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Highlight Review

Carotenoid Radicals: Cryptochemistry of Natural Colorants

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Abstract

This review examines the properties and uses of carotenoid radicals. Their mechanisms of action on the molecular level play a crucial role in electron transfer and light regulation processes in photosynthesis, in the design of improved artificial solar cells, and in the scavenging of toxic free radicals. There is a remarkable increase in the quantum yield and the lifetime of the charge-separated states of the carotenoid radical cations in the supramolecular "hosts" β-glycyrrhizic acid (GA) and arabinogalactan (AG).

♦ Introduction

About 750 various carotenoids are known at this moment.[1] A few examples of natural and synthesized carotenoids **I–XII** are given in Figure 1. About 20 carotenoids have been found in human tissues. Carotenoids provide the color of foliage, fruits, vegetables, fish, birds, and insects: β-carotene (**I**) (carrot, crustaceans, sponges, locusts, bug lice), lycopene (**VIII**) (tomato), zeaxanthin (**III**), violaxanthin (**IV**), lutein (**XI**), 9′-*cis*-neoxanthin (**XII**) (pea, spinach, green leaves), astaxanthin (**V**) (algae, salmon, shrimp, aphids), canthaxanthin (**II**) (flamingo feathers, moths, crustaceans), and so on. Furthermore, 8′-apo-carotenal (**VII**) is widely used as food colorant such as in cheddar cheese. Absence of carotenoids in animals and fish hinders reproduction.

Carotenoids also play an important role in light harvesting, energy, and electron transfer in photosynthesis.[2,3] Light absorbed by carotenoids in the blue-green region is transferred to bacteriochlorophyll (BChl) or chlorophyll (Chl) via singlet–singlet energy transfer with variable efficiency.[2–4] The carotenoid cofactor in the photosystem II reaction center can act as an alternative electron donor to reduce $P680^+$ under condition where the primary electron-transfer pathway is blocked.[5,6] In addition carotenoids protect the photosynthetic apparatus from excess solar light by quenching the triplet state of BChl or Chl, and singlet oxygen.[7,8] Furthermore, formation of the zeaxanthin–chlorophyll ($Zea^{\bullet+}\cdots Chl^{\bullet-}$) charge-transfer complex in intact thylakoid membranes causes the excess energy from intense sunlight to be dissipated through the C–C vibrational modes of the radical cation.[9] Thus, photosynthetic light harvesting is regulated. This charge-transfer quenching mechanism to form a radical cation has also been observed in all light-harvesting complexes II (LHC-II) CP24, CP26, and CP29.[10–12]

Why carotenoids were chosen by nature for the utilization of light energy and participating in electron-transfer processes in photosynthesis—is a topic of great current research through out

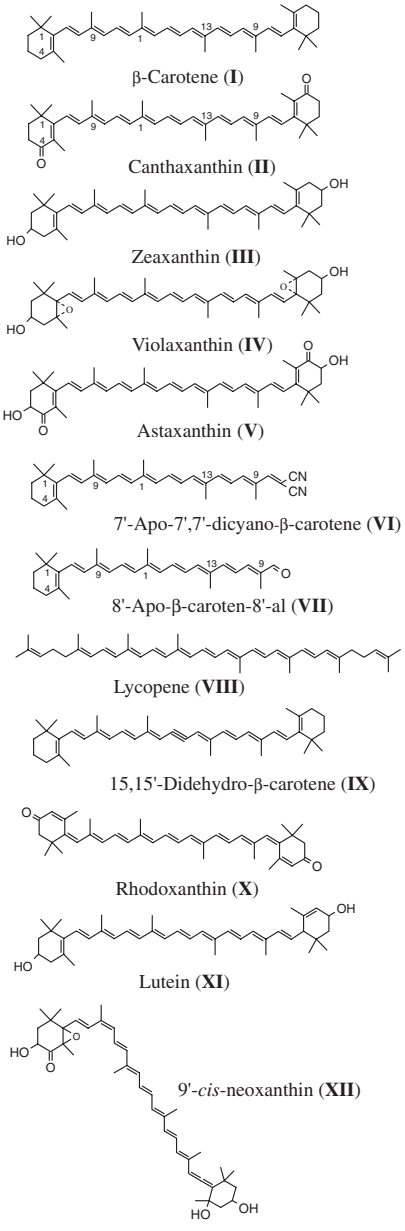

Figure 1. A few examples of natural (**I–V**, **VII**, **VIII**, **X**, **XI**, and **XII**) and synthesized (**VI** and **IX**) carotenoids.

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the world. Of special interest is what structural features of carotenoids are important for their biological activity? In this review we will show that the key to their activity may lie in the unique features of the carotenoid radicals.

