

Model systems for observing photoredox reactions of carotenoids

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Abstract: Two approaches to eliciting photoelectrochemical reactions from carotenoids are being pursued. One uses LB films of amphipathic carotenoids deposited on semiconducting electrodes which are immersed in electrolytes containing electron donors or acceptors. Photocurrents are observed and their action spectra implicate the excited carotenoid pigment, possibly in an aggregated form, as the photoactive species. An example of the second approach is a molecular dyad consisting of a carotenoid covalently linked to C₆₀. Excitation of the carotenoid moiety generates a charge-separated species in high yield. More complex systems include a molecular triad (C-P-Q) containing a porphyrin moiety (P) linked to a quinone (Q) and to a carotenoid (C). Triads of this type have been incorporated unidirectionally into lipid bilayer membranes. Time-resolved fluorescence experiments reveal that excitation of C-P-Q in the membrane leads to electron transfer from ¹P to give C-P^{•+}-Q^{•-}. A subsequent electron transfer from C to P^{•+} yields C^{•+}-P-Q^{•-}. In this species the carotenoid pigment acts as a molecular wire to conduct charge across the membrane. In the presence of appropriate cofactors, this system translocates protons across the bilayer lipid membrane and generates proton motive force.

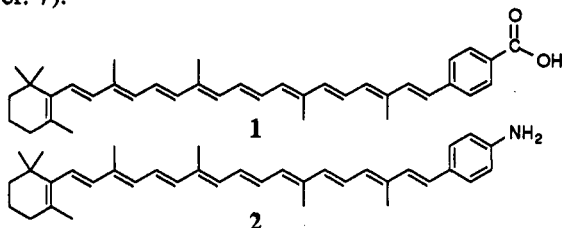
INTRODUCTION

Blue light is responsible for many developmental and phototropic effects in higher plants. Even though the identity of certain blue-light photoreceptors remains unknown, both flavins and carotenoids have been suggested as the likely chromophores, (ref. 1). For example, studies of blue-light-dependent coleoptile phototropism and stomatal opening yield action spectra having close similarity to the absorption spectra of carotenoids in solution, (ref. 2). Additionally, β-carotene and other carotenoids including the main components of the xanthophyll cycle, violaxanthin and zeaxanthin, have been isolated from cell extractions of these organs. Moreover, blue-light-induced chlorophyll fluorescence quenching from chloroplasts of guard cells as well as coleoptile tips have action spectra with maxima at 450 nm and minor peaks at 470 and 420 nm, matching quite closely the absorption of zeaxanthin in solution, (ref. 3). These observations and others have been used to point to carotenoids as putative blue-light photoreceptors. However, this hypothesis had stirred controversy due to the fact that carotenoid pigments in solution are generally not photochemically reactive. Presumably, this lack of reactivity is due both to the very short lifetime of the S₁ and S₂ excited states (< 50ps and < 200fs, respectively), which precludes diffusion-controlled bimolecular processes, and to the vanishingly low yield of intersystem crossing to the longer-lived triplet species, (ref.4-6). However, ultrafast processes in biological systems are generally not bimolecular processes, as exemplified by the pseudounimolecular photoinduced electron transfer process of photosynthesis, photoinduced isomerizations in vision, and the light-driven proton pump in the purple bacterium, *Halobacterium halobium*.

In view of the possibility of the photochemical involvement of carotenoids as photoreceptors, it is of interest to explore conditions under which photochemical reactions of carotenoids can be elicited. One plausible way to overcome the kinetic limitation imposed by the short singlet excited state lifetime is to incorporate the carotenoid into a structure in which an ultrafast photoinduced electron transfer reaction can occur. This might be accomplished by forming an organized assembly of the carotenoid pigments in contact with a conducting or semiconducting electrode so that rapid charge transfer between the pigment and the electrode effectively competes with the decay of the carotenoid excited state. Another strategy is to attach the carotenoid covalently to an appropriate electron acceptor via a linkage that allows sufficient electronic coupling for electron transfer on the picosecond or sub-picosecond time scale. Both approaches are being pursued in our laboratories and examples which demonstrate that photoinduced electron transfer can indeed occur from excited carotenoid species are included in this review.

