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Simultaneous Electrochemical and Electron Paramagnetic Resonance Studies of Carotenoid Cation Radicals and Dications

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Comproportionation equilibrium constants have been determined from simultaneous electrochemical and EPR measurements for the carotenoid cation radicals ($CAR^{\bullet+}$) and dications (CAR^{2+}) of β-carotene (I), β-apo-8′-carotenal (II), and canthaxanthin (III). $K(I)_{com} = 2.4 \times 10^{-2}$, $K_{com}(II) = 1.8 \times 10^{-2}$, $K_{com}(III) = 2.1 \times 10^{3}$. These indicated that, upon oxidation of III, 96% $CAR^{\bullet+}$ would be formed while 99.7% CAR^{2+} would be formed for I and II if the oxidation potential was 100 mV anodic of the first observed voltammetric wave. This explains the reason for the strong EPR spectrum observed for III and the weak EPR spectra observed for I and II. Rotating disk experiments confirm that oxidation of carotenoids occurs by an EE rather than by an ECE mechanism and are highly quasireversible systems. The second oxidation peak in the CV spectrum of II has been shown not to be due to a dication analogous to the CV of III but to a radical apparently from the oxidation of a decay product of the dication from II.

Introduction

Carotenoids are present in the chloroplasts of photosynthetic green plants[1–4] and serve as photoprotect devices and as antenna pigments. Light-driven electron-transport processes across a bilayer membrane are an important component of converting solar energy into chemical energy. Because of this, studies have been undertaken of photoinitiated charge separation in model carotenoid–porphyrin–quinone[5–7] (C–P–Q), carotenoid–diporphyrin–quinone (CPPQ),[8] and carotenoid–porphyrin–diquinone (CPQQ)[9] molecules. The final charge-separated states $C^{\bullet+}$–P–$Q^{\bullet-}$, $C^{\bullet+}$–P–P–$Q^{\bullet-}$, and $C^{\bullet+}$–P–Q–$Q^{\bullet-}$ have been found to have a lifetime of microseconds[5–9] and up to 340 μs in C–P–P–Q_A–Q_B molecules.[10] It has been shown using these complex tetrads that the presence of additional electron-transfer steps each occurring over a short distance lead to an increased quantum yield[8–10] and an increase in the lifetime[10] of a high-energy charge-separated state. Since the carotenoid cation radical is formed as part of the charge-separated state, a detailed study of the carotenoid cation radical may be helpful in designing an even more stable charge-separated state.

Carotenoid cation radicals have also been detected as a flash-induced optical absorption after phenolic herbicides have been added to suspensions of oxygen-evolving photosystem II.[11] In addition, electron transfer in photosystem II, either light induced or under oxidizing conditions, gives rise to an absorption increase attributed to a carotenoid radical cation.[12] Electrochemical studies[13–16] of the oxidation of the carotenoids I, II, and III (see Figure 1) reveal that cation radicals are not the only important species that can be formed, but dications, cations (loss of H^+ from dications), and neutral radicals, upon reduction of the cations, are formed as well. It follows that detailed knowledge of the carotenoids and their oxidation products is important to the overall understanding of electron transfer involving carotenoids.

The cyclic voltammetry (CV) for III (Figure 2) consists of two oxidation peaks where $E_{1/2}$'s are separated by 196 mV while the CV for II (Figure 2) consists of two less resolved oxidation peaks.[13] Only one oxidation peak is observed for β-carotene[13–15] (Figure 2). Bulk electrolysis[16] in a CH_2Cl_2 solvent shows that the oxidation process involves the transfer of two electrons and thus the two oxidation peaks for III were assigned to the cation radical and the dication, respectively. It was presumed that the single oxidation peak for I was assigned to the overlap of both the radical and dication CV peaks. Electrochemical oxidation of the carotenoids was studied by EPR[13] and showed[16] that the EPR signal lifetime was in the order III > I > II. Upon deuteration the lifetime of the β-carotene cation radical increases by an order of magnitude. Furthermore, the rate of formation of radical cations by diffusing dications reacting with carotenoids varies according to the measured diffusion coefficients.