Carotenoid radicals can be generated chemically (via reaction with electron acceptors, for example, quinones[13] or metal ions[14–19]), photochemically (via photoreaction with electron acceptors[18,20] or even general solvents[21]), and electrochemically.[22–24] Radical ions and neutral radicals of carotenoids can be detected by optical absorption techniques[13,14,16,17] and electron paramagnetic resonance spectroscopy (EPR).[13,15,18–21,25] Some highlights of these studies are presented below to show that the properties of the carotenoids and their radicals can be modified significantly by their incorporation into supramolecular "hosts." Incorporation of carotenoids into the "host" macromolecules β-glycyrrhizic acid[26] and arabinogalactan[27] has the following advantages: enhancing bioavailability of the carotenoids; protection against sunlight, temperature, oxidation, and hydrolysis; preventing ingredient interactions; simplify handling.

♦ Electrochemical Generation of the Carotenoid Radical Cations, Dications, and Neutral Radicals

First of all, carotenoids exhibit low oxidation potentials and are thus good electron donors. Simultaneous electrochemical and EPR studies (SEEPR) of a series of carotenoids have demonstrated that carotenoid radical cations are formed during the first oxidation step (eq 1) followed by the formation of diamagnetic dications upon transfer of the second electron (eq 2).[22] A very important feature of the carotenoid radical cations is the existence of an equilibrium between the radical cation, dication, and the original carotenoid in solution as given in eq 3. The SEEPR measurements of the carotenoid oxidation show that the following processes take place in dichloromethane solution:

$$\mathrm{Car} \overset{E^0_{1OX}}{\rightleftharpoons} \mathrm{Car}^{+\bullet} + \mathrm{e}^- \quad (1)$$

$$\mathrm{Car}^{+\bullet} \overset{E^0_{2OX}}{\rightleftharpoons} \mathrm{Car}^{2+} + \mathrm{e}^- \quad (2)$$

$$\mathrm{Car} + \mathrm{Car}^{2+} \overset{K_{com}}{\rightleftharpoons} 2\mathrm{Car}^{+\bullet} \quad (3)$$

where Car is the neutral carotenoid species, $\mathrm{Car}^{+\bullet}$ is the radical cation, and Car^{2+} is the dication. The equilibrium constant depends on the electron donor (cyclohexane ring or methyl end groups) or acceptor substituents (F-substituted phenyl, keto, or dicyano) of the carotenoid. For example, step 1 for canthaxanthin occurs at an oxidation potential, $E^0_{1OX} = 0.77\,\mathrm{V}$ and step 2 at $E^0_{2OX} = 0.97\,\mathrm{V}$ (electron acceptor, keto groups) versus SCE (standard calomel electrode).[23] The comproportionation equilibrium constant (K_{com}, step 3) was found to be equal $K_{com} = 2.2 \times 10^3$, which favors the presence of radical cations in solution for canthaxanthin (strong acceptor substitutent). The same measurements for β-carotene (cyclohexene ring, electron donor) showed a single oxidation wave and maximum EPR intensity to occur at a higher potential than the maximum of the cyclic voltammetric (CV) plot. Extensive and very careful electrochemical studies demonstrated, surprisingly, that two-electrons are transferred in the first oxidation step so that the formation of the β-carotene dications occurs at a lower oxidation potential ($E^0_{2OX} = 0.60\,\mathrm{V}$ vs. SCE) than the formation of the radical cations ($E^0_{1OX} = 0.63\,\mathrm{V}$ vs. SCE) due to the solvation energy of the dication.[23] K_{com} for β-carotene equals 0.3, thus favoring the presence of the diamagnetic dications rather than the paramagnetic radical cations. The equilibrium given by eq 3 was directly observed by EPR measurement.[22] When SEEPR experiments were carried out for canthaxanthin dication in the presence of an excess of the canthaxanthin, the EPR intensity increased as a function of the diffusion rate of neutral canthaxanthin in the CH_2Cl_2 solvent, indicating electron transfer to the canthaxanthin dication forming paramagnetic radical cations. The electrochemical properties of wide range of carotenoids have been examined and summarized.[22–24]

Another important feature of the carotenoid radical cation and dication is a tendency to lose a proton, H^+, to form a neutral carotenoid radical or a cation according to the following reactions:

$$\mathrm{Car}^{2+} \overset{K_{dp}}{\rightleftharpoons} \#\mathrm{Car}^{+} + \mathrm{H}^+ \quad (4)$$

$$\mathrm{Car}^{+\bullet} \overset{K'_{dp}}{\rightleftharpoons} \#\mathrm{Car}^{\bullet} + \mathrm{H}^+ \quad (5)$$

$$\#\mathrm{Car}^{+} + \mathrm{e}^- \overset{E^0_3}{\rightleftharpoons} \#\mathrm{Car}^{\bullet} \quad (6)$$

Here $\#\mathrm{Car}^+$ is the deprotonated cation and $\#\mathrm{Car}^\bullet$ is the deprotonated neutral carotenoid radical.[24] The site of proton loss depends on the terminal substituents of the carotenoid. For β-carotene radical cation the deprotonation of 5-CH_3 or 4-CH_2 groups at the terminal double bond of the cyclohexene ring produces the most stable neutral radical because of the extent of the π-conjugated system, while the deprotonation at 9-CH_3 and 13-CH_3 methyl groups causes significant reduction of the conjugation.[28,29] The magnitude of pK_{dp} ($pK_{dp} = -\log K_{dp}$) or pK'_{dp} reflects the tendency to lose a proton. pK_{dp} values near -2 and pK'_{dp} values from 4 to 7 were deduced for various carotenoids.[24] These examples indicate that proton removal from Car^{2+} is much easier than from $\mathrm{Car}^{\bullet+}$.

