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Oxidation of carotenoids by free radicals: relationship between structure and reactivity

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Abstract

The relationship between structure and reactivity is reported for a collection of carotenoids in solution reacted with oxidants generated by a modified Fenton process or with peroxyl radicals generated via the azo-initiators AMVN and AIBN. The initial rates of oxidation were in the order: lycopene > β,β-carotene, zeaxanthin > echinenone, isozeaxanthin > astaxanthin, canthaxanthin.

The oxidative degradation caused rapid bleaching, due to disruption and breakdown of the polyene chromophore. A number of reaction mechanisms are likely to be involved. Isozeaxanthin, canthaxanthin and astaxanthin, in which the C-4 and C-4′ positions are occupied by functional groups, react more slowly than β,β-carotene and zeaxanthin, in which this position is free. Products such as the 4-methoxy (or 4-ethoxy) and 4,4′-dimethoxy (or 4,4′-diethoxy) derivatives were isolated from reactions of β,β-carotene with peroxyl radicals in the presence of methanol or ethanol. Electron density calculations suggest that the different reactivities cannot be attributed solely to differences in electron distribution along the polyene chain of the different chromophores, which would alter the susceptibility to free-radical addition to the conjugated double-bond system. Other reactions must therefore be considered, including hydrogen abstraction from positions allylic to the polyene chain (C-4 of β,β-carotene and its derivatives, and of lycopene). Lycopene, lutein and zeaxanthin all reacted rapidly with oxidising agents, so these dietary carotenoids must also be considered as potential antioxidants. © 1997 Elsevier Science B.V.

Keywords: Carotenoid; Antioxidant; Free radical; Reactivity; Structural determinant

1. Introduction

For many years it was thought that the only biological role of carotenoids ingested and absorbed by man was as precursors of vitamin A [1]. It now appears that important actions can be attributed to β,β-carotene itself and other carotenoids that are normally present in the human diet. Evidence suggests that β,β-carotene and other carotenoids may reduce rates of development of some human cancers [2]. This evidence has been acquired from epidemiological surveys [3,4], from experiments with cell cultures in vitro [5] and from animal models [6,7]. Increased intake and serum levels of carotenoids are associated

Abbreviations: AIBN, 2,2′-azobis-isobutyronitrile; AMVN, 2,2′-azobis(2,4′-dimethylvaleronitrile).

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with reduced risk of cardiovascular and ocular diseases [8,9]. Several reports imply significant beneficial effects of β,β-carotene on parameters of the immune system [10–12] and other cell–cell interactions such as gap junction communication [13,14]. The chemical mechanisms of these various actions remain unknown, but the common view is that the carotenoids act mostly by interfering with reactions of damaging oxidising agents and free radicals.

β,β-Carotene is an excellent physical quencher of singlet oxygen, $^1O_2$, and the triplet states of photosensitisers such as porphyrins which can generate $^1O_2$ [15]. This has led to the clinical use of β,β-carotene to ameliorate skin erythema in patients with erythropoietic protoporphyria [16]. β,β-Carotene also protects lipids against oxidation induced by $^1O_2$ generated in organic solution [17–19] or in liposomal model membranes [20–22], again by direct physical quenching.

β,β-Carotene may also act as a radical-trapping antioxidant. To protect tissues against damage caused by oxidising agents or free radicals, carotenoids must in some way interrupt the harmful oxidation by free-radical mediated processes. The classical view of an antioxidant is of a substance that can break a free-radical chain reaction. The major lipid-soluble chain-breaking antioxidant is vitamin E, tocopherol [23], but Burton and Ingold reported that, in a simple chemical system, β,β-carotene is an antioxidant at oxygen partial pressures of 150 Torr (air) or less, being particularly effective at low oxygen partial pressures (~15 Torr) which are typical of many tissues [24].

Since Burton and Ingold's report appeared, a number of studies have indicated an antioxidant role for carotenoids in solution or in model membranes [18,21,25–43]. Many of these investigations have been reviewed comprehensively [44]. The suggestion of Button and Ingold that the reaction of β,β-carotene involves addition of a peroxyl radical to the polyene chain of the carotenoid remains a hypothesis, although it has been invoked to explain results obtained under a wide variety of conditions which may be far removed from the chemical systems originally studied.

