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CAROTENE–OXYGEN RADICAL INTERACTIONS

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All-trans β-carotene radical anion efficiently transfers an electron to oxygen but the reverse reaction is not observed and, instead we suggest the formation of a β-carotene-superoxide radical addition complex. On the other hand, all-trans lycopene undergoes a reversible electron transfer with the superoxide radical. This distinctive behaviour may be related to the anti-cancer properties of these molecules.

KEY WORDS: Carotene, lycopene, oxy-radical, superoxide.

INTRODUCTION

There is epidemiological evidence that the incidence of cancer may be lower for individuals with high intake of all-trans β-carotene[1] and there are current trials in progress to further test this possibility.

It is well known that carotenoids are effective quenchers of singlet oxygen ($^1O_2$) and also of the $^1O_2$ precursors, such as porphyrin and chlorin triplet states.[2–5] These quenching reactions are pivotal in the role of all-trans β-carotene (hereafter known as β-carotene) in ameliorating the skin photosensitivity associated with the hereditary disease known as erythropoietic protoporphyria and in protecting the photosynthetic apparatus from photosensitised damage.

The role of β-carotene in the prevention of cancer (in the absence of light) is less likely to involve singlet oxygen but may be related to its ability to trap activated oxy-species[6–8] which otherwise may cause DNA damage within cells leading to carcinogenesis.[9] Thus, Burton and Ingold[10] used indirect evidence to show that β-carotene interacts with peroxyl radicals arising from lipid peroxidation. We now present, for the first time, evidence for an interaction between both β-carotene and its open chain analogue, lycopene with superoxide, $O_2^{\cdot-}$, and report studies of the reverse reaction for lycopene (for structures of carotenoids investigated see Figure 1).

MATERIALS AND METHODS

The hexane used was obtained from Aldrich as spectroscopic quality, water was

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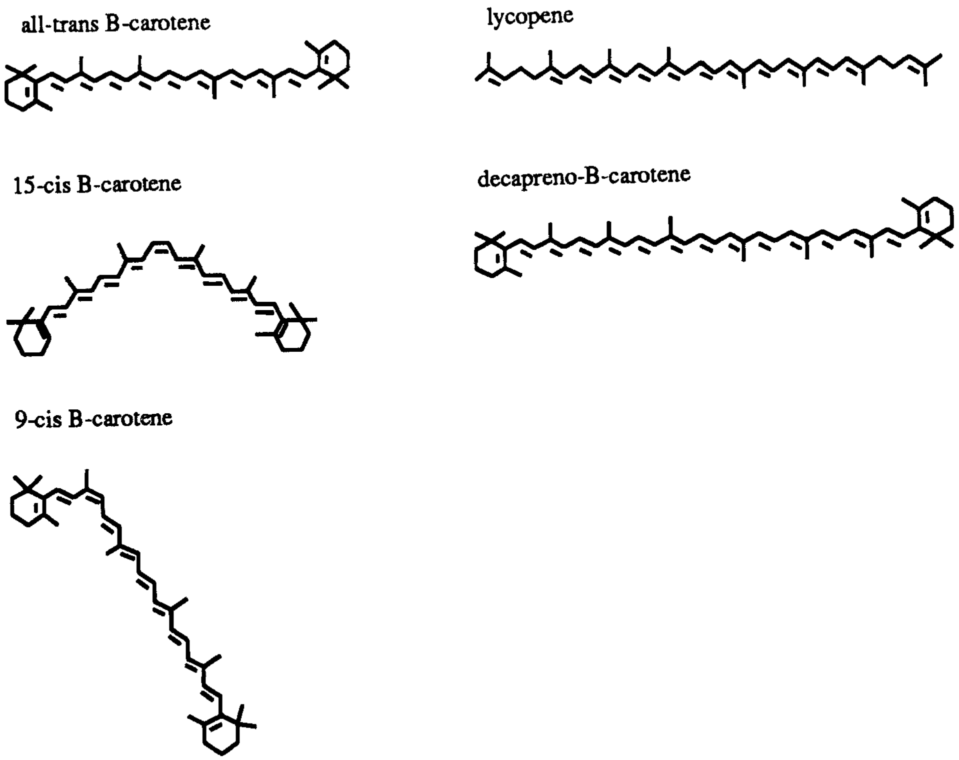

FIGURE 1 Carotenoid structures.

re-distilled from alkaline permanganate. Triton X-100 was obtained from Fluka and all carotenoids from Hoffmann-La Roche, these were used as supplied.