Electrochemical reversibility and the stability of the radical cations and dications can be approximated from the ratio of the cathodic and anodic currents. If the ratio is 1, the dications or radical cations are relatively stable and have a relatively long lifetime so reactions (1) and (2) are quasi-reversible. However, the cathodic peaks are often less than 50% of the anodic current (see example, Figure 2) which indicates that the dications and cation radicals are not stable and decay at ambient temperatures (Figure 2).[30]

If traces of water (ca. 0.1 mM) are present, reaction (4) can predominate and only a CV peak due to reaction (6) will appear and almost no cathode current for the reduction of Car^{2+} will appear.

Electrochemical measurements of carotenoids where the substituents are varied from electron donors like cyclohexene in β-carotene to groups containing strong electron acceptors like the dicyano ($-C(CN)_2)_2$, aldehyde (–CHO), or keto group showed that the oxidation potential increased by about 200 mV from **I** (donor) to **II** (acceptor).[31] Similar studies of acetylenic carotenoids (those containing one central triple bond), show increased oxidation potentials from **I** by ca. 250 mV for **IX**.[32] Addition of the central triple bond in **I** to form **IX** decreases the concentration of the radical cation by an order of magnitude, due to rapid electron-transfer rates (as deduced electrochemically) to form dications. The long polyene chain accommodates the

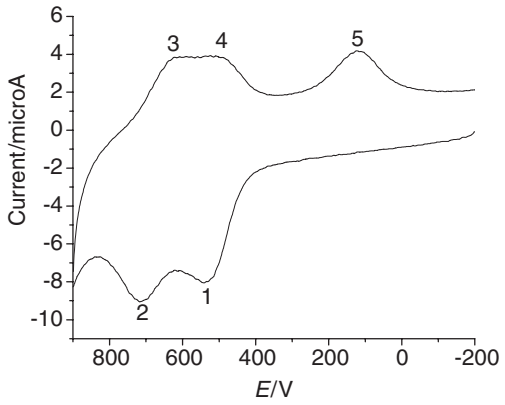


Figure 2. CV plot of the thiol-substituted carotenoid, 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene on a gold electrode. Peaks 1 and 2 are due to formation of radical cation and dication, and peak 5 is from neutral radical.

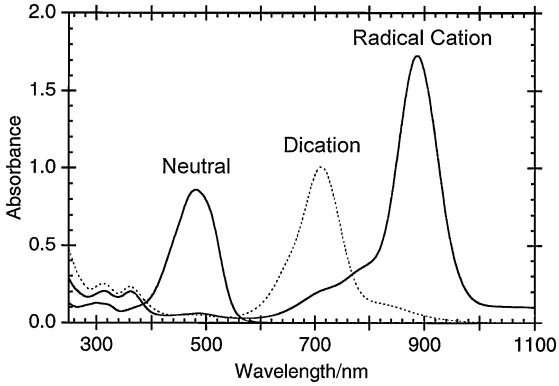


Figure 3. Optical absorption spectra of the canthaxanthin molecule, radical cation and dication detected in dichloromethane in the presence of different concentrations of ferric chloride.

distribution of two positive charges in the two halves of the molecule. Deprotonation of the dication of **IX** is much faster than that of **I**. SEEPR studies of keto and hydroxy carotenoids show that K_{com} varies by four orders of magnitude, from 2 for zeaxanthin (**III**) to 10^4 for rhodoxanthin (**X**).[24] Little change in oxidation potential occurs upon substitution of one hydroxy group at the 4- or 4′-position of **I**; however, substitution of a keto group at the 4- or 4′-position increased the E_{ox} by 175 mV. It also has been shown that an increase in chain length results in the decrease of the first and second oxidation potentials for series of ester, aldehyde and cyano-substituted carotenoids.[33]

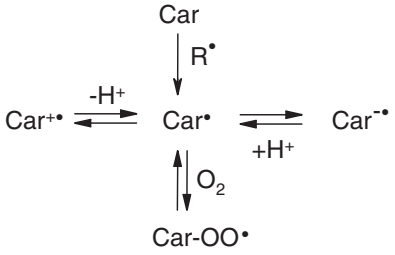
♦ *Optical Study of the Carotenoid Radicals in Solution*

Due to the presence of the long polyene chain, carotenoid radical cations are quite stable in anhydrous solvents at low concentrations (less than 10^{-5} M) even at room temperature, which made it possible to detect their absorption spectra. As an example, the optical spectra of the radical cations of **I** and **II** consist of a $D_0 \rightarrow D_1$ transition at 1425 and 1310 nm respectively and a $D_0 \rightarrow D_2$ transition at 990 and 885 nm, respectively. The optical absorption spectra of their dications in dichloromethane have been also reported (Figure 3).[34] The molar extinction coefficients of radical cations and dications were determined because they did not decay appreciably during the measurement (<1 min).[34]