Liebler [45] has also evaluated much of the experimental work in terms of the underlying chemistry. Antioxidant, auto-oxidation and even pro-oxidant processes are possible, and the relative efficiency of these processes in any system will determine whether carotenoids have a beneficial antioxidant action. The complexity of the chemistry of carotenoid oxidation is well illustrated by reference to the many different oxidation reactions that have been reported and used over the years, especially in the earlier period of carotenoid structure elucidation. A variety of oxidising agents were used in this work to prepare different carotenoid derivatives, demonstrating that several parts of the molecule are susceptible to attack and a number of different mechanisms for oxidative attack on the carotenoid chromophore may be possible [46–53]. The reactions and mechanisms must therefore be understood as the basis of evaluating the importance of carotenoids as protective antioxidants in vivo.

Carotenoids other than β,β-carotene are also present in the human diet and must also be considered as possible protective antioxidants. We now report a comparative study of the free-radical mediated oxidation of a range of carotenoids in organic solution and discuss the influence of some structural features on the reactions.

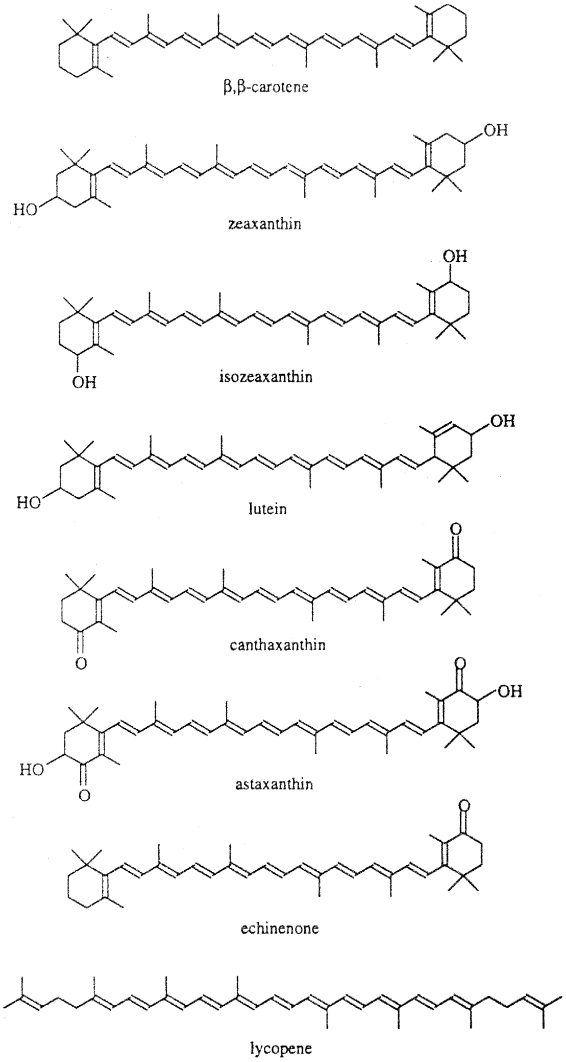
2. Materials and methods

2.1. Materials

Lycopene, β,β-carotene, zeaxanthin, lutein, canthaxanthin, astaxanthin and echinenone (as the all-*E* isomers) were gifts from F. Hoffmann-La Roche and Co. Ltd, Basel, Switzerland. Their structures are given in Fig. 1. Isozeaxanthin was prepared by reduction of canthaxanthin with sodium borohydride [54]. Samples were purified by thin layer chromatography prior to use. 2,2′-Azo*bis*-isobutyronitrile (AIBN) was obtained from BDH Ltd., Dorset, UK and 2,2′-azo*bis*(2,4′-dimethylvaleronitrile) (AMVN) from Park Scientific Ltd., Northampton, UK. All solvents were of the highest purity available. Diethyl ether, light petroleum, ethanol, methanol and acetone were redistilled prior to use.

2.2. Carotenoid manipulations

Manipulations were performed under subdued lighting to limit isomerisation. Concentrations of



Structures of carotenoids tested.

