astaxanthin; phenoxyl radical; antioxidant synergism; carotenoid radicals*

INTRODUCTION

The last few years have seen a growing interest in the antioxidant properties of carotenoids (Krinsky, 1994; Palozza and Krinsky, 1994). Carotenoids may act as antioxidants by quenching singlet oxygen and triplet excited states (Palozza and Krinsky, 1992a), a mechanism by now fairly well-understood. Carotenoids' possible role as a chain-breaking antioxidant, i.e., as quencher of free radicals, is less well-understood (Jørgensen and Skibsted, 1993). More than a decade ago it was suggested that β-carotene reacted with peroxyl radicals by forming an adduct (Burton and Ingold, 1984). However, until very recently solid evidence of this mechanism did not exist, when adducts between β-carotene and free radicals were detected by mass spectrometry (Liebler and McClure, 1996). Until then, only degradation products of carotenoids had been identified. These were primarily epoxides and carbonyls (Palozza and Krinsky, 1994). However, very recent kinetic studies (Böhm et al., 1995; Everett et al., 1995, 1996; Hill et al., 1995; Mortensen and Skibsted, 1996a,b) and product analysis (Liebler and McClure, 1996) have shown that the mechanism of scavenging of free radicals by carotenoids is more complex than a simple adduct formation between free radicals and carotenoids. Carotenoids react with free radicals by at least two parallel pathways: (i) formation of an adduct between carotenoid and free radical and (ii) electron transfer yielding the carotenoid radical cation.

Phenolic compounds are widely abundant in food systems. Many antioxidants, e.g., vitamin E and flavonoids, are phenols, and phenols are also present in proteins (tyrosine). Phenoxylic radicals may be generated by reaction between a phenolic compound and a lipid-derived free radical. This is the method of action of phenolic antioxidants. Tyrosyl radicals have been detected in a number of proteins (Qin and Wheeler, 1995). This may lead to modification of the protein via cross-linking with another tyrosine in the same or another protein (Tew and Ortiz de Montellano, 1988) or with DNA (Simic and Dizdaroglu, 1985). The reaction between phenoxylic radicals and carotenoids is therefore of great interest in relation to prevention of oxidative modification to biomolecules (like proteins) and in relation to synergism between phenolic antioxidants and carotenoids. The flavor development in black teas is also related to oxidative degradation of carotenoids initiated by polyphenol oxidase activity and with phenoxylic radicals as reactive intermediates. A better understanding of the effect of carotenoid structure, including length of conjugated system and presence and nature of substituents, on the reaction with phenoxylic radicals is clearly of importance for exploitation of antioxidant synergism in food protection and for dietary recommendations. The current focus on the health aspects of an increased intake of carotenoids other than β-carotene like lycopene (Gerster, 1997) clearly demonstrates the need for improved understanding of the relation between structure and reactivity of carotenoids.

Following our initial observation that β-carotene reacts with phenoxyl radicals by two parallel pathways, we now report on the influence the structure of the carotenoid has on these reactions.

MATERIALS AND METHODS

Materials. Astaxanthin, canthaxanthin, echinenone, β-apo-8′-carotenal, lutein, zeaxanthin, lycopene, and β-carotene (Figure 1) were supplied by Roche A/S (Hvidovre, Denmark) sealed in ampules under argon and were used without further purification. Phenol (p.a.) from Merck (Darmstadt, Germany), benzene (pronalys) from May and Baker Ltd. (Dagenham, England), and di-*tert*-butyl peroxide from Merck-Schuchardt (Hohenbrunn bei München, Germany) were all used as received. The solutions were made up from stock solutions of 10^{-4} M carotenoid in benzene and 2.5 M phenol in di-*tert*-butyl peroxide yielding a concentration of carotenoid ranging from