To examine the CV assignment of the radical species, simultaneous electrochemical–EPR (SEEPR) experiments are described here using the method and cells described previously.[17,18] Com-

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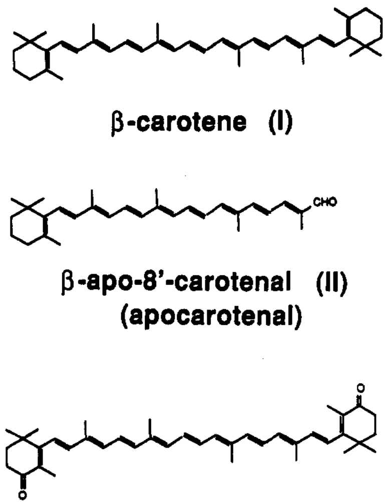

β-carotene (I)

β-apo-8'-carotenal (II)
(apocarotenal)

Canthaxanthin (III)

Figure 1. Molecular structures of β-carotene (I), β-apo-8'-carotenal (apocarotenal (II)), and canthaxanthin (III).

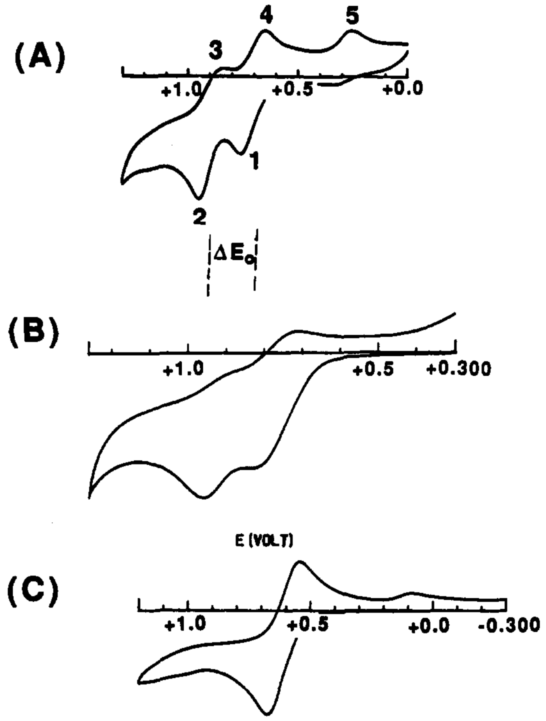

Figure 2. The CV's for (A) canthaxanthin, (B) apocarotenal, and (C) β-carotene, scan rate of 100 mv/s.

proportionation equilibrium constants for dications and cation radicals are determined. CV peaks previously misassigned have been corrected and this study demonstrates once again[18] that the SEEPR method is necessary to accurately assign the oxidation peaks to either dication or radical cation species.

Experimental Section

Apparatus. SEEPR experiments were carried out with an IBM enhanced electrolytic cell, ER/164 ECA, using a Varian E-12 EPR spectrometer and a V-4533 rotating cylindrical cavity. A hollow Teflon cylinder was placed along the cavity axis to lower the coaxial resonator frequency to 9.5 GHz. The CV's were obtained by using this cell with a BAS-100 A electrochemical analyzer simultaneously recording the EPR spectra during the CV scan. The data was transferred to a JAMECO-PC and stored on floppy disks. The magnetic field was measured with a Bruker EPR 035 M gaussmeter and the microwave frequency was measured with a Model HP 5245 L frequency counter.

The working electrode was a 6 cm long, 6 mm diameter helical coil of 0.020 in. diameter platinum wire with a surface area of 10 cm^2. The auxiliary electrode was also a platinum wire and a silver wire was used as a pseudoreference electrode. The silver wire was left for several hours in the solution of the carotenoid to be measured to ensure more consistent readings.

Chemicals. Unopened bottles of HPLC grade dichloromethane (CH_2Cl_2) from Aldrich were transferred under nitrogen in a drybox to a round-bottom flask. Solvent was transferred by syringe from this flask to a volumetric flask containing a measured amount of carotenoid. The solution was syringed into the IBM electrolytic cell where the solution was further degassed with dry N_2.