Fig. 1. Structures of carotenoids.

carotenoids were determined by spectrophotometry using published coefficients [54]. The carotenoids were then evaporated under reduced pressure or under a stream of N_2, and redissolved in the reaction solvent to the desired concentration. The UV/visible absorption spectra of the solutions showed no evidence of carotenoid aggregation. In all cases, reactions were monitored by following the loss of absorbance of the solution at the wavelength maximum of the carotenoid under study.

2.3. Apparatus

UV–visible absorption spectra were determined and reactions followed in a Cecil Instruments CE593 scanning spectrophotometer with quartz cuvettes (3 cm^3; 1 cm pathlength) held in a thermostatically controlled Cecil CE245 cuvette holder. Data were recorded on a Cecil CE399 chart recorder. Electron impact mass spectra were obtained with a VG Micromass 70-70H mass spectrometer.

2.4. Generation of oxidising species by the modified Fenton reaction

Carotenoids were dissolved in a mixture of benzene/methanol/diglyme/isopropanol (1 : 1 : 1 : 3; v/v) and allowed to equilibrate for 5 min at 25°C in a cuvette. A mixture of freshly prepared $FeCl_3$, sodium ascorbate and hydrogen peroxide was then rapidly added to initiate oxidant formation. Specific reactant conditions are given in Fig. 2a.

2.5. Oxidation of carotenoids by free radicals generated from AIBN

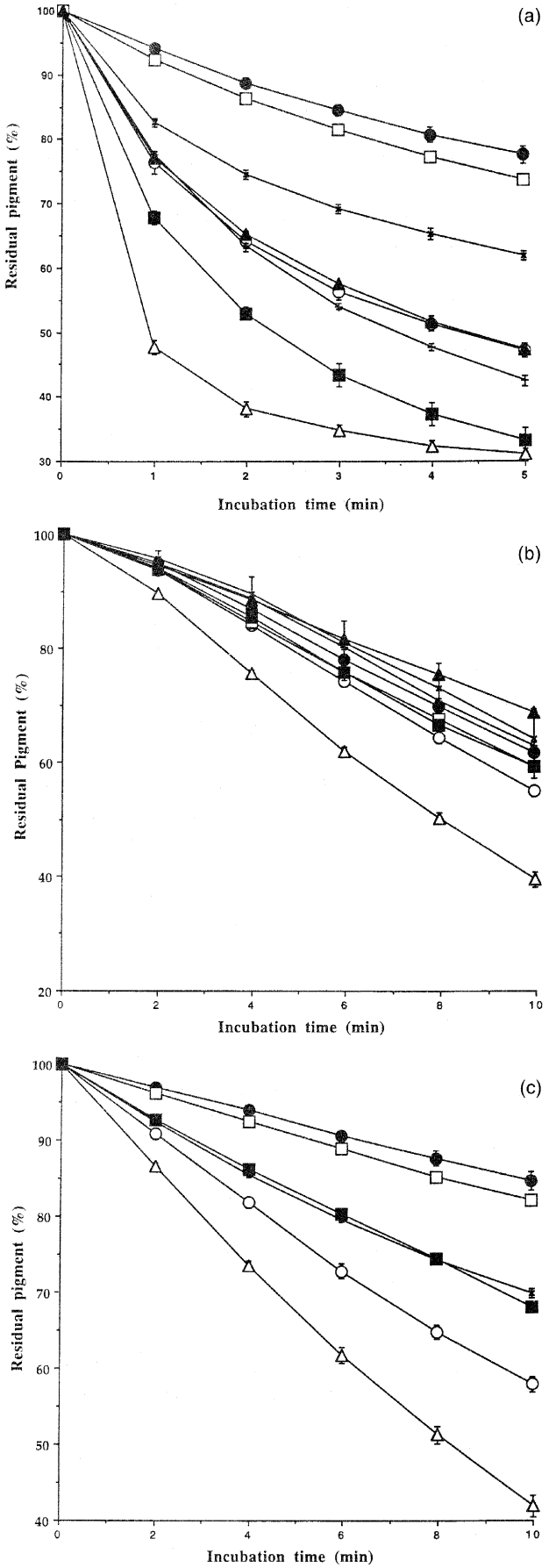
Carotenoids were redissolved in chlorobenzene and preincubated for 5 min at 30°C. A freshly prepared solution of AIBN in chlorobenzene was then added to initiate the reaction [24]. Specific reactant concentrations are given in Fig. 2b.