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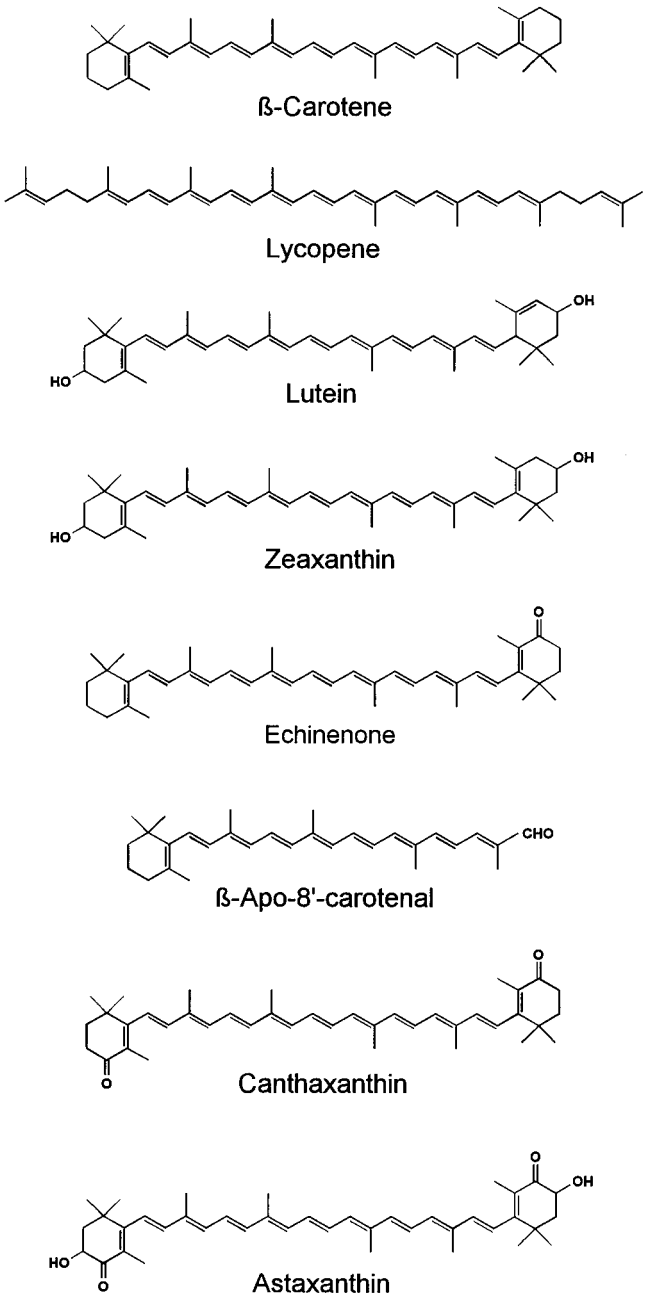

Figure 1. Structures of the eight carotenoids examined in this study.

5 to 30 μM and a concentration of phenol of 1.75 M in di-*tert*-butyl peroxide/benzene (70/30, v/v). The solutions were stored in the dark at 0 °C and were used the same day as they were made. Oxygen was removed from some of the samples prior to measurements by three freeze−pump−thaw cycles.

Spectroscopic Methods. Laser flash photolysis experiments were carried out with an LKS.50 laser flash photolysis spectrometer from Applied Photophysics Ltd. (Leatherhead, U.K.). The third harmonic at 355 nm of a pulsed Q-switched Nd-YAG laser, Spectron Laser Systems (Rugby, U.K.), 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. 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 carotenoid by the Xe arc lamp used for monitoring, whereas a UV cutoff 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. Absorption spectra were recorded on an HP 8452 UV−vis diode array spectrophotometer (Hewlett Packard, Palo Alto, CA).

Time traces were analyzed by nonlinear least-squares fitting using the Levenberg−Marquardt algorithm (Origin 4.1 from Microcal Software, Inc., Northampton, MA). Transient absorption spectra were constructed from time traces at every 10 nm in the visible region and at every 20 nm in the near-infrared region.

RESULTS

Photochemical generation of free radicals by homolysis of a peroxide allowed real-time kinetic investigation of the reaction of carotenes and xanthophylls with free radicals using laser flash spectroscopy. Oxygen was found to have no influence on the observed kinetics.

Kinetic Scheme. Laser flash photolysis of di-*tert*-butyl peroxide with UV excitation results in the formation of *tert*-butoxyl radicals (Gilbert and Baggott, 1991):

$$(CH_3)_3COOC(CH_3)_3 \rightarrow 2(CH_3)_3CO^{\bullet} \quad (1)$$

These free radicals are very rapidly quenched (within 10 ns) by the large excess of phenol present in the solutions used for the experiments:

$$(CH_3)_3CO^{\bullet} + C_6H_5OH \rightarrow (CH_3)_3COH + C_6H_5O^{\bullet} \quad (2)$$

The phenoxyl radical, identified by its strong absorption around 400 nm (Johnston et al., 1993), decays by what seems to be largely a second-order process:

$$2C_6H_5O^{\bullet} \rightarrow P_1 \quad (3)$$

though the fit to a second-order decay is not perfect. This could be due to interference by the following equilibrium:

$$2C_6H_5O^{\bullet} \rightleftarrows P_2 \quad (4)$$

as has been proposed previously based on thermodynamic data (Mahoney and Weiner, 1972). P_1 and P_2 may represent a number of nonradical products.

In the presence of carotenoids, the phenoxyl radical formed may, in competition with the second-order decay, react with the carotenoid:

$$Car + C_6H_5O^{\bullet} \rightarrow \text{intermediate(s)} \quad (5)$$

Neglecting the reversible dimerization of the phenoxyl radical, the reaction scheme is described by the coupled differential equations:

$$-\frac{d[C_6H_5O^{\bullet}]}{dt} = 2k_3[C_6H_5O^{\bullet}]^2 + k_5[C_6H_5O^{\bullet}][Car] \quad (6)$$

$$-\frac{d[C\ ar]}{dt} = k_5[C_6H_5O^{\bullet}][Car] \quad (7)$$

In the absence of phenol no reaction with the carotenoid is observed, probably because the reactive *tert*-butoxyl radical (present in low concentration) disappears before reacting with the carotenoid (also at low concentration). The lack of reaction is hence not necessarily due to an