All carotenoids were purified on a column packed with silica gel (Davison Chemicals, Davisil 62). β-carotene (I) (Sigma) was eluted with carbon tetrachloride (CCl_4), β-apo-8'-carotenal (II) (Fluka) with a mixture of petroleum ether and acetone (20:1, v/v), and canthaxanthin (III) (Fluka) with a CCl_4 acetone mixture (20:1, v/v). The purity of each compound was verified by thin-layer chromatography. All carotenoids were stored in evacuated sealed vials until use when they were opened under N_2 in the drybox. All glassware was washed in a KOH/EtOH bath, rinsed several times with distilled water, and dried in an oven at 110 °C. The glassware was removed just before use and cooled in a N_2 or Ar atmosphere. Tetra-*n*-butylammonium hexafluorophosphate (TBAHFP), polarographic grade, was used as supplied from Fluka as supporting electrolyte. TBAHFP was kept inside an oven at 110 °C for 1 day, pumped in the antichamber of the drybox for several hours, and then stored inside the N_2 atmosphere of the drybox. A check was made for the absence of electroactive impurities.

Results

Previous experiments[16] showed that the oxidation of canthaxanthin involves the transfer of two electrons. So far it has not been determined[13] definitely whether after the first electron transfer at the electrode surface (E) there followed a second electron transfer at the electrode surface (E) or whether the product of the first oxidation step undergoes a chemical (C) reaction before the second electron-transfer step. The first electrokinetic mechanism is designated by the symbols, EE, while the second is known as an ECE mechanism.[19] These two mechanisms can be differentiated by carrying out a rotating disk experiment. As the rotational rate of the disk electrode is varied, the limiting current for both oxidation peaks increases linearly with rotational rate if an EE mechanism is operative. When an ECE mechanism occurs, the limiting current for the first oxidation peak linearly increases with rotational rate; however, the limiting current for the second peak varies at a measurable slower rate, due to chemical reactions occurring between the two electron-transfer steps. Carrying out such a study indicates that the oxidation of canthaxanthin follows an EE mechanism.

The heterogeneous transfer rate constant, k_0, can also be deduced[20] from rotating disk experiments by plotting 1/current vs (rotation rate)$^{-1/2}$ at different potentials in the kinetic region of the anodic scan (see Figure 3 for β-carotene). The intercept of the lines at zero rotational rate gives $1/i_k$, the reciprocal of the exchange current at a particular potential. Then $k(E)$ can be deduced from $i_k = nFAk(E)C$. Plots of ln $k(E)$ vs $(E - E°)$ gives k_0 as the intercept by solving $k(E) = k_0 \exp[(1 - \alpha)nF(E - E°)]/RT$. The heterogeneous transfer rates deduced were equal to 2×10^{-2}, 0.8×10^{-2}, and 1.7×10^{-2} cm/s for I, II, and III, respectively. This suggests that the three carotenoids exhibit relatively high electron-transfer rates and approach Nernstian behavior i.e.: $\Delta E = 0.059/n$, where $\Delta E = E_{p_a} - E_{p_c}$, n being the number of electrons transferred, E_{p_a} the oxidation peak potential, and E_{p_c} the reduction peak potential. If $\Delta E > 0.059/n$, the system

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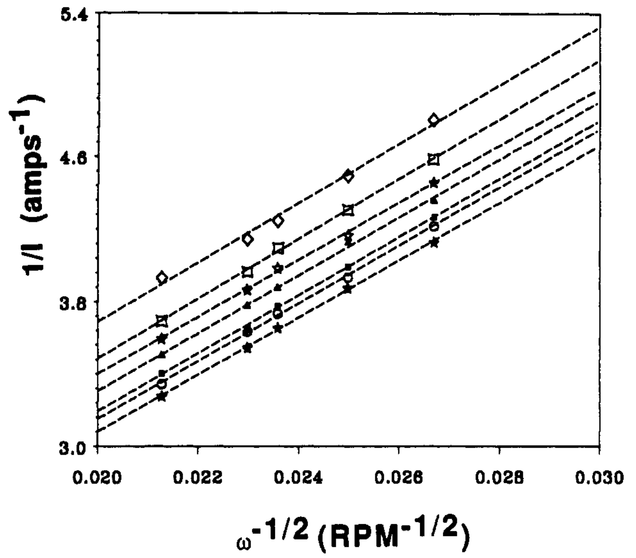

Figure 3. Plot of I^{-1} vs $\omega^{-1/2}$ at (E vs Ag reference) (◇) 680, (□) 700, (☆) 720, (▲) 740, (■) 780, (○) 800, (★) 820 mV for β-carotene.

