

Cation radicals of xanthophylls

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Abstract Carotenes and xanthophylls are well known to act as electron donors in redox processes. This ability is thought to be associated with the inhibition of oxidative reactions in reaction centers and light-harvesting pigment–protein complexes of photosystem II (PSII). In this work, cation radicals of neoxanthin, violaxanthin, lutein, zeaxanthin, β -cryptoxanthin, β -carotene, and lycopene were generated in solution using ferric chloride as an oxidant and then studied by absorption spectroscopy. The investigation provides a view toward understanding the molecular features that determine the spectral properties of cation radicals of carotenoids. The absorption spectral data reveal a shift to longer wavelength with increasing π -chain length. However, zeaxanthin and β -cryptoxanthin exhibit cation radical spectra blue-shifted compared to that of β -carotene, despite all of these molecules having 11 conjugated carbon–carbon double bonds. CIS molecular orbital theory quantum computations interpret this effect as due to the hydroxyl groups in the terminal rings selectively stabilizing the highest occupied molecular orbitals of preferentially populated *s-trans*-isomers. The data are expected to be useful in the analysis of spectral results from PSII pigment–protein complexes seeking to understand the role of carotene and xanthophyll cation radicals in regulating excited state energy flow, in protecting PSII reaction centers against photoinhibition, and in dissipating excess light energy absorbed by photosynthetic organisms but not used for photosynthesis.

Keywords Absorption spectroscopy · Carotenoid · Energy levels · Molecular orbitals · Near-infrared spectra · Quantum computations

Abbreviations

CIS	Configuration interaction singles
DDQ	2,3-dicyano-5,6-dichloro-parabenzoquinone
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
LUMO	Lowest unoccupied molecular orbital
NIR	Near-infrared
PSII	Photosystem II
TCNQ	7',8,8'-tetracyanoquinodimethide
THF	Tetrahydrofuran

Introduction

Carotenoids have extended π -electron conjugated chains that make them inclined to engage in redox chemical reactions (Isler 1971). Radical cations of carotenoids may be readily formed chemically by the addition of an oxidizing agent (Ioffe et al. 1976; Ding et al. 1988; Jeevarajan et al. 1996; Gao et al. 1997; Wei et al. 1997; Krawczyk 1998), electrochemically in a cell having a sufficiently positive voltammetric potential (Grant et al. 1988; Khaled et al. 1990; Khaled et al. 1991; He and Kispert 1999a; He and Kispert 1999b; Liu and Kispert 1999; Deng et al. 2000; Hapiot et al. 2001), photochemically upon light absorption in photosynthetic pigment–protein complexes (Schenk et al. 1982; Rivas et al. 1993; Polívka et al. 2004; Holt et al. 2005), or in synthetic covalently linked carotenoid donor/electron acceptor molecular systems (Gust and Moore 1991; Gust et al. 1993).

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In animals, carotenoid radical formation may be associated with the action of the molecules as free radical quenchers or biological antioxidants (Burton and Ingold 1984; Jørgensen and Skibsted 1993; Palozza and Krinsky 1994; Böhm et al. 1995; Everett et al. 1996; Woodall et al. 1996; Mortensen and Skibsted 1997). The optical spectroscopic signature of a carotenoid radical is a strong absorption band in the 800–1,100 nm near-infrared (NIR) region of the electromagnetic spectrum (Sorenson 1965; Adams 1969; Ioffe et al. 1976; Ding et al. 1988; Jeevarajan et al. 1996; Gao et al. 1997). In an analysis of the reactivity of carotenoids having various π -electron chain lengths with phenoxyl radicals, Mortensen and Skibsted (1997) reported a correlation between the susceptibility of a carotenoid to degradation and the position of the maximum absorption wavelength of its cation radical NIR spectrum. They showed that the longer wavelength absorbing carotenoids were exponentially more effective at scavenging the phenoxyl free radicals (Mortensen and Skibsted 1997).

In plants, carotenoid cation radical formation may be associated with a mechanism of photoprotective adaptation against the deleterious effects of excess light absorption (Holt et al. 2005). Carotenoids are well known for their role as energy donors in light-harvesting complexes (van Grondelle et al. 1994; Frank and Christensen 1995; Kramer et al. 1995; Connelly et al. 1997; Desamero et al. 1998; Trissl et al. 1999; Bassi and Caffarri 2000; Gradinaru et al. 2000; Krueger et al. 2001; van Amerongen and van Grondelle 2001; Das and Frank 2002; Croce et al. 2003; Papagiannakis et al. 2003; Cogdell et al. 2004), but their ability to act as electron donors, and therefore as potential antioxidants, in photosynthesis has only recently become known through experiments on various light-harvesting pigment–protein complexes from photosynthetic bacteria and on photosystem II (PSII) protein preparations from higher plants, algae, and cyanobacteria (Polívka et al. 2002; Frank and Brudvig 2004; Polívka et al. 2004; Holt et al. 2005). Understanding the controlling features by which carotenoids transfer electrons and relate to their ability to inhibit oxidative reactions is critical toward revealing how plants have adapted to various environmental stresses.

