Communications to the Editor

Does β-Carotene Really Protect Vitamin E from Oxidation?

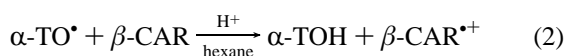Luca Valgimigli,[*,†] M. Lucarini,[†] G. F. Pedulli,[†] and K. U. Ingold[*,‡]

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Many *in vitro* studies have demonstrated that vitamin E (mainly α-tocopherol, α-TOH) and provitamin A (β-carotene, β-CAR, and other carotenoids) can act as free-radical trapping antioxidants under appropriate conditions (homogeneous,[1] heterogeneous,[2] radical flux,[3] oxygen partial pressure,[4] etc). These two classes of compounds are therefore presumed to function as antioxidants *in vivo* even when (as with carotenoids) this is clearly not their prime role. Attention has also focused on interactions between antioxidants, such as the "regeneration" of α-TOH from its radical, α-TO•, by ascorbate, AH−, reaction 1, under biomimetic (heterogeneous) conditions.[2,3,5] In this

$$\alpha\text{-TO}^\bullet + \text{AH}^- \rightarrow \alpha\text{-TOH} + \text{A}^{\bullet-} \qquad (1)$$

Journal it was recently claimed[6] that α-TOH could also be regenerated from α-TO• by β-CAR (and other carotenoids), reaction 2, at close to the diffusion-controlled limit ($k_2 = 1.0$

$$\alpha\text{-TO}^\bullet + \beta\text{-CAR} \xrightarrow[\text{hexane}]{\text{H}^+} \alpha\text{-TOH} + \beta\text{-CAR}^{\bullet+} \qquad (2)$$

$\times 10^{10}$ M^{-1} s^{-1}). This claim was based on pulse radiolysis of hexane solutions of α-TOH and β-CAR with time resolved monitoring of transients attributed to β-CAR•+ in the near infrared. We decided to check whether reaction 2 occurred at all (let alone at the diffusion-controlled rate) for three reasons: (i) there is a current high level of interest in oxidative stress and biological antioxidants,[7] (ii) mechanistic conclusions should not be based solely on the optical monitoring of transient intermediates, and (iii) it has already been demonstrated that β-CAR can be protected against autoxidation by α-TOH[8] (rather than the reverse). We chose EPR spectroscopy because this allows α-TO• to be identified and monitored in an unambiguous manner.

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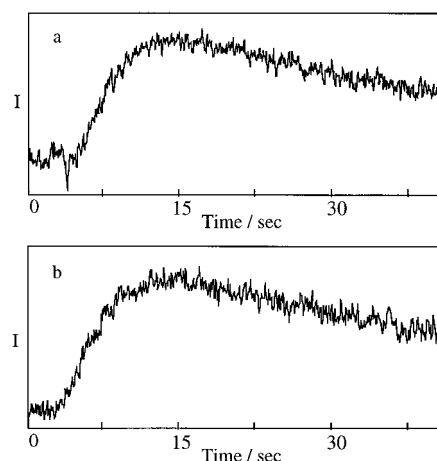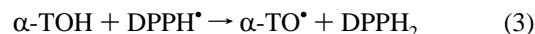

Figure 1. Time evolution of α-TO• generated in anisole/BOOB (9:1, v/v) from α-TOH in the absence (a) and in the presence (b) of 1.0 mM β-CAR after injection of DPPH•. EPR signal intensities (I) are reported on the same scale.

In the first set of experiments,[9] α-TO• was generated within the cavity of an EPR spectrometer by reaction of α-TOH (1.35 mM) with the 1,1-diphenyl-2-picrylhydrazyl radical, DPPH• (1.3 $\times$ 10^{-5} M) at 25 °C, reaction 3.[10,11] In hexane and in benzene reaction 3 was too fast to be monitored, but the maximum

$$\alpha\text{-TOH} + \text{DPPH}^\bullet \rightarrow \alpha\text{-TO}^\bullet + \text{DPPH}_2 \qquad (3)$$

concentrations of α-TO• (ca. 1.2 $\times$ 10^{-5} M), and the decay traces were the same with or without 0.5 or 1 mM β-CAR. The solvent was changed to anisole to reduce the rate of reaction 3,[11] and in an otherwise identical matched pair of experiments the rates of growth of α-TO•,[12] the maximum concentration of α-TO• (ca. 1.0 $\times$ 10^{-5} M), and the concentration/time curves were identical with or without 1 mM β-CAR (see Figure 1).