2.6. Oxidation of carotenoids by free radicals generated from AMVN

Carotenoids were dissolved in hexane and preincubated for 5 min at 37°C. A freshly prepared solution of AMVN in hexane was then added to initiate the reaction. A series of reactions was also performed with the carotenoid and the AMVN in the solvent mixture (hexane-isopropanoltetrahydrofuran, 6 : 5 : 2, v/v) used by Terao [25]. Specific reactant concentrations are given in Fig. 2c.

2.7. Reaction of β,β-carotene with AIBN-induced free radicals in the presence of methanol or ethanol

The reaction of β,β-carotene with AIBN was carried out on a larger scale to provide reaction



products in sufficient quantities for isolation. β,β-Carotene (0.186 μmoles) was reacted with AIBN (90.5 μmoles) in 1 ml chlorobenzene containing 50 μl of methanol or ethanol for 30 min at 30°C under air. Reaction products were extracted with ethanol/diethyl ether and the ethereal solution evaporated to dryness under reduced pressure, redissolved in hexane and applied to a column of Brockmann grade III neutral alumina (5×0.25 cm). Fractions eluted with 100% diethyl ether and 100% ethanol were collected and analysed by reversed phase HPLC on a Spherisorb-ODS 2 column (5 μm, 25×0.46 cm) with a linear gradient of ethyl acetate in acetonitrile–water (9:1), 0–100% over 25 min. UV/visible absorption spectra were determined on-line by means of a Waters 990 photodiode array detector. The main products were collected for mass spectral analysis.

2.8. Computational methods

The geometry of β,β-carotene and astaxanthin was modelled with standard geometrical parameters and subsequently optimised with PCMODEL (PCM version 3.2) by the SCF method. Electronic charge densities as well as final geometry were obtained by energy minimisation of this optimised structure with the MOPAC (version 93) program package using the AM1 Hamiltonian [55].

Fig. 2. Time course of oxidation of carotenoids by free radicals; β,β-carotene (○), lycopene (△), zeaxanthin (■), isozeaxanthin (▲), echinenone (+), lutein (×), astaxanthin (□), canthaxanthin (●). Data points are the means ± s.e. of five experiments. (a) Oxidation by free radicals generated by non-specific Fenton reaction. The reaction system consisted of the carotenoid (10 μM) in benzene/methanol/diglyme/isopropanol (1:1:1:3) and iron (II) chloride (1 mM), sodium ascorbate (1 mM) and hydrogen peroxide (0.03% v/v), added simultaneously to final concentrations shown and incubated under air at 25°C. (b) Oxidation by peroxyl radicals initiated via AIBN. The reaction system consisted of carotenoid (8.6 μM) and AIBN (72.5 mM) in chlorobenzene, incubated at 30°C under air. (c) Oxidation by peroxyl radicals initiated via AMVN. The reaction system consisted of carotenoid (10 μM) and AMVN (10 mM) in hexane, incubated at 37°C under air.

2.9. Statistical analysis

All data are expressed as mean ± standard error. Statistical analysis of results was assessed by Student's *t*-test. A value of $P < 0.05$ was considered significant.

3. Results and discussion

The objective of this work was to study the reactivity of a range of carotenoids towards oxidising agents and especially to compare the reactivities of some β,β-carotene derivatives.

3.1. Reactivity of carotenoids with an oxidation system based on the Fenton reaction

The reaction conditions used, with iron (III) chloride, ascorbate and hydrogen peroxide, provide a convenient system with which to examine the overall susceptibility of carotenoids to destruction by a range of oxidising agents. The system is non-specific but the hydroxyl radical, ($\cdot$OH), is likely to be among the oxidants produced. The oxidation of carotenoids was monitored conveniently by following the loss of absorbance at the λ_{max} of the carotenoid. This method of monitoring carotene oxidation is well established and has been used previously in many studies [40,53,56–58].

The reaction produces a rapid burst of oxidant generation. This caused rapid bleaching of carotenoids, so that approximately 25% of β,β-carotene was destroyed in 1 min and more than 50% in 5 min. The time courses of destruction of several different carotenoids under these conditions are illustrated in Fig. 2a. All the carotenoids had suffered substantial destruction at the end of the 5 min reaction period. Relative susceptibilities of different carotenoids to oxidation were compared by their initial rates of oxidation, determined from the initial tangent to the time-course curves. The results, listed in Table 1, column (a), show substantial differences between the various carotenoids.

