Relative One-Electron Reduction Potentials of Carotenoid Radical Cations and the Interactions of Carotenoids with the Vitamin E Radical Cation

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Abstract: Pulse radiolysis studies have been used to determine the electron-transfer rate constants between various pairs of carotenoids, one of which is present as the radical cation. These dietary carotenoids include those of importance to vision, namely zeaxanthin and lutein. These results have suggested the order of relative ease of electron transfer between six carotenoids. Additional experiments, involving electron transfer between astaxanthin (ASTA), β-apo-8′-carotenal (APO), and vitamin E (TOH), lead to the following order in terms of relative ease of electron transfer for the seven carotenoid radical cations studied: astaxanthin > β-apo-8′-carotenal > canthaxanthin > lutein > zeaxanthin > β-carotene > lycopene, such that lycopene is the strongest reducing agent (the most easily oxidized) and astaxanthin is the weakest, and the radical cations of the visual carotenoids, lutein (LUT) and zeaxanthin (ZEA), are reduced by lycopene (LYC) but not by β-carotene (β-CAR). Work on 7,7′-dihydro-β-carotene (77DH) and vitamin E allows us to better understand the interaction of the vitamin E radicals with carotenoids.

Introduction

The role of carotenoids as antioxidants and their possible switch to prooxidant behavior is of much current interest.[1−3] One aspect of this is the possibility that singlet oxygen quenching by carotenoids (CAR) is partly responsible for their antioxidant behavior. It has been claimed that in some environments lycopene (LYC) is a more efficient singlet oxygen quencher than β-carotene (β-CAR).[4,5] Nevertheless, we have shown[5] that singlet oxygen is quenched only about 1.3 times faster by lycopene compared to β-carotene in both benzene and toluene and this small difference is unlikely to be of any significance in either chemical or biological systems. However, the major role of carotenoids with respect to antioxidant (and prooxidant) behavior may well involve radical scavenging processes including electron transfer and consequent free radical production. For example, an oxygen-centered radical such as a peroxyl radical ($RO_2^{\bullet}$) may react either via electron transfer[6] to produce a carotenoid radical cation

$$RO_2^{\bullet} + CAR \rightarrow RO_2^{-} + CAR^{\bullet+} \quad (1)$$

or via other processes, such as, an addition reaction to a carbon−carbon double bond

$$RO_2^{\bullet} + CAR \rightarrow [RO_2\text{- - -}CAR]^{\bullet} \quad (2)$$

and hydrogen-atom transfer

$$RO_2^{\bullet} + CAR \rightarrow ROOH + CAR(-H)^{\bullet} \quad (3)$$

The electron-transfer reaction 1 will clearly be favored where the R groups are electron withdrawing (e.g., $CCl_3O_2^{\bullet}$). Clearly, the ease of oxidation of the carotenoids will be an important factor governing the relative rate constants of these radical scavenging processes. In the present paper, we investigate the rates of electron transfer between pairs of carotenoids, one of which is present as the radical cation, to establish the order of the ease of electron transfer of a series of carotenoid radical cations:

$$CAR1^{\bullet+} + CAR2 \rightarrow CAR1 + CAR2^{\bullet+} \quad (4)$$

We have recently reported[7] the formation of carotenoid radical cations following quenching of a vitamin E radical in hydrocarbon solvents. In the present paper, we also use pulse radiolysis to further investigate the vitamin E radical reaction with 7,7′-dihydro-β-carotene (77DH) and show that the vitamin E radical quenched is, in fact, the radical cation $TOH^{\bullet+}$. The structures of the carotenoids studied, including several xanthophylls (XAN), are given in Figure 1.