In the second set of experiments,[13] α-TO• was generated within the cavity of an EPR spectrometer by reaction of α-TOH (1.3 mM) with photochemically generated *tert*-butoxyl radicals, BO•, at 25 °C in hexane/di-*tert*-butyl peroxide (BOOB) (9:1, v/v) and in benzene/BOOB (9:1, v/v). On cutting off the light, the α-TO• EPR signal decayed, reaction 5, with second-order

(9) Solutions containing 1.35 mM α-TOH, without β-CAR or with 0.5 mM β-CAR in hexane or 1 mM β-CAR in benzene or anisole, in quartz EPR tubes within the EPR cavity were held at 25 °C and stirred by a fine stream of nitrogen bubbles. An aliquot of a concentrated stock solution of DPPH in the same solvent was quickly (ca. 0.1 s) injected to give in solution [DPPH] = 1.3 $\times$ 10^{-5} M (mixing time 0.5−1 s). The intensity of a clear EPR line of α-TO• (i.e., unblocked by DPPH•) was monitored continuously with the spectrometer locked at the appropriate (predetermined) field-frequency value. There was no reaction between DPPH• and β-CAR.
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(12) In both cases, $k_3 = 2 \times 10^3$ M^{-1} s^{-1} in good agreement with the value of 1.4 $\times$ 10^3 M^{-1} s^{-1} at 25 $\pm$ 2 °C obtained previously in anisole.[11]
(13) Solutions containing 1.3 mM α-TOH in hexane/BOOB (9:1, v/v), benzene/BOOB (9:1, v/v) or CH$_3$CN / BOOB (9:1, v/v) in quartz EPR tubes within the EPR cavity were held at 25 °C and stirred with N$_2$. Irradiation with the unfiltered light from a 500 W high-pressure mercury lamp was continued until the α-TO• signal reached steady state (ca. 15−20 s). The light was cut-off with a mechanical shutter, and the α-TO• decay was monitored. The experiment was then repeated with an injection of a concentrated stock solution of β-CAR or ArOH being made a few seconds after shuttering the light.
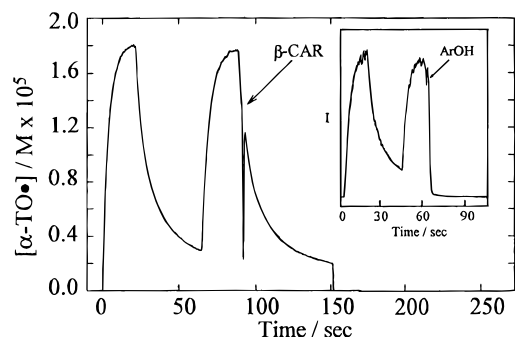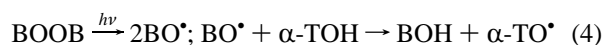
Figure 2. Second-order decay of photolytically generated α-TO$^\bullet$ in benzene/BOOB (9:1, v/v) in the absence and presence of 1.0 mM β-CAR (main) and of 0.25 M ArOH (inset). The EPR signal in the main plot has been converted to absolute radical concentrations after calibration of the spectrometer response.

kinetics, $2k_{obs} = 6.6 \times 10^3$ M^{-1} s^{-1}, in excellent agreement with earlier work[14] using the same sample of α-TOH. (A minor

$$\text{BOOB} \xrightarrow{h\nu} 2\text{BO}^\bullet; \text{BO}^\bullet + \alpha\text{-TOH} \rightarrow \text{BOH} + \alpha\text{-TO}^\bullet \quad (4)$$

$$\alpha\text{-TO}^\bullet + \alpha\text{-TO}^\bullet \xrightarrow{k_{obs}} \text{nonradical products} \quad (5)$$