A simple energy diagram depicting highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals associated with the ground and excited singlet state configurations of butadiene as an example of a typical π -electron conjugated system is shown in Fig. 1. The figure shows that the ground and first excited singlet states, S_0 and S_1 , both have gerade (g) symmetry in the idealized C_{2h} point group. In the figure, the S_1 state is formed from a simple HOMO \rightarrow LUMO electronic transition. However, this is not the case for polyenes and carotenoids, where configuration interaction between more energetic, single

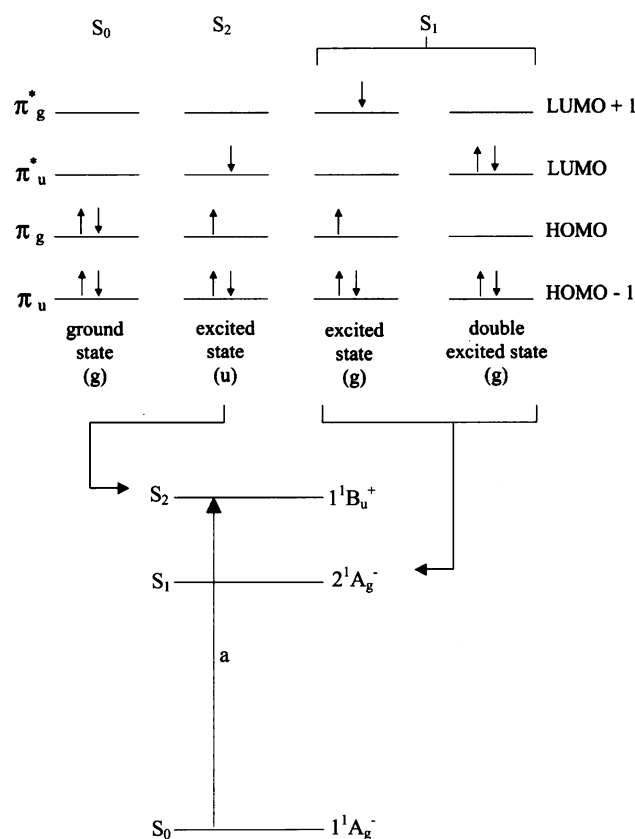


Fig. 1 A molecular orbital diagram (not to scale) depicting the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals and three low-lying electronic states of butadiene. g, gerade; u, ungerade. The bottom part shows an energy level diagram depicting the strongly allowed absorption (a) of carotenoids and polyenes

HOMO \rightarrow LUMO + 1 (Fig. 1) and doubly excited HOMO \rightarrow LUMO electronic configurations of like (g) symmetry cause a state of $1^1A_g^-$ symmetry to be formed at lower energy than the low-lying $1^1B_u^+$ state (Schulten and Karplus 1972; Schulten et al. 1976; Ohmine et al. 1978; Tavan and Schulten 1979, 1986; Serrano-Andrés et al. 1993; Starcke et al. 2006). Figure 1 also shows the now well-accepted state-ordering diagram for carotenoids and polyenes, and depicts the S_0 ($1^1A_g^-$) \rightarrow S_2 ($1^1B_u^+$) transition associated with the strongly allowed absorption transition.

The formation of radical cations of carotenoids can be understood from a molecular orbital standpoint. If an electron is removed from the HOMO of a neutral carotenoid in its ground state via a chemical, electrochemical, or photochemical process, the remaining electron is left in a molecular orbital of either g or u symmetry depending on the number of conjugated π -electron double bonds. Upon light absorption the system may undergo a $g \rightarrow u$ or $u \rightarrow g$ transition to form an excited state radical cation; the state designations being D_0 for the ground state, and D_1

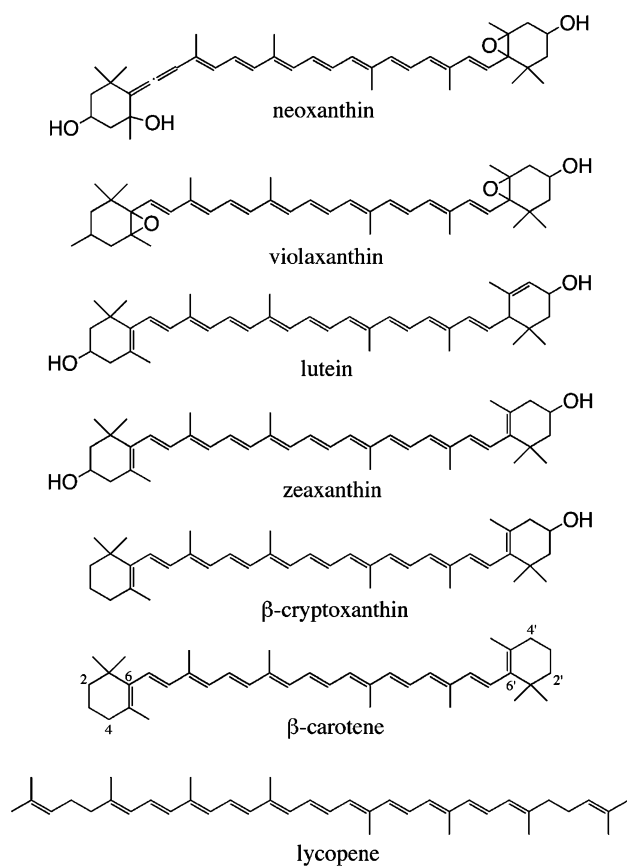


Fig. 2 Molecular structures of xanthophylls, β -carotene, and lycopene

and D_2 for the two lowest excited states (Jeevarajan et al. 1996).

$S_0 \rightarrow S_2$ and $D_0 \rightarrow D_2$ spectra are associated with strongly allowed $\pi\pi^*$ transitions in the visible and NIR regions, respectively, and the dependence of the energies of these transitions on π -electron chain length can be understood on the basis of simple molecular orbital theory (Schulten and Karplus 1972; Andrews and Hudson 1978; Kohler 1990, 1991). The theory predicts that the energies of the transitions can be described by the relationship $\Delta E = A + B/N$, where N is the effective number of conjugated double bonds (Kohler 1990, 1991; Christensen 1999; Christensen et al. 2004). More quantitative agreements between theoretical and experimental energies require inclusion of extensive configuration interaction and consideration of solvent interactions, which has been prohibitively computationally intensive.