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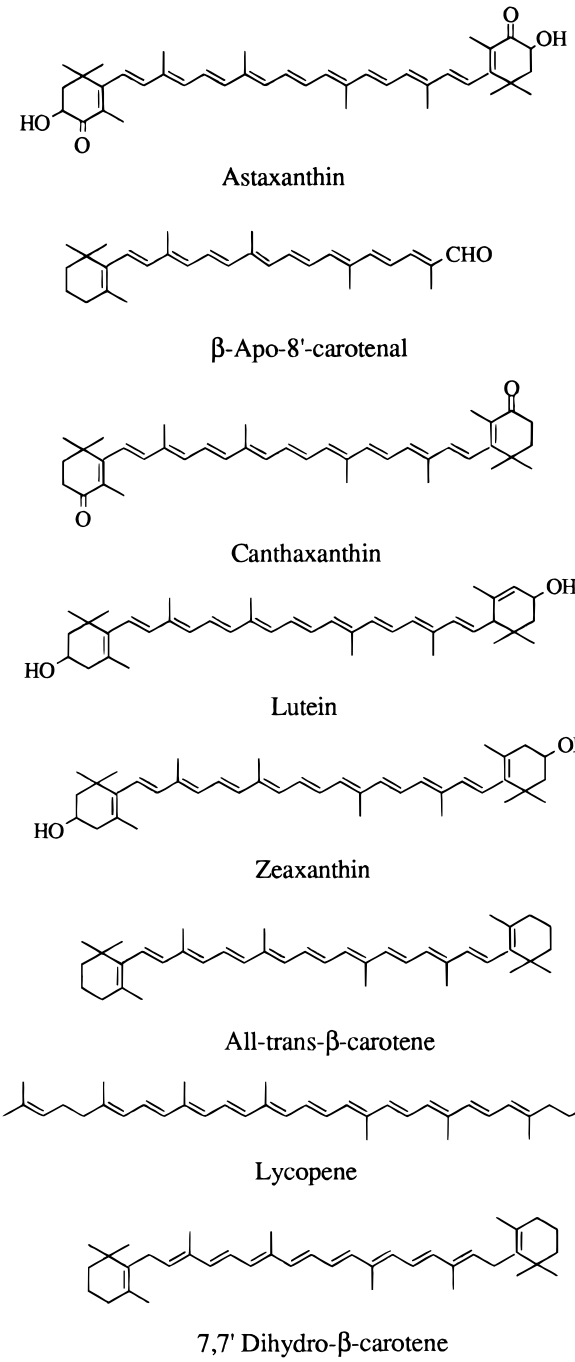

Figure 1. Structures of carotenoids and xanthophylls.

Methods

Experiments were carried out using a 9−12-MeV Vickers linear accelerator as previously described,[8,9] using 50−200-ns pulses. Solutions were studied using quartz flow-through cells with an optical path length of 2.5 cm and an internal volume of 3 cm^3. The vitamin E was supplied by Sigma and purified, via spinning plate chromatography, to >99.5% pure (by HPLC) before use. The carotenoids were a gift from Hoffmann-La Roche and were used as supplied, after confirming their purity by HPLC (>99.5%). The lutein sample, however, contained about 4% zeaxanthin. The benzene and hexane were super purity solvents from Romil.

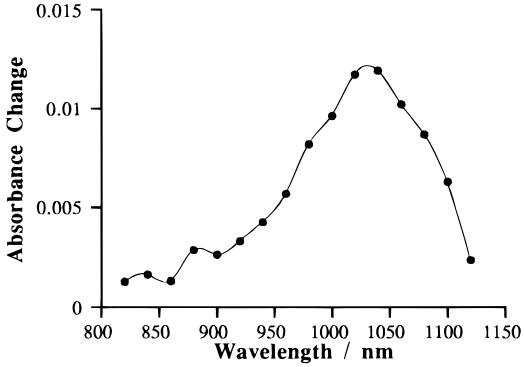

Figure 2. Spectrum of β-carotene radical cation in argon-flushed benzene. The spectrum is taken at a long enough time for the radical anion to have completely decayed.

Table 1. Wavelengths of the Carotenoid Radical Cation Absorption Band Maxima (λ_{Max}) in Benzene and Hexane

carotenoid	$\lambda_{max\ in\ benzene}$/nm	$\lambda_{max\ in\ hexane}$/nm
astaxanthin	920[a]	940[b]
β-apo-8'-carotenal	880[a]	890[a]
canthaxanthin	940[a]	960[c]
lutein	950[a]	973[b]
zeaxanthin	1000[a]	1040[b]
β-carotene	1020[a]	1040[d]
lycopene	1050[a]	1070[d]

[a] This work. [b] Reference 6. [c] Reference 10. [d] Reference 11.

