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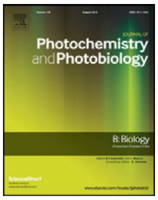

Radicals formed from proton loss of carotenoid radical cations: A special form of carotenoid neutral radical occurring in photoprotection

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ABSTRACT

In an organized assembly in *Arabidopsis thaliana* plant, proton loss from the radical cation of zeaxanthin (Zea$^{\bullet+}$) was found to occur under intense illumination, a possible component in photoprotection. A stable neutral radical is formed because of the favorable proton loss at C4(4′) position(s) of the terminal ends of Zea$^{\bullet+}$ that extends the unpaired spin density distribution (notation Zea•(4) or Zea•(4′) by symmetry). Proton loss from the radical cation of β-carotene (β-car$^{\bullet+}$) to available proton acceptors was also detected in a PSII sample upon irradiation. A controversial optical absorption peak at 750 nm predicted by DFT was attributed to β-carotene neutral radical formed by proton loss at C4(4′) position(s) situated on the terminal end(s) (notation β-car•(4) or β-car•(4′) by symmetry), also detected in solid copper-containing MCM-41 molecular sieves (Cu-MCM-41) and by hydrogen atom transfer from β-carotene to hydroxyl radical. Unlike the PSII organized assembly where proton loss occurs from the terminal ends of the radical cation, in the Cu-MCM-41 unorganized assembly proton loss occurs from all positions including the methyl groups on the polyene chain forming neutral radicals with different EPR couplings, different unpaired spin density distribution and different optical absorption peaks. We emphasize here the properties of these neutral radicals for various carotenoids formed under high-light conditions and in different media (solution, solid siliceous materials, and photosynthetic samples).

1. Introduction

Carotenoids are integral components of light-harvesting centers and photochemical reaction centers in photosynthetic organisms. A variety of functions have been proposed for carotenoids including scavenging of free radicals, auxiliary light-harvesting pigments, and protective agents to quench and dissipate excess energy present under high-light conditions when photosynthetic process is saturated. In non-polar solvents peroxyl radical (ROO•) addition to carotenoids (Car) occurs exclusively to give ROOCar• addition radicals and adsorption occurs only in the visible region around 500 nm like that of parent carotenoid [1]. This addition is favored by the long conjugated length of carotenoids such those given in Scheme 1.

In polar solvents however, due to their antioxidant property, carotenoids react by an electron transfer mechanism generating the radical cation Car$^{\bullet+}$ and ROO^{-}. This property of carotenoids in polar media has been used to form and stabilize radical cations Car$^{\bullet+}$ for long term study (days, rather than the short transient lifetime in solution) on electron acceptor matrices such as silica-alumina, and the catalysts MCM-41 and metal-substituted MCM-41 [2–9].

EPR measurements show that Car$^{\bullet+}$ is formed in the absence of light by electron transfer to the Lewis acid sites of the matrix surface, and upon light irradiation, neutral radicals (Car•) were detected. There was an order of magnitude increase in the concentration of carotenoid radicals, including both radical cations and neutral radicals, when a metal was present [10]. A possible cause for increasing neutral radical concentration [10] is the formation of superoxide radical anion $O_2^{\bullet-}$ from peroxyl anions O_2^{2-} reacting with the photo-generated electrons trapped (e_{tr}) at the metal-oxygen (M-O) bonds. The superoxide radical anion $O_2^{\bullet-}$ then can abstract a proton form Car$^{\bullet+}$ to form Car• according to: $O_2^{\bullet-} + Car^{\bullet+} \rightarrow Car^{\bullet} + OOH$. The superoxide radical anion $O_2^{\bullet-}$ could also be formed by reaction of O_2^{2-} with holes or hydroxide radical, or by reduction of the molecular oxygen on the surface by the photo-generated electrons [11].