ORGANIZED ASSEMBLIES OF CAROTENOID PIGMENTS IN CONTACT WITH SEMICONDUCTING ELECTRODES

Synthetic carotenoids 7'-apo-7'-(4-carboxyphenyl)- β -carotene (**1**) and 7'-apo-7'-(4-aminophenyl)- β -carotene (**2**) were employed in these studies. These amphipathic carotenoids were prepared by Wittig reactions of 8'-apo- β -carotenal and 4-carbomethoxybenzyltriphenylphosphonium bromide or 4-(N-acetylamino)benzyltriphenylphosphonium bromide, followed by basic hydrolysis of the ester or amide, (ref. 7).



Langmuir-Blodgett (LB) films of **1** and **2** were deposited on semiconducting transparent electrodes of indium-tin oxide (ITO) coated borosilicate glass. The subphase was water which contained cadmium nitrate, sodium acetate and acetic acid (pH = 8.6) for the case of **1** and water with phosphate buffer (pH = 5.2) for the case of **2**. Surface pressure measurements on monolayers were used to

determine average values of the area per molecule occupied by **1** and **2** at the air-water interface. The limiting area per molecule is 42-46 Å², which is consistent with the ~ 40 Å² area for the polar head of these molecules, estimated by molecular mechanics calculations, (ref.8). This area per molecule is similar to those found for carotenoids structurally related to **1**. X-ray diffraction measurements were performed on a multilayer stack of 20 monolayers of **1**. A spacing of 50 Å between the polar heads was obtained, which is consistent with separation of the polar head groups by approximately two molecular lengths. Molecular mechanics modeling of a Y-type deposition, (head to head and tail to tail or bilayer structure) with the polyenic axis tilted 45° to the surface of the glass yields a spacing of ~ 50 Å between alternate layers of polar headgroups. The electronic absorption spectra of films of **1** and **2** show λ_{\max} at ~400 nm, which is significantly blue-shifted from the monomeric absorption. Moreover, the long-wavelength absorption tails of the spectra extend to longer wavelengths than for **1** or **2** in solution (see Figure 1).

The loss of the fine structure and the widening of the spectrum suggest that these carotenoids form aggregates in the LB film on the ITO. However, a linear relationship between the absorbance at 400 nm and the number of monolayers transferred was observed, indicating that there are no interactions between the layers that significantly affect the extinction coefficient. Thus, the aggregation must occur within individual monolayers, (ref. 9).

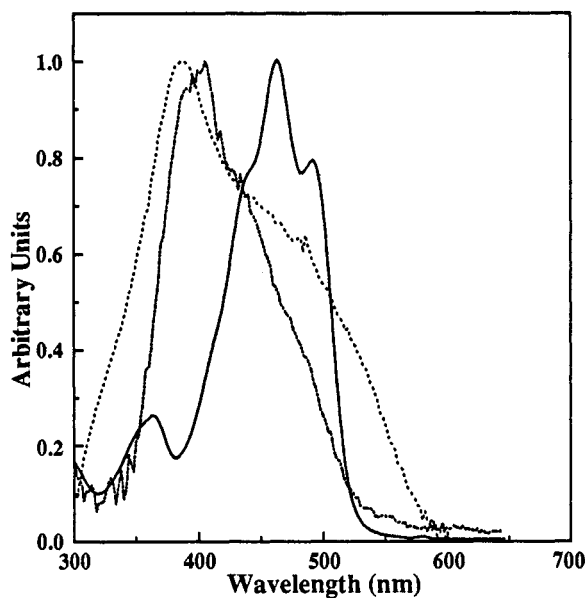


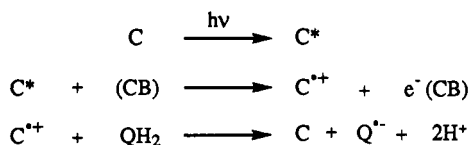
Figure 1. Action spectrum for photocurrent of one-layer LB film of **2** on ITO (·····), absorption spectrum of the same LB film (-----), and absorption spectrum of **2** in methylene chloride solution (—).

