Carotenoids Enhance Vitamin E Antioxidant Efficiency

Fritz Böhm,[†] Ruth Edge,[‡] Edward J. Land,[§]
David J. McGarvey,[‡] and T. George Truscott[‡,*]

Department of Dermatology (Charité)
Humboldt University, 10117 Berlin, Germany
Department of Chemistry
Keele University, ST5 5BG, UK
CRC Department of Biophysical Chemistry
Paterson Institute for Cancer Research
Christie Hospital NHS Trust, Manchester, M20 9BX, UK

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The principal role of vitamin E (of which α-tocopherol is the major component) is that of a free radical scavenging antioxidant, and there is evidence that a high intake of carotenoids (particularly *β*-carotene) and α-tocopherol may prevent cancer[1] and other diseases. However, some trials indicate that *β*-carotene can increase the incidence of lung cancer amongst heavy smokers.[2] In addition, there is some evidence that other carotenoids may be more efficient antioxidants than *β*-carotene.[3,4]

We report here results of pulse radiolysis and laser flash photolysis studies of the reactions of the α-tocopheroxyl radical with a range of carotenoids and of the interaction of ascorbic acid with the carotenoid radical cations. The results of this work are used to support a proposed molecular mechanism for the antioxidant protection of human lymphoid cells from the $NO_2^{\bullet}$ radical, to be reported elsewhere.

Carotenoid and α-tocopherol radicals were generated in hexane by pulse radiolysis[5] using a 9−12 MeV Vickers linear accelerator with pulses of 20 ns duration and a dose of 0.3 nC. Solutions were nitrous oxide saturated (to capture the electron) and were irradiated in quartz flow-through cells with internal volumes of either 0.7 or 3 cm^3 and a monitoring optical path length of 2.5 cm. With a solution of α-tocopherol alone (10 mM) the solvent radicals react with the α-tocopherol producing a species with an absorption maximum at 420 nm assigned to the α-tocopheroxyl radical.[6] In methanol, carotenoid radical cations were generated using laser flash photolysis via electron transfer quenching ([CAR] = 10 μM) of the 1-nitronaphthalene triplet state, as described previously.[7]

For both carotenoid alone and carotenoid/α-tocopherol mixtures (100 μM α-tocopherol and 10 μM carotenoid) in hexane, the carotenoid radical cation formation was monitored in the near infrared (800−1100 nm).[8,9] The growths of the radical cations of all the carotenoids studied were observed at their respective absorption maxima, and the rate constants for these growths were much lower than in the absence of α-tocopherol where the $CAR^{\bullet+}$ is produced directly from the solvent radical cation. Typical traces are shown in Figure 1A for 7,7′-dihydro-*β*-carotene (77DH). These observations show that electron transfer occurs **from** carotenoids (CAR) **to** the α-tocopheroxyl radical ($TO^{\bullet}$).

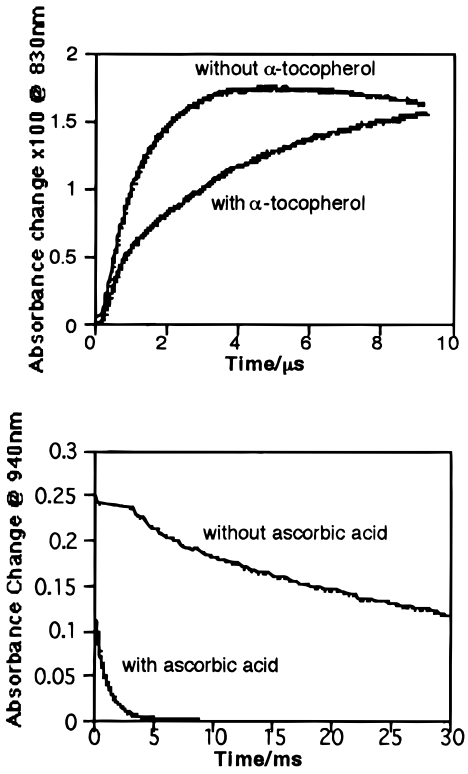

Figure 1. (A, top) Representative kinetics profile of the formation of $77DH^{\bullet+}$ following pulse radiolysis of 10 μM 77DH in the absence and presence of 100 μM α-tocopherol. (B, bottom) Representative kinetics profile of the decay of *β*-$CAR^{\bullet+}$ following pulse radiolysis of 100 μM *β*-CAR in the absence and presence of 100 μM ascorbic acid in aqueous Triton X 100, pH 7.

$$TO^{\bullet} + CAR \xrightarrow{H+} TOH + CAR^{\bullet+} \quad (1)$$

It has been proposed that α-tocopherol protects *β*-carotene.[10] However, except for astaxanthin, see below, our results show that the reverse of reaction 1 does not occur. Therefore α-tocopherol is unlikely to protect *β*-carotene by a repair mechanism.

The second-order rate constants (±10%) for reaction 1 were calculated from the pseudo-first-order rate constants of the formation of the carotenoid radical cations in the presence of α-tocopherol[11] using $k = k_{formation}/[CAR]$. All of the second-order rate constants of reaction 1 are close to diffusion controlled

* To whom correspondence should be addressed.
[†] Department of Dermatology (Charité).
[‡] Keele University.
[§] Christie Hospital NHS Trust.

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(8) The weakly absorbing α-tocopheroxyl radical could not be monitored in the mixture due to strong carotenoid ground state absorption in that region (e.g. *β*-carotene has $\epsilon_{420} = 90\,000\ M^{-1}\ cm^{-1}$).