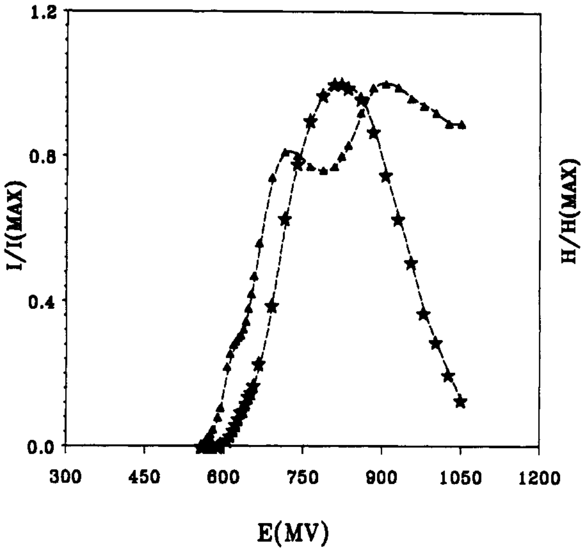

Figure 4. Normalized current (I/I(max)), (▲) from cyclic voltammetry of canthaxanthin versus oxidation potential (E (mV)). Superimposed is the normalized EPR height (★) recorded simultaneously versus oxidation potential (Ag reference) at 2 mV/s.

is referred to as quasireversible. Since the carotenoids approach Nernstian behavior, they are considered "high quasi" or nearly reversible systems.

An SEEPR study of III shows that (Figure 4) the EPR signal is maximum at the average of the two half-wave potentials of the CV plot, proving that the first oxidation results in a radical cation. $\text{EPR}_{\max}$ at equilibrium always comes at $(E_1{}^0 + E_2{}^0)/2$ but lags voltage in swept experiments more and more as the sweep rate increases.[18] If $K_{\text{com}} \ll 1$, i.e., an apparent two-electron oxidation, then $(E_1{}^0 + E_2{}^0)/2 = E_{1/2}$ in a dc polarographic experiment. For CV experiments, $E_p - E_{1/2} = 28$ mV/n for a reversible system. Since $n = 2$, ideally at an infinitely slow sweep rate, $\text{EPR}_{\max}$ should *precede* E_p by 14 mV but because of the finite sweep rate, $\text{EPR}_{\max}$ and E_p often coincide within experimental error as they do in our experiment. In conclusion, whether $\text{EPR}_{\max}$ leads or lags E_p depends completely on sweep rate—which $\text{EPR}_{\max}$ is sensitive to but E_p is not (for reversible system). Combining rotating disk experiments with SEEPR results confirms the previous assignment of the second peak as due to dication, formed by an EE mechanism.

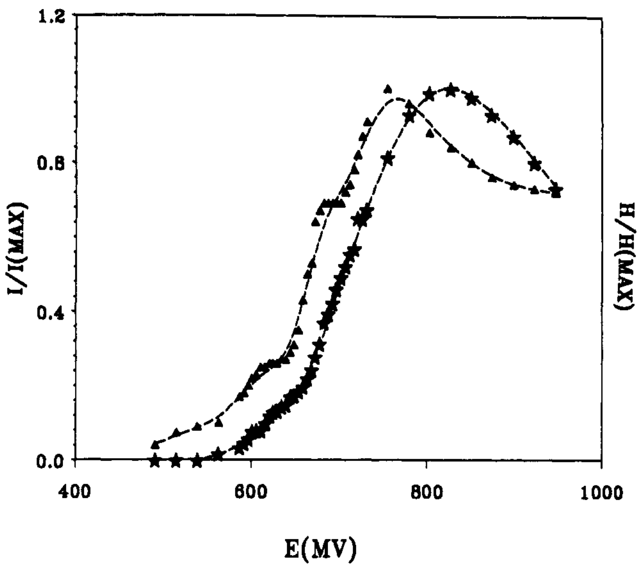

Figure 5. Same as Figure 4 except for β-carotene.

