

Carotenoids in Antioxidant Networks. Colorants or Radical Scavengers

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ABSTRACT: Optical and electronic properties of carotenoids as also reflected in their colors have been fine-tuned through evolution, resulting in a structural diversity important for carotenoid properties as radical scavengers and as quenchers of electronically excited states. Carotenoids form antioxidant networks based on one-electron transfer with other carotenoids depending on the balance between ionization energy and electron affinity of the individual carotenoids as has been demonstrated by real-time kinetic studies and later confirmed by quantum mechanical calculations. The more hydrophilic xanthophylls serve as molecular wiring across membranes in these networks through anchoring in water/lipid interfaces resulting in synergism with more lipophilic carotenoids. Radical scavenging of such networks seems to be thermodynamically controlled according to a two-dimensional classification of potential antioxidants. Carotenoids in birds' plumage, as reflected by their color and color intensity, seem to be indicators of good antioxidant status and health of the bird, and such antioxidant networks appear to be in "equilibrium". Carotenoids are under other conditions involved in networks with other types of antioxidants as in egg yolk and in some fish. For the more hydrophilic (iso)flavonoids and their glycosides, antioxidant synergism through regeneration of the lipophilic carotenoids active as radical scavengers becomes kinetically controlled at interfaces. Carotenoids appear accordingly, and also in food, as antioxidants under two types of conditions: (i) in "equilibrium" with other antioxidants in *thermodynamically* controlled networks serving as color indicators of good antioxidant status and (ii) as antioxidants active through radical scavenging in networks with *kinetically* controlled regeneration.

KEYWORDS: *antioxidant synergism, carotenoids, radical scavenging, polyphenols, tocopherols*

■ INTRODUCTION

Carotenoids are lipophilic pigments with conjugated double bonds almost exclusively with *trans*-configuration in contrast to the unsaturated lipids, which have nonconjugated double bonds mainly of *cis*-configuration. Carotenoids first emerged through evolution in *archaeobacteria* (see Figure 1). Carotenoids are as polyenes rigid and difficult to bend. Carotenoids reinforce accordingly cell membranes in bacteria and animals due to their rigid backbone and their proper length corresponding to the hydrophobic zone of such membranes.¹ Carotenoids with polar end groups (i.e., the xanthophylls) are further anchored in the water/lipid interface and are of special importance for membrane stability. Carotenoids play a crucial role in photosynthesis in bacteria and plants, the primary event for appearance of all aerobic life on Earth, in light harvesting, and in electron transfer followed by dissipation of excess energy in the form of heat during intense sunlight exposure.² Carotenoids are not synthesized in animals, but despite this fact they have important functions in animals by protecting sensitive tissue and organelles during oxidative stress and excessive exposure to light. The structural diversity of carotenoids and hydrophilic/lipophilic balance allow specific orientation in membranes resulting in synergistic effects for combinations of carotenoids as antioxidants as was demonstrated in certain organized media such as liposomes.³ In less organized media, the individual carotenoids most resistant to oxidative deterioration have been found to protect unsaturated lipids against oxidation more efficiently than the less resistant carotenoids.⁴

Astaxanthin, the red-colored C₄₀-carotenoid synthesized in phytoplankton, is transferred in the marine feed chain to krill

and further to fish and shellfish and concentrates in eggs of marine organisms such as salmon, but astaxanthin has also been found to have antioxidant properties in humans.⁵ Lutein and other yellow C₄₀-carotenoids are transferred from leafy vegetables such as spinach and alfalfa to land-based animals and to birds. For birds, the yellow carotenoids, which are notably more reducing than the red carotenoids including astaxanthin, as evidenced by their electrochemical potentials, are found in the egg yolk depending on the birds' foraging.⁶ In birds, carotenoids are also the basis of the ornamental pigmentation important in sexual signaling to conspecifics.⁷

The role of carotenoids as *in vivo* antioxidants in animals active through scavenging of reactive oxygen species, and lipid radicals, has often been challenged. For birds, the presence of unbleached carotenoids has been suggested rather to reflect a healthy status of the individual bird.⁸ Control of oxidative stress by other and more reducing antioxidants as a feature of good health accordingly should leave carotenoids as part of an "antioxidant equilibrium" being important in the honesty of carotenoid-based signals in trade-offs and sexual selection among birds and other animals.⁹ This leaves carotenoids as color indicators of the status of other and more reducing antioxidants rather than the active radical scavengers. Recent theoretical calculations have, however, shown that carotenoids of deep red color are less efficient in reductive scavenging of

Received: December 14, 2011

Revised: February 14, 2012

Accepted: February 16, 2012

Published: February 16, 2012

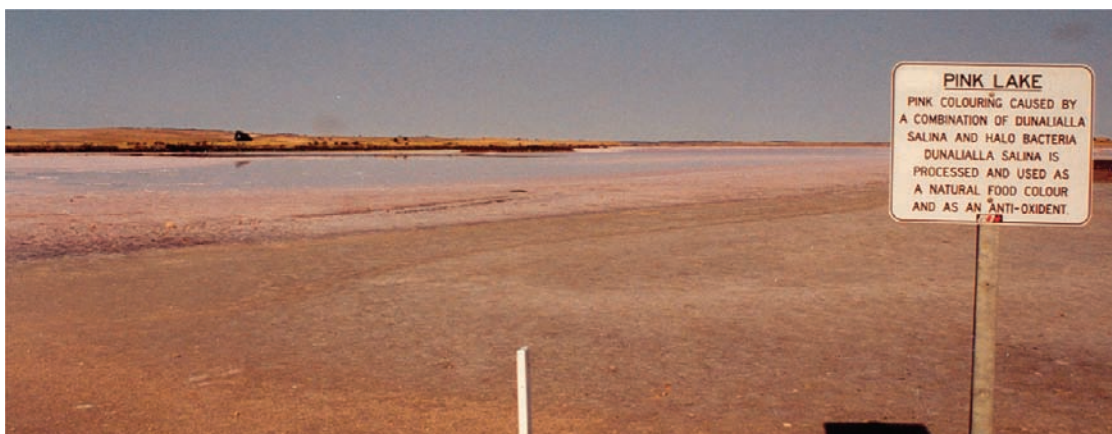


Figure 1. Carotenoids appeared during evolution first in *archaeobacteria* and may be found in microorganisms living under extreme conditions. They are striking colorants, but are they also antioxidants? (Photo 2002, Southern Australia, by Lisbet Snedstrup Christensen; reproduced with permission.)