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(11) The lifetime of $EO^{\bullet}$ in the absence of *β*-carotene is long compared to the lifetime in the presence of the carotenoid. Thus, the rate equation $k_{formation} = k_{decay} + k[CAR]$ can be simplified to $k_{formation} = k[CAR]$ (where $k_{formation}$ is the rate constant for the formation of $CAR^{\bullet+}$ and k_{decay} is the rate constant for the decay of $EO^{\bullet}$ other than by electron transfer from the carotenoid).

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(21×10^9 M^{-1} s^{-1} in hexane). The values in hexane, obtained at the λ_{max} of each carotenoid radical cation,[12] are (10^9 M^{-1} s^{-1}) 5.3, 8.8, 10, 13, 14, 18, and 26 for lutein, canthaxanthin, β-carotene, septapreno-β-carotene, lycopene, 7,7′-dihydro-β-carotene, and zeaxanthin, respectively.

Of course, the rate constants reported here are not measured in a biological environment, but the high values obtained do indicate the direction of electron transfer, from carotene to $TO^{\bullet}$, and, therefore, the repair of the α-tocopheroxyl radical by the carotenoids. Doubling the β-carotene concentration did not affect the amount of radical cation formed, but the rate of formation doubled indicating complete scavenging. Hence, all the carotenoids studied are capable of regenerating α-tocopherol from its radical. However, in a recent experiment, we have found that astaxanthin does not recycle α-tocopherol, but it may be recycled by α-tocopherol, and this will be the subject of further work.

Our investigations of the reactivity of ascorbic acid ($AscH_2$) with carotenoid radical cations, using pulse radiolysis and laser flash photolysis, have shown an enhanced $CAR^{\bullet+}$ decay rate in the presence of ascorbic acid (see Figure 1B), consistent with the following reactions in methanol (2) and water, Triton X 100 pH 7 (3):

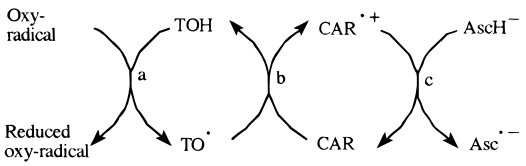
$$CAR^{\bullet+} + AscH_2 \rightarrow CAR + AscH^{\bullet} + H^{+} \qquad (2)$$

$$CAR^{\bullet+} + AscH^{-} \rightarrow CAR + Asc^{\bullet-} + H^{+} \qquad (3)$$

These reactions, for β-carotene, have second-order rate constants (±10%) of 9.4×10^6 M^{-1} s^{-1} in aqueous 2% Triton X 100 detergent micelles, pH 7, and 0.35×10^9 M^{-1} s^{-1} in methanol. The corresponding rate constants in methanol for the other carotenoids studied are (10^9 M^{-1} s^{-1}) 1.3, 1.1, 1.4, 0.91, and 7.7, for lutein, canthaxanthin, septapreno-β-carotene, 7,7′-dihydro-β-carotene, and zeaxanthin, respectively. These were calculated from a plot of concentration of ascorbic acid (0−100 μM) against the pseudo-first-order rate constants for the decay of $CAR^{\bullet+}$. Unfortunately, due to solubility problems we could not obtain a value for lycopene.

A synergistic effect we have observed in cell protection by β-carotene and vitamins E and C[13] may be related to the fact that β-carotene is not only quenching oxy-radicals but is also repairing the α-tocopheroxyl radical (b in Scheme 1), which is produced when α-tocopherol scavenges an oxy-radical (a in Scheme 1). Such a synergistic mechanism requires that the $CAR^{\bullet+}$ is reconverted to CAR (c in Scheme 1). Of course, in hexane the β-carotene and α-tocopherol simply compete in scavenging any radicals present. However, a synergistic effect

Scheme 1



may reflect differing cellular locations of β-carotene and α-tocopherol. It is generally accepted that β-carotene is more lipophilic than α-tocopherol[14] and will be more likely to be in the interior of a membrane. However, the β-carotene radical ($CAR^{\bullet+}$) is a charged species, while the α-tocopherol radical ($TO^{\bullet}$) is uncharged. Thus, the $CAR^{\bullet+}$ may reorientate so that the charge is near the polar interface of the cell membrane and hence be more accessible to aqueous phase antioxidants such as ubiquitous ascorbic acid. Indeed, the disscussion of Pryor *et al.*[15] also suggests the role of carotenoids as antioxidants depends on their environment. We are currently using time-resolved resonance Raman spectroscopy of carotenoid radical ions to investigate such effects.[7]

Our findings are in agreement with Palozza and Krinsky[10] who showed that β-carotene and α-tocopherol can act synergistically in the membrane system, rat liver microsomes; however, we differ in that our findings show that α-tocopherol protects β-carotene and not vice versa. Willson[16] proposed that α-tocopherol and β-carotene have similar electron donor abilities, while Tappel[17] suggests β-carotene is the weaker antioxidant. Our results show clearly that most carotenoids (including β-carotene) are better electron donors than α-tocopherol. Furthermore, this scheme allows us to suggest that the low levels of antioxidants, such as ascorbic acid, in smokers compared to nonsmokers, may be related to the apparent failure of β-carotene to offer any benefit to this group, as reported recently.[2]

In conclusion, we show that carotenoids could substantially enhance vitamin E/C protection, and our results suggest a molecular mechanism (Scheme 1) for this effect.

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