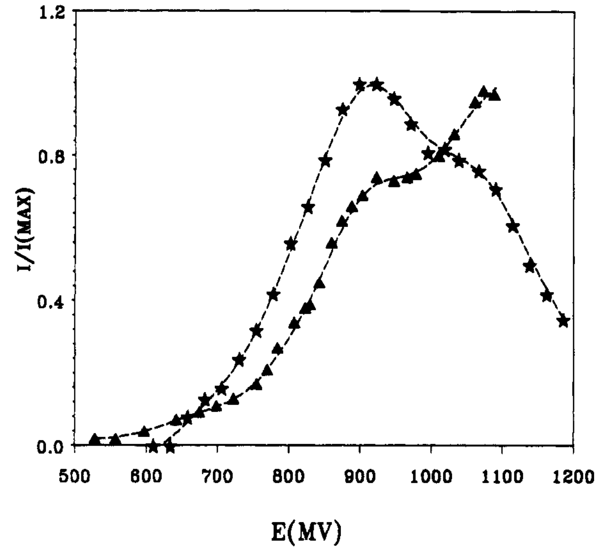

Figure 6. Same as Figure 4 except for apocarotenal.

An SEEPR study of β-carotene shows the EPR maximum to occur at a higher potential than the maximum of the CV plot (Figure 5). Surprisingly, an SEEPR study of apocarotenal shows not one but two maxima in the EPR intensity, the maxima of each CV and EPR plot both occurring at the same potential (Figure 6). Furthermore, a plot of the EPR intensity as a function of scan rate (Figure 7) shows that the peak at higher potential decays faster than that at lower potential. It is interesting to note that the maxima in the EPR and CV spectra occur at the same potential for II but the EPR maximum for I lags the CV maxima. This difference is attributed to the differences in the lifetime of the radicals and to the systems not being in equilibrium. It is also noteworthy that the potentials recorded during the SEEPR experiments are in slight disagreement with those generally accepted for I, II, and III. We attribute this to the difficulty of using a Ag wire pseudoreference electrode.

If the concentration of II (1.2 mM) is lowered by a factor of 2, the SEEPR spectrum exhibits only the peak at lower potential and the second maximum at higher potential is not observed. Examination of the integrated EPR intensity as the concentration of carotenoid in solution was increased by a factor of 2 indicated the EPR intensity followed linearly the increase in initial carotenoid concentration, suggesting that no dimers were formed. An important observation is to compare the absolute EPR intensity for the three carotenoids (Figure 8) using similar starting concentrations. The EPR intensity of III recorded after complete electrolysis is an order of magnitude more intense than that of I and II (if concentration differences are considered).

The SEEPR system was calibrated with a known concentration of tanol, a stable free radical called 4-hydroxy-TEMPO in the

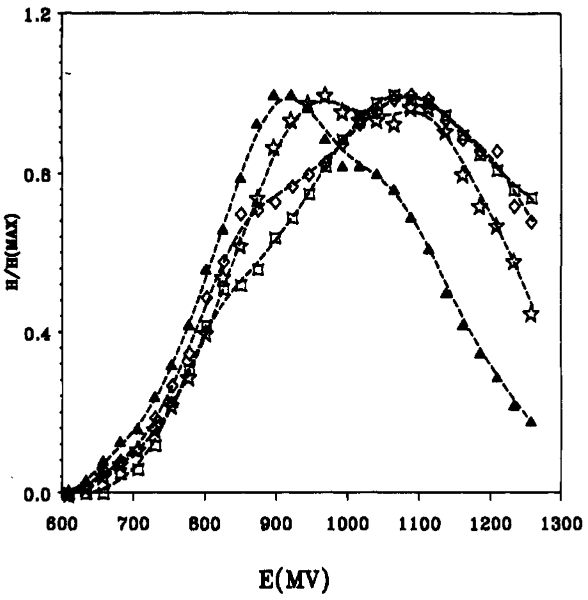

Figure 7. Normalized EPR height for apocarotenal versus potential (E (mV)) at a scan rate of (▲) 2, (☆) 4, (◇) 8, (□) 16 mV/s (Ag reference).