Much less attention is paid to carotenoid radical anions as compared with their radical cations. The radical anions of carbonyl-containing carotenoids **II**, **V**, and **VII** were prepared by pulse radiolysis in aqueous Triton-X100 and methanol solutions.[35] The absorption maxima of these species are in the range of 590–750 nm in aqueous Triton-X100 solution and 550–620 nm in methanol. The radical anions are rapidly transformed to its protonated forms via reaction with the solvent with rate constants 10^3–10^5 M^{-1} s^{-1}. Corresponding neutral radicals of the carotenoids have absorption maxima at 510–570 nm in aqueous Triton-X100 solution and in methanol. The pK_a value of **II**-H$^\bullet$ was estimated as 11.7 ± 0.2 and of **V**-H$^\bullet$ and **VII**-H$^\bullet$ as 10.5 ± 0.5. In addition, investigation of the reactions of carotenoids with reducing radicals in aqueous Triton-X100 reveals that the reduction potentials for astaxanthin, canthaxanthin, and β-apo-8′-carotenal are near -1450 mV, and that those for β-carotene and zeaxanthin are in the range -1950 to -2100 mV.[36] We can conclude that carotenoid radical cations are strong oxidizing agents, but their radical anions are very strong reducing agents.

There are also a few published examples of the absorption spectra of carotenoid neural radicals produced in the reaction of carotenoids with free radicals. For example, long-lived species with absorption maximum in the 750–850-nm range were attributed to the radical adducts of carotenoids with neutral peroxyl radicals.[37] In 1984, Burton and Ingold suggested that β-carotene scavenges peroxyl radicals by addition to the conjugated system of double bonds, yielding a resonance-stabilized carbon-centered radical (ROOCar$^\bullet$).[38] This feature of the carotenoid radicals has significant importance for their application in medicine since free radicals are suggested to be an important factor contributing to the development of various diseases, including infarction, cerebral thrombosis, and tumors.[39]

The important feature of carbon-centered carotenoid radicals is their reaction with oxygen.[40,41] Stern–Volmer analysis of the variation of the decay rate of carotenoid radicals with oxygen concentration leads to rate constants for oxygen addition from 10^3 to 4×10^4 M^{-1} s^{-1} for different carotenoids in benzene. Although oxygen additions to carbon-centered radicals are usually diffusion-controlled ($K \approx 10^9$ M^{-1} s^{-1}),[42] increased resonance stabilization of carbon-centered radicals leads to a reduction in the rate constant for oxygen addition.[43] Another conclusion of these studies was reversibility of oxygen addition to the carbon-centered radicals. We can propose the following scheme for the reactions of carotenoid radicals:



(7)

Here Car$^\bullet$ is a resonance-stabilized carbon-centered radical. The main decay pathway of the carotenoid radicals in the absence of

oxygen is the formation of dimers.[44] On the other hand, the formation of Car–OO$^{\bullet}$ radicals has been assumed as a source of pro-oxidant effect of some carotenoids. The role of carotenoid radicals in antioxidant and pro-oxidant activity of carotenoids is a subject of several reviews and research articles.[36,45–49]

♦ Electron Transfer Mediated cis–trans Isomerization of Carotenoids

Since carotenoids have a number of double bonds, *cis–trans* isomerization should be one of the main reaction pathways for their transformation in photochemical processes, as it was earlier observed for a wide series of substituted ethylenes and short polyenes.[50,51] In these examples *cis–trans* isomerization mainly occurs via singlet or triplet excited states of the molecules. However, in the case of carotenoids, isomerization occurs through the formation of their radical cations and dications.[52,53] Isomers of carotenoids are formed by chemical, electrochemical, and photo oxidation,[17,54,55] as well as by acid-induced isomerization.[56] Also *cis* isomers of carotenoids were found in fresh and processed fruit and vegetable products.[57]

Cis isomers of **I** and **II** were observed to form in the presence of even 0.1 mM of acid in HPLC grade dichloromethane.[56] The presence of this concentration of acid results in protonation of the carotenoid molecule and will induce isomerization, forming 9-*cis* and 13-*cis* isomers in greater yield than other *cis* isomers. AM1 molecular orbital calculations predict that free rotation would occur around the carbon atom with the more positive charge density, meaning the formation of the 9-*cis*, 13-*cis*, 15-*cis*, 13′-*cis*, 9′-*cis*, and 7′-*cis* isomers, respectively.[42] However, the 9-*cis* and 13-*cis* isomers are more energetically favorable. AM1 calculations showed that multiple protonation sites for the carotenoid polyene chain are possible. Optical spectra of Car–H_2^{++} exhibit peaks in the range of 700–1020 nm, close to the optical spectra of the corresponding radical cations.