3.2. Reactions of carotenoids with peroxyl radicals generated via azo-initiators

Reactions and scavenging of peroxyl radicals are of particular interest because of the prominent role of such radicals in lipid peroxidation. Azo-compounds as radical generators are commonly used to study interactions between carotenoids and peroxyl radicals [24,25,42,43]. These compounds are stimulated by heat or UV light to generate peroxyl radicals by unimolecular decomposition in a controlled process.

Table 1
Initial rates of carotenoid oxidation with free radicals

Carotenoid	Initial rate of carotenoid destruction (μM min^{-1}		
	(a)	(b)	(c)
β,β-Carotene	3.346 ± 0.20	0.406 ± 0.007	0.421 ± 0.009
Lycopene	12.95 ± 1.13 [a]	0.576 ± 0.016 [a]	0.582 ± 0.046 [a]
Canthaxanthin	0.581 ± 0.01 [b]	0.364 ± 0.002 [b]	0.152 ± 0.046 [b]
Astaxanthin	0.813 ± 0.06 [b]	0.363 ± 0.009 [b]	0.177 ± 0.020 [b]
Echinenone	2.257 ± 0.28 [b]	0.360 ± 0.006 [b]	0.294 ± 0.030 [b]
Zeaxanthin	4.070 ± 0.30 [c]	0.390 ± 0.007 [c]	0.310 ± 0.013 [b]
Lutein	2.571 ± 0.05 [b]	0.342 ± 0.007 [b]	n.d.
Isozeaxanthin	2.882 ± 0.07 [b]	0.281 ± 0.011 [b]	n.d.

Data are mean ± S.E. of five separate experiments.
(a): Oxidation by free radicals generated by the Fenton reaction (conditions as in Fig. 2a).
(b): Reaction with peroxyl radicals generated by AIBN (conditions as in Fig. 2b).
(c): Reaction with peroxyl radicals generated by AMVN (conditions as in Fig. 2c).
[a] Oxidation rate of carotenoid significantly greater than that of β,β-carotene ($P < 0.05$).
[b] Oxidation rate of carotenoid significantly less than that of β,β-carotene ($P < 0.05$).
[c] Oxidation rate of carotenoid is not significantly different to that of β,β-carotene ($P > 0.05$).
n.d. = experiment not done.

Several of these initiators are available and two, AIBN and AMVN, which are suitable for work in organic solvents, were used.

3.3. Reaction with AIBN

AIBN was used in the seminal work of Button and Ingold [24] and was therefore used under similar conditions in the present work to compare the reactivities of carotenoids. At constant temperature, peroxyl radicals are generated at a constant rate by homolytic thermal decomposition of the azo-initiator to give a carbon-centred radical which then reacts with O_2 to give the corresponding peroxyl radical. These conditions lead to an approximately linear degradation of β,β-carotene when this is included in the reaction system. The rate of degradation observed was rather slower than with the Fenton-based system, but about 50% of the β,β-carotene was lost in 10 min.

The time courses for the destruction of the various carotenoids by peroxyl radicals generated via AIBN are compared in Fig. 2b. At the end of the 10 min reaction period, lycopene had undergone the greatest amount of destruction. Of the dicyclic carotenoids. β,β-carotene showed the greatest overall loss, isozeaxanthin the smallest, but the differences observed were not great. As with the Fenton-based system, the reactivity is better indicated by the initial rates of the reactions, as listed in Table 1, column (b). In the case of β,β-carotene, post-reaction HPLC analysis with UV/visible detection showed the presence of no more than trace amounts of other light absorbing substances that might be reaction products. A separate experiment involving reactions on a larger scale gave sufficient quantities of these products to allow a range of apocarotenals and ketones to be isolated and identified by their UV/vis absorption spectra and mass spectra, by the spectra of their alcohol products after reduction with $NaBH_4$ and, when possible, by comparison with authentic samples [59]. Small amounts of other compounds such as epoxides were also detected. This confirms the identification of products of β,β-carotene oxidation reported by others [60]. Although they give an indication of the reactions that may have occurred, these compounds were detected in only trace amounts and represent only a small fraction of the total carotene lost. It is unlikely that they are the primary products of the initial reaction between carotene and the oxidant, rather that they are simply the stable products that remain after a complex series of reactions. The diversity of the products found indicates that reactions can occur at all parts of the carotenoid molecule, both along the polyene chain and at the endocyclic double bond. No attempts were made to identify such trace products from the reactions of any of the other carotenoids.