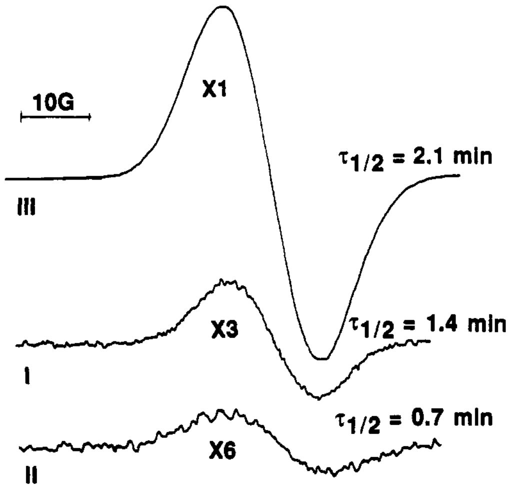

Figure 8. EPR spectra recorded for the carotenoid cation radicals prepared electrochemically from III (0.75 mM), I (1.2 mM), and II (0.49 mM) using the SEEPR cell.

TABLE I: SEEPR Concentration Measurement for Carotenoids I, II, and III

compound	I	II	III
$[CAR]_m$	1.2×10^{-3}	1.2×10^{-3}	7.5×10^{-4}
$[CAR^{\bullet+}]_{max}$	8.6×10^{-5}	7.7×10^{-5}	2.5×10^{-4}
$[CAR^{\bullet+}]_{max}/[CAR]_m$	0.072	0.064	0.33
K_{com}	2.4×10^{-2}	1.9×10^{-2}	2.1×10^{3}[b]
ΔE	−0.1 V[a]	−0.1 V[a]	196 mV

[a] ΔE for I and II calculated from SEEPR K_{com}. [b] K_{com} calculated from experimental ΔE (K_{com} from SEEPR experiment ≈ 1).

Aldrich catalog or officially 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, in a CH_2Cl_2 solution. Integration of the tanol EPR spectrum and the EPR spectrum for I, II and III resulted in the radical concentrations given in Table I. The ratio of the maximum radical concentration, $[CAR^{\bullet+}]_{max}$, to the starting carotenoid concentration, $[CAR]_m$, for the three carotenoids I, II, and III are 0.072, 0.064, and 0.33, respectively. For the comproportionation equilibrium

$$CAR^{2+} + CAR \rightleftarrows 2CAR^{\bullet+} \qquad K_{com} = \frac{[CAR^{\bullet+}]^2}{[CAR][CAR^{2+}]}$$

it is shown in Appendix A that

$$K_{com} = \left[\frac{2[CAR^{\bullet+}]/[CAR]_m}{1 - \dfrac{[CAR^{\bullet+}]}{[CAR]_m}}\right]^2 \tag{1}$$

giving K_{com} equal to 2.4×10^{-2}, 1.9×10^{-2}, and 1.0 for I, II, and III. K_{com}'s calculated from SEEPR experiments and eq 1 should be considered as lower limits only. The derivation of eq 1 assumes that at the potential being considered all species are at their equilibrium concentrations. This is not strictly true in the experiment since the potential is being swept. The SEEPR cell takes at least 20 s to approach equilibrium at a fixed potential, so ideally, the potential sweep rate should be slow enough that each data point takes 20 s. On the other hand, as shown in Figure 8, the radicals decay with half-lives of approximately 100 s so signal intensity is lost by this mechanism if the potential sweep is slow enough to meet the equilibrium requirement. The sweep rates and diffusion coefficients for the three carotenoids reported here are sufficiently similar that the relative K_{com}'s should be accurate to 50% but their absolute values may be much higher than reported.