Electrochemical oxidation of *all-trans* **I** and **II** leads to significant *trans* to *cis* isomerization (ca. 50% products formed).[52,54] The ratio of isomers detected by HPLC technique is 50:4:21:21:4 for *all-trans*, 15-*cis*, 13-*cis*, 9-*cis*, and 9,13-*dicis* isomers, respectively. The reason for this is that the bond lengths of the double bonds in the polyene lengthen upon formation of the radical cation. This lowers the energy barrier to isomerization. Even greater bond lengthening and reduction in rotation barrier occurs for the dications.[52–54] Subsequently, *cis*-dications are converted by the comproportional equilibrium eq 3 to *cis*-radical cations which are then transformed to neutral *cis*-carotenoids by exchanging one electron with the neutral *trans*-carotenoids. The reaction of carotenoids with metal ions or other electron acceptors results in the formation of *cis*-isomers from *all-trans* materials according to the same mechanism as outlined for electrolysis of carotenoids.[17]

The difference in isomer distribution was detected when the reaction of electron transfer occurs in organized media, in particular MCM-41 molecular sieves.[55] It was discovered that large yields of 13-*cis* isomer of **I** are formed in MCM-41 and metal-substituted MCM-41 molecular sieves. This is in contrast to the distribution of isomers that are formed by electrolysis or in photoinitiated electron-transfer processes. The 13-*cis* isomer of **I** forms because it is the lowest-energy conformation that can exist oriented around the cylinder of the sieve. The EPR technique provides direct evidence for the reduction of metal ions in metal-substituted MCM-41 during the photolysis in the presence of carotenoids.[55]

♦ Advanced EPR Study (ENDOR, ESEEM, and HYSCORE) and DFT Calculation of the Carotenoid Radical Structures

Advanced EPR techniques[58] such as ENDOR (electron nuclear double resonance), ESEEM (electron spin-echo envelope modulation), and HYSCORE (hyperfine sublevel correlation spectroscopy) have been successfully used to elucidate the magnetic properties of carotenoid radicals as well as to examine complexation and electron transfer between carotenoids and the surrounding media in which the carotenoid is located.[18,28,59]

Due to large number of proton couplings, the EPR spectrum of carotenoid radicals is a broad unresolved line of 13 ± 0.5 Gauss peak-to-peak line width with $g = 2.0027$. To resolve the hyperfine couplings, ENDOR measurements have been carried out. For each set of equivalent protons, only two ENDOR lines separated by the hyperfine coupling constant occur instead of multiple EPR lines. HYSCORE is a two-dimensional technique which provides correlation between nuclear frequencies originating from different electron manifolds. The α-proton anisotropic couplings were detected by this technique.[18,28]

By using these techniques the evidence of neutral carotenoid radical formation in photochemical processes was obtained.[28] DFT calculations confirmed that the isotropic β-methyl proton hyperfine couplings do not exceed 9 MHz for the carotenoid radical cation, Car$^{\bullet+}$. DFT calculations of neutral carotenoid radicals, #Car$^{\bullet}$, formed by proton loss from the radical cation, predicted isotropic β-methyl proton couplings up to 16 MHz.[28] These results explain the large isotropic couplings observed by ENDOR measurements for methyl protons in UV irradiated carotenoids supported on silica gel, Nafion films, silica–alumina matrices or incorporated in molecular sieves.[60] It has now been shown that the neutral carotenoid radical is not formed in absence of light. According to the DFT calculations of the zeaxanthin (Zea) neutral radical, the most energetically favorable is the loss of a proton from 4-CH_2 group (#Zea$^{\bullet}$(4)). In the case of violaxanthin (Vio) radical cation, loss of a 9-CH_3 proton produces the most energetically favorable neutral radical (#Vio$^{\bullet}$(9)). Examples of the unpaired spin distribution of the radical cations and neutral radicals are depicted in Figure 4.[28]

Proton loss from the 4-CH_2 and 5-CH_3 groups of violaxanthin radical cation forms structures higher in energy than the most stable neutral radical, #Vio$^{\bullet}$(9) and would generate neutral radicals that are not experimentally observed in the EPR spectrum and apparently are not formed. This difference (the absence or presence of an epoxide group) between zeaxanthin and violaxanthin in the location of proton loss has been shown to be critical to the quenching of chlorophyll excited states in the light harvesting center in the presence of excess light.[28]

Could neutral radicals of zeaxanthin and violaxanthin be important in understanding photosynthesis? There are a number of different photoprotection mechanisms at work in photosynthetic systems, and one class, nonphotochemical quenching (NPQ), involves the dissipation of energy absorbed in the light-