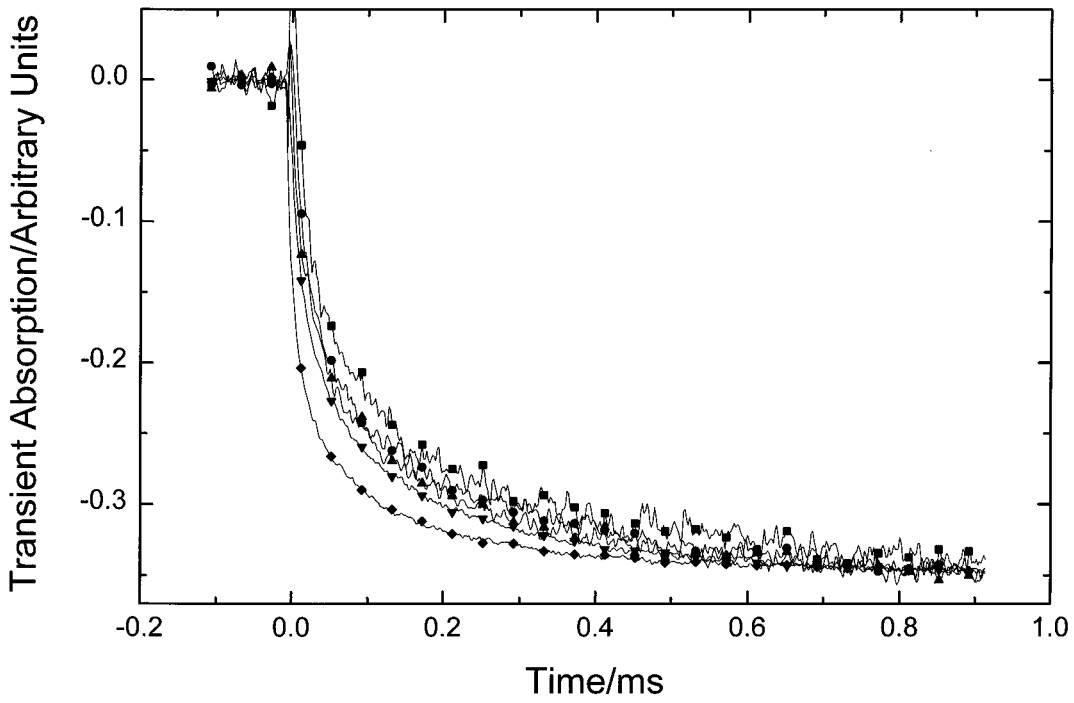

Figure 2. Time traces of absorption changes of 1.0×10^{-5} M lycopene (◆, at 500 nm), β-carotene (▼, at 500 nm), zeaxanthin (▲, at 500 nm), lutein (●, at 490 nm), and echinenone (■, at 490 nm) following a single laser flash (~60 mJ at 355 nm for 8 ns). The time traces have been scaled to the same transient absorption.

inability of the carotenoid to scavenge the *tert*-butoxyl radical.

Although the lack of reaction between the *tert*-butoxyl radical and the carotenoid simplified the rate expression, the coupled differential eqs 6−7 cannot be solved analytically, and they represent anyway a simplification because the reversible reaction of eq 4 has not been taken into account. The concentration of phenoxyl radical produced in a single laser flash is of the order of 10^{-4} M based on published extinction coefficients (Neta and Schuler, 1975). Phenoxyl radical is hence formed in excess compared to carotenoid. However, eqs 6 and 7 cannot be approximated by pseudo-first-order kinetics (setting $[C_6H_5O^{\bullet}]$ constant) because eq 3 is quite significant [$2k_3$ is on the order of 10^9 M^{-1} s^{-1} (Tripathi and Schuler, 1984; Weiner, 1972)] and the largest fraction of phenoxyl radicals will disappear by eq 3. Therefore, a different approach was chosen in which a first-order decay was fitted to the time traces of absorbance (a second-order decay did not improve the quality of the fit significantly). This procedure gave an acceptable description, although the rate constants thus obtained are not meaningful in a quantitative way but may still be used to compare the relative reactivity of different carotenoids reacting under similar conditions. This comparison was done by setting the first-order rate constant of β-carotene equal to 1, and the first-order rate constants for the other carotenoids were taken relative to this.