3.4. Reaction with AMVN

The presence of chlorobenzene, which may scavenge peroxyl radicals directly in the AIBN reaction mixture as used by Burton and Ingold, is a considerable disadvantage for studies to compare reactivities in different solvent media or to evaluate antioxidant activity, so the related azo-initiator AMVN was used in subsequent work in different solvents as undertaken by other workers [25]. Our initial study was undertaken with hexane as solvent. The loss of β,β-carotene in the reaction with AMVN at 37°C was similar to that with AIBN at 30°C; about 50% of the carotenoid was destroyed in 10 min, but some differences were seen in the relative rates for the different carotenoids in comparison with the rates obtained with AIBN. With the Fenton-based system or with AIBN, there was great similarity between the reaction rates of β,β-carotene and zeaxanthin. In the reaction with AMVN in hexane, however, β,β-carotene appeared to be much more reactive than zeaxanthin. This apparent discrepancy may be attributed to differences in solubility. The first two reactions are carried out in solvent mixtures in which the carotenoids should all be freely soluble. In hexane, however, β,β-carotene is reasonably soluble but the solubility of zeaxanthin is poor. The zeaxanthin concentration in solution in the reaction medium is therefore low and much of the zeaxanthin may be in aggregated form which may be less accessible to attack by peroxyl radicals. When the same reaction with AMVN was performed in a mixed medium in which both are soluble, namely hexane–isopropanol–tetrahydrofuran (6:5:2) as used by Terao [25], β,β-carotene and zeaxanthin gave similar rates of reaction (0.38, 0.40 $\mu M\ min^{-1}$ respectively). All the carotenoids examined showed an approximately linear rate of degradation (Fig. 2c). The calculated initial rates of carotenoid

oxidation are listed in Table 1, column (c). The UV/vis absorption spectrum of the reaction mixture for the treatment of β,β-carotene with AMVN in hexane was also determined at various time intervals (not shown). The characteristic spectrum of β,β-carotene rapidly decreased in intensity but, apart from the appearance of a slight shoulder at 460–480 nm in the early stages and a slight increase in UV absorption, there was no evidence of the appearance of any light-absorbing reaction products which could interfere with the reaction.

3.5. Relationship between structure and reactivity

Comparison of the initial reaction rates for the different carotenoids shows that, in all three oxidising systems studied, lycopene was the most reactive compound, followed closely by β,β-carotene, whereas the diketo compounds canthaxanthin and astaxanthin were the least reactive. In the following discussion of the results, emphasis is placed on the relationships between structure and reactivity, and what these indicate about the possible mechanisms of the reactions.

β,β-Carotene, zeaxanthin and isozeaxanthin all have the same chromophore, i.e. the nine conjugated double bonds of the polyene chain plus a contribution from the two double bonds of the β-rings, although these are not coplanar with the polyene chain. This is illustrated by their identical absorption spectrum maxima. Isozeaxanthin and zeaxanthin are isomers, only differing in the position of the two hydroxy groups on the β-ring by one position and both possess great similarities in solubility. Any reaction involving the conjugated double bond system, in particular the addition of peroxyl radicals according to the Burton and Ingold hypothesis [24] or electron capture by the chromophore [58], should therefore be similar for all these compounds, so similar rates of reaction would be expected. This was the case with β,β-carotene and zeaxanthin, but the rate of reaction with isozeaxanthin was significantly slower than either of the other two. Clearly, the influence of the presence and position of the hydroxyl group at the 4-position modifies the reactivity of the carotenoid. This suggests that some factor other than free-radical addition to the polyene chain or electron capture by the chromophore is involved, a conclusion supported by results obtained with the ketocarotenoids. In spite of their longer chromophore, canthaxanthin and astaxanthin reacted much more slowly than β,β-carotene and its simple hydroxy derivatives.