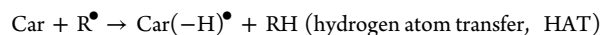
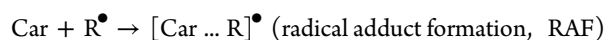
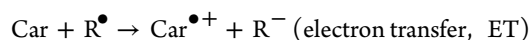
radicals than the less red and more yellow carotenoids.¹⁰ For birds, these yellow carotenoids are actively concentrated in the egg yolk in what has been called a “high carotenoid investment” by the female parent to fulfill specific protective roles under the conditions of oxidative stress in the vulnerable, lipid-rich tissues of developing embryos and during hatching.⁶ In the human eye, lutein and zeaxanthin are selectively incorporated in the yellow spot despite higher concentrations of other carotenoids in the blood, and dietary supplementation together with zinc is increasingly being recommended for protection against age-related macular degeneration (AMD).^{11,12} Certain dietary flavonoids have now been shown to protect human retinal pigment epithelial cells from oxidative damage and death.¹³ For the developing embryos of birds, interaction of carotenoids with vitamin E has been recognized.⁶ Carotenoid interaction with tocopherols such as vitamin E and with plant polyphenols such as (iso)flavonoids and their glycosides may hold the key to a better understanding of the often disputed role of carotenoids as protectors against oxidative stress other than that emerging from singlet oxygen and other electronic excited state.¹⁴ Carotenoid interaction with vitamin antioxidants such as the tocopherols and vitamin C and with nonvitamin antioxidants such as (iso)flavonoids will accordingly be discussed with special emphasis on an understanding of the thermodynamic and kinetic aspect of the free radical interaction between these groups of dietary antioxidants.

■ CAROTENOID ANTIOXIDANT HIERARCHIES

Nature has fine-tuned the optical and electronic properties of carotenoids through evolution, resulting in a rich structural diversity as illustrated by the structure of carotenoids selected in Figure 2 for further discussion of the role of carotenoids as antioxidants.¹⁵ Carotenoids have important functions as quenchers of singlet oxygen in light-exposed tissue where photosensitizers (type II) such as chlorophyll may activate ground-state oxygen. The quenching of singlet oxygen depends on the energy difference between the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) and on the ionization energy, and a quantitative structure–activity relationship (QSAR) has been established for the singlet oxygen quenching rate.¹⁶ Whereas quenching of singlet oxygen approaches the diffusion limit primarily as a function of the number of double bonds present in the polyene chain in these molecules,¹⁷

carotenoids do not quench triplet (or singlet) excited states of riboflavin, which is a relevant example of an important photosensitizer present in the skin and eye. Such type I photosensitization is extremely damaging, especially to proteins, during light exposure at low oxygen pressure,^{18,19} under which singlet oxygen is not formed through type II photosensitization. Carotenoid structures also have an impact on radical scavenging as shown for phenoxyl radicals.²⁰ The scavenging rate was found to increase for an increasing number of coplanar conjugated double bonds in the carotenoid, whereas the presence of hydroxyl and especially keto groups as in astaxanthin and canthaxanthin decreased the rate. Quantum mechanical calculations now seem to indicate that the nature of the radical is of equal or even more importance than the structure of the carotenoids for scavenging efficiencies.¹⁰

As for scavenging of oxidizing radicals by carotenoids, mainly three reaction pathways have been considered:



Carotenoid radical cations resulting from ET reactions have a lifetime belonging to the millisecond time frame and absorb in the near-infrared region around 1000 nm.^{20–22} The carotenoid radical adducts absorb around 520 nm and will, according to recent theoretical calculations, scavenge an additional free radical, leading to the formation of nonradical products and consequently terminate the free radical chain reaction characteristic of lipid oxidation.²³ Hydrogen atom transfer is characteristic for lipid oxidation initially forming carbon-centered radicals of lipids, and HAT has often been suggested for carotenoids as antioxidants.^{20,21} Car[•] has now been characterized spectrally and was found to have an absorption maximum at 750 nm for β -carotene and a short lifetime of 150 ns under anaerobic conditions.²⁴ Whereas carotenoid adducts may quantitatively be the most important products of carotenoid radical scavenging, the neutral carotenoid radical seems to be related to conditions of extreme oxidative stress with a high hydroxyl radical flux.²⁴ The tendency of one-electron transfer of carotenoids to oxidizing radicals to form the carotenoid radical cation is related at least to some degree to the reduction potential of the radical cation, and experimental values for CH_2Cl_2 as