Results and Discussion

Relative One-Electron Reduction Potentials of Carotenoid Radical Cations. The radical cations of a range of carotenoids have previously been characterized in hexane,[6,10,11] dichloromethane,[12] and more recently by Skibsted and co-workers in chloroform.[13] In the present work benzene is used as the solvent because it affords a higher solubility of carotenoids than hexane, especially those containing oxygen (the xanthophylls). Also, the radiation chemistry of benzene is well understood, and Figure 2 shows the absorption spectrum of the β-carotene radical cation in this solvent. For comparison, the corresponding wavelengths (nm) of the radical cation absorption band maxima in benzene and hexane are given in Table 1. The solvent polarizabilities for benzene and hexane (based on refractive indices of 1.501 and 1.375, respectively) and the observed solvent shifts for carotenoid radical cations fit the trends noted by Andersson et al.[14] for carotenoid ground states.

Figure 3 shows the changes in spectra with time on pulse radiolysis of 1×10^{-4} M ASTA in the presence of 1×10^{-5} M LYC. This illustrates positive charge transfer from $ASTA^{\bullet+}$ to LYC, i.e. electron transfer from LYC to $ASTA^{\bullet+}$. Similar data have allowed the electron-transfer second-order rate constants to be determined for 10 such pairs as given in Table 2. In general, these pulse radiolysis kinetic studies show that lycopene (LYC) efficiently quenches (reduces) the radical cation of all the oxy-containing carotenoids studied

$$XAN^{\bullet+} + LYC \rightarrow XAN + LYC^{\bullet+} \qquad (5)$$

whereas β-carotene only reduces the radical cation of astaxanthin (ASTA), canthaxanthin (CAN), and β-apo-8′-carotenal (APO),

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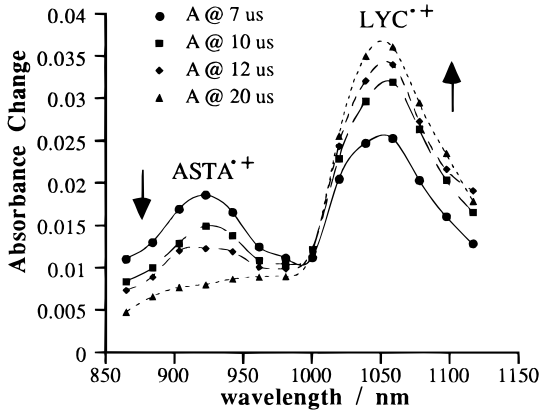


Figure 3. Spectra obtained on pulsing 1×10^{-4} M ASTA with 1×10^{-5} M LYC in argon-flushed benzene.

Table 2. Bimolecular Rate Constants for Electron Transfer between Carotenoid Pairs ($CAR1^{\bullet+} + CAR2 \rightarrow CAR1 + CAR2^{\bullet+}$)

carotenoid radical cation	rate constant (±10%)/10^9 M^{-1} s^{-1}		
	lycopene	β-carotene	zeaxanthin
$ASTA^{\bullet+}$	9	8	5
$APO^{\bullet+}$	11	6	8
$CAN^{\bullet+}$	8	5	<1
$ZEA^{\bullet+}$	7	<1	—
$LUT^{\bullet+}$	5	<1	<1

(see Table 2). However, for several pairs, spectral overlap precluded the observation of the electron-transfer process.

It is noteworthy that the radical cations arising from the two carotenoids in the human macular [lutein (LUT) and zeaxanthin (ZEA)] are both repaired efficiently by lycopene but not by β-carotene. The retina is the only organ in the body which is continually exposed to high levels of focused radiation and is in a highly oxygenated environment. This combination of light and oxygen, together with the presence of photosensitizers, gives a potential for oxy free radical and singlet oxygen generation. Lutein and zeaxanthin both contain terminal hydroxyl groups (see Figure 1) which may allow one or both of them to span the outer segment membrane. Hence, they may play a particularly efficient role in antioxidant processes by being more accessible to species, such as vitamin C in the extracellular environment, so as to regenerate the carotenoid from its radical cation. While it is well established that the retina does not contain high concentrations of hydrocarbon carotenoids such as β-carotene and lycopene, nevertheless Mares-Perlman et al.[15] have shown a correlation between age-related macular degeneration (in which the yellow spot of the macular, which contains the xanthophylls, may be photodamaged) and low levels of serum lycopene. Also, there is other evidence that dietary factors, involving xanthophylls and other carotenoids, may well be related to age-related macular degeneration.[16,17]