The action spectra for the photocurrent of films of **1** and **2** on ITO were recorded from 300 to 700 nm. A conventional Pyrex glass three-electrode cell was used. Negligible photocurrents (< 2 pA/cm²) were obtained when ITO electrodes coated with films of **1** or **2** were immersed in solutions containing only the supporting electrolyte. Addition of electron acceptors such as benzoquinone and Fe(CN)₆³⁻, or donors such as hydroquinone and Fe(CN)₆⁴⁻ to the electrolyte solution resulted in a substantial increase in the photocurrent in the case of **1**. The largest responses in the case of **1** were obtained with hydroquinone. In the case of **2**, on the other hand, responses above the base line were only obtained in the presence of electron acceptors. A typical photocurrent response as a function of excitation wavelength for a one-

layer film of **2** with benzoquinone in the electrolyte solution is shown in Figure 1, together with the absorption spectrum of the monolayer film and the absorption spectrum of **2** in solution. The spectra have been normalized at the maxima for comparison. The measured value of the photocurrent at 400 nm was $\sim 2 \mu\text{A}/\text{cm}^2$. The action spectrum resembles the absorption spectrum, although the long-wavelength absorbing species do not contribute proportionally to the photocurrent. A similar experiment on a bare ITO electrode shows negligible response. Thus, **2** must be responsible for the observed photocurrent, but all spectral forms of **2** do not contribute equally to the photocurrent generation.

The maximum absorbance and photocurrent are directly proportional to the number of layers deposited. The values of $\Phi_\lambda \times Pi_\lambda$, where Pi_λ is the number of incident photons per s and Φ_λ is the quantum yield, were 8.5×10^{10} for one and three monolayers and 8.0×10^{10} for five-layer films of **1** (Pi_λ was constant in these measurements). Thus, within experimental error all three LB films give the same value of Φ_λ . Therefore, each light-absorbing molecule has the same probability of producing photocurrent, which means that photocurrent is produced not only by the molecules at the electrode interface, but also by those in the more distant layers. Hence, there must be efficient charge transfer and/or energy transfer between the layers which renders the quantum yield independent of the distance between the pigment which initially absorbs the photon and the semiconductor electrode. This result is unique to these carotenoid films and

contrast to observations for saturated long chain acids, which act as insulators in similar experiments (ref. 9,10).



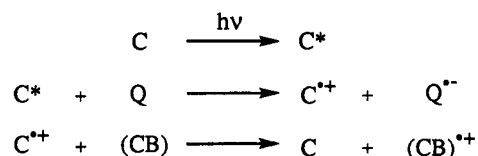
The direction of the photocurrent was investigated as a function of the bias potential as well as the type and concentration of the redox couple in the surrounding electrolyte.

Typical results for **1**-ITO electrodes with **1**,

3, and **5** stacked monolayers exhibit anodic photocurrents when hydroquinone was present in the surrounding solution. These results are in agreement with the scheme shown below.

The carotenoid excited state C^* donates an electron to the conduction band (CB) of ITO. The resulting $\text{C}^{*\cdot}$ radical cation is then reduced by the donor in solution, hydroquinone (QH_2). The excited state C^* is presumably the first excited singlet state (S_1). This state has a lifetime of < 50 ps for monomeric carotenoids in solution which implies that, in order to be efficient, electron transfer must be much faster than 50 ps, (ref. 4-6). On the other hand, it is conceivable that the aggregated pigment in the LB films has a longer S_1 lifetime, which would kinetically enhance its reactivity. With increasing bias on the electrode, the electric field increases so that successful injection into the electrode is highly favored and the photocurrent increases until a saturation current is reached.