The pulse radiolysis experiments were performed with a 9–12 MeV Vickers linear accelerator, as previously described,[11,12] using 50–200 ns pulses. Solutions were studied using quartz flow-through cells, optical pathlength 2.5 cm, of either 0.7 or 3 cm^3 internal volume.

Pulse radiolysis generates high concentrations of relatively long lived radicals in polar solvents such as water. In oxygen-saturated aqueous sodium formate solutions, all the primary radicals are converted to $O_2^{\cdot -}$ as follows:

$$O_2 + e_{aq}^- \rightarrow O_2^{\cdot -},$$

$$OH^{\cdot} + HCO_2^- \rightarrow H_2O + CO_2^{\cdot -}$$

$$H^{\cdot} + HCO_2^- \rightarrow H_2 + CO_2^{\cdot -}$$

$$CO_2^{\cdot -} + O_2 \rightarrow O_2^{\cdot -} + CO_2$$

$$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot} \quad (pK_a = 4.7),$$

while for the interaction of $O_2^{\cdot -}$ with the carotenoids we used an aqueous system. In the

$$S + e_{aq}^- \rightarrow S^{\cdot -}$$

Thus we are able to generate $O_2^{\cdot -}$ within a microsecond and monitor its interaction

TABLE I
Second-order rate constants $(k)^a$ for the reaction of radical anions with oxygen in hexane

Carotenoid	n^{++}	$k/10^8\,M^{-1}\,s^{-1}$
β-Carotene	11	25 ± 5
15-cis-β-Carotene	11	20 ± 5
9-cis-β-Carotene	11	20 ± 5
Lycopene[b]	11	2 ± 1
Decapreno-β-Carotene	15	1 ± 0.5

n^{++} = number of conjugated double bonds.

[a] Second-order rate constants were calculated from measurements of the pseudo first-order rate constants for the decay of radical anions over a range of oxygen concentrations.

[b] Lycopene has two additional, non-conjugated double bonds.

with carotenoids, and also to generate carotenoid radical anions and estimate the rate constants for their reaction with oxygen using nitrogen/oxygen mixtures ranging from 0.4% to 2.4% oxygen. In the experiments reported we used two solvent systems. The majority of the carotenoid radical interactions with oxygen were studied in hexane, while for the interaction of $O_2^{\overline{\cdot}}$ with the carotenoids we used an aqueous system. In the latter system the carotenoids were solubilised using the neutral detergent Triton X-100 as described previously.[13] The $O_2^{\overline{\cdot}}$ was then generated in the aqueous phase with the concentrations of the carotenoids and Triton X-100 chosen such that double occupancy of a micelle was unimportant (< 10%). In this work the macroscopic pH was 7.0 so that less than 1% of the $O_2^{\overline{\cdot}}$ radicals would be protonated.

RESULTS AND DISCUSSION

Carotenoid Radical Anions and their Interaction with Oxygen

We have previously characterised the radical anions (and cations) of a wide range of carotenoids[14] in hexane as solvent. In such hydrocarbon solvents the yield of radicals observed is rather low, but the molar absorption coefficients of the radicals of the carotenoids are so high ($> 10^5\,M^{-1}\,cm^{-1}$) that it is quite easy to monitor such species. In general, the radicals absorb well to the red of the parent molecule, e.g., for all-trans β-carotene the wavelength maxima are at 450 nm, 880 nm and 1040 nm for the parent, radical anion and radical cation, respectively. One aspect of the present study is the interaction of the radical anions with molecular oxygen. Table I gives the second-order rate constants obtained for such interactions over a range of oxygen concentrations from $6.2 \times 10^{-4}\,M$ to $3.7 \times 10^{-3}\,M$. These reactions are almost certainly due to electron transfer processes of the type:

$$C^{\overline{\cdot}} + O_2 \rightarrow C + O_2^{\overline{\cdot}}$$

with the carotenoid radical anions ($C^{\overline{\cdot}}$) being converted back to the parent molecule, which is consistent with the lack of new absorption bands.