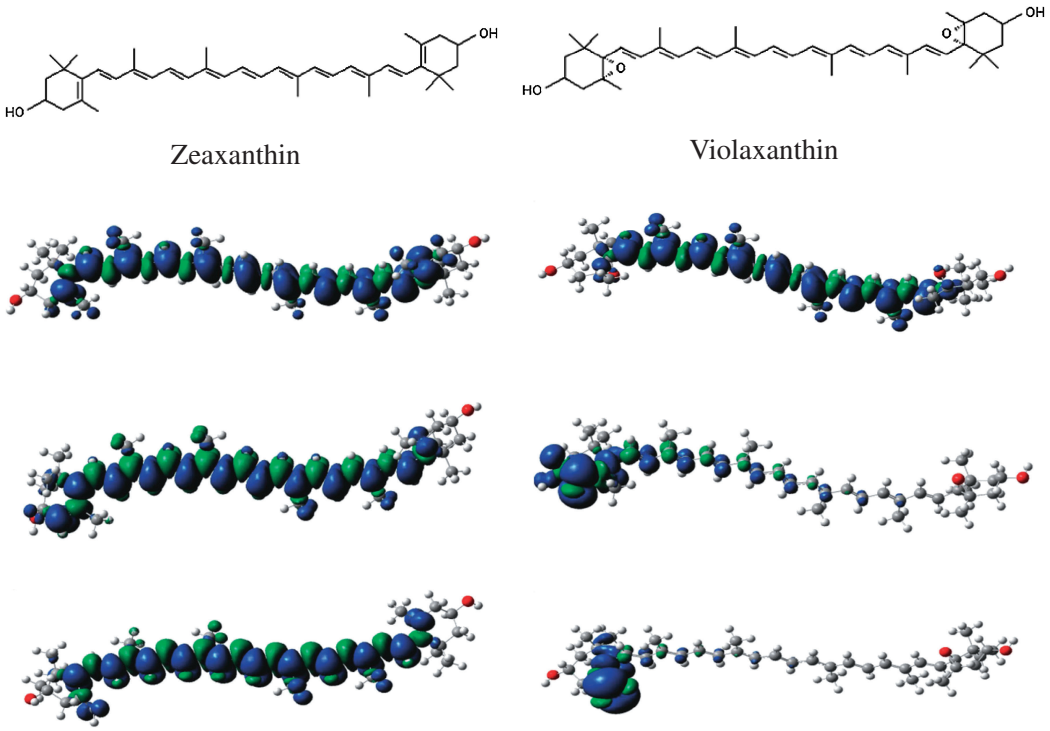
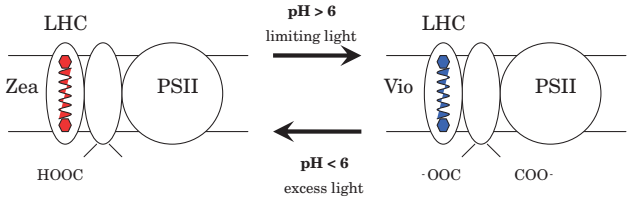


Figure 4. Unpaired spin distribution for zeaxanthin (left) and violaxanthin (right) radicals. From up to down: $Zea^{\bullet+}$, $\#Zea^{\bullet}$(4), $\#Zea^{\bullet}$(5) and $Vio^{\bullet+}$, $\#Vio^{\bullet}$(4), $\#Vio^{\bullet}$(5), respectively. The blue represents excess α and the green excess β unpaired spin density. The carbon atoms are gray, the oxygen atoms are red, and the hydrogen atoms are white.



Scheme 1.

harvesting apparatus into thermal energy.[61] These mechanisms involve carotenoids of the xanthophyll cycle (Scheme 1) specifically zeaxanthin and violaxanthin. A pH gradient is generated across the thylakoid membranes in the presence of excess light. The cycle provides the replacement of Vio by Zea in the light-harvesting complex II (LHC-II) under high light conditions. The presence of Zea leads to dissipation of excess excitations in LHC-II by forming a charge-transfer complex $Zea^{\bullet+}\cdots Chl^{\bullet-}$ on the femtosecond time scale.[62] The excess energy is dissipated in the vibrational modes of $Zea^{\bullet+}$.

Once $Zea^{\bullet+}$ is formed, loss of H^+ from the 4 methylene position and the 5-CH_3 group of $Zea^{\bullet+}$ would form the neutral radical $\#Zea^{\bullet}$ in the polar region of LHC-II according to the follow equations.

$$Zea\cdots Chl \xrightarrow{h\nu} Zea^{\bullet+}\cdots Chl^{\bullet-} \quad (8)$$

$$Zea^{\bullet+} \xrightarrow{h\nu} \#Zea^{\bullet} + H^+ \quad (9)$$

$$\#Zea^{\bullet}\cdots Chl^{*} \longrightarrow \#Zea^{\bullet}\cdots Chl \quad (10)$$

The presence of the epoxide prevents the formation of the neutral radical $\#Vio^{\bullet}$ in the polar region of LHC-II. In the context of NPQ, formation of the neutral radical would have two significant consequences. First, collapse of the charge-transfer state to regenerate Zea and Chl is blocked because the product would be the conjugate base of Zea, a highly unstable product. Second, free radicals are very efficient quenchers of both singlet and triplet excited states, so that $\#Zea^{\bullet}$ would be a very efficient quencher of excitation in LHC-II. The neutral radical of Zea would effectively shut down the input of energy to photosystem II (PS II).