Redox couples with electron acceptors in the aqueous phase and **1**-ITO or **2**-ITO electrodes exhibited cathodic photocurrent. The only responses detected with **2**-ITO electrodes were cathodic photocurrents and, as mentioned above, the effect was largest with benzoquinone (Q) in solution. The most straightforward interpretation for the generation of cathodic photocurrents involves an electron transfer from the excited carotenoid aggregate to Q with a subsequent electron transfer from the conduction band of the ITO to the holes residing in the carotenoid film, as indicated in the scheme below.



Because the carotenoid S_1 has a lifetime of only a few tens of picoseconds, this scheme for cathodic currents cannot depend upon diffusional processes to bring together C^* and Q. A complex between C and Q may form prior to excitation. This might be a charge-transfer complex or a less-specific association resulting from partitioning of Q between the aqueous solution and the LB film. In preliminary

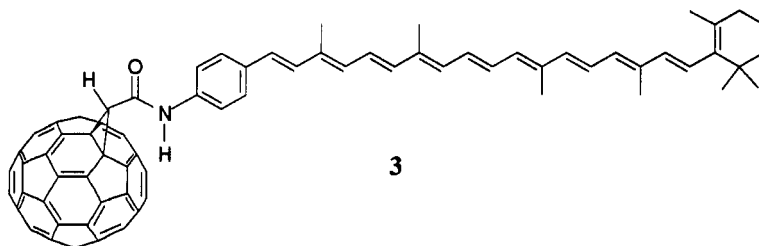
experiments, nonlinearity in absorbance vs. concentration plots of mixtures of C and Q in solution has been observed, which is suggestive of complexation between these species in solution.

In summary, photoinduced electron transfer between aggregated carotenoids within a monolayer would in principle generate $\text{C}^{*\cdot}$. The resulting electron and hole could migrate through the monolayer stack to the interfaces to give either anodic or cathodic photocurrents. The photocurrent flow depends on the nature of the redox component in the electrolyte solution. In the presence of redox couples favoring electron donation, the photoelectrode exhibited anodic photocurrent, while with electron acceptors in the aqueous phase it exhibited cathodic photocurrent. Although the unusually short excited singlet state lifetimes of carotenoid pigments severely limit their reactivity in diffusion-controlled reactions, these experiments demonstrate that when incorporated into organized structures they can participate as the photoactive species in photoinduced electron transfer processes, (ref. 9).

PHOTOINDUCED ELECTRON TRANSFER IN COVALENTLY LINKED SYSTEMS

Caroteno-C₆₀ dyad.

In order to explore intramolecular photochemical processes involving carotenoid polyenes and fullerenes, we have synthesized **3**, a C-C₆₀ dyad. The precursor methanofullerene-carboxylic acid was prepared according to the method described by Diederich *et al.*, (ref. 11). Reaction of C₆₀ and (ethoxycarbonyl)methyl diazoacetate yielded the methanofullerene ester, which was hydrolyzed by treatment with boron tribromide. Dyad **3** was synthesized by a dicyclohexylcarbodiimide-mediated condensation of the methanofullerenecarboxylic acid and the aminocarotenoid **2** in bromobenzene in the presence of catalysts, (ref. 12).



The absorption spectrum in toluene of dyad **3** is nearly a linear combination of the spectra of an appropriate model fullerene and carotenoid. The intense S₀ to S₂ carotenoid absorption band is centered at 475 nm. The absorption spectrum of **3** clearly illustrates the increased absorption cross section in the visible region provided by the carotenoid S₀ to S₂ transition. In the dyad, this band is slightly blue-shifted from that of the model carotenoid, 7'-apo-7'-(4-acetamidophenyl)-β-carotene. No fluorescence readily discernible from the background was observed in steady state fluorescence excitation and emission experiments using carefully purified samples of **3** in CS₂ or toluene solution. Excitation of a solution of **3** in CS₂ at 600 nm with 150 fs laser pulses gave a transient spectrum with a prominent band at 1068 nm. The rise of the transient absorption at 1068 nm is a first order process with a time constant of 800 fs; its decay is also first order with a lifetime of 534 ps.