In this article, we report the radical cation absorption spectra of neoxanthin, violaxanthin, lutein, zeaxanthin, β -cryptoxanthin, β -carotene, and lycopene (Fig. 2) formed by chemical oxidation. Quantum computations are used to interpret the spectral observations. Given that carotenoids have the potential to act as potent biological antioxidants in

both plants and animals, and that their spectral properties are correlated with their reactivity to free radicals (Jørgensen and Skibsted 1993; Everett et al. 1995, 1996; Hill et al. 1995; Mortensen and Skibsted 1996, 1997), it is important to explore in detail the structural factors that determine their spectral properties.

Materials and methods

Violaxanthin, neoxanthin, and lutein were extracted from spinach leaves using 50:50 (v/v) acetone:methanol. The solution was then filtered and evaporated to dryness using a rotary evaporator. The remaining residue was redissolved in acetonitrile. High-performance liquid chromatography (HPLC) was then carried out to separate and purify the xanthophylls (Niedzwiedzki et al. 2006). β -carotene was purchased from Sigma-Aldrich. β -cryptoxanthin, zeaxanthin, and lycopene were obtained as gifts. β -carotene and β -cryptoxanthin were purified by HPLC utilizing a YMC Carotenoid S5 μ column (4.6 mm \times 250 mm) with acetone as the mobile phase and a flow rate of 0.5 ml/min. Lycopene was purified using a C18 column (3.9 mm \times 300 mm) with 50:50 (v/v) acetone:water as the mobile phase at a flow rate of 1.0 ml/min. Zeaxanthin was purified as previously described (Niedzwiedzki et al. 2006).

Cation radicals of the molecules were generated by reaction with anhydrous ferric chloride in anhydrous dichloromethane following a previous method (Jeevarajan et al. 1996). The optical density of the molecules was adjusted to between 1.1 and 1.5 at the maximum absorption wavelength in a 1 cm pathlength cuvette, except for violaxanthin where an optical density of ~ 2.4 was used to improve the signal-to-noise ratio. The solutions were then freeze–pump–thawed for three cycles to remove all dissolved gases. For β -carotene, β -cryptoxanthin, zeaxanthin, lycopene, and lutein, approximately 50 μ l of a saturated, degassed, anhydrous ferric chloride solution was added to the freeze–pump–thawed solutions, and absorption spectra were recorded on a Cary 50 spectrophotometer. For neoxanthin and violaxanthin, a dilute ($\leq 50 \mu$ M) solution of anhydrous ferric chloride solution was added dropwise until a peak corresponding to a cation radical was observed in the near-IR region. The use of dilute ferric chloride for the experiments on neoxanthin and violaxanthin inhibited the degradation of the molecules and the excessive formation of a peak in the 650–750 nm region thought to be associated with the removal of a second electron to form a dication (Jeevarajan et al. 1996). In all cases, ferric chloride was present in a molar excess compared to the carotenoids. The resulting optical densities of the cation radical signals in the NIR were ~ 0.4 for lutein, zeaxanthin, β -cryptoxanthin, β -carotene, and lycopene, and ~ 0.02 for

neoxanthin and violaxanthin. Gaussian deconvolution of the absorption spectral lineshapes was used to obtain the λ_{max} values (Josue and Frank 2002).

For the quantum computations, the structures of the molecules were constructed in a virtual vacuum environment using Polak–Ribiere algorithm in HyperChem 5.1 (Hypercube, Inc.). The coordinates from HyperChem 5.1 were imported into Anamol 5.2.9 (Robert Birge, University of Connecticut, program available upon request) to generate the input file, and the structures were optimized using Gaussian 03 (Gaussian, Inc.) employing B3LYP density functional methods and a 6-31G(d) basis set. The $D_0 \rightarrow D_2$ transition energies were computed using CIS molecular orbital theory with full single configuration interaction over the π system of the polyene.

Results

The $S_0 \rightarrow S_2$ absorption spectra of the molecules in dichloromethane red-shift with increasing number of conjugated carbon–carbon double bonds (Fig. 3). The absorption spectra of zeaxanthin, β -cryptoxanthin, and β -carotene are very similar due to all three carotenoids having 11 conjugated carbon–carbon double bonds and two terminal rings. Lycopene also has 11 conjugated carbon–carbon double bonds but unlike zeaxanthin, β -cryptoxanthin, and β -carotene, it does not have terminal rings which causes its absorption spectrum to be red-shifted by ~ 20 nm relative to β -carotene (Fig. 3). As is typical for these molecules at

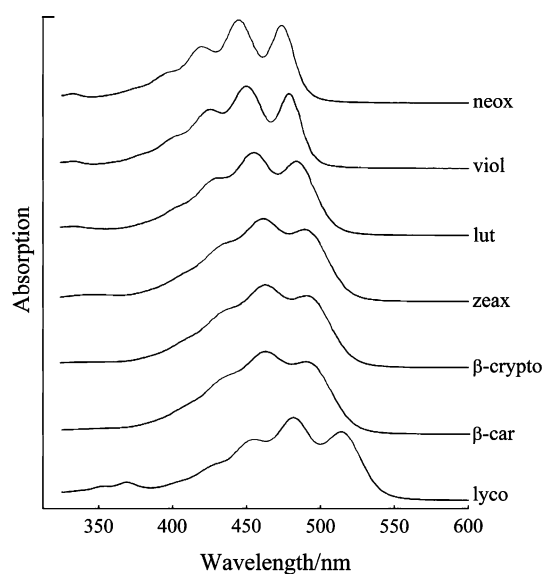


Fig. 3 Steady-state absorption spectra of the xanthophylls, neoxanthin (neox), violaxanthin (viol), lutein (lut), zeaxanthin (zeax), and β -cryptoxanthin (β -crypto), and the carotenes, β -carotene (β -car) and lycopene (lyco). The spectra were offset vertically for clarity

