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Review

Interactions of dietary carotenoids with singlet oxygen ($^1O_2$) and free radicals: potential effects for human health

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The dietary carotenoids provide photoprotection to photosynthetic organisms, the eye and the skin. The protection mechanisms involve both quenching of singlet oxygen and of damaging free radicals. The mechanisms for singlet oxygen quenching and protection against free radicals are quite different — indeed, under some conditions, quenching of free radicals can lead to a switch from a beneficial anti-oxidant process to damaging pro-oxidative situation. Furthermore, while skin protection involves β-carotene or lycopene from a tomato-rich diet, protection of the macula involves the hydroxyl-carotenoids (xanthophylls) zeaxanthin and lutein. Time resolved studies of singlet oxygen and free radicals and their interaction with carotenoids *via* pulsed laser and fast electron spectroscopy (pulse radiolysis) and the possible involvement of amino acids are discussed and used to (1) speculate on the anti- and pro-oxidative mechanisms, (2) determine the most efficient singlet oxygen quencher and (3) demonstrate the benefits to photoprotection of the eye from the xanthophylls rather than from hydrocarbon carotenoids such as β-carotene.

Key words: dietary carotenoids; free radicals; singlet oxygen; redox potentials

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INTRODUCTION

Reactive Oxy-Species (ROS) comprise singlet oxygen (SO) and a range of oxidising free radicals. The interaction of carotenoids with such species is important for the understanding of many important aspects of life such as photosynthesis, vision, various medical treatments from dermatology to cancer, as well as understanding possible deleterious reactions affecting man and also for commercial reasons, such as, investigations into the stability of carotenoids used as food colourants.

Ground state molecular oxygen has a spin multiplicity of 3 (i.e. it is in a triplet state, $^3\sum_{g-}$) with its two unpaired electrons in the degenerate pair of π^* orbitals. The two lowest electronic excited states of oxygen are singlet states ($^1\Delta_g$ and $^1\sum_{g+}$) with the $^1\Delta_g$ state being the lower lying and as such it is commonly referred to as singlet oxygen. The SO quenching by carotenoids is mainly dependent on the triplet energy level of the carotenoid and is extremely efficient (typically $\approx 10^{10}$ M^{-1} s^{-1}) for most carotenoids with 11 conjugated double bonds, at least in simple solvents (Edge *et al.,* 1997*)*. However, for lutein with only 10 conjugated double bonds, there is some reduction in SO quenching efficiency suggesting its main role in protecting the eye is related to free radical quenching. For lycopene, the red pigment in tomatoes, all 11 conjugated double bonds are involved in the electron delocalisation whereas for all other dietary carotenoids the terminal rings may lead to a loss of planarity of the two terminal double bonds and a consequent small increase in triplet energy level compared to lycopene. The consequence being that lycopene is the most efficient SO quencher. However, this is a minor effect compared to the overall very efficient SO quenching by all dietary carotenoids. However, carotenoids can often aggregate in various ways and this leads to a marked loss of efficiency in quenching SO. Such aggregation/stacking may well arise for oxy-containing carotenoids (xanthophylls) and explain the lack of SO quenching by zeaxanthin and lutein observed at higher concentration in lipid membranes. This may be of importance in the use of such supplements to protect the macula of the retina.

A possibly surprising observation is that for heterogeneous environments such as lipid membranes the site of generation of the SO is not important with respect to the quenching efficiency by the carotenoid. That is, water soluble and lipid soluble sensitisers of SO lead to similar efficiency of SO quenching by dietary carotenoids. (Cantrell *et al.,* 2003)

Overall, because the SO quenching is quite well understood, (Edge & Truscott, 2010 and references therein) this review mainly concerns the more complex reactivity of oxy-radicals with the dietary carotenoids.

CAROTENOID RADICAL CATIONS

These are the most studied carotenoid radicals. The reduction potentials of carotenoid radical cations, E_0 (CAR^+/CAR) are important to understand the role of carotenoids in free radical quenching processes. We obtained relative potentials using pulse radiolysis of N_2O saturated benzene solutions which contained two dietary carotenoids in a ratio of at least 10:1. This allowed the carotenoid present in the larger amount to react preferentially with benzene radical cation formed during the pulse, producing the radical cation of this carotenoid. We could then determine the rate of reaction of this radical cation with the second carotenoid, i.e.

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Abbreviations: $AscH_2$, ascorbic acid; NN, 1-nitronaphthalene; ROS, reactive oxy-species; SO, singlet oxygen.