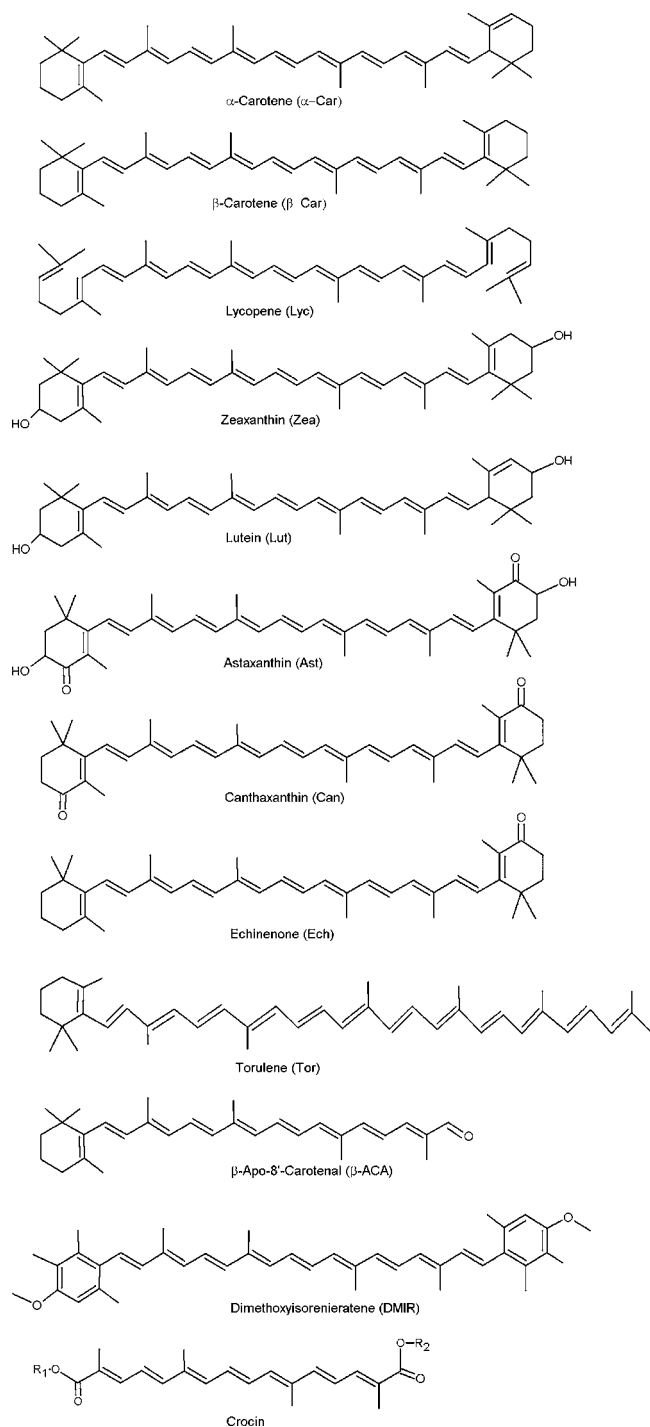


Figure 2. Structures of carotenes (α -Car, β -Car (+ β -ACA), Lyc, Tor), xanthophylls (Zea, Lut, Ast, Can, Ech, DMIR), and the water-soluble carotenoid sugar ester, crocin ($R_1 = R_2 =$ gentobiose with β -linkage).

solvent varies between 0.84 V for β -carotene and 0.97 V for astaxanthin versus NHE.^{15,26} Notably, carotenoids may also be reduced, forming radical anions.²⁵ The balance between electron donation and electron acceptance forming radical cations and radical anions, respectively, varies among the carotenoids and is of importance for their role in antioxidant networks. In Figure 3 spectra are shown for the three different simple β -carotene radicals. Real time kinetic studies using oxidative conditions for which the carotenoid radical cations are formed have established

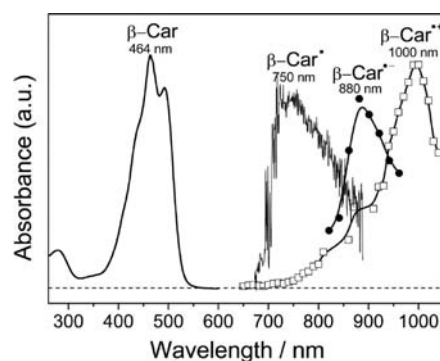
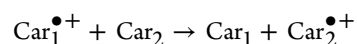
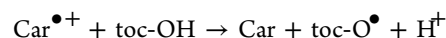


Figure 3. Absorption spectra of β -carotene and simple radicals of β -carotene.^{22,24,25} Absorption is not shown to scale.

an antioxidant hierarchy among carotenoids according to the reaction



In chloroform as an electron-accepting solvent, $\text{Car}^{\bullet+}$ is easily formed following light absorption, and the formation and the decay of radical intermediates could be monitored by transient absorption spectroscopy, also including the four homologous tocopherols (see Figure 4). The ordering of carotenoids and tocopherols according to their tendency to reduce oxidizing radicals including one-electron-oxidized antioxidants is shown in Figure 5 for chloroform as solvent. Notably, lycopene is, according to this antioxidant hierarchy, the most efficient radical scavenger and comparable in efficiency with the tocopherols. It may be questioned whether these results obtained for chloroform also are valid in biological systems. Theoretical calculations have, however, confirmed this general ordering among the carotenoids with the carotenes lycopene and β -carotene as the most efficient radical scavengers through electron transfer from the carotenoid.¹⁰ The theoretical calculations have further identified the less common insect carotene torulene, which contains 13 conjugated double bonds (Figure 1), as even more efficient.²⁷ Torulene should accordingly be further studied for its properties as a radical scavenger. The interaction of carotenoids with the tocopherols and the tocotrienols is of special interest due to the role of carotenoids and compounds of the vitamin E family in protection of membranes against oxidation:^{28–30}



It has been discussed whether α -tocopherol may be regenerated by carotenoids from the phenoxyl radical formed during scavenging of lipid radicals or whether carotenoids are regenerated from their radical cations by vitamin E as shown above. Clearly the acidity becomes important at membrane interfaces to water, as the tocopherol radical cation $\text{toc-OH}^{\bullet+}$ initially formed is a strong acid. However, also other interfacial phenomena should be considered in relation to membranes as has been demonstrated by chemical modification of carotenoids to increase their hydrophilicity and contact to the aqueous phase and hydrophilic antioxidants, in effect increasing their rate of electron transfer by up to a factor of 4.³¹

■ ANTIOXIDANT HIERARCHY IN TWO DIMENSIONS

Astaxanthin ranks together with canthaxanthin among the least efficient reductive scavengers of radicals according to both theoretical calculations and real time kinetic studies (cf. Figure 5).