The absence of detectable fluorescence from **3** following excitation into the fullerene absorption bands indicates that the C₆₀ S₁ level of the dyad is strongly quenched. Because the yield of triplet is only a few percent, the quenching cannot be assigned to increased intersystem crossing. Energetic considerations (*vide infra*) rule out singlet-singlet energy transfer to the carotenoid. The quenching is therefore attributed to photoinduced electron transfer to yield C^{•+}-C₆₀^{•-}. This state is energetically accessible from C⁻¹C₆₀ at 1.76 eV since the energy level of C^{•+}-C₆₀^{•-} lies only 1.01 eV above the ground state (estimated from cyclic voltametry). C^{•+}-C₆₀^{•-} is also accessible from ¹C-C₆₀, which is above 1.90 eV (*vide infra*).

Additional evidence for electron transfer quenching comes from the transient absorption results. Excitation of **3** at 600 nm yields mainly C⁻¹C₆₀, as the polyene has negligible absorption at that wavelength. The transient spectrum observed 3 ps after 600 nm excitation is consistent with contributions from both C₆₀^{•-} and C^{•+}, (ref. 13-15). Although photoinduced electron transfer must yield equal populations of both radical ions, the spectrum is assigned primarily to C^{•+} because the extinction coefficient at 1068 nm of C^{•+} is estimated to be *ca* ten-fold higher than that of the fullerene radical anion, (ref. 13-15). As the lifetime of the excited singlet state of C₆₀ model is *ca* 1 ns, the absence of observable fluorescence from **3** suggests that the quantum yield of C^{•+}-C₆₀^{•-} must be essentially unity.

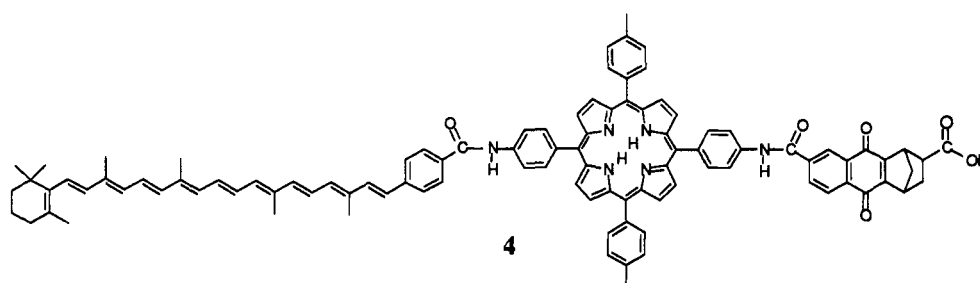
Because of its electric-dipole-forbidden nature, the S₀ to S₁ absorption of the carotenoid chromophore is not observed and hence the exact energy of the S₁ level is not known, (ref. 4,5). However, in previous studies the S₁ level of 7'-apo-7'-(4-acetamidophenyl)-β-carotene was found to be above 1.9 eV and to have a lifetime of 16 ps, (ref. 16). The S₁ state of the fullerene in **3** is located at 1.76 eV and it is therefore the lowest excited singlet state in **3**. The S₁ level of the carotenoid is populated by internal conversion from the S₂ level. In order for the excitation of the carotenoid to contribute to the photochemistry of dyad **3** there must be either efficient singlet energy transfer to the fullerene, or photoinduced electron transfer directly from the S₁ level of the carotenoid moiety. Due to the extremely low fluorescence quantum yield of **3** it was not possible to assay the energy transfer efficiency from the fluorescence excitation spectrum, and therefore determine the relative yields of these two processes.

Experimental data relevant to the question of relaxation pathways of the carotenoid and fullerene S_1 levels come indirectly from measurements of the yield of the lowest triplet species in **3**, the carotenoid triplet. The triplet yield upon excitation of the dyad at 515 nm, where >99% of the light is absorbed by the carotenoid, was compared to the yield upon excitation at 355 nm, where the fullerene absorbs *ca* 67% of the light. The yields were comparable in toluene solution. In other experiments, it has been shown that the carotenoid triplet state arises not by intersystem crossing of either $^1C-C_{60}$ or $C-^1C_{60}$, but rather by charge recombination of $C^{*+}-C_{60}^{*-}$. This being the case, the triplet quantum yield data can only be accommodated if light absorbed by either chromophore, carotenoid or C_{60} , produces a high yield of $C^{*+}-C_{60}^{*-}$.