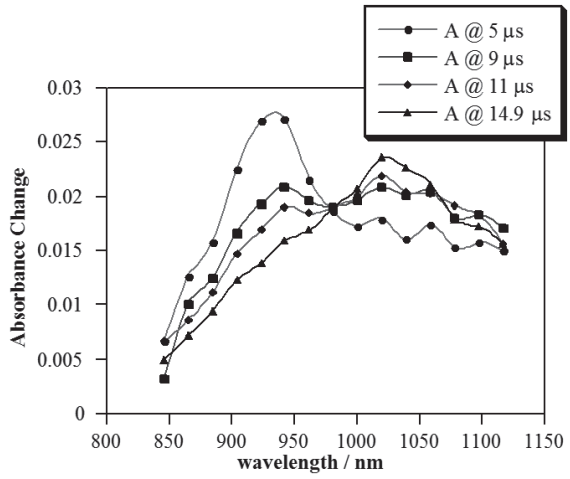


Figure 1. Spectra obtained on pulsing 1×10^{-4} M astaxanthin with 1×10^{-5} M lycopene in argon flushed benzene, which show a decreasing absorbance of astaxanthin radical cation matching an increasing absorbance of lycopene radical cation.

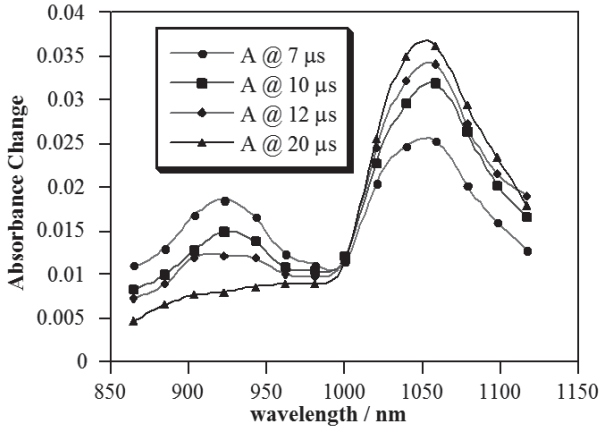


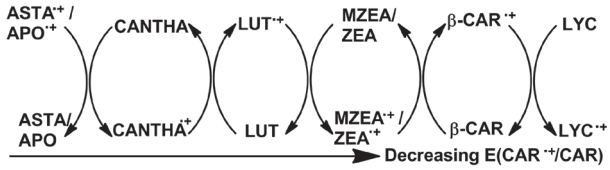
Figure 2. Spectra obtained on pulsing 1×10^{-4} M canthaxanthin with 1×10^{-5} M β-carotene in argon flushed benzene, which show a decreasing absorbance of canthaxanthin radical cation matching an increasing absorbance of β-carotene radical cation.

$$\text{Carotenoid1}^{\bullet+} + \text{carotenoid2} \rightarrow \text{carotenoid1} + \text{carotenoid2}^{\bullet+}$$

The following results (Edge *et al.,* 1998) are typical.

Figure 1 shows the changes in spectra with time on pulse radiolysis of 1×10^{-4} M astaxanthin in the presence of 1×10^{-5} M lycopene and Fig. 2 shows similar data for the canthaxanthin /β-carotene pair.

These figures illustrate that positive charge transfer occurs from astaxanthin$^{\bullet+}$ to lycopene and from canthaxanthin$^{\bullet+}$ to β-carotene, i.e. electron transfer from lycopene to astaxanthin$^{\bullet+}$ and from β-carotene to canthaxanthin$^{\bullet+}$. Similar data allowed rate constants to be determined for many such pairs and results in the Scheme 1.



Scheme 1.

Table 1. The reduction potentials of some dietary carotenoids in Triton X micelles.

Carotenoid$^{\bullet+}$	E_0 (CAR$^{\bullet+}$/CAR)/mV
β-Carotene	1060
Canthaxanthin	1041
Zeaxanthin	1031
Astaxanthin	1030
Lycopene	980

In general, such kinetic studies show that lycopene efficiently quenches (reduces) the radical cations of all the xanthophylls studied, β-carotene only reduces the radical cations of astaxanthin, canthaxanthin and β-apo-8'-carotenal, i.e. the xanthophylls containing carbonyl groups.

The absolute values of the reduction potentials of the carotenoid radical cations were obtained by establishing equilibria with substrates of known redox potential, such as tryptophan radical (Edge *et al.*, 1998). Determination of the equilibrium constant, together with use of the Nernst equation leads to the data shown in Table 1.

These, together with the rather long inherent lifetimes we have obtained for such radical cations (not discussed in this review, but see Burke *et al.,* 2001) are sufficiently oxidising to damage biosubstrates such as some amino acids (e.g. tyrosine and cysteine at pH7, E_7=930 mV and 940 mV, respectively) (Harriman, 1987; Bensasson *et al.*, 1983), and hence probably lead to oxidation of proteins.

So, while carotenoids are generally accepted as 'antioxidants', the reactivity/lifetime of such carotenoid radicals may lead to a switch from anti- to pro-oxidant behaviour and such redox-controlled reactions may lead to deleterious as well as beneficial health effects.

However, the relative values shown above suggest that in a mixture of carotenoids all carotenoid radical cations will be converted by lycopene back to the parent carotenoid and the lycopene may be sacrificed. It is noteworthy that the radical cations arising from the three carotenoids present in the human macular (lutein, zeaxanthin and mesozeaxanthin) are all repaired efficiently by lycopene but not by β-carotene. A possible example of such processes may be an explanation of the work of Mares-Perlman *et al.* (1995) and Gouranton *et al.* (2008) showing a correlation between age-related macular degeneration and low levels of serum lycopene (even though such hydrocarbon carotenoids do not accumulate in the macula). Oxidation of dietary lutein and zeaxanthin to their radical cations being repaired by lycopene allowing these xanthophylls, essential for the protection of the eye, to reach the macula.