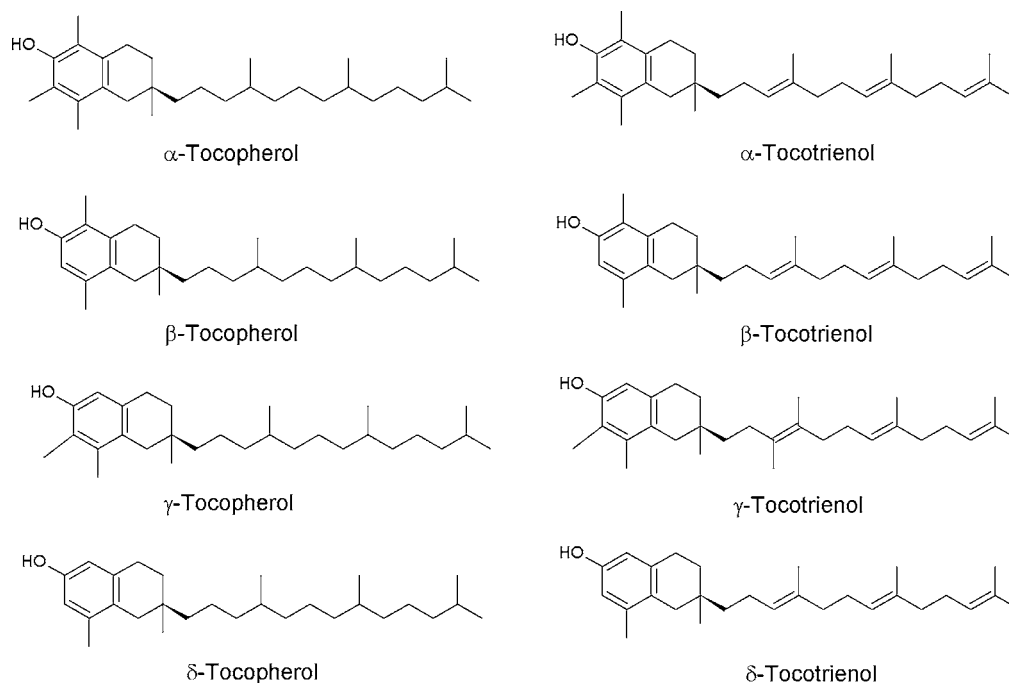


Figure 4. Homologous tocopherols and tocotrienols.

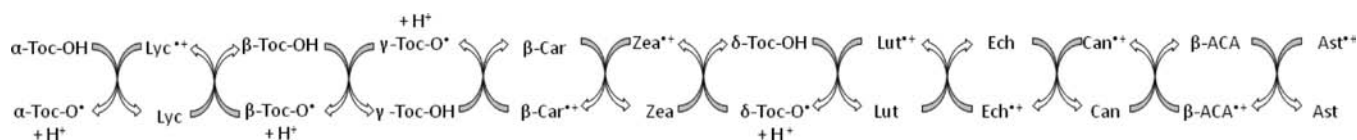
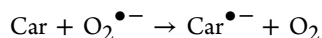
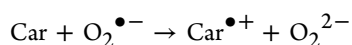


Figure 5. Antioxidant network including carotenoids and the homologous tocopherols according to their tendency to reduction of their one-electron-oxidized forms as determined by real-time kinetic methods.²²

Still, astaxanthin is found in an increasing number of studies to have excellent antioxidant properties.^{5,32–35} Carotenoids should also be considered to be potential electron acceptors, forming the carotenoid radical anion (Figure 3) through reduction³⁶



as well as electron donors under oxidative stress:



The antioxidant activity of carotenoids when reacting with the superoxide radical anion through oxidation of this radical to oxygen lies in the capacity to prevent the formation of peroxides ($\text{O}_2^{2-}/\text{H}_2\text{O}_2$) and other reactive oxygen species such as the hydroxyl radical derived from peroxides through Fenton-type reactions.

The antioxidant ordering of carotenoids in oxidative scavenging is to a high degree the opposite of the ordering according to reductive scavenging. However, quantum mechanical calculations show that it is misleading simply to correlate the radical-scavenging function of carotenoids (and other antioxidants) with their reduction potentials.³⁷ Electron acceptor properties and electron donor properties should be considered simultaneously, leading to a donor–acceptor map or a two-dimensional antioxidant hierarchy. An electron acceptor index, R_a , and an electron donor index, R_d , have been defined relative to fluorine and sodium, respectively, and the acceptor/donor classification shown in Table 1 identifies compounds that are both good acceptors and good donors as the overall best radical scavengers. The most efficient chain-breaking antioxidants in

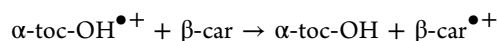
Table 1. Classification of Radical Scavengers as Electron Donors and Electron Acceptors^a

R_d	R_a	
	low electron acceptor index (bad acceptors)	high electron acceptor index (good acceptors)
low electron donation index (good donors)	good radical scavengers good antioxidants example: vitamin E	best radical scavengers example: β -carotene
high electron donation index (bad donors)	poor radical scavengers example: vitamin C	good radical scavengers good “antireductants” example: astaxanthin