Other examples also exist: DFT and EPR measurement[63] show that once the lutein (**XI**) radical cation is formed, proton loss from the C6′ methyl group is likely, generating a neutral radical (#Lut(4) and #Lut(5)). Carotenoid neutral radicals were detected in PS II.[64] On the other hand 9′-*cis*-neoxanthin radical cations were shown not to form neutral radicals because of the presence of expoxy and alkene groups which disrupts the conjugation needed to form neutral carotenoid radicals.[63]

♦ *The Effect of Supramolecular "Host" on the Properties of Carotenoid Radicals*

It is well known that the wide practical application of carotenoids is substantially hampered by their hydrophobic properties, instability in the presence of oxygen and metal ions, and high photosensitivity. Application of the "host–guest" inclusion complexes was first related to an attempt to minimize the aforementioned disadvantages of carotenoids when these compounds are used in food processing (colors and antioxidant capacity) as well as for production of therapeutic formulations considering the better solubility and consequently higher bioavailability.[65–69]

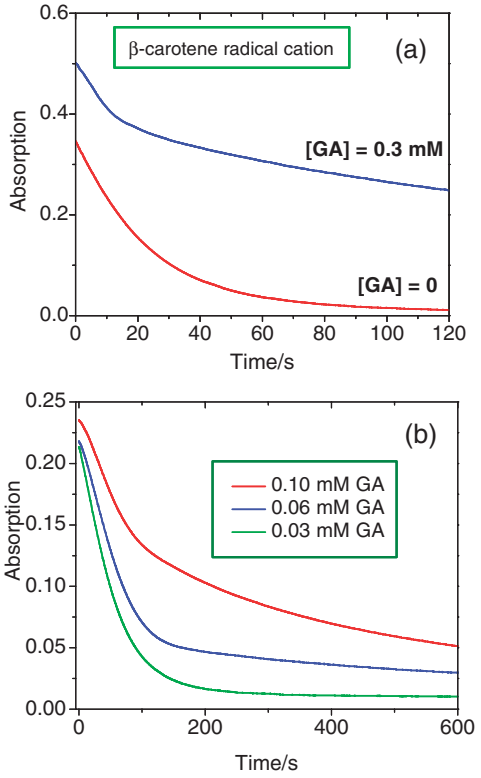


Figure 5. Kinetics of the decay of β-carotene radical cation at 935 nm. (a) 4 μM β-carotene + 4 μM DDQ with and without GA; (b) 2 μM β-carotene + 5 μM DDQ for various GA concentrations.

Stability of Carotenoid Radical Cations in Solution

Recently it was demonstrated that complexation significantly affects also the reactivity of carotenoids as well as the properties of carotenoid radical ions.[26,27,70] As an example, inclusion complexes of carotenoids **II** and **VI** with natural triterpene glycoside, β-glycyrrhizic acid (GA) show enhanced ability (in orders of magnitude) to scavenge free radicals.[26,70] A very important observation is determining the effect of GA on the oxidation potential of carotenoids.[26]

It was demonstrated that incorporation of carotenoids into a supramolecular "host" results in stabilization of carotenoid radicals due to the reduced recombination rate of the radical ion pair.[27,70] Figure 5a demonstrates the difference in the decay kinetics of the β-carotene radical cation in homogeneous acetonitrile solution and in the GA complex in the reaction with quinone at room temperature.[70] Dichlorodicyanobenzoquinone (DDQ) was used as a model system, which, owing to its low reduction potential, reacts with carotenoids without additional initiation by either light or temperature. Whereas for a free carotenoid, the radical cation signal decays exponentially with a half-life time τ of ca. 20 s, in the presence of GA bimodal decay was observed. The fast process exhibits exponential decay with $\tau \approx 20$ s, and the slow process is described by eq: $I = a/(1 + t/\tau)$, with $\tau \approx 1000$ s.

The slow component increases with increasing GA concentration (Figure 5b). It was suggested that the slow decay component is due to the radical cation in the complex. This feature of carotenoid complexes might be useful in the design of the artificial photosynthetic devices and solar cells. Some attempts have been made to increase the quantum yield and the lifetime of the charge-separated state in order to design an efficient model of the natural photosynthetic center.[71] The advantage of the carotenoids is the presence of a long unsaturated chain which provides charge distribution and stabilization of their radical cations.

Stability of Carotenoid Radicals on Titanium Dioxide Nanoparticles

An unique stability was demonstrated by the canthaxanthin radical cation in the solid-state natural polysaccharide arabinogalactan (AG) complex adsorbed on the surface of titanium dioxide nanoparticles.[27] Among the semiconductors, titanium dioxide is the most suitable for many environmental applications. Due to its ability to absorb light, TiO_2 is widely used in photocatalysis and in artificial solar cells.[72–74]

Figure 6 provides the schematic illustration of the photocatalytic reactions of carotenoid (Car) adsorbed on the surfaces of TiO_2 nanoparticles.

Canthaxanthin–AG complex irradiated on TiO_2 at $T = 77$ K by visible light ($\lambda > 350$ nm) shows (Figure 7) a significant increase in the intensity of the EPR signal (plot c) with $g = 2.0027 \pm 0.0002$ and $\Delta H_{pp} = 13.0 \pm 0.5$ G, which is characteristic of a carotenoid radical cation, compared to that of the pure carotenoid (plot b).[27]

It is suggested that the low yield of the charge-separated state in the absence of AG might be due to efficient back electron transfer on semiconductor materials. The "redox cycling," where a product of the hole transfer acts, in turn, as scavenger for the photogenerated electrons, appears as a frequent cause of weak photocurrents.[75] The isolation of the carotenoid radical cation from the TiO_2 surface by incorporation into the polysaccharide matrix allows more efficient charge separation, reducing the rate of back electron transfer. This is an important property, useful in preparing improved artificial solar cells.