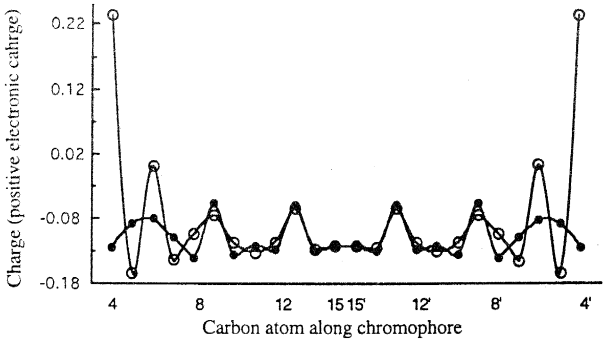


Fig. 3. Calculated positive electronic charge on the carbon atoms in the chromophore of β,β-carotene (●) and astaxanthin (○), as determined by semi-empirical molecular orbital calculations.

A similar result was obtained by Terao for the reaction with AMVN [25]. Terao attributed this result to an electron-withdrawing effect of the keto-groups, which would leave the polyene chain comparatively electron deficient and thus less susceptible to attack. However, semiempirical calculations (Fig. 3) show that the electron distribution in astaxanthin does not differ substantially from that in β,β-carotene or zeaxanthin except in and near to the cyclic end groups. Large differences are seen in electron density at C-4, C-5 and C-6, with smaller differences at C-7 and C-8. The central part of the molecule is essentially unaltered, so a large decrease in the rate of addition of peroxyl radicals to the polyene chain of the diketo derivatives would not be expected. Any reactions in or close to the ring may be affected, however. This is supported by consideration of the chemical reactions of carotenoids with peroxidising agents such as monoperoxyphthalic acid; the initial addition reaction occurs preferentially to the C-5,6 double bond in the ring β,β-carotene but at the C-10,11 double bond in the polyene chain of canthaxanthin [48].

Canthaxanthin, astaxanthin and isozeaxanthin all have substituent groups at C-4 and C-4′, whereas β,β-carotene and zeaxanthin do not. The C-4 and C-4′ positions, allylic to the chromophore, are reactive positions which readily undergo dehydrogenation by N-bromosuccinimide and also undergo facile hydroxylation [31]. Enzymatic co-oxidation of 13-cis

retinoic acid gives the corresponding 4-hydroxy-retinoid, suggesting a hydrogen abstraction mechanism, but was not observed in chemical systems [43]. Free-radical abstraction of a hydrogen atom from the C-4 position of a β-ring to give a resonance-stabilised carbon-centred radical should be favoured because of delocalisation of the unpaired electron over the polyene chain. Hydrogen abstraction from other positions in the β,β-carotene molecule would be energetically unfavourable; the other CH_2 groups in the ring are not allylic and are therefore much less reactive, and the C=C–H bonds are too strong to allow H abstraction from positions along the polyene chain by peroxyl radicals [61]. In this respect, the allylic CH_2 group at C-4 of β,β-carotene and zeaxanthin, and that at C-4 of lycopene, which are allylic to the polyene chromophore, may be considered to resemble the allylic CH_2 groups in polyunsaturated fatty acids, from which hydrogen abstraction occurs readily to initiate and propagate peroxidising chain reacting. A similar reaction might be possible for these carotenoids. The CH_2 group at C-3 of lycopene is allylic only to the isolated C-1,2 double bond and would not be expected to be very reactive.

When the reaction of β,β-carotene with AIBN was performed in the presence of a small amount of methanol, small amounts of products of low polarity were isolated and identified by their chromatographic properties, UV–visible spectra and mass spectra. The two main products, which had UV/vis absorption spectra identical to that of β,β-carotene, were identified by mass spectrometry as methoxy derivatives. The major compound had molecular weight 596 and major fragment ions at m/z 564 and 532, due to losses of one and two molecules of methanol, respectively, by elimination of allylic methoxy groups. The minor component had molecular weight 566 and a major fragment ion at m/z 534 due to facile elimination of one molecule of methanol, analogous to the loss of one molecule of water from the allylic hydroxy compound isocryptoxanthin. The data are consistent with the identification of these compounds as 4,4′-dimethoxy-β,β-carotene and 4-methoxy-β,β-carotene, respectively. When ethanol was included in the reaction mixture in place of methanol, a similar set of products was isolated, and the main ones were identified by mass spectrometry as the corresponding ethoxy derivatives, 4,4′-diethoxy-β,β-carotene (molecular weight 624) and 4-ethoxy-β,β-carotene (molecular weight 580). Fig. 4 shows the structures of these products. The amounts of the compounds available were too small to allow confirmation of the structure by NMR, so these identifications should be considered tentative. Other products, present in smaller amounts after the two reactions, were also shown by mass spectrometry to be methoxy derivatives of β,β-carotene, but with UV–vis absorption spectra indicative of shorter chromophores, consistent with a break in conjugation due to the addition of methanol to various positions in the polyene chain. These products were not further characterised.