Bleaching. Reaction between phenoxyl radical and carotenoid leads to permanent bleaching in the blue-green spectral region (400−530 nm) where the carotenoids absorb strongly. Figure 2 shows the time traces of absorption change of a number of carotenoids, and in Figure 3 is shown an example of a transient spectrum of lutein at two different times. Positive transient absorption at short wavelengths (Figure 3) is due to the formation of the phenoxyl radical which absorbs strongly around 400 nm. The bleaching is largely complete

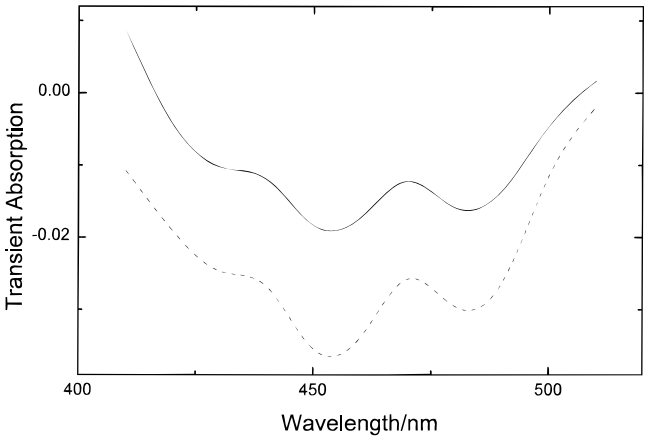

Figure 3. Transient absorption spectra of 1.0×10^{-5} M lutein and 1.75 M phenol in benzene/di-*tert*-butyl peroxide (3/7, v/v) 40 μs (—) and 890 μs (− − −) after the laser flash (~60 mJ at 355 nm for 8 ns).

within 1 ms (Figure 2). At longer times recurrence of absorption may be observed at the blue side of the absorption band due to formation of degradation products with shorter conjugated systems (*vide infra*). It is possible qualitatively to estimate the ability of the carotenoids to scavenge phenoxyl radicals by looking at the extent of bleaching caused by a single laser pulse. Table 1 shows the results of this analysis for experiments with different carotenoids under conditions similar to those used for lutein (Figure 3). Notably, astaxanthin does not react at all. Canthaxanthin and β-apo-8′-carotenal do react with phenoxyl radicals, but only to a very small extent (≪1%). Figure 2 shows that the various carotenoids bleach at different rates. The relative "first-order rate constants" calculated as indicated above are also given in Table 1.

Near-IR-Absorbing Intermediates. Concurrent with the bleaching of carotenoid is the formation of one or more transient species absorbing in the near-infrared

Table 1. Degradation of Carotenoids by a Single Laser Flash and Relative "First-Order Rate Constants" for Reaction of Carotenoid with (Excess) Phenoxyl Radical at 20 °C in Benzene/Di-*tert*-butyl Peroxide

carotenoid	extent of degradation[a] (%)	relative first-order rate constant[b]
lycopene	38	1.66
β-carotene	10	1
zeaxanthin	4.2	0.79
lutein	3.5	0.70
echinenone	2.0	0.68
canthaxanthin	≪1	
β-apo-8′-carotenal	≪1	
astaxanthin	∼0	

[a] Determined for experimental conditions as described in the text. [b] Relative to β-carotene.

spectral region. Figure 4 shows the transient absorption spectra of the carotenoids examined in this study which exhibited formation of transient species upon reaction with phenoxyl radicals. It is evident that the rate of spectral changes is not the same across the entire absorption band, i.e., more than one transient species is formed. From an inspection of the spectral changes it was found that formation of two transient species could account for the time evolution of spectral changes. The absorption maxima of the two species are given in Table 2 for the individual carotenoids. The spectral data for the intermediates derived from canthaxanthin and β-apo-8′-carotenal are due to low extent of reaction poorly defined.

The maximum concentration of intermediates is attained after approximately 200 μs (Figure 5A). The intermediates only decay slowly, still being observable after 100 ms (Figure 5B).

DISCUSSION

Reactivity toward Phenoxyl Radicals. As seen from Table 1 there is a significant difference in the ability of the various carotenoids to scavenge phenoxyl radicals. Lycopene is the most effective followed by β-carotene, zeaxanthin, lutein, and echinenone, the latter three showing only slight differences in their ability to scavenge phenoxyl radicals. Canthaxanthin and β-apo-8′-carotenal only react with phenoxyl radicals to a very small extent (≪1%), and astaxanthin does, perhaps surprisingly, not react at all. The relative "first-order rate constants" show the same order of reactivity toward phenoxyl radicals though these values should not be interpreted as representing true rates (*vide supra*). The large differences observed are a consequence of structure as discussed below.

Formation and Decay of Intermediates. The kinetics of formation of the two near-infrared-absorbing intermediates is just as complex as the bleaching of the carotenoid (eqs 6 and 7). Figures 2 and 5A show that formation of intermediate is, as expected, on the same time scale as bleaching of carotenoid. The intermediates decay rather slowly, i.e., tens of milliseconds (Figure 5B). This slow decay can be ascribed to the high stability of these intermediates, making the carotenoids effective antioxidants as they form relatively stable radical products by reaction with free radicals. The carotenoid free radical intermediates can be expected to decay by second-order kinetics as in eq 3 for the phenoxyl radical. However, there is a small but clear deviation from second-order kinetics as shown in the insert of Figure 5B (in some cases the deviation is more pronounced). Since two intermediates (I_1 and I_2) are formed, three termination reactions may be envisaged:

$$2I_1 \rightarrow \text{products} \quad (8)$$

$$2I_2 \rightarrow \text{products} \quad (9)$$

$$I_1 + I_2 \rightarrow \text{products} \quad (10)$$

giving the differential equations

$$-\frac{d[I_1]}{dt} = 2k_8[I_1]^2 + k_{10}[I_1][I_2] \quad (11)$$

$$-\frac{d[I_2]}{dt} = 2k_9[I_2]^2 + k_{10}[I_1][I_2] \quad (12)$$

Termination reactions between these intermediates and phenoxyl radicals can be excluded due to the short lifetime (completely gone in 0.1 ms) of the phenoxyl radical. The differential equations show that deviation from second-order kinetics is to be expected.