It is also established that canthaxanthin can accumulate in the retina after high-dose ingestion. While the presence of canthaxanthin does not appear to have any significant clinical consequence, we find that both lycopene and β-carotene undergo

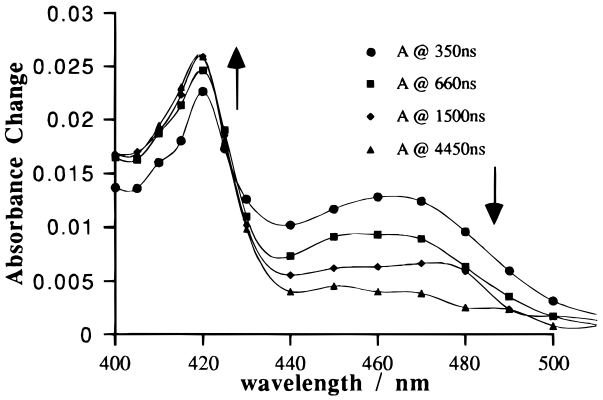
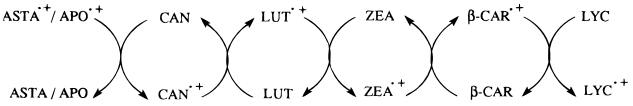


Figure 4. Transient spectra of 1×10^{-2} M vitamin E in N_2O-flushed hexane.

electron transfer to $CAN^{\bullet+}$. Indeed, we find that the introduction of the oxygen heteroatom into a carotenoid (to give a XAN) leads to a radical cation which is a stronger oxidizing species and this is especially so if the oxygen is present as part of a carbonyl group. Consistent with this, we find that ZEA reduces the radical cations of ASTA and APO (see Table 2).

Based on these studies of pairs of carotenoids, we propose that the carotenoid radical cations can be placed in the following relative order in terms of reduction potential:



It should be noted that this is just an order of reduction potentials and in some cases the difference may be small, leading to only very slow reactions (e.g., ZEA/β-CAR pair).

Reactions of Carotenoids with the Vitamin E Radical Cation ($TOH^{\bullet+}$). In a previous paper,[7] we reported that seven carotenoids underwent analogous electron-transfer reactions leading to $CAR^{\bullet+}$ formation from the vitamin E radical (interpreted as $TO^{\bullet}$ but shown below to be $TOH^{\bullet+}$). Also, we suggested that this reaction did not occur with astaxanthin. We now confirm this result for astaxanthin and have also shown that TOH accelerates the decay of $ASTA^{\bullet+}$ so that the reverse process $ASTA^{\bullet+} + TOH \rightarrow ASTA + TOH^{\bullet+}$ is suggested. These studies were performed in hexane rather than in benzene as solvent due to the more prominent triplet absorptions in benzene which interfere at the wavelengths of interest.

Figure 4 shows the transient absorption spectrum at various times following pulse radiolysis of 1×10^{-2} M vitamin E in N_2O-flushed hexane. As can be seen, there are two peaks (420 and 460 nm maxima) but the kinetics associated with these absorption maxima are clearly quite different. The species with $\lambda_{max} = 420$ nm does not decay on the time scale of our experiments. However, the species at 460 nm decays with two lifetimes of about 250 ns and 6 μs. The shorter of these is not of interest in the present work. On the basis of previous work,[18−21] we interpret the species absorbing at 460 nm as the radical cation ($TOH^{\bullet+}$) and that at 420 nm as the neutral radical