When $K_{com} \ll 1$, the other principle error in this method is in determining the ratio $[CAR^{\bullet+}]/[CAR]_m$ which relies on double integration on the carotenoid and standard EPR signals a procedure which is only accurate to 50%, although the precision can be much higher, so again, relative K_{com}'s are more reliable. When $K_{com} \geq 1$, the ratio $[CAR^{\bullet+}]/[CAR]_m$ approaches 1 so the denominator in eq 1 becomes even more uncertain so $K = 1$ is only a lower limit and other ways should be sought to measure K_{com}.

If $K_{com} > 1$, the two electrochemical oxidation waves may be resolved and thus K_{com} can be calculated from

$$K_{com} = \exp\left(\frac{\mathcal{F}\Delta E}{RT}\right) \tag{2}$$

where ΔE is the separation between the two half-wave potentials. Since the oxidation waves are resolved for III, ΔE is measured to equal 196 mV. Such a calculation shows $K_{com} = 2.1 \times 10^3$, indicating 96% of CAR_m is converted to $CAR^{\bullet+}$ upon oxidation. If compounds I and II are exhaustively oxidized at the potential corresponding to the maximum EPR signal, only 6% of the initial carotenoid is converted to the radical cation, which explains the difference in the intensity of the EPR signals.

The equilibrium amounts of CAR, $CAR^{\bullet+}$, and CAR^{2+} are given in Table II for a theoretical exhaustive electrolysis at the average peak potential, $(E_1 + E_2)/2$, calculated from equations in the Appendix. Table II is possibly misleading with respect to the fractions of the various components because, in the natural system, the oxidizing agent has a single oxidizing potential which is not varied to produce the maximum EPR signal. A more realistic example to illustrate the difference in the species present after a 1 e^- versus a 2 e^- oxidation is to assume the oxidation takes place at a potential 0.1 V more anodic than the first observed voltammetric wave (see Figure 9). The fractions calculated with the equations in the Appendix are given in Table III. Thus under similar electrolysis conditions, III is almost entirely $CAR^{\bullet+}$ while I and II are converted quantitatively to CAR^{2+}. If we return to Figure 6, it is now clear that the first peak is both the oxidation peak to the radical cation and to the dication for carotenoid II. The second maximum at higher potential is due to the oxidation of the decay product of the dication.

The radical responsible for the second EPR peak of II at more anodic potential (Figures 6 and 7) has not been identified. However, some features should be noted. The first EPR peak was shown in an earlier section to be due to the formation of the apocarotenal cation radical resulting from the comproportionation reaction between the dication of II and II. If the species responsible for the second peak is formed from the dication of II at a slow rate, the EPR peak will occur at a fixed time later; in fast scans it will appear to be shifted to more anodic potentials (Figure 7) but in slow scans, it will appear at the second CV peak (Figure 6). In fact, this is what is observed.

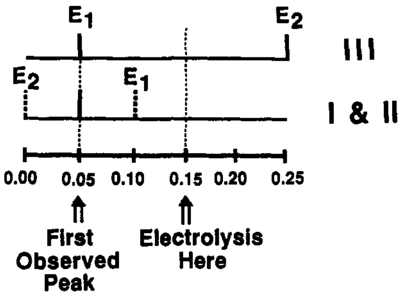

Figure 9. Electrolysis at 0.1 V more anodic than the first observed voltammetric wave.

TABLE II: Equilibrium Concentrations (%) of CAR, $CAR^{\bullet+}$, and CAR^{2+} after Exhaustive Electrolysis at $(E_1 + E_2)/2$

	CAR	$CAR^{\bullet+}$	CAR^{2+}
I and II	47	6	47
III	2	96	2

TABLE III: Equilibrium Concentrations (%) of CAR, $CAR^{\bullet+}$, and CAR^{2+} at 0.1 V More Anodic Than the First Voltammetric Wave

	CAR	$CAR^{\bullet+}$	CAR^{2+}
I and II	0.04	0.3	99.7
III	2	96	2

It is also noted that the radical corresponding to the second peak has a shorter lifetime than that of the first peak. The relative intensity of the second peak is dependent on concentration and thus it is proposed that a dimer of II is formed by a slow reaction (eq 3) after which $(CAR)_2$ is oxidized at the second CV peak to

$$2CAR^{2+} \rightarrow (CAR)_2 + 4H^+ \qquad (3)$$

give a radical cation. Product analysis, which is needed to confirm this proposal, has not been carried out.