The final stable products of reaction between carotenoids and phenoxyl radicals have not been identified. However, there is an increase in absorption on the blue side of the carotenoid absorption band indicating that the final products consist of conjugated systems which are only slightly shorter than the parent carotenoid, i.e., 1−2 double bonds shorter.

Nature of Intermediates. Based on previous assignments in the literature, the intermediate absorbing at the shorter wavelength was tentatively identified as an adduct between phenoxyl radical and β-carotene and the intermediate absorbing at the longer wavelength as the β-carotene radical cation (Mortensen and Skibsted, 1996b). The present results do not warrant any reinterpretation of these assignments. The structure of these intermediates is shown in Figure 6 with echinenone as an example. Both structures are characterized by a long odd-electron-conjugated system which explains the relative high stability of these intermediates. The second-order plot for absorbance changes at 740 nm for lutein (Figure 5) thus mainly corresponds to decay of the radical adduct.

Structure vs Reactivity. The carotenoids examined in this study may be subdivided into three categories according to their structure: the carotenes lycopene and β-carotene (hydrocarbons), the hydroxycarotenoids (xanthophylls) zeaxanthin and lutein, and the ketocarotenoids (xanthophylls) astaxanthin, canthaxanthin, echinenone, and β-apo-8′-carotenal. The order of reactivity of these carotenoids toward phenoxyl radicals is carotenes > hydroxycarotenoids > ketocarotenoids. Within each group there are large differences as may be seen from Table 1. Lycopene is far more reactive than β-carotene, zeaxanthin is more reactive than lutein, and echinenone is much more reactive than canthaxanthin and β-apo-8′-carotenal, whereas astaxanthin is inactive. Some of these results may be easily rationalized. Both lycopene and β-carotene have 11 conjugated double bonds (Figure 1). However, in the case of β-carotene two of these bonds are in cyclohexene rings which are not planar with the rest of the molecular backbone thereby reducing the effective length of the conjugated system. This results in the absorption maximum of lycopene being red-shifted by 21 nm compared to that of β-carotene (Table 2). The resulting conjugated system of the carotenoid free radicals is therefore effectively longer and hence more stable in the