As can be seen, the electron transfer rate constants listed for the isomers of β-carotene are of the order of $10^9\,M^{-1}\,s^{-1}$ whilst those for lycopene and decapreno-β-carotene are an order of magnitude less.

The explanation of the low rate constants for lycopene and decapreno-β-carotene

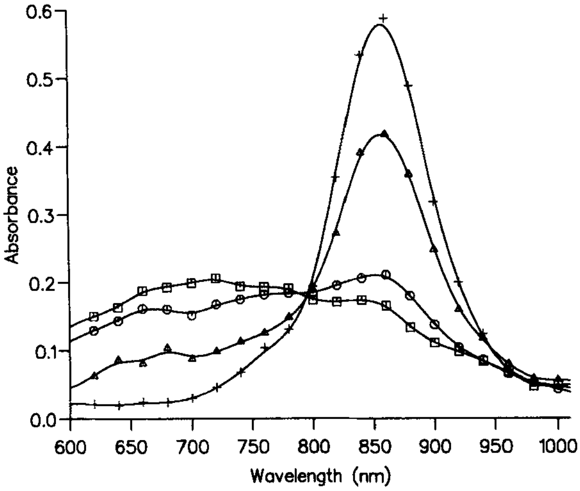

FIGURE 2 Absorption changes due to the formation of β-carotene radical anion from the hydrated electron. +, 14 μs; Δ, 4.2 μs; 0, 800 ns; □, 160ns after pulse. [β-carotene] = 1 × 10^{-4} M in argon flushed water containing 2% Triton X-100 and 10^{-2} M sodium formate. Dose ~4 Gy, absorption normalised to 10 Gy.

is not clear. They both contain additional double bonds compared to β-carotene, decapreno-β-carotene four additional conjugated carbon–carbon double bonds and lycopene two additional carbon–carbon double bonds. These additional double bonds appear to stabilise the carotenoid radical anions and reduce the tendency to release an electron to oxygen. This presumably reflects differences in 1-electron reduction potential which may be somewhat surprising for lycopene where the additional double bonds are not part of the conjugated system.

These results contrast our recent findings[2] that the quenching of singlet oxygen ($^1O_2$) by carotenoids to yield the carotenoid triplet state (^3C), $^1O_2 + C \rightarrow O_2 + {}^3C$, is faster for those carotenoids with additional carbon–carbon double bonds. For example, the rate constant for decapreno-β-carotene was higher than that for β-carotene (2.0×10^{10} and $1.3 \times 10^{10}\,M^{-1}s^{-1}$ respectively in benzene).

Superoxide Radical Anion Interaction with β-Carotene

Many diseases, including cancer, may be related, at least in part, to the formation of activated forms of oxygen. The species inducing such oxidative stress may include $^1O_2$, $O_2^{\cdot-}$ (and its conjugate acid $HO_2^{\cdot}$), the hydroxyl radical $OH^{\cdot}$ and peroxyl radicals as well as the various non-radical species such as H_2O_2. In the present study we have generated $O_2^{\cdot-}$ and monitored the time-resolved spectral changes consequent upon its interaction with spore β-carotene. Figure 2 shows typical time resolved spectra of β-carotene in argon saturated Triton X-100. As can be seen there is a broad structureless absorption with a peak at around 700 nm which decreases with time — this is due to the hydrated electron. A much stronger band grows in as the electron decays. The wavelength maximum of this band is near 860 nm, which is quite close to that of the radical anion of β-carotene in hexane[14] and is assigned to this radical in Triton X-100.