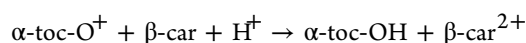
^a R_a and R_d are relative to fluorine and sodium as standards and based on vertical electron affinity and vertical ionisation energy, respectively.³⁷

lipid oxidation, such as the tocopherols, are found among compounds that are good electron donors but bad electron acceptors, in effect scavenging lipid radicals through reduction. Notably, R_a and R_d have no simple relationship to each other, justifying the novel two-dimensional classification.³⁷ Astaxanthin is a good electron acceptor but a bad electron donor, in agreement with the ranking seen in Figure 4, and accordingly not a chain-breaking antioxidant in lipid oxidation. The role of carotenoids as both electron donor forming $\text{Car}^{\bullet+}$ and electron acceptor forming $\text{Car}^{\bullet-}$ (cf. Figure 3 for β -carotene) seems unique and may explain their importance in antioxidant networks.

The interaction between carotenoids and α -tocopherol (vitamin E) and ascorbate (vitamin C) has been of special interest.³⁰ According to the antioxidant hierarchy established (Figure 5) for aprotic solvents, α -tocopherol should be capable of regenerating β -carotene from the radical cation of β -carotene. Ascorbate ($E^\circ = 0.22$ V versus NHE) being even more reducing than α -tocopherol ($E^\circ = 0.80$ V) should also regenerate β -carotene ($E^\circ = 0.84$ V) depending on contact at lipid/water interfaces.²⁶ The reverse reaction may, however, be of importance under some conditions for vitamin E,²⁸ as the reaction



is at least electrochemically feasible. A regeneration of vitamin E by β -carotene will clearly be important in membrane antioxidant networks. However, evidence is now available which indicates that the regeneration reaction may be a two-electron transfer instead of a one-electron process:²⁹



The diamagnetic cation of α -tocopherol is, however, not the initial oxidation product when α -tocopherol acts as a chain-breaking antioxidant, but may be formed under strongly oxidizing conditions or possibly by disproportionation of the tocopheryl radical (Figure 6). For conditions of "homogeneous

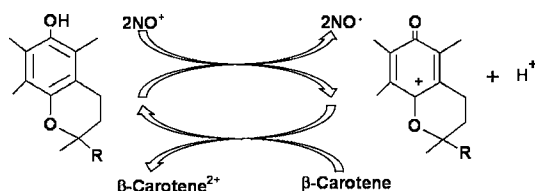


Figure 6. Two-electron-oxidized α -tocopherol is reduced back to active vitamin E by β -carotene.²⁹

solution" as in the lipids of animals, thermodynamics may be in control of such processes, providing a theoretical framework for the concept of "antioxidant equilibrium" as used in evolutionary ecology to explain the role of carotenoids in sexual signaling for birds.^{7,9}

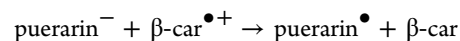
In red palm oil in which both α -carotene and β -carotene (Figure 1) are present together with tocotrienols and tocopherols (Figure 4), the carotenes became depleted upon heating when the oil was used for frying and apparently protecting the tocotrienols and tocopherols, suggesting a role of the carotenes as primary antioxidants.³⁸ However, the carotenes had no effects as antioxidants when added alone to palm oil mildly depleted of antioxidants by "stripping", but showed a clear synergistic effect with the tocotrienols as antioxidants in liposomes, protecting the unsaturated fatty acids against oxidation, suggesting that carotenes rather than the tocotrienols are the primary scavengers of lipid-derived radicals during oxidation in red palm oil. A regeneration of the carotenes by tocopherols or tocotrienols as evident from Figure 5 seems to explain the antioxidant synergism observed for the liposomes. However, during storage at ambient conditions of mixtures of fish oil and palm oil, as a potential functional food, the carotenes were found to have prooxidative effects,³⁹ confirming the original suggestion that carotenoids are prooxidants for high oxygen concentration, as in the mixture of fish oil and palm oil during ambient storage, but antioxidants for low concentrations of oxygen, as in palm oil where oxygen is expelled during frying.⁴⁰

The carotenoid network depending on electron transfer from a more reducing carotenoid to a radical cation of a less reducing carotenoid will approach a situation of steady state, which may be considered as an "antioxidant equilibrium", which also includes the chromanols for homogeneous solution with low oxygen concentration, as the electron transfer processes are all fast.^{22,26} However, in heterogeneous systems, other effects clearly become important as evidenced by the antioxidant synergism demonstrated in liposomes.^{3,38} In phosphatidylcholine liposomes the combination of β -carotene (or lycopene as shown in Figure 7) with astaxanthin shows a clear antioxidant synergism for lipid oxidation in the lipid phase.⁴¹ Astaxanthin anchored in the water/lipid interface is suggested to scavenge radicals, and an electron is transferred to the radical cation of astaxanthin (a good electron acceptor according to the classification of Table 1) from β -carotene (or lycopene), which has superior electron donor properties as compared to astaxanthin. Spatial organization and interaction between carotenoids with different balances between donor and acceptor properties for electrons become important and may accordingly result in antioxidant synergism.