According to earlier published results, carotenoid radical cations generated on the surface of TiO_2 are stable at 77 K but disappear when the temperature is increased above 250 K.[76] The most important design feature of the carotenoid–AG complex is the significant increase in stability of the carotenoid radical cation. Increasing the temperature up to room temperature does

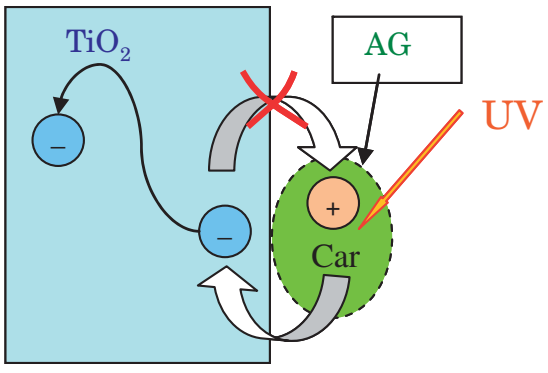


Figure 6. Schematic illustrations of the photocatalytic processes on the surfaces of TiO_2 nanoparticles in the presence of carotenoid (Car). This scheme illustrates the situation of the light absorption by carotenoid.

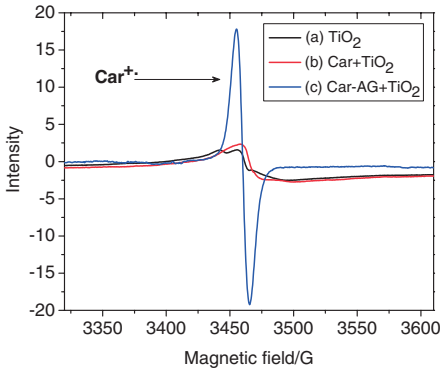


Figure 7. EPR spectra detected at 20 K after irradiation of TiO_2 powder (a); TiO_2 in the presence of canthaxanthin (b); and TiO_2 in the presence of canthaxanthin–AG complex in solid state (c).

not lead to disappearance of the EPR spectrum. The increase in lifetime of such molecular devices opens up wide possibilities for their use in molecular electronics as nanosized means of communication and data processing as well as in sensors. Simultaneously, the AG matrix protects the carotenoid from degradation decay.

♦ Conclusions and Outlook

In solution equilibrium exists between the carotenoid radical cation, dication, and the original carotenoid. This equilibrium shifts toward the formation of the radical cation for acceptor substituents and toward the dication for donor substituents. The oxidation potential is 0.60 V vs. SCE for β-carotene (donor) and increases by 200 mV for acceptor substituents. Carotenoid radical cations are weak acids with $pK_a = 4$–7, and the dications are much stronger acids with $pK_a \approx -2$. They tend to form neutral radicals or cations in the presence of trace of water by loss of a proton from the 4(4′)-CH_2 or 5(5′)-CH_3 groups stabilized by the extended π-conjugated system from such deprotonation. This feature of carotenoid radicals is important for their roles in the light-harvesting center of plants as well as in antioxidant activity.

A zeaxanthin radical cation is generated in the light-harvesting complex LHC-II when a charge-transfer complex is formed with a half-life of 100 fs in the presence of excess light between the carotenoid zeaxanthin and the excited chlorophyll. This is one of the ways that the excess energy not needed in high light conditions by the plant can be quenched by the polyene C–C vibrational modes. The unpaired electron density distribution predicted by DFT calculations is distributed along the polyene chain, forming a π-radical cation and characterized by an EPR spectrum at $g = 2.0027$. $Car^{\bullet+}$ can also be formed in the photosystem II (PS II) reaction center when the carotenoid cofactor acts as an alternative electron donor to reduce $P680^{\bullet+}$ when the primary electron pathway is blocked. Furthermore, the carotenoid neutral radical formed by proton loss of the radical cation is observed in PS II.

The optical absorption of the radical cations typically occurs at 885 to 990 nm with weaker transitions at 1300–1400 nm, while that for the dication occurs at lower (ca. 800 nm) wavelength. The absorption bands of the radical anions are typically observed at 550 to 750 nm.

Cis–trans isomerization of carotenoids occurs through electron transfer forming either a radical cation or a dication, because of the decrease barrier to the isomerization species. DFT calculations, confirmed by advanced EPR studies, predict the π-unpaired electron distribution for carotenoid radical cation.

Supramolecular complexes of carotenoids enable an enhanced bioavailability; increase stability to UV light, temperature, oxidation, and hydrolysis; prevent interaction with foreign materials; and simplify handling by reducing volatility and converting liquids to powders. These advantages open new possibilities to control reactivity of carotenoids in living systems and reduce the required dosage of antioxidants in medical preparations. The increase in stability of carotenoid radicals may permit their use in molecular electronics.

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