Summary

Rotating disk experiments confirm that oxidation of carotenoids occurs by an EE rather than by an ECE mechanism and are highly quasireversible systems. Upon oxidation of III, approximately 96% radical cations would be formed, and for I and II, 99.7% dications would be formed if the oxidation occurs at a potential 0.1 V anodic of the first observed voltammetric wave. This explains the strong EPR signals for radical cations of III and the weaker signals for II and I. While the first oxidation for III produces electrochemically stable radical cations and the second oxidation peak produces dications, this is not the case for II where the first peak produces largely dications. The second peak of II is attributed to a radical formed from the oxidation of the decay of dications.

The significance of this work is that carotenoids containing ketone substituents can exhibit an apparent long-lived radical cation EPR spectra upon oxidation while dications are more prevalent for other carotenoids such as I and II. Thus the appearance or absence of carotenoid radical cation spectra in a photosystem may be reflective of the particular carotenoid. The absence of detectible EPR spectra for carotenoids may be related to the two-electron-transfer character of carotenoids.

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Appendix

Following Male et al.,[18] let

$$CAR + CAR^{2+} \rightleftarrows 2CAR^{\bullet+}; \quad K_{com} = \frac{[CAR^{\bullet+}]^2}{[CAR][CAR^{2+}]}$$

and

$$CAR_m = CAR + CAR^{\bullet+} + CAR^{2+}$$

For the oxidation

$$CAR \xrightarrow{-e^-} CAR^{\bullet+} \xrightarrow{-e^-} CAR^{2+}$$

the Nernst equation gives

$$\frac{[CAR]}{[CAR^{\bullet+}]} = \exp\left[\frac{-\mathcal{F}(E - E_1^0)}{RT}\right] = \xi_1$$

$$\frac{[CAR^{\bullet+}]}{[CAR^{2+}]} = \exp\left[\frac{-\mathcal{F}(E - E_2^0)}{RT}\right] = \xi_2$$

thus $K_{com} = \xi_2/\xi_1$. Thus at any potential

$$\frac{[CAR]}{[CAR]_m} = \frac{\xi_1\xi_2}{1 + \xi_2 + \xi_1\xi_2}$$

$$\frac{[CAR^{\bullet+}]}{[CAR]_m} = \frac{\xi_2}{1 + \xi_2 + \xi_1\xi_2}$$

$$\frac{[CAR^{2+}]}{[CAR]_m} = \frac{1}{1 + \xi_2 + \xi_1\xi_2}$$

and at $E = (E_1^0 + E_2^0)/2$, the potential giving the maximum EPR signal,

$$\frac{[CAR]}{[CAR]_m} = \frac{1}{2 + (K_{com})^{1/2}} = \frac{[CAR^{2+}]}{[CAR]_m}$$

$$\frac{[CAR^{\bullet+}]}{[CAR]_m} = \frac{(K_{com})^{1/2}}{2 + (K_{com})^{1/2}}$$

thus

$$K_{com} = \left[\frac{2[CAR^{\bullet+}]/[CAR]_m}{1 - [CAR^{\bullet+}]/[CAR]_m}\right]^2$$

Registry No. I, 7235-40-7; $I^{\bullet+}$, 57793-67-6; I^{2+}, 113301-75-0; II, 1107-26-2; $II^{\bullet+}$, 67509-73-3; II^{2+}, 113301-76-1; III, 514-78-3; $III^{\bullet+}$, 113349-54-5; III^{2+}, 113301-77-2; TBAHFP, 3109-63-5; 4-hydroxy-TEMPO, 2226-96-2; Pt, 7440-06-4; CH_2Cl_2, 75-09-2.