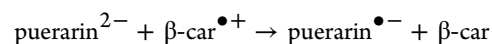
■ POLYPHENOL/CAROTENOID NETWORK

Antioxidants are often classified according to their nutritive value (nonvitamin/vitamin) or their lipophilic/hydrophilic balance (lipid- or water-soluble). Interaction between carotenoids and plant polyphenols as lipophilic and hydrophilic nonvitamin antioxidants, respectively, has been less investigated than the five other binary combinations of the four groups of antioxidants. Such interactions between natural nonvitamin antioxidants seem, however, important as a few examples of hybrids between carotenoids and polyphenols are found in nature such as the extremely efficient antioxidant 3,3'-dihydroxyisorenieratene (DMIR, see Figure 1).⁴² Interaction between flavonoids and carotenoids may also be important for eye health.¹³

During oxidative stress, carotenoid radical cations formed in the lipid phase of liposomes have been found to be regenerated by plant phenols such as puerarin. Puerarin is the water-soluble C-glycoside of the isoflavonoid daidzein isolated from the roots of *Puerarin lobata*, which is used in Chinese herbal medicine.⁴³ Notably, this regeneration occurring at the water/lipid interface (see Figure 8) was found to depend on pH and only the conjugated base of puerarin, which has $pK_{a1} = 7.2$ and $pK_{a2} = 9.8$, was capable of regenerating β -carotene:⁴³



(shown in Figure 7)



However, the opposite process in which a plant phenol is regenerated by a carotenoid may be relevant at low pH. The regeneration process is fast approaching the diffusion-controlled limit (Table 2), and the dianion of the isoflavonoid diadzein and its C-glycoside, puerarin, reacts with similar rates. Astaxanthin and canthaxanthin react more rapidly than β -carotene and zeaxanthin, and clearly, astaxanthin reacts more rapidly than canthaxanthin, identifying the keto group as most important for electron transfer (cf. Figure 1), and a linear free energy relationship was established between driving force (reaction free energy, ΔG°) and rate of electron transfer (activation free energy, ΔG^\ddagger).⁴³ Carotenoid keto groups as present in astaxanthin: (i) facilitate accordingly electron transfer to carotenoids radical

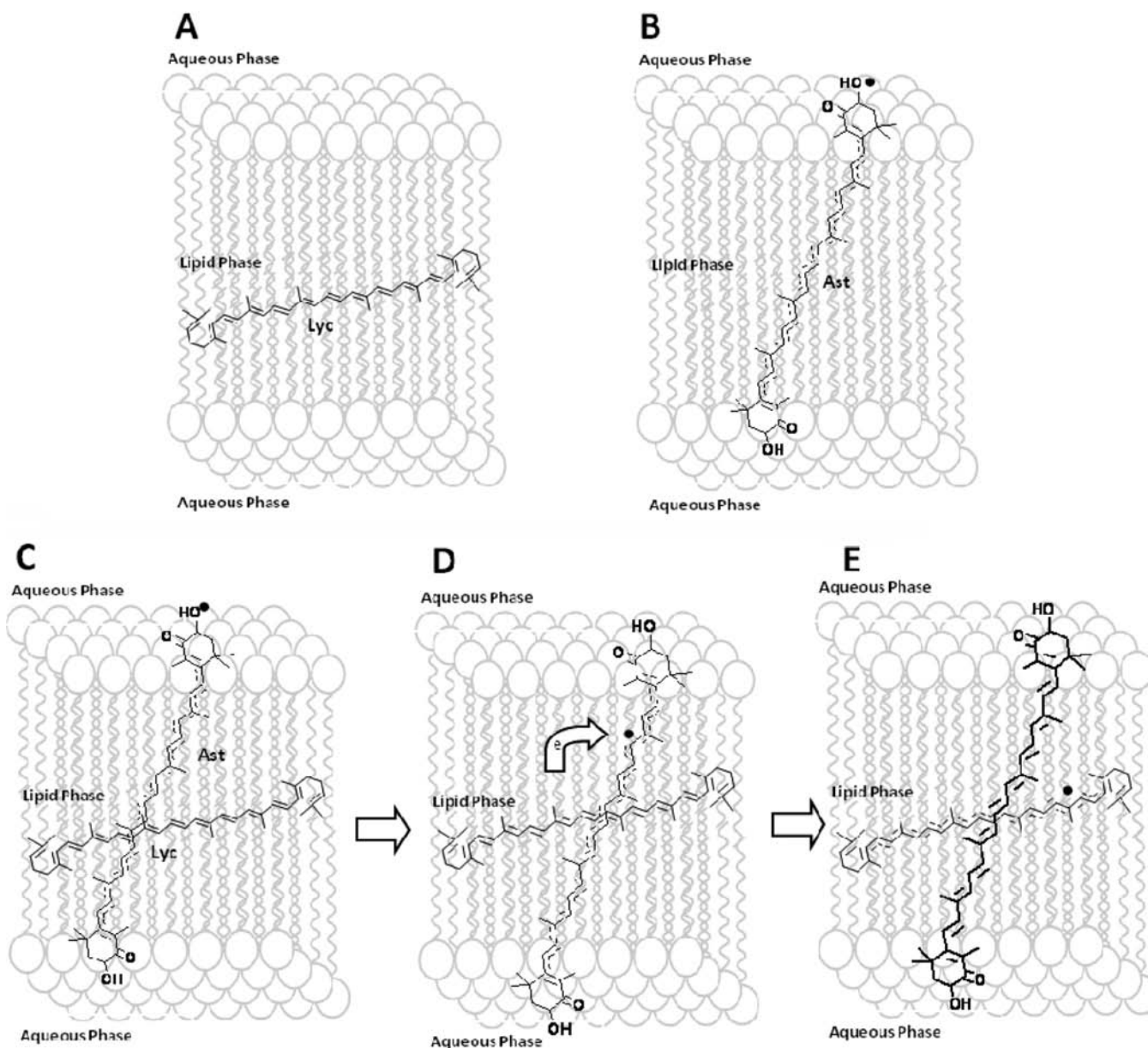


Figure 7. Astaxanthin and lycopene in a liposomal membrane.⁴¹ The lipid-soluble radical initiator 2,2'-azobis(2,4-dimethylvaleronitrile) (AMV) was used to generate radicals in the water/lipid interface (the cleaved AMV is hydrophilic and moves toward the water/lipid interface). Lycopene has little contact with initially formed radicals (A), whereas astaxanthin is a poor scavenger (B). Together the two carotenoids were found to provide antioxidant synergism as the radical cation of astaxanthin is reduced by lycopene with the astaxanthin polyene backbone serving as a molecular wire (C–E).