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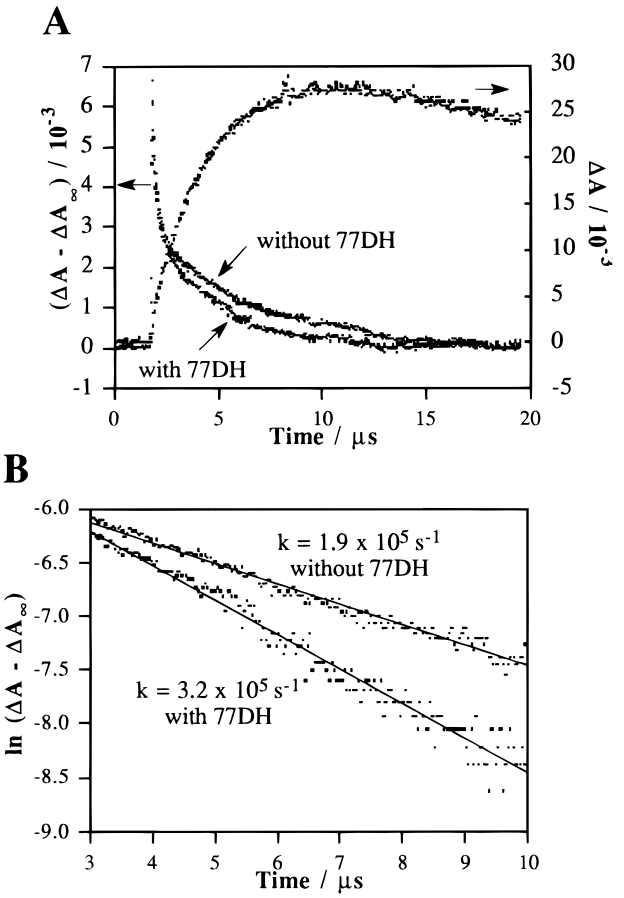

Figure 5. (A) Decay trace of $TOH^{\bullet+}$ at 460 nm with and without 77DH (1×10^{-5} M) and formation of $77DH^{\bullet+}$ at 830 nm in N_2O-flushed hexane. For the traces at 460 nm, the residual absorption ($A_\infty = 0.0021$ and 0.00124 for the traces with and without, respectively) has been subtracted out. (B) Plot of $\ln(\Delta A - \Delta A_\infty)$ for the decay of $TOH^{\bullet+}$ with and without 77DH.

($TO^{\bullet}$). Our results are consistent with the decay of the $TOH^{\bullet+}$ being, in part, due to deprotonation with a corresponding growth in the species absorbing at 420 nm.

On adding 77DH (10μM) the decay rate of the slow component of the species absorbing at 460 nm is markedly increased with a second-order rate constant of $\sim 10^{10}$ M^{-1} s^{-1}. (The ground-state absorption spectrum of this carotenoid allows us to monitor the $TOH^{\bullet+}$ at 460 nm, whereas this is not so for the other carotenoids.) Figure 5 compares the kinetics of the decay of the species at 460 nm with and without 77DH and shows the formation of the 77DH radical cation at 830 nm in the presence of 1×10^{-2} M vitamin E. Figure 5c shows that the rate of formation of the 77DH radical cation occurs at the same rate as the enhanced decay of the slow component at 460 nm. We interpret this as the electron-transfer process:

$$TOH^{\bullet+} + CAR \rightarrow TOH + CAR^{\bullet+} \qquad (6)$$

While we can monitor $TOH^{\bullet+}$ (at 460 nm), unfortunately, spectral overlap with the strongly absorbing carotenoid ground state prevents us from monitoring the effects of the carotenoid on $TO^{\bullet}$ (at 420 nm) by optical methods. However, the ESR work of Ingold and co-workers[22] has clearly shown that $TO^{\bullet}$ is unaffected by β-carotene.

We have also studied the interaction of APO with $TOH^{\bullet+}$ and found that this xanthophyll behaves like all the other carotenoids studied, except ASTA. This allows us to distinguish between ASTA and APO in the above scheme, such that $ASTA^{\bullet+}$ has the higher reduction potential. Also, it is interesting to note that the lifetime of $TOH^{\bullet+}$ in nonpolar environments is markedly longer than had been previously suggested.[19] It is also noteworthy[23] that $ASTA^{\bullet+}$, a carotenoid radical cation containing both carbonyl and hydroxyl groups, has a reduction potential that is higher than $E(TOH^{\bullet+}/TOH)$.

In conclusion, while there is no direct evidence for the production of carotenoid radical cations in vivo as a result of radical scavenging, the relative ease of oxidation of the carotenoids is worthy of future study and may well be a significant parameter in their dietary antioxidant role.

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