EPR and DFT studies have identified the radicals formed on these matrices, both Car$^{\bullet+}$ and Car•, for numerous carotenoids including symmetrical β-carotene, zeaxanthin, violaxanthin, astaxanthin, canthaxanthin, and asymmetrical lutein in Scheme 1. Upon light illumination, the matrix surfaces are activated forming proton acceptors. Also electrochemical studies present [12] radical cations as being weak acids,

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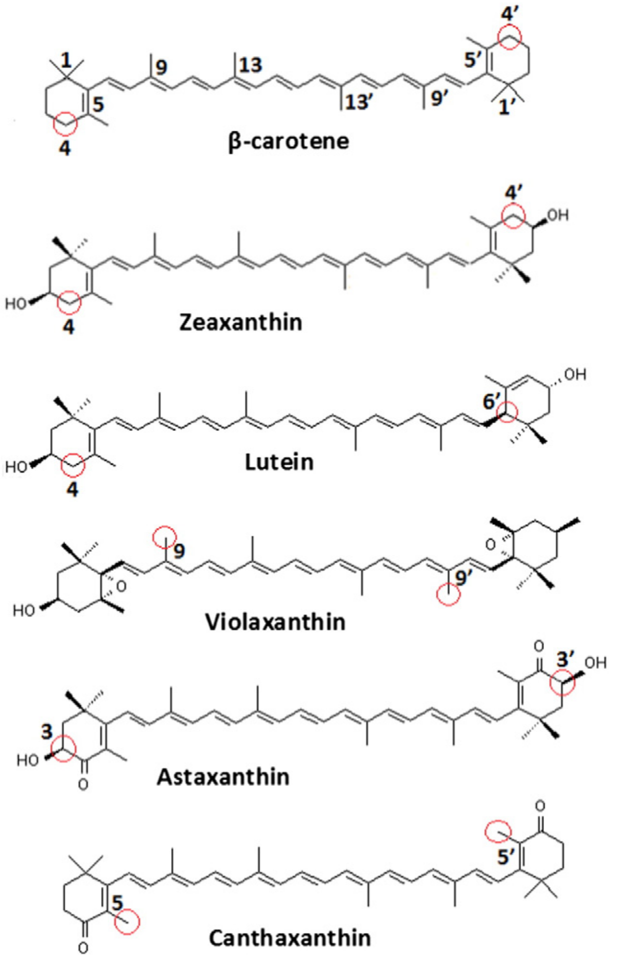

Scheme 1. Carotenoid structures (circled in red are the positions for the most favorable proton loss from the radical cation-predicted by DFT calculations).

enabling proton donation and thus forming the carotenoid neutral radical Car•. In such assemblies like the solid matrices described above or in solution, proton loss detected by advanced EPR occurs from different positions of the radical cation (see Table 1). DFT calculations predict [2–9] the preferred location of the proton loss from the radical cation given by the minimum energy and other higher energy proton loss locations (Table 1). For example, for zeaxanthin radical cation Zea•$^{+}$ the preferred proton loss (indicated by the minimum energy $\Delta E = 0$) occurs from the C4, or by symmetry at C4′ methylene positions on the cyclohexene rings (see Scheme 1, zeaxanthin), followed by loss of the methyl proton attached at the C5(or C5′), C9(or C9′) or C13(or C13′) positions on the polyene chain with relative energies $\Delta E = 3.15$, $\Delta E = 8.39$ and $\Delta E = 10.21$ kcal/mol, respectively [4] (see relative energies for zeaxanthin neutral radicals in Table 1). The relative energies increase from proton loss at C4(4′) methylene to C5(5′) to C9(9′) to C13(13′) methyl group, similarly to β-carotene, for example. We use notations Zea•(4) or Zea(4′), Zea•(5) or Zea(5′), Zea•(9) or Zea(9′), Zea•(13) or Zea(13′) for the specific neutral radicals formed, proton loss position being indicated in parenthesis.

In *Arabidopsis thaliana* model plant proton loss from Zea•$^{+}$ to form Zea• occurred only from one position, the methylene position at either C4 or at C4′ by symmetry, thus at one end of the radical cation [13]. In solution, proton loss from a chemically-generated radical cation Zea•$^{+}$ to form Zea• was shown to occur at both ends suggesting that *A. thaliana* plant is an organized assembly allowing proton loss only at one end. The neutral radical species formed has been shown [13] to be involved in the photo protection of the plant in the intense sun light on a clear sunny day. Without Zea•$^{+}$ and Zea• being formed in the presence of the intense sunlight, the photoprotection mechanism needed for photosynthesis would be destroyed.