room temperature, the Franck-Condon maximum corresponds to the (0–1) band of the $S_0 \rightarrow S_2$ transition (Isler 1971; Christensen et al. 1999). Also, the resolution of the vibronic bands decreases with increasing extent of π -electron conjugation. This is due to spectral inhomogeneity induced by the presence of the terminal rings in the conjugated chain (Christensen and Kohler 1973; Kohler 1995).

The $D_0 \rightarrow D_2$ absorption spectra of the cation radicals appear in the near-IR region, and like the $S_0 \rightarrow S_2$ spectra, red-shift with increasing number of carbon–carbon double bonds (Fig. 4). Neoxanthin and violaxanthin have nine conjugated carbon–carbon double bonds and exhibit a main peak at ~ 850 nm. Lutein has 10 conjugated carbon–carbon double bonds and a cation radical peak at 950 nm. Interestingly, zeaxanthin, β -cryptoxanthin, and β -carotene have the same number of π -electron conjugated double bonds and two terminal rings, but their cation radical signals occur at different wavelengths; 977 nm, 981 nm, and 989 nm, respectively. This corresponds to an ~ 45 cm^{-1} difference between zeaxanthin and β -cryptoxanthin, and an ~ 80 cm^{-1} difference between β -cryptoxanthin and β -carotene. This influence of the terminal ring hydroxyl groups on the positions of the spectral bands of the cation radicals of zeaxanthin versus β -carotene has been previously reported (Mortensen and Skibsted 1997; Edge et al. 1998; Amarie et al. 2007). Lycopene also has 11 conjugated

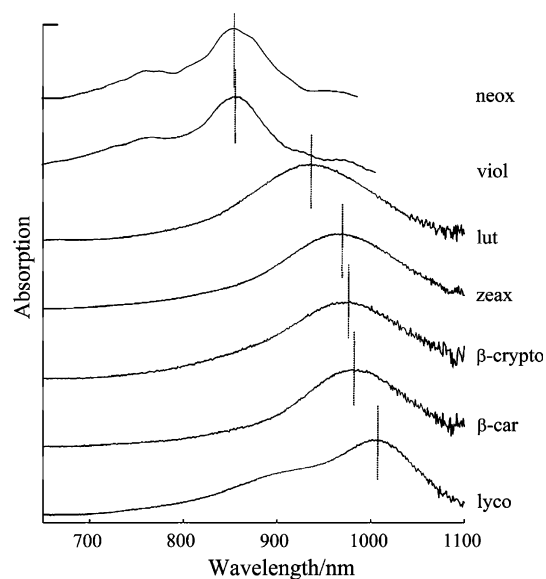


Fig. 4 Steady-state absorption spectra of the cation radicals of the xanthophylls, neoxanthin (neox), violaxanthin (viol), lutein (lut), zeaxanthin (zeax), and β -cryptoxanthin (β -crypto), and the carotenes, β -carotene (β -car) and lycopene (lyco). The peak in the near-IR region for neoxanthin was smoothed using binomial Savitzky–Golay filter method. For violaxanthin, a fast Fourier-transformation filter method with 70% degree of smoothing was utilized. The spectra were offset vertically for clarity. The vertical lines depict the λ_{max} of the absorption

carbon–carbon double bonds, but its cation radical signal occurs at 1,014 nm, which is $\sim 250 \text{ cm}^{-1}$ lower in energy than β -carotene.

It was found that a freeze–pump–thawed solution produced a lesser amount of cation radical signal compared to a solution not treated in this manner. For example, the β -carotene cation radical signal produced by adding 50 μl of a saturated solution of ferric chloride to a freeze–pump–thawed solution had an intensity ~ 5 times less than that produced from a solution of β -carotene not freeze–pump–thawed. Another finding attributable to freeze–pump–thawing and the oxygen-free environment of our samples is that the cation radical spectra appeared red-shifted compared to those that were not treated in this manner. Also, zeaxanthin, β -cryptoxanthin, β -carotene, and lutein produced a single peak in the near-IR spectrum upon the addition of ferric chloride, whereas neoxanthin, violaxanthin, and lycopene displayed a shoulder at shorter wavelength in addition to the main peak (Fig. 4). This shoulder is most likely attributable to a dication signal that increased in intensity relative to the main band when additional ferric chloride solution was added (Jeevarajan et al. 1996). The fact that the dication signal is observed only for neoxanthin, violaxanthin, and lycopene, three molecules without terminal rings in the conjugated π -electron system, suggests that the presence of the rings somehow inhibits the removal of a second electron by the oxidizing agent.