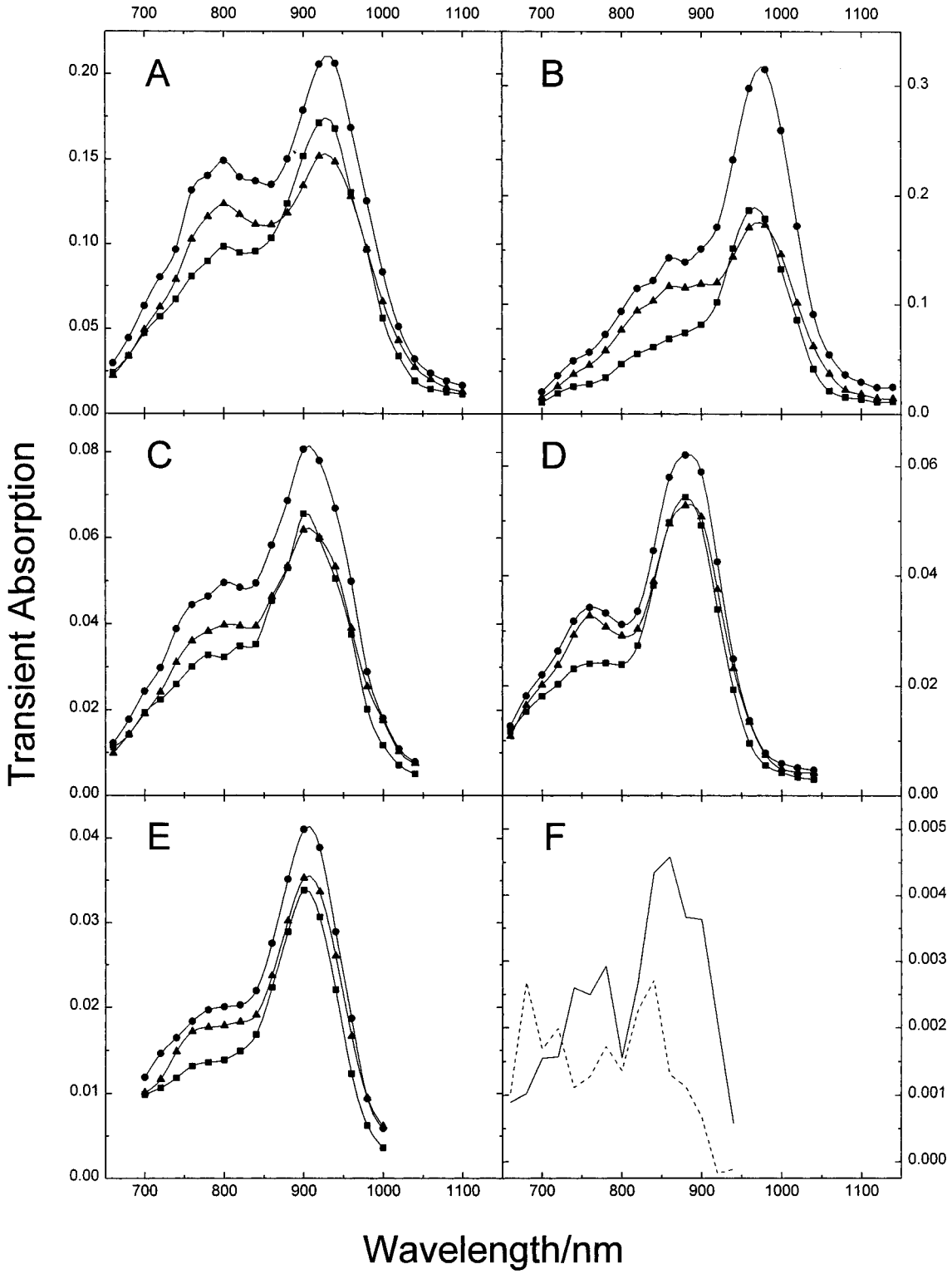

Figure 4. Transient absorption spectra (note the different absorbance scales) of 3.0×10^{-5} M β-carotene (A), lycopene (B), zeaxanthin (C), lutein (D), echinenone (E), (—) canthaxanthin (F), and (– – –) β-apo-8′-carotenal (F) and 1.75 M phenol in benzene/di-*tert*-butyl peroxide (3/7, v/v) 40 μs (■), 190 μs (●), and 890 μs (▲) after the laser flash (~60 mJ at 355 nm for 8 ns, except for lycopene: ~5 mJ). The spectra in panel F are shown at 89 μs. The concentrations of transients of canthaxanthin and β-apo-8′-carotenal are low compared to the other carotenoids, and the spectral resolution is comparably lower.

case of lycopene than in the case of β-carotene, which also shows as an even larger red shift in the absorption maximum of lycopene free radicals compared to β-carotene free radicals (Table 2). The same line of argument can be applied to zeaxanthin and lutein, lutein having only one cyclohexene double bond in conjugation (Figure 1) whereas zeaxanthin has two, as is also reflected in the position of the absorption maximum of the two carotenoids and of the free radicals derived from these two carotenoids (Table 2).

Looking at the structures of echinenone, canthaxanthin, astaxanthin, and β-apo-8′-carotenal, it is, however, not easily realized why the structural differences give the order of reactivity mentioned above. Astaxanthin and canthaxanthin have the longest conjugated system: 11 double bonds and two carbonyl groups, and

Table 2. Absorption Maxima of Carotenoids and Near-Infrared-Absorbing Transient Species Formed by Reaction with Phenoxyl Radical

carotenoid	parent (nm)	radical cation (nm)	radical adduct (nm)
lycopene	480	975	860
β-carotene	459	930	800
zeaxanthin	459	907	800
lutein	453	882	760
echinenone	478	907	800
canthaxanthin	490	860	
β-apo-8′-carotenal	482	840	