Carotenoid neutral radicals of β-carotene (β-car•) were also observed in PSII sample where the organized assembly provided the aspartic acid [6] as the proton acceptor, and detected by the presence of an optical spectrum at 750 nm. Further confirmation of the 750 nm spectrum was reported [14] for the hydrogen atom transfer from β-carotene to the hydroxyl radical to form the carotenoid neutral radical. This species had a lifetime of approximately 15 ns consistent with the lifetime of the zeaxanthin neutral radical lifetime found in *Arabidopsis thaliana* [13] estimated to be longer than 150 ps but less than the microsecond timescale for which reactions with diffusing oxygen occur. Careful preparation of an optical sample containing the β-carotene neutral radicals formed on copper-containing MCM-41 silicate (Cu-MCM-41) produced the experimental spectrum displayed in Fig. 1a which has been deconvoluted in Fig. 1b into the optical peaks due to β-carotene (β-car) molecule at 520 nm, neutral radical β-car•(4) due to proton loss of the methylene proton from the radical cation at 752 nm, and neutral radicals, β-car•(5), β-car•(9) and β-car•(13) formed by loss of the methyl protons attached at the C5(5′), C9(9′) and C13(13′) positions of the radical cation occurring at 715, 675, and 536 nm respectively. No radical cation β-car•$^{+}$ was detected indicating complete hydrolysis of the radical cation from the preparation. The arrows indicate the optical positions predicted by DFT, confirming the assignment.

The different absorption maxima for the different β-carotene neutral radicals formed on Cu-MCM-41 are consistent with the difference in delocalization length (conjugation length) (Fig. 2). Proton loss at C4(4′) gives a neutral radical with longer conjugation length (24C atoms) has

Table 1
Relative energies ΔE(n) in kcal/mol (calculated relative to the energy minimum) of carotenoid neutral radicals formed by proton loss from the radical cation. Letter "n" indicates the position from which the proton was lost. For example, ΔE(4) is the relative energy for Car•(4), the neutral radical formed by proton loss from the C4 position and ΔE(5) is the relative energy for Car•(5), the neutral radical formed by proton loss from the methyl group attached at C5 position. In parenthesis below each relative energy, delocalization length is indicated by counting the number of C atoms over which the spin is delocalized. For proton loss at a methyl group (attached at positions C5, C9 and C13 and by symmetry at C5′, C9′ and C13′), the number of carbon atoms was counted from the methyl group in the direction of delocalization.

Car	ΔE(3)	ΔE(4)	ΔE(5)	ΔE(9)	ΔE(13)	ΔE(13')	ΔE(9′)	ΔE(6′)	ΔE(5′)	ΔE(4′)	ΔE(3′)
β-carotene [2,6]	–	0 (24)	4.92 (24)	10.29 (19)	12.18 (15)	12.18 (15)	10.29 (19)	–	4.92 (24)	0 (24)	–
Zeaxanthin [4]	–	0 (24)	3.15 (24)	8.39 (19)	10.21 (15)	10.21 (19)	8.39 (15)	–	3.15 (24)	0 (24)	–
Lutein [3]	–	6.68 (22)	6.70 (22)	15.16 (18)	17.06 (14)	17.04 (14)	14.72 (18)	0 (23)	22.69 (4)	45.44 (4)	–
Violaxanthin [4]	–	15.33 (4)	19.43 (4)	0 (17)	1.78 (13)	1.78 (13)	0 (17)	–	19.4 (4)	15.33 (4)	–
Astaxanthin [9]	0 (26)	–	13.7 (24)	14.0 (19)	15.9 (16)	15.9 (16)	14.0 (19)	–	13.7 (24)	–	0 (26)
Canthaxanthin [3]	–	–	0 (24)	4.32 (19)	5.83 (15)	5.83 (15)	–	–	4.32 (19)	0 (24)	–