Discussion

The reactions that take place after ferric chloride addition to β -carotene, canthaxanthin, and ethyl *all-trans*-8'-apo- β -caroten-8'-oate have been described previously (Gao et al. 1997; Gao and Kispert 2003). Kispert and co-workers (Gao et al. 1997) proposed that after addition of the reagent at room temperature, an equilibrium exists between the neutral molecule, the cation radical, a cation radical dimer, a deprotonated dimeric radical, and a deprotonated dimeric cation. Another study on β -carotene and ethyl *all-trans*-8'-apo- β -caroten-8'-oate at room temperature by the same group proposed an equilibrium involving the neutral molecule, the cation radical, the dication, and the inorganic species Fe^{3+} , Fe^{2+} , and Cl^- (Gao and Kispert 2003). Peroxide derivatives of the carotenoids were also formed if the reaction was done in the presence of oxygen (Gao and Kispert 2003). Peroxide derivatives of β -carotene and ethyl *all-trans*-8'-apo- β -caroten-8'-oate are characterized by a visible absorption spectrum that occurs $\sim 30 \text{ nm}$ to shorter wavelength relative to the $\text{S}_0 \rightarrow \text{S}_2$ (0–0) band. In addition, peroxide derivatives have a broad NIR peak $\sim 200 \text{ nm}$ blue-shifted with respect to the main band of the $\text{D}_0 \rightarrow \text{D}_2$ transition. In the present experiments, the

solutions were freeze–pump–thawed and thus, are devoid of oxygen. Consequently, neither blue-shifted visible spectra nor NIR absorption features of this sort attributable to peroxide derivatives were expected nor were they observed. However, neoxanthin, violaxanthin, and lycopene cation radical signals were accompanied by the formation of a shoulder ~ 80 – 110 nm to shorter wavelength relative to the main peak of the $\text{D}_0 \rightarrow \text{D}_2$ transition (Fig. 4). This shoulder is most likely associated with a dication as previously described (Jeevarajan et al. 1996). Kispert et al (Jeevarajan et al. 1996) showed that in the presence of more than 2 molar equivalents of ferric chloride, neutral canthaxanthin is converted to a dication. Bulk electrochemical oxidation confirmed the formation of a dication radical (Jeevarajan et al. 1996). Simultaneous electrochemical and electron paramagnetic studies (Khaled et al. 1991; Jeevarajan et al. 1994) further supported the interpretation. In the present experiments, ferric chloride was added in excess (>2.5 molar equivalents) to bring about the oxidation of the molecules. Thus, any blue-shifted peaks or shoulders are very likely attributable to dications. Kispert and co-workers (Gao et al. 1997) also observed another NIR peak for canthaxanthin and β -carotene blue-shifted $\sim 100 \text{ nm}$ compared to the main band, and assigned it to a deprotonated dimeric cation. This species was evident when a vast molar excess (>20 molar equivalents) of the carotenoid over ferric chloride was used. Such samples were not used in the present work. Thus, dimerization of the molecules is not likely. Table 1 contains a summary of the λ_{max} values of carotenoid cation radical spectra in solution from the present work and those generated by various other techniques reported in the literature.

Figure 5 shows a plot of the $\text{D}_0 \rightarrow \text{D}_2$ versus $\text{S}_0 \rightarrow \text{S}_2$ transition energies of all the carotenoids examined here except lycopene, which does not have terminal rings. The plot indicates an approximate linear relationship ($R = 0.95647$) between the two different transition energies. Although the $\text{S}_0 \rightarrow \text{S}_2$ transition energies of β -carotene, β -cryptoxanthin, and zeaxanthin all lie within the uncertainty of the measurements, the $\text{D}_0 \rightarrow \text{D}_2$ transition energies clearly shift to higher energy when hydroxyl groups are introduced into the rings (Figs. 4, 5). To explore the origin of this effect, quantum computations were performed to obtain the ground state geometric configurations and excited state energies and spectra of the cation radicals.

The D_2 state energies and oscillator strengths of the 6-*s-cis* and 6-*s-trans* conformers of zeaxanthin cation radical (zeax^+) and β -carotene cation radical ($\beta\text{-car}^+$) were obtained using configuration interaction singles (CIS) molecular orbital theory (Table 2). Although this level of theory excludes double configuration interaction that is necessary to properly describe transition energies in polyene systems (Schulten and Karplus 1972; Schulten et al. 1976;

Table 1 Carotenoid cation radicals generated by different methods reported in the literature. *N* is the number of π -electron carbon–carbon conjugated bonds unless otherwise indicated. The sample column heading indicates either the solvent or pigment–protein complex prepared from biological materials. Uncertainties were determined in the present work from the instrument resolution