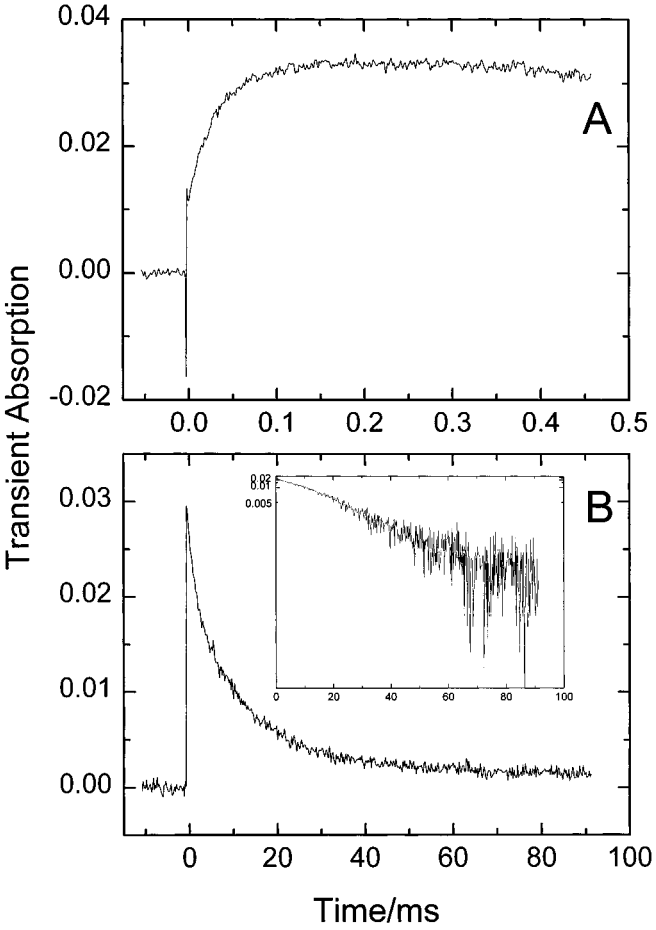

Figure 5. Time traces of 3.0×10^{-5} M lutein and 1.75 M phenol in benzene/di-*tert*-butyl peroxide (3/7, v/v) at 740 nm at which wavelength the radical adduct dominates the absorption (Table 2). The insert in panel B shows the data plotted with a reciprocal ordinate (second-order plot).

should, *a priori*, be expected to provide the most stable free radical intermediates upon one-electron oxidation. Echinenone with 11 conjugated double bonds and one carbonyl group should, considering only the length of the conjugated system of the intermediates, be better at scavenging free radicals than β-carotene but less effective than astaxanthin and canthaxanthin. The opposite order of reactivity is observed. β-Apo-8′-carotenal follows the expectation in that it is not a good antioxidant with only nine conjugated double bonds and one carbonyl group.

In the reaction with free radicals derived from chloroform (Mortensen and Skibsted, 1997a) and with the ABTS radical cation (Miller et al., 1996), the same order of reactivity was observed, i.e., carotenes > hydroxycarotenoids > ketocarotenoids. Very recently it was reported that β-carotene and zeaxanthin reduced susceptibility to oxidant stress in chicken liver whereas

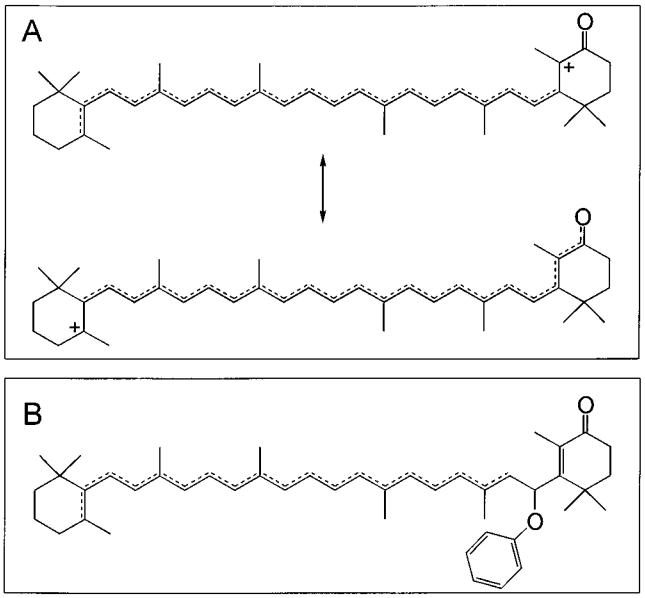

Figure 6. Structures of the radical cation of echinenone (A) and the adduct between phenoxyl radical and echinenone (B).

canthaxanthin did not (Woodall et al., 1996), a finding supporting the present results, i.e., ketocarotenoids are less efficient scavengers of free radicals than carotenes and hydroxycarotenoids. However, in cells it was observed that canthaxanthin and lutein apparently scavenged phenoxyl radicals faster than β-carotene and lycopene (Tyurin et al., 1997).

It has been found that the free radical intermediates of ketocarotenoids were less stable than the corresponding intermediates of the two other classes of carotenoids, as evidenced by a blue shift of the absorption maximum and a shorter lifetime of the former class of carotenoid intermediates (Mortensen and Skibsted, 1997a). The carbonyl group hence raises the energy of the intermediate and the transition states for the reactions with phenoxyl radicals, thereby making ketocarotenoids less able at scavenging free radicals probably due to a higher energy of activation. Notably, the presence of a carbonyl group also changes the spectroscopic and photophysical properties of C_{40}-carotenoids more significantly than the presence of a hydroxy group (Nielsen et al., 1996). The, albeit small, reactivity of echinenone may be explained as follows. If the phenoxyl radical adds to the conjugated system close to the cyclohexenone ring as in Figure 6B, the carbonyl group will not be part of the odd-electron-conjugated system and the intermediate hence more stable. This hypothesis is corroborated by the observation that the absorption maxima of the adducts of β-carotene and echinenone are the same (Figure 4 and Table 2), showing that the carbonyl group is not part of the conjugated system. In the case of astaxanthin and canthaxanthin with two carbonyl groups, one carbonyl group will always be part of the odd-electron-conjugated system of the adduct, thereby lowering the stability of these adducts. The same line of argument applies to β-apo-8′-carotenal if the phenoxyl group adds next to the cyclohexene ring and not next to the carbonyl group. In the case of the radical cation of echinenone, one resonance form does not involve the carbonyl group (Figure 6A), whereas this is not the case as far as astaxanthin and canthaxanthin are concerned. As a consequence, the absorption maximum of the echinenone radical cation is only slightly blue-shifted from that of the β-carotene radical cation as compared