Figure 3 shows some typical transient absorption changes for β-carotene in oxygen

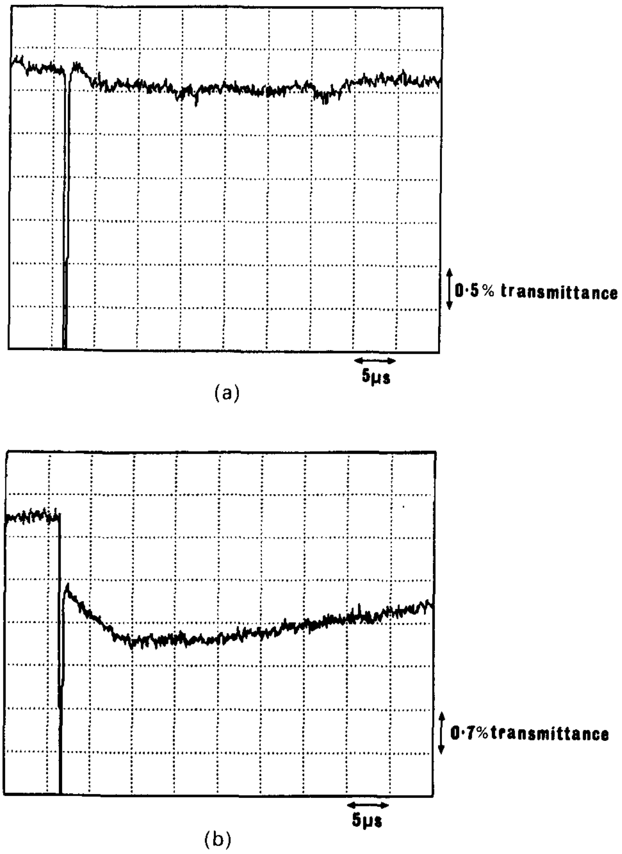

FIGURE 3 Typical changes in transmittance with time for β-carotene (concentration = 1×10^{-4} M) in oxygen saturated water containing 2% Triton X-100 and 10^{-1} M sodium formate, Dose ~ 25 Gy. (a) 700 nm; (b) 960 nm.

saturated Triton X-100 solutions. As can be seen in Figure 3a a short lived absorption predominates at 700 nm, the spectrum of this species corresponds to that of the solvated electron. Figure 3b (at 960 nm) shows both this electron and a slower growing new species which itself decays over tens of microseconds.

Figure 4 shows the absorption spectrum of this new species. As can be seen the wavelength maximum is near 950 nm that is, about 90 nm red shifted from that of the radical anion in Triton X-100. Thus the radical anion of β-carotene is not being produced via an electron transfer from $O_2^{\cdot -}$. Also, the spectrum produced in the oxygenated solutions is considerably less sharp than that of the radical anion in argon flushed solution. We postulate, following the work of Burton and Ingold[10] on the interaction of β-carotene with peroxyl radicals, that $O_2^{\cdot -}$ forms an addition complex with the β-carotene. It seems reasonable that the $O_2^{\cdot -}$ can be located at more than one region of the conjugated chain leading to several overlapping spectra and hence rather a broad overall spectrum. It is just conceivable that the apparent reaction of $O_2^{\cdot -}$ with β-carotene proceeds via its protonated form, $HO_2^{\cdot}$.

Unfortunately, it is much more difficult to solubilise lycopene (or decapreno-

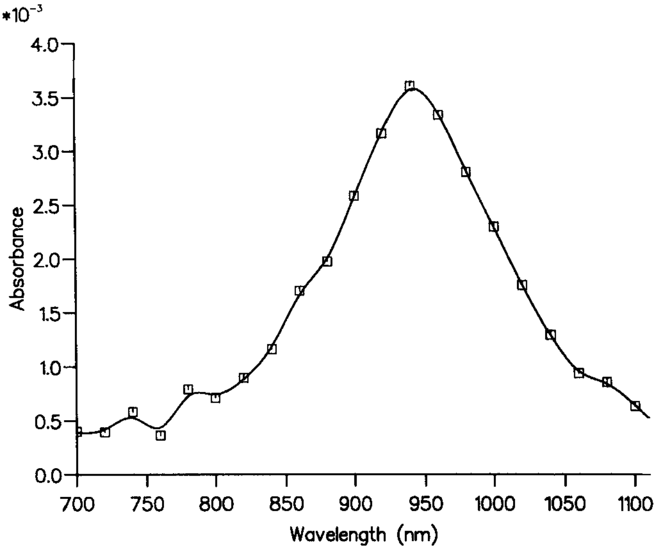

FIGURE 4 Absorption spectrum of proposed O_2^{-}-β-carotene addition product obtained 22 μs after pulse radiolysis of the solutions described in the legend to Figure 3. Dose ~25 Gy, absorption normalised to 10 Gy.