Carotenoid	<i>N</i>	Method	λ_{\max} (nm)	Sample	Reference
Septatreno- β -carotene	9	Pulse radiolysis	915	Hexane	Bensasson et al. (1983)
		Pulse radiolysis	850	Triton X-100	Hill et al. (1995)
β -apo-8'-carotenal	9	Electrochemical	840	Dichloromethane	Grant et al. (1988)
		Electrochemical	842	Dichloroethane	Grant et al. (1988)
		Iodine	850	Dichloroethane	Ding et al. (1988)
		DDQ	820	Dichloroethane	Ding et al. (1988)
		TCNQ	850	Dichloroethane	Ding et al. (1988)
		Bromine	860	Dichloromethane	Ding et al. (1988)
		Flash photolysis	840	Benzene	Mortensen and Skibsted (1997)
		Pulse radiolysis	880	Benzene	Edge et al. (1998)
		Pulse radiolysis	890	Hexane	Edge et al. (1998)
8'-apo- β -carotene-8'-nitrile	9	Ferric chloride	841	Dichloromethane	Deng et al. (2000)
Neurosporene	9	photoexcitation	925, 1200	LH2	Polívka et al. (2004)
		Electrochemical	841	Ethanol	Wang et al. (2005)
Neoxanthin	9	Ferric chloride	852 \pm 2	Dichloromethane	This work
Violaxanthin	9	Photoexcitation	830	Ethanol	Amarie et al. (2007)
		Photoexcitation	909	LHCII	Amarie et al. (2007)
			856 \pm 2	Dichloromethane	This work
β -zeacarotene	9	Potassium ferricyanide	898	PSII core	Bautista et al. (2005)
Ethyl 8'-apo- β -caroten-8'-oate	9 (+1 C=O)	Ferric chloride	855	Dichloromethane	Deng et al. (2000)
8'-apo- β -caroten-8'-al	9 (+1 C=O)	Ferric chloride	848	Dichloromethane	Deng et al. (2000)
7'-Cyano-7'-ethoxycarbonyl-7'-apo- β -carotene	10	Ferric chloride	890, 1329	Dichloromethane	Jeevarajan et al. (1996)
7',7'-Dimethyl-7'-apo- β -carotene	10	Ferric chloride	934	Dichloromethane	Jeevarajan et al. (1996)
6'-apo- β -carotene-6'-nitrile	10	Ferric chloride	890	Dichloromethane	Deng et al. (2000)
Spheroidene	10	Photoexcitation	~900 nm	Methanol	Polívka et al. (2002)
		Photoexcitation	960	LH2	Polívka et al. (2002)
		Photoexcitation	960, 1400	LH2	Polívka et al. (2004)
		Electrochemical	883	Ethanol	Wang et al. (2005)
Lutein	10	Flash photolysis	973	Hexane	Hill et al. (1995)
		Flash photolysis	900	Triton X-100	Hill et al. (1995)
		Flash photolysis	882	Benzene	Mortensen and Skibsted (1997)
		Photoexcitation	880	Ethanol	Amarie et al. (2007)
		Pulse radiolysis	950	Benzene	Edge et al. (1998)
		Ferric chloride	950 \pm 2	Dichloromethane	This work
Ethyl 6'-apo- β -caroten-6'-oate	10 (+1 C=O)	Ferric chloride	902	Dichloromethane	Deng et al. (2000)
6'-apo- β -caroten-6'-al	10 (+1 C=O)	Ferric chloride	895	Dichloromethane	Deng et al. (2000)
Carotenoid-porphyrin-quinone triads	11	Pulse radiolysis	1025	Benzene	Land et al. (1987)
Carotenoid-porphyrin-quinone triads	11	Pulse radiolysis	970	Dichloromethane	Land et al. (1987)
Carotenoporphyrin ester	11	Pulse radiolysis	1025	Benzene	Land et al. (1987)
Carotenoporphyrin dyad	11	Laser excitation	975	Butyronitrile	Hermant et al. (1993)

Table 1 continued

Carotenoid	<i>N</i>	Method	λ_{\max} (nm)	Sample	Reference		
<i>β</i> -carotene	11	Pulse radiolysis	1040	Hexane	Dawe and Land (1975)		
		Flash photolysis and pulse radiolysis	1040	Hexane	Bensasson et al. (1983)		
		Photoexcitation	920	Ethanol	Amarie et al. (2007)		
		Electrochemical	955	THF	Grant et al. (1988)		
		Electrochemical	1000	Dichloromethane	Grant et al. (1988)		
		Electrochemical	1025	Dichloroethane	Grant et al. (1988)		
		Iodine	818	Dichloroethane	Ding et al. (1988)		
		Iodine	875	Dichloroethane	Ding et al. (1988)		
		Iodine	950	Dichloroethane	Ding et al. (1988)		
		Iodine	1030	Dichloroethane	Ding et al. (1988)		
		DDQ	800	Dichloroethane	Ding et al. (1988)		
		TCNQ	755, 860	Dichloroethane	Ding et al. (1988)		
		Chloranil	790	Dichloroethane	Ding et al. (1988)		
		Bromine	925 to 775	Dichloroethane	Ding et al. (1988)		
		Electrochemical	990, 1425	Dichloromethane	Jeevarajan et al. (1994)		
		Ferric chloride	970, 1425	Dichloromethane	Jeevarajan et al. (1996)		
		Electrochemical	999	Dichloromethane	Jeevarajan et al. (1996)		
		Iodine	1025	Not specified	Jeevarajan et al. (1996)		
		Potassium ferricyanide	~990	PSII	Tracewell and Brudvig (2003)		
		Potassium ferricyanide	984	PSII core complex	Bautista et al. (2005)		
		Ferric chloride	970	Dichloromethane	Gao et al. (1997)		
Flash photolysis	930	Benzene	Mortensen and Skibsted (1997)				
Pulse radiolysis	1020	Benzene	Edge et al. (1998)				
Pulse radiolysis	936	Triton X-1000	Hill et al. (1995)				
Ferric chloride	989 \pm 2	Dichloromethane	This work				
Lycopene	11	Flash photolysis	1070	Hexane	Dawe and Land (1975)		
		Flash photolysis	975	Benzene	Mortensen and Skibsted (1997)		
		Electrochemical	945	Ethanol	Wang et al. (2005)		
		Pulse radiolysis	1050	Benzene	Edge et al. (1998)		
		Ferric chloride	1014 \pm 2	Dichloromethane	This work		
4'-apo- β -carotene-4'-nitrile	11	Ferric chloride	939	Dichloromethane	Deng et al. (2000)		
β -cryptoxanthin	11	Ferric chloride	981 \pm 2	Dichloromethane	This work		
Zeaxanthin	11	Pulse radiolysis	1040	Hexane	Hill et al. (1995)		
		Flash photolysis	907	Benzene	Mortensen and Skibsted (1997)		
		Pulse radiolysis	1000	Benzene	Edge et al. (1998)		
		Pulse radiolysis	936	Triton X-100	Hill et al. (1995)		
		Photoexcitation	900	Ethanol	Amarie et al. (2007)		
		Photoexcitation	983	LHCII	Amarie et al. (2007)		
		Illumination	~1000	Thylakoid membrane	Holt et al. (2005)		
		Ferric chloride	977 \pm 2	Dichloromethane	This work		
		Ferric chloride	948	Dichloromethane	Deng et al. (2000)		
		Ethyl 4'-apo- β -caroten-4'-oate	11 (+1 C=O)	Ferric chloride	948	Dichloromethane	Deng et al. (2000)