ASCORBIC ACID

It has been established for many years that the oxidised radical of vitamin E reacts with (water soluble) vitamin C to regenerate the parent vitamin E (Packer *et al.* 1979; Bisby & Parker, 1995). We considered it important to establish if such reactions also may arise with the dietary carotenoids.

We generated the carotenoid$^{\bullet+}$ *via* 355nm laser flash photolysis of 1-nitronaphthalene (NN) in methanol.

$$\text{NN} \rightarrow {}^3\text{NN}$$

$$^3\text{NN} + \text{Carotenoid} \rightarrow \text{NN}^{\bullet-} + \text{Carotenoid}^{\bullet+}$$

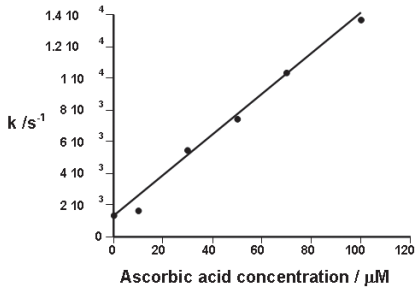


Figure 3. Rate constant for the decay of lycopene radical cation as a function of ascorbic acid concentration.

Table 2. Second order rate constants for the reaction of carotenoid radical cations with ascorbic acid in methanol.

Carotenoid	k_q /10^8 $M^{-1}s^{-1}$
Lycopene	1.3
β-Carotene	3.5
Zeaxanthin	7.7
Mesozeaxanthin	8.2
Canthaxanthin	11
Lutein	13
Astaxanthin	13
β-apo-8'-carotenal	15

In the presence of ascorbic acid (10–100 mM) we observed an enhanced decay of the carotenoid$^{\bullet+}$ which increased with the ascorbic acid concentration, as shown in Fig. 3 for lycopene.

Thus dietary carotenoid radical cations react with ascorbic acid regenerating the parent carotenoid, in a similar way to the reaction of ascorbic acid with vitamin E radicals.

For the reaction:

$$\text{Carotenoid}^{\bullet+} + \text{AscH}_2 \rightarrow \text{Carotenoid} + \text{AscH}^{\bullet} + \text{H}^+$$

we obtained a range of rate constants as shown in Table 2. The order of the rate constants obtained for this reaction suggests a similar ordering of the reduction potentials of the carotenoid radical cations in methanol as obtained in the study on pairs of carotenoids in benzene. For example, lycopene reacts slowest with ascorbic acid suggesting its cation has the lowest reduction potential and β-apo-8'-carotenal reacts fastest, suggesting its radical cation has the highest reduction potential.

We have observed similar reactions both in micellar environments and when the carotenoids are in unilamellar liposomes. We assume the radical cations of the hydrocarbon carotenoids re-orientate to be nearer the micellar surface in order to react with the water soluble vitamin C.

DISCUSSION

A number of speculations can arise from the results presented. It is generally accepted that free radical quenching is a key requirement for an efficient antioxidant. However, the antioxidant capacity does not depend solely on the efficiency of quenching/removal of the oxidizing free radical but also on the reactivity and the lifetime of the products of the quenching reaction. For a strong oxidizing radical such as NO_2• the product is the radical cation of the carotenoid and carotenoid radical cations are themselves strong oxidizing species and can have a relatively long lifetime. To avoid oxidative damage from such species it may be that a high concentration of vitamin C is needed to remove the carotenoid radical cation and the oxidative damage it can cause.

This can be used to explain the well-publicized lung-cancer effects of β-carotene in heavy smokers (The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group, 1994). This sub-population are known to have low vitamin C levels and hence damaging smoke components, such as NO_2, can produce β-carotene$^{\bullet+}$ which will reach the lung and initiate further damage. In non-smokers, the vitamin C is likely to be present in sufficient concentration to preclude the damaging process due to the carotenoid radical cation.

Another speculation concerns the claim that lycopene can protect the macula even though it does not accumulate significantly in the eye (Mares-Pearlman *et.al.*, 1995; Gouranton *et al.,* 2008). Possibly, dietary lutein and zeaxanthin in the diet are oxidised to their corresponding radical cations but can then be re-converted back to the useful parent carotenoid (by lycopene) and hence accumulate in the macula.

Finally, for such processes to be extremely efficient, as would certainly be necessary to protect the macula, the carotenoid$^{+\bullet}$ and the vitamin C need to be in close proximity. Because zeaxanthin and lutein have terminal −OH groups they are fixed to the lipid–water interface leading to a "super-efficient" antioxidant system. Other hydrocarbon carotenoids such as lycopene and β-carotene would need to re-orientate to interact with vitamin C possibly reducing the efficiency of the antioxidant system. Such considerations may explain why the macular only accumulates the xanthophylls even though other carotenoids are present at greater concentration in our diet.

Acknowledgements

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