In summary, the carotenoid in dyad **3** participates in ultrafast photoinduced electron transfer to generate $C^{*+}-C_{60}^{*-}$ and greatly increases the absorption cross section for photoinduced electron transfer in the visible region of the spectrum, (ref. 12).

Caroteno-porphyrin-quinone triad.

More complex systems with as many as five covalently linked components (porphyrins, quinones, carotenoid polyenes) that mimic photosynthetic antenna function and undergo photoinduced electron transfer to produce energetic, long-lived charge-separated states in high quantum yield have been prepared, (ref. 17,18). One of these artificial reaction centers, a molecular triad **4**, was prepared by linking a synthetic tetraarylporphyrin (P) to both a naphthoquinone (Q) moiety, which is fused to a norbornene system bearing a carboxylic acid group, and a carotenoid polyene (C), (ref. 19).



Upon excitation, triad **4** dissolved in various solvents undergoes photoinduced electron transfer from the excited singlet state of the porphyrin moiety to yield the intermediate charge-separated species $C-P^{*+}-Q^{*-}$ in less than 100 ps and with a quantum yield of ~ 1 . Subsequent electron transfer from the carotenoid to the porphyrin radical cation competes with charge recombination to yield $C^{*+}-P-Q^{*-}$ with a quantum yield up to 0.15 and a lifetime of >300 ns.

We have previously shown that triads similar to **4** can be inserted into lipid bilayer membranes where they photoconduct electrons across the bilayer. In that case the appropriate electron donors and acceptors were present in the aqueous phases on either side of the membrane (ref. 20). In a significant variation on this theme, triad **4** was inserted into the bilayer of liposomes with a view toward using the carotenoid to anchor the triad to the lipid component of the bilayer. Photochemical formation of $C^{*+}-P-Q^{*-}$ would generate intramolecular redox potential distributed within the bilayer as a redox gradient. However, vectorial redox potential distributed within the lipid bilayer would require asymmetric insertion of **4** into the liposomal bilayer membrane. This was accomplished by addition of a tetrahydrofuran solution of **4** into an aqueous solution containing preformed liposomes, a procedure which delivers **4** to the outside surface of the bilayer. The direct insertion of the lipophilic carotenoid of **4** into the bilayer is expected because it avoids the energetic cost of moving the polar, carboxylate-bearing quinone through the low dielectric medium. Thus, the carotenoid serves to direct the asymmetric self-assembly of this system.

Liposomes prepared with **4** and an auxiliary, lipophilic quinone (Q_s , 2,5-diphenylbenzoquinone) in the bilayer demonstrate photochemical translocation of protons from the external aqueous phase to the interior aqueous phase. The vectorial redox potential provided by excitation of **4** and subsequent electron transfers was coupled to the transmembrane proton transfer process by Q_s . Upon accepting an electron from Q^{*-} , the reducing site of the photochemically generated $C^{*+}-P-Q^{*-}$ species, Q_s is reduced to its anion radical. This species then accepts a proton from the exterior aqueous phase to form the semiquinone (Q_sH^{\bullet}) which diffuses to the site of oxidation potential, the carotenoid cation radical. Oxidation of Q_sH^{\bullet} yields Q_sH^+ which rapidly deprotonates to the nearby internal aqueous phase. This process generates a ΔpH and a membrane potential in much the same way that light energy is converted to proton motive force in natural photosynthetic membranes.

In conclusion, when tightly coupled to electron acceptors, carotenoids participate readily in photoelectrochemical reactions. The generation of charge-separated species within membranes and the translocation of protons across membranes upon absorption of light could represent the initial steps of a phototransduction mechanism. Observation of such effects in model systems reinforces the view that carotenoid pigments can indeed be blue light photoreceptors.

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