impurity present in all α-TOH samples plays a major role in determining the rate of reaction 5.[10]) Injection of a concentrated solution of β-CAR in the same solvent mixture (to give [β-CAR] = 0.5 or 1 mM) immediately after shutting off the light did not change the α-TO$^\bullet$ decay rate (see, e.g., Figure 2). Since electron transfer reactions (putative reaction 2) are favored in more polar solvents, the same experiment was performed in acetonitrile/BOOB (9:1, v/v). Once again, the injection of β-CAR to a final concentration of ca. 1 mM did not change the rate of decay of α-TO$^\bullet$ (see Supporting Information). Finally, and to validate this method for detecting accelerated α-TO$^\bullet$ decay, a concentrated solution of 2,6-di-*tert*-butyl-4-methylphenol (ArOH, final concentration 0.25 M) was injected immediately after cutting off the light. This produced an "instantaneous" (<1 s) loss of the α-TO$^\bullet$ signal (see insert in Figure 2), reaction 6. From

$$\alpha\text{-TO}^\bullet + \text{ArOH} \rightarrow \alpha\text{-TOH} + \text{ArO}^\bullet \quad (6)$$

literature data,[15] k_6 can be estimated to be $\leq 5 \times 10^3$ M^{-1} s^{-1}, corresponding to a pseudo-first-order decay rate constant for α-TO$^\bullet \leq 1.3 \times 10^3$ s^{-1}. This value is very much lower than the calculated pseudo-first-order rate constant of 10^7 s^{-1} which would be produced by 10^{-3} M β-CAR if reaction 2 occurred with its proposed rate constant of 1×10^{10} M^{-1} s^{-1}.[6]

We conclude that β-CAR, and in all probability other carotenoids, do not regenerate α-TOH from α-TO$^\bullet$ and that reaction 2 does not occur. The mechanism by which the transients observed by Truscott and co-workers[6] were formed remains to be elucidated. Our work points up the "danger" of drawing mechanistic conclusions based solely on the UV, visible, or near IR spectra of transients.

The procedures we employed to disprove reaction 2 rely on the fact that α-TO$^\bullet$ is persistent. They cannot, therefore, be applied to check a somewhat more reasonable claim by Mortensen and Skibsted[17] that the transient and much more reactive phenoxyl radical reacts with β-CAR, though the reported rate constants appear to us to be improbably large.[18]

Addendum (by Truscott, T. G.; Edge, R.; Land, E. J.; McGarvey, D. J.; Dodd, N. Department of Chemistry, Keele University, Keele ST5 5BG, U.K.): We have confirmed the EPR results reported above which demonstrated that the rate of decay of α-TO$^\bullet$ in hexane and in benzene is not influenced by β-CAR. However, pulse radiolysis (PR) of α-TOH + β-CAR in both these solvents indicates that β-CAR$^{\bullet+}$ is formed very rapidly. Some possible explanations for the PR observations (e.g., that α-TOH$^{\bullet+}$ rather than α-TO$^\bullet$ is involved) will be explored, and the results will be reported in a full paper.

Supporting Information Available: A detailed experimental section and kinetic traces for the decay of α-TO$^\bullet$ in hexane and in acetonitrile in the presence and absence of β-CAR (4 pages). See any current masthead page for ordering and Internet access instructions.

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(15) For the α-TO$^\bullet$ reaction with a "look alike" α-TOH dimer, $k = 2.5 \times 10^5$ M^{-1} s^{-1} at 37 °C (after statistical correction for the two OH groups in the dimer).[10] This value was decreased by a factor of 50 to allow for the ca. 2.3 kcal/mol endothermicity of reaction 6,[14,16] but the resulting 5×10^3 M^{-1} s^{-1} will still probably be an upper limit because of steric protection of the phenolic H-atom in ArOH.

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(18) Two reaction channels were reported[17] with ca. 10^{-5} M β-CAR: (i) PhO$^\bullet$ + β-CAR → adduct, $k = (1-1.5) \times 10^4$ s$^{-1}/10^{-5}$ M = $(1-1.5) \times 10^9$ M^{-1} s^{-1} and (ii) PhO$^\bullet$ + β-CAR → PhO$^-$ + β-CAR$^{\bullet+}$, $k = (2-3) \times 10^4$ s$^{-1}/10^{-5}$ M = $(2-3) \times 10^9$ M^{-1} s^{-1} in 1.75 M PhOH in BOOB/c-C$_6$H$_{12}$ (70:30, v/v) at 20 °C.