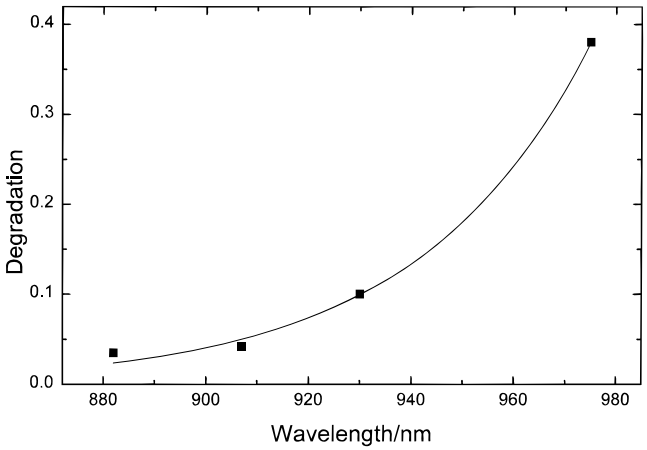

Figure 7. Degree of degradation of 1.0×10^{-5} M lycopene, β-carotene, zeaxanthin, and lutein following reaction with phenoxyl radical in 1.75 M phenol in benzene/di-*tert*-butyl peroxide (3/7, v/v) plotted against the absorption maximum of the carotenoid radical cations. The curve is an exponential fit to the data.

to the large blue shift of the canthaxanthin radical cation (Figure 4 and Table 2).

The difference in reactivity between β-carotene and zeaxanthin cannot be explained by differences in the length of the conjugated system but must be due to the more polar nature of zeaxanthin, with its two hydroxy groups making the radical cation less stable as evidenced by a lower absorption maximum compared to the β-carotene radical cation (Table 2).

Tables 1 and 2 show that the better the carotenoid is at scavenging phenoxyl radicals, the higher is the absorption maximum of the radical cation, if ketocarotenoids are disregarded (as shown above, the carbonyl group completely alters the antioxidant charateristics of carotenoids). Figure 7 shows that there is an approximate exponential relationship between the degree of degradation and the absorption maximum of the radical cation. The absorption maximum can hence be used as a measure of the ability of a carotenoid as a scavenger of free radicals.

Regeneration of Phenol. As discussed above, carotenoids react with phenoxyl radicals by two parallel pathways: (i) formation of an adduct and (ii) electron transfer yielding the carotenoid radical cation and, perhaps most important, regenerating phenol. This parallel mechanism is the same as has been proposed based on product analysis studies (Liebler and McClure, 1996). The fact that carotenoids may reduce one-electron-oxidized phenol, a model compound for tyrosine, back to phenol shows that at least some carotenoids are able to prevent oxidative modifications of proteins, adding further support to the observation of a very fast reaction between the carotenoid crocin and a protein radical derived from myoglobin (Jørgensen et al., 1997). The results may also indicate that at least some of the carotenoids may play a role in recycling other one-electron-oxidized phenolic antioxidants like tocopherols. However, it has been suggested that α-tocopherol protects β-carotene from being oxidized and not the other way around (Palozza and Krinsky, 1992a,b). 2,2,5,7,8-Pentamethyl-6-hydroxychromane, a tocopherol homologue, has been found to protect β-carotene against free radical-induced oxidation in liposomes (Tyurin et al., 1997). Very recent studies (Mortensen and Skibsted, 1997b) show that one-electron-oxidized lycopene can be reduced by α-tocopherol whereas one-electron-oxidized δ-tocopherol can be reduced by lycopene. One-electron-oxidized β- and γ-tocopherol are in equilibrium with lycopene, i.e., the lycopene radical cation may be reduced by β- and γ-tocopherol, and the β- and γ-tocopheroxyl radicals may be reduced by lycopene depending on the concentration of the various species. On the other hand, it has been claimed that a number of carotenoids are able to reduce the α-tocopheroxyl radical (Böhm et al., 1997). Clearly, more work is needed to elucidate the hierarchy of antioxidant efficiency.

CONCLUSIONS

The rather long lifetime of the carotenoid free radicals (adduct and radical cation) indicates that these free radicals are relatively stable and the parent carotenoids may hence act as chain-breaking antioxidants. However, the carotenoids are not equally good at scavenging phenoxyl radicals. The carotenes lycopene and β-carotene are best followed by the hydroxycarotenoids zeaxanthin and lutein and the monoketocarotenoid echinenone. Xanthophylls with two keto groups or an aldehyde group are inefficient scavengers of phenoxyl radicals. A correlation exists between the absorption maximum of the radical cation and the extent of bleaching of carotenoid. The absorption maximum of the carotenoid radical cation may hence be used as an indicator of the ability of the carotenoid as free radical scavenger.

ACKNOWLEDGMENT

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