β-carotene) than β-carotene in Triton X-100. However, we have studied lycopene in oxygen saturated hexane. In this environment there is a growth of species whose absorption maximum (Figure 5) is at the same wavelength as that of the lycopene radical anion (950 nm). Linking this with the much less efficient quenching of the radical anion of lycopene by oxygen, compared to β-carotene, leads to our main conclusion. This is that the reduction potential of β-carotene is such that the equilibrium with respect to oxygen is strongly in favour of O_2^{-} whereas this is not so for lycopene (nor decapreno-β-carotene). Thus, in hexane, the only reaction we detected for β-carotene radical anion is an electron transfer to oxygen to produce O_2^{-}. For lycopene, this reaction is much less efficient, and electron transfer can be detected in both directions. The major consequence is that for β-carotene the inefficient electron transfer from O_2^{-} to β-carotene in Triton X-100 allows an alternative reaction to compete, namely an addition between O_2^{-} and β-carotene. Consistent with this interpretation we have shown in hexane that β-carotene readily transfers an electron to lycopene ($k \sim 4 \times 10^{10} M^{-1} s^{-1}$) whereas lycopene^{-} does not transfer an electron to β-carotene ($k \leqslant 10^{9} M^{-1} s^{-1}$).

Overall, our major conclusions are that while for all carotenoids studied to date there are only minor variations in their rate of quenching of singlet oxygen, the same is not true with respect to radical reactions involving O_2 where we have demonstrated that the reaction pathways themselves vary allowing both electron transfer and addition complexes to arise in some cases. Thus, it is to be expected that different carotenoids (arising for example from different diets/dietary supplements) will vary significantly in potential beneficial effects with respect to radical quenching and hence to disease prevention.

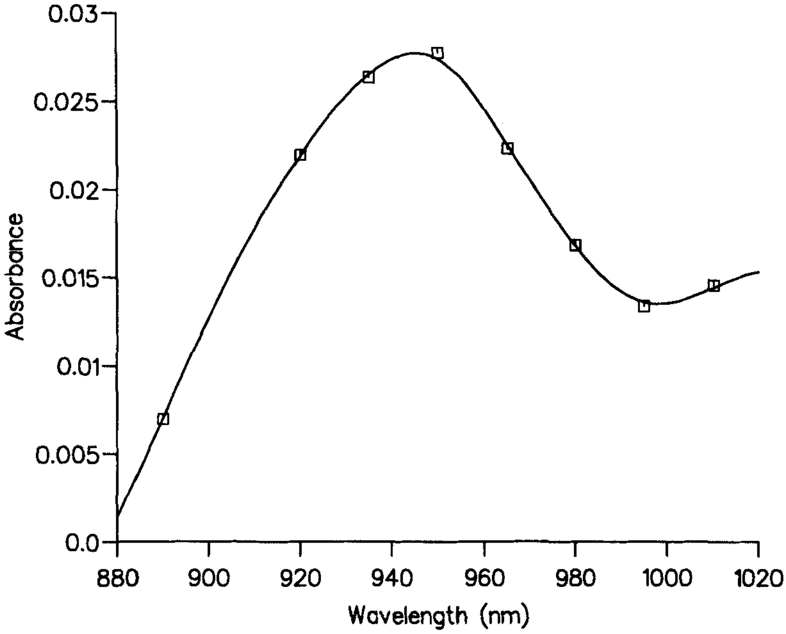

FIGURE 5 Absorption spectrum due predominantly to lycopene radical anion generated from $O_2^{\cdot-}$ using oxygen-saturated hexane. The solution contained 0.5×10^{-4} M lycopene. Dose ~4 Gy, absorption normalised to 10 Gy.

Acknowledgements

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