Table 1 continued

Carotenoid	<i>N</i>	Method	λ_{\max} (nm)	Sample	Reference
4'-apo- β -caroten-4'-al	11 (+1 C=O)	Ferric chloride	944	Dichloromethane	Deng et al. (2000)
Echinenone	11 (+1 C=O)	Flash photolysis	907	Benzene	Mortensen and Skibsted (1997)
Astaxanthin	11 (+2 C=O)	Pulse radiolysis	940	Hexane	Hill et al. (1995)
Astaxanthin	11 (+2 C=O)	Pulse radiolysis	920	Benzene	Edge et al. (1998)
		Pulse radiolysis	875	Triton X-100	Hill et al. (1995)
Canthaxanthin	11 (+2 C=O)	Pulse radiolysis	960	Hexane	Lafferty et al. (1977)
		Pulse radiolysis	960	Hexane	Bensasson et al. (1983)
		Electrochemical	890	Dichloromethane	Grant et al. (1988)
		Electrochemical	890	Dichloroethane	Grant et al. (1988)
		Iodine	930	Dichloroethane	Ding et al. (1988)
		DDQ	900	Dichloroethane	Ding et al. (1988)
		TCNQ	869	Dichloroethane	Ding et al. (1988)
		Bromine	925	Dichloromethane	Ding et al. (1988)
		Electrochemical	885, 1310	Dichloromethane	Jeevarajan et al. (1994)
		Ferric chloride	887, 1310	Dichloromethane	Jeevarajan et al. (1996)
		Flash photolysis	860	Benzene	Mortensen and Skibsted (1997)
		Pulse radiolysis	940	Benzene	Edge et al. (1998)
		Pulse radiolysis	862	Triton X-100	Hill et al. (1995)
Anhydrorhodovibrin	11	Electrochemical	1007	Ethanol	Wang et al. (2005)
Spirilloxanthin	13	Electrochemical	1049	Ethanol	Wang et al. (2005)
Dodecapreno- β -carotene	19	Flash photolysis and pulse radiolysis	1480	Hexane	Bensasson et al. (1983)

Ohmine et al. 1978; Tavan and Schulten 1979, 1986; Serrano-Andrés et al. 1993; Starcke et al. 2006), it is adequate for obtaining relative energies and establishing trends for the strongly allowed 1B_u -like states. To examine the importance of double CI on the excitation energies, we carried out CIS(D) calculations on the 6-*s-trans* cation species of β -carotene and zeaxanthin. The perturbation calculations indicated that the inclusion of doubles and triples lowers the energy of the strongly allowed $^1B_u^+$ -like state by 0.156 eV in both carotenoids. This red-shift did bring both excitation energies in closer agreement with experiment, but we concluded that the additional computational effort was not providing new insight into the role of the hydroxyl group in blue-shifting the cation transition energy. Our discussion is therefore limited to the CIS results. The use of CIS methods also avoided the pitfalls of size-dependent problems that we encountered in using time-dependent density functional theory and spin contamination errors that prevented our using SACCI methods. It is also important to note that the calculations were performed in the absence of solvent. Therefore, the energy values presented in this work are overestimated. The ~250 nm difference in the $D_0 \rightarrow D_2$ transition energy of carotenoid cation

radicals in solution obtained experimentally versus those determined from quantum computations has been previously discussed for canthaxanthin, β -carotene, 7'-cyano-7'-ethoxycarbonyl-7'-apo- β -carotene, and 7,7'-dimethyl-7'-apo- β -carotene (Jeevarajan et al. 1996). The difference was attributed largely to the stabilization of the D_2 state due to solvent interaction.

Quantum computations carried out in the present work indicate that in the excited state, the molecules possessing terminal rings exist as a distribution of conformers formed primarily due to rotations about the 6 and 6' carbon-carbon single bonds (see β -carotene in Fig. 2). The primary components are 6,6'-*s-cis* and 6,6'-*s-trans* conformers. Thus, two factors determine the observed $D_0 \rightarrow D_2$ transition energy of the xanthophyll cation radicals: Stabilization of the HOMO energy and conformer composition. These also explain the blue-shift of $zeax^+$ and β - $crypto^+$ relative to β - car^+ . First, the $D_0 \rightarrow D_2$ energy of 6,6'-*s-trans-zeax^+ is predicted to be blue-shifted 6 nm relative to 6,6'-*s-trans- β -car^+ (Table 2). The blue-shift is caused by the hydroxyl group in each ring preferentially stabilizing the HOMO. Figure 6 shows that the electron density in the HOMO extends toward the oxygen atom of**