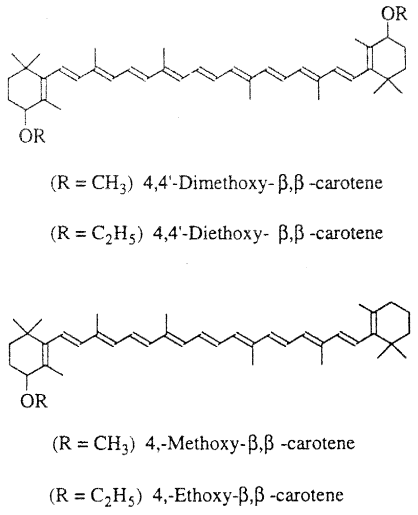


Fig. 4. Structures of reaction products of β,β-carotene with peroxyl radicals in the presence of reactant amounts of methanol and ethanol.

These results show that the allylic C-4 position in the ring of β,β-carotene is a reactive position and the available evidence is consistent with its being susceptible to hydrogen abstraction by free radicals, including peroxyl radicals. The reactivity of the C-4 position was recognised by El-Tinay and Chichester [53] In canthaxanthin, astaxanthin and isozeaxanthin, the possibility of hydrogen abstraction from C-4 and C-4′ is removed or reduced.

The high reactivity of lycopene could be attributed to the easy addition of peroxyl radicals to the longer conjugated polyene chain, but hydrogen abstractions may also be a major feature in this case. Lycopene has an extended chromophore compared with β,β-carotene; in the case of the latter pigment, the termi-

nal conjugated double bonds present in the β-rings are not coplanar with the remainder of the polyene chain as the β-rings are twisted out of plane due to steric hindrance. This would reduce the π-electron overlap between the 5,6 C=C bond and the remainder of the polyene chain, lowering the effectively ability to delocalise an unpaired electron formed at C-4 into the polyene chain. With lycopene, in which all the C=C bonds are coplanar, this problem does not occur and unpaired electrons formed at the allylic C-4 positions would be readily delocalised. Lycopene and lutein are major carotenoid components of the human diet and are potential dietary antioxidants which could be important in disease prevention [62]. Lutein also reacts rapidly in the Fenton-based system and with peroxyl radicals but its reactivity was rather lower than that of zeaxanthin.

4. Conclusions

The main interest in relation to the reactions of carotenoids with oxidising agents and free radicals is whether the carotenoids can be effective antioxidants in vivo and thus give protection against diseases mediated by oxidative stress. It is shown in this paper that carotenoids are readily and rapidly oxidised by a range of oxidants. In a complex system this rapid reaction with carotenoids could reduce the availability of free radicals to react with other molecules such as unsaturated lipids, and therefore reduce the level of damage caused to these other molecules and thus to structures such as membranes. The results presented here show that hydrogen abstraction should be considered as one of the possible mechanisms that occur when carotenoids are exposed to peroxyl radicals and other oxidising agents. We do not suggest that this is the only mechanism; indeed, peroxyl radical addition and electron capture also occur. However, the available evidence presented in this paper also suggest that allylic hydrogen abstraction may be a factor which influences carotenoid reactivity with free radicals.

Understanding the fundamental chemistry of the reactions of carotenoids with oxidising free radicals is essential. Elucidation of the nature of the extremely rapid primary reactions is especially important and detailed investigations to identify transient species formed by the reactions with known free radicals generated under controlled conditions, e.g. by pulse radiolysis, will be required to prove reaction mechanisms [30,40]. Only when the chemistry is fully understood can the real value of carotenoids as protective antioxidants be assessed.

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