cations and seem important for kinetic control of antioxidant networks, and (ii) favor electron acceptor properties over electron donor properties ranking astaxanthin at an extreme in thermodynamically controlled networks. Together these two properties may explain the unique antioxidant properties of astaxanthin often encountered.⁵ For one of the few water-soluble carotenoids, crocin from saffron, the rate of reduction of hypervalent iron by the carotenoid, in effect deactivating hydrogen peroxide, was also found to depend on the driving force (ΔG°), as the perferrylmyoglobin was found to react more quickly than the less oxidizing ferrylmyoglobin⁴⁴ (see Figure 9). As a new development, daidzein has been covalently linked with a retinal analogue C₂₂ aldehyde through a C–C bond to understand the importance of spatial orientation in the water/lipid interface.⁴⁵ This “dyad” showed improved antioxidant

activity compared to a combination of the isoflavonoid and the polyene (retinol acetate), especially at high oxidative stress. For noncovalently linked (iso)flavonoids and their glycosides, the effective rate of the bimolecular reaction with the β -carotene radical cation regenerating β -carotene depends on the penetration of the polyphenols into the lipid phase and the association with the radical cation rather than on the bond dissociation energy of the phenol O–H bond, confirming the importance of kinetic effects for antioxidant synergism of carotenoids and polyphenols in heterogeneous systems.⁴⁶

■ CAROTENOIDS AND NITRIC OXIDE

Carotenoids scavenge reactive nitrogen species such as nitrogen dioxide (a molecule of relevance to lung diseases).^{47,48} Electron transfer from β -carotene is thermodynamically favored in polar

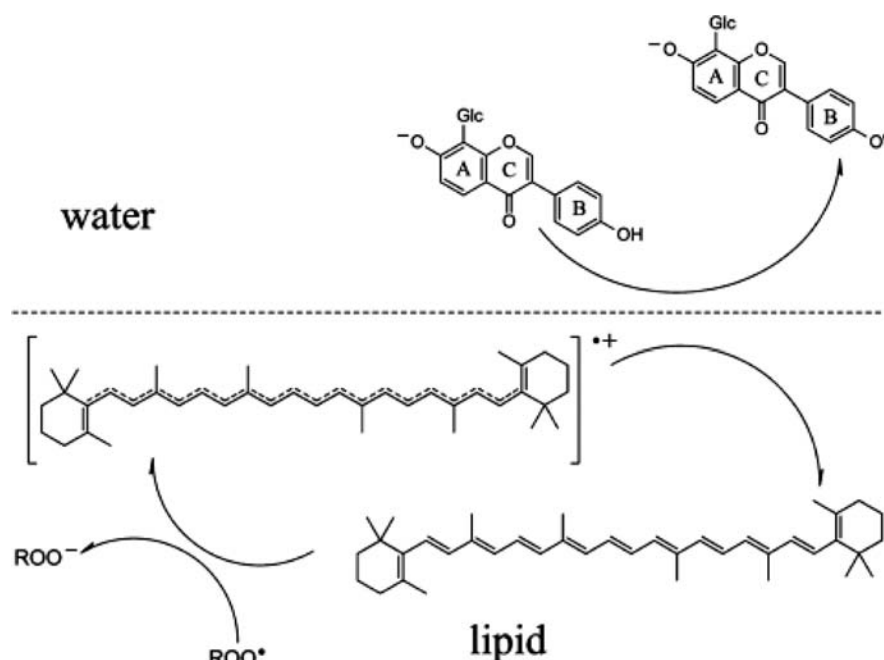


Figure 8. Regeneration of β -carotene radical cation formed in the lipid phase during oxidative stress by the anion of the water-soluble C-glycoside of the isoflavonoid daidzein. Reproduced from ref 46.

Table 2. Second-Order Rate Constants for Electron Transfer from Daidzein Dianion or from the Dianion of Its C-Glycoside Puerarin to Carotenoid Radical Cation in Methanol/Chloroform (1:9) at 25 °C Together with Standard Reduction Potentials versus NHE and Electron Acceptor Capacities, R_a , and Electron Donor Capacities, R_d , of the Carotenoid^{37,44}

	second-order rate constant ($L mol^{-1} s^{-1}$)		E°/V	R_a	R_d
	daidzein ²⁻	puerarin ²⁻			
β -carotene	5.8×10^9	5.5×10^9	0.84	0.46	1.40
zeaxanthin	8.3×10^9	8.5×10^9	0.85	0.49	1.44
canthaxanthin	5.7×10^{10}	6.5×10^{10}	0.95	0.82	1.93
astaxanthin	9.2×10^{10}	11.1×10^{10}	0.97	0.94	2.10

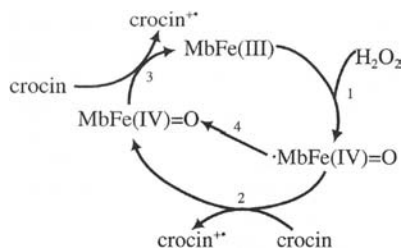


Figure 9. Metmyoglobin as a pseudoperoxidase oxidizes the water-soluble carotenoid crocin from saffron when activated by hydrogen peroxide.⁴⁴ The rate of reduction of perferrylmyoglobin (2) is faster than the reduction of ferrylmyoglobin (3).

solvents, whereas hydrogen atom abstraction occurs in both polar and apolar solvents as shown by calculation using density function theory accounting also for solvent effects.