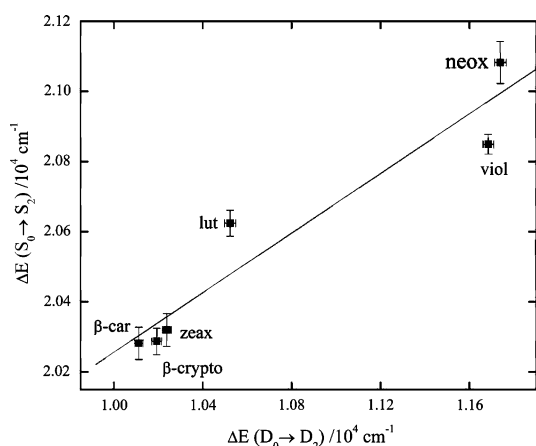


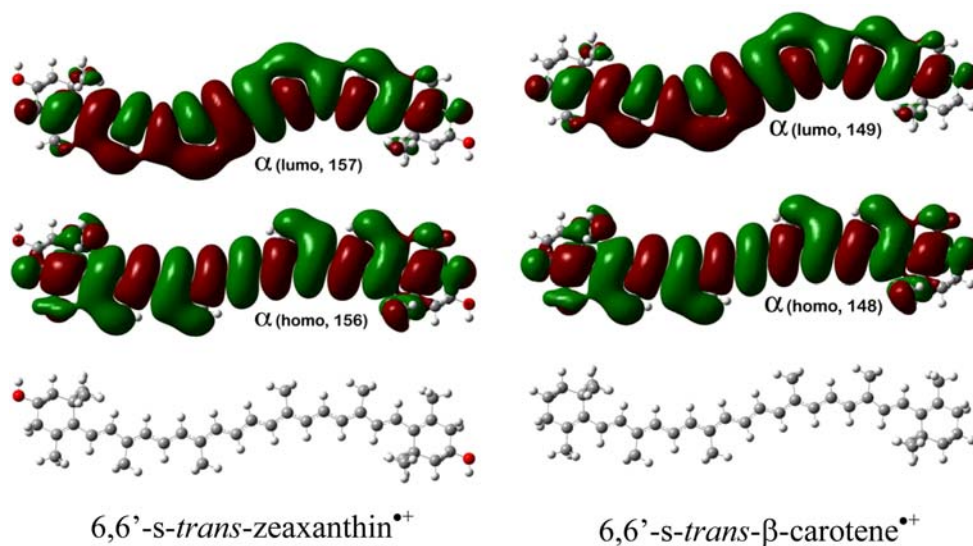
Fig. 5 Plot of the $D_0 \rightarrow D_2$ versus $S_0 \rightarrow S_2$ transition energies of neoxanthin (neox), violaxanthin (viol), lutein (lut), zeaxanthin (zeax), β -cryptoxanthin (β -crypto), and β -carotene (β -car). The uncertainties in the numbers were determined from the instrument resolution. The plot was fit to a straight line with slope, 0.42, and y-intercept, 16,010. The correlation coefficient was 0.95647

Table 2 Computed electronic transition wavelengths (in nm) of the $D_0 \rightarrow D_2$ transition for zeaxanthin (zeax^+) and β -carotene ($\beta\text{-car}^+$) cation radicals. The oscillator strengths for the $D_0 \rightarrow D_2$ transition are given in parentheses

Cation radical	Conformer	
	6,6'-s-cis	6,6'-s-trans
Zeax^+	680 (0.59)	691 (0.95)
$\beta\text{-car}^+$	680 (0.37)	697 (0.95)

the hydroxyl group. The extension is less for the LUMO. The low-lying strongly allowed $D_0 \rightarrow D_2$ transition has a HOMO \rightarrow LUMO contribution of 88%. Therefore the interaction with hydroxyl groups will generate a blue-shift

Fig. 6 Electronic distributions of the HOMOs and LUMOs of the 6,6'-s-trans conformers of the zeaxanthin and β -carotene cation radicals



of the spectrum of zeax^+ compared to $\beta\text{-car}^+$. The stabilization caused by the hydroxyl groups is not computationally predicted for 6,6'-s-cis- zeax^+ . This may be due to less interaction between the hydroxyl groups and the π -conjugated system owing to non-planarity between the terminal rings and the π -electron chain. The terminal rings are $\sim 34^\circ$ out of plane relative to the π -electron chain.

The second factor important in determining the $D_0 \rightarrow D_2$ transition energy of the molecules is that the conformer composition of the samples is predicted to be different. The ratio of the 6,6'-s-cis to 6,6'-s-trans population of zeax^+ is larger by a factor of 1.2 compared to $\beta\text{-car}^+$. The fact that the blue-shifted 6,6'-s-cis isomer is predicted to be present in a larger amount in zeax^+ compared to $\beta\text{-car}^+$, and in addition, the prediction that the oscillator strength of 6,6'-s-cis- zeax^+ is larger than that of 6,6'-s-cis- $\beta\text{-car}^+$ (Table 2), contribute to the overall blue-shifted lineshape.

Conclusions

A series of xanthophyll cation radicals were generated in solution and were studied by steady-state absorption spectroscopy. The data show that the absorption spectra shift to longer wavelength with increasing π -chain length. In addition, a blue-shift is observed in going from $\beta\text{-car}^+$ to $\beta\text{-crypto}^+$ to zeax^+ , all of which have 11 conjugated carbon-carbon double bonds but differ in the number of hydroxyl groups. CIS molecular orbital theoretical calculations were carried out and reveal this effect to be due to the hydroxyl groups in the terminal rings of 6,6'-s-trans- zeax^+ preferentially stabilizing the HOMO, and causing a shift to higher energy relative to 6,6'-s-trans- $\beta\text{-car}^+$. Furthermore, the blue-shifted 6,6'-s-cis isomer of

zeax⁺ is predicted to be present in a larger amount compared to the 6,6'-s-trans isomer of zeax⁺ which is not the case for β -car⁺. This also contributes to the overall blue-shifted spectral profile of zeax⁺ relative to β -car⁺.

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