Nitric oxide is also a radical, but it reacts quite differently with β -carotene, as it adds to the conjugate polyene backbone forming nitroxides.^{49,50} Whether such addition reactions, also known for sulfur radicals,^{24,51} which have been followed by electron spin resonance spectroscopy, are reversible is unclear.

Carotenoids are bleached in the presence of nitric oxide under anaerobic conditions, but clearly the reaction leading to a stable nitroxide as shown in Figure 10 depends on hydrogen atom abstraction, which could result from traces of NO_2 in the NO gas. NO is in the water phase an important antioxidant in animals and reversibly bound in nitrosylmyoglobin, MbFe(II)-NO. Nitroxides are stable radicals and may be a reservoir of NO in membranes or may act directly as an antioxidant.

RESEARCH PERSPECTIVES

Carotenoids may, depending on the balance between ionization energy and electron affinity for the individual carotenoid rather than on the reduction potential of the carotenoids, affect oxidative processes in biological systems including food by radical scavenging (i) through reduction as in the case of hydroxyl radicals, alkoxy, and peroxy lipid radicals and phenoxy radicals or (ii) through oxidation as in the case of the superoxide anion. Radical adduct formation with carotenoids competes with electron transfer from carotenoid to the radical and with hydrogen atom transfer. Neutral carotenoid radicals formed by hydrogen atom transfer exhibit nanosecond lifetimes, which are much shorter lived than carotenoid radical adducts, carotenoid radical anions, and especially carotenoid radical cations with millisecond lifetimes. The longer lived carotenoid radical cations formed by radical scavenging through electron transfer react with other antioxidants in one-electron regeneration reactions including other carotenoids, chromanol derivatives such as α -tocopherol, ascorbate, or plant polyphenols, establishing a basis for antioxidant system hierarchies. For hydrophilic antioxidants kinetically controlled interfacial phenomena become important for antioxidant synergism with the more lipophilic carotenoids, as demonstrated for carotenoid/polyphenol combinations, and may be of importance for eye (and skin) health. Synergism between xanthophylls such as astaxanthin and carotenes such as lycopene as antioxidants in membranes depends on anchoring of the xanthophylls in the water/lipid interface, where especially carotenoid hydroxyl and keto groups facilitate electron transfer with the xanthophylls serving as

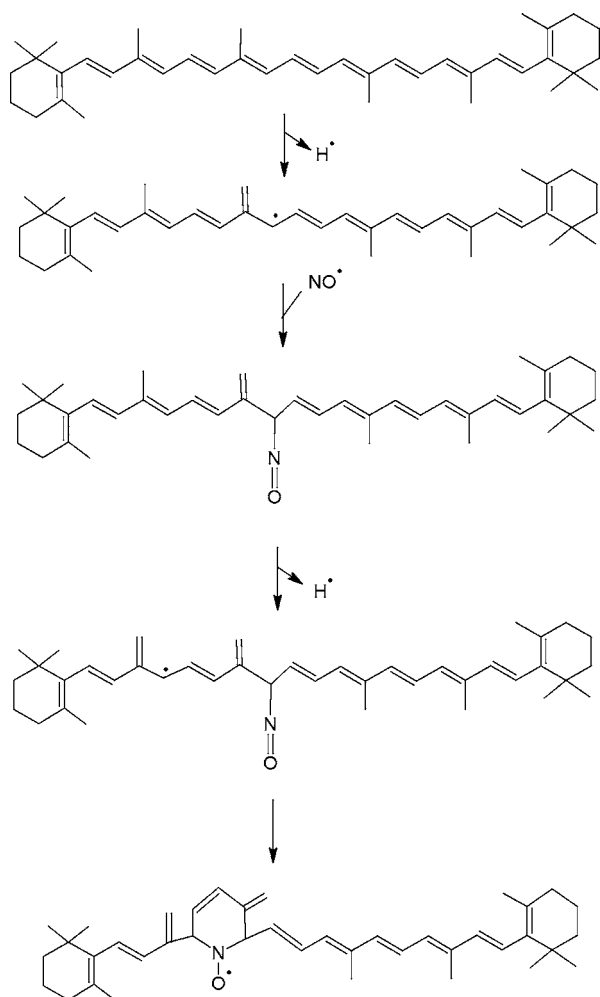


Figure 10. Nitric oxide bleaches carotenoids, forming stable free radicals identified as nitroxides.^{49,50} Nitrogen dioxide (or hydroxyl radicals) may be involved in hydrogen atom abstraction from the carotenoid prior to addition of nitric oxide.^{24,47}

“molecular wiring” across the membrane. Antioxidant equilibrium with thermodynamic control may, in contrast, be important in carotenoid-based signalization of health in sexual selection for some animals. A physiological role of interaction between carotenoids and nitric oxide resulting in stable nitroxides as potential lipid-based antioxidants is less clear.

Future studies should focus on the role of carotenoids as molecular wiring in membranes and other biological structures using improved time resolution to follow intramolecular electron transfer in addition to intermolecular electron transfer. Quantum mechanical calculations should further be expanded to model transition states for electron transfer between different carotenoids and between carotenoids and other potential antioxidants. The results of such calculations should be followed by experimental studies in which mixtures of carotenoids are compared with individual carotenoids for their protective effects against oxidative stress including the role of carotenoids as protectors of proteins against carbonyl formation and oxidative cross-linking.

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Funding

Continuing support from the Danish Research Councils, currently the Danish Council for Independent Research – Technology and Production, as project “Redox communication in the digestive tract” (09-065906/FTP) is gratefully acknowledged.

Notes

The author declares no competing financial interest.

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