

## NEW TRENDS IN PHOTOBIOLOGY (Invited Review)

### THE PHOTOPHYSICS AND PHOTOCHEMISTRY OF THE CAROTENOIDS

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#### Summary

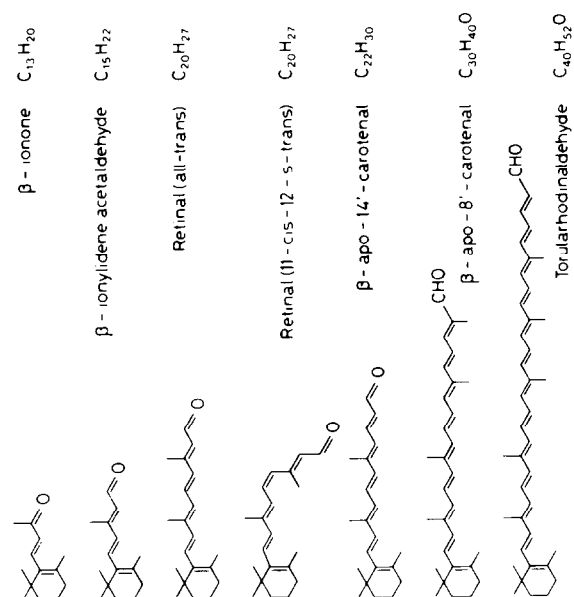
Considerable interest has been shown in the carotenoids for many years due to their wide ranging roles in photochemistry, photobiology and photomedicine and their possible use as a chemopreventative treatment for cancer. Studies about 20 years ago identified the triplet–triplet absorption of  $\beta$ -carotene; this work was of importance in understanding the protective role of these molecules in photosynthesis and bacterial photosynthesis and in porphyric disease treatment. Recently, attention has turned to the very weak fluorescence of these systems using picosecond measurements; results are related to the antenna role of carotenoids in photosynthesis. Photoisomerization continues to be studied with many of the recent developments based on pump-probe measurements using time-resolved resonance Raman spectroscopy for monitoring both the triplet state and the forbidden ( $^1\text{Ag}^{-*}$ ) excited singlet state. Interest is also currently centred on the interaction of singlet oxygen with carotenoids; these studies are of value in photophysics (*e.g.* determination of the carotenoid lowest triplet energy level) and in photomedicine (*e.g.* evaluation of the use of carotenoids as chemopreventative drugs).

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#### 1. Introduction

The two major groups of carotenoids of photobiological interest are the  $\text{C}_{40}$  pigments related to  $\beta$ -carotene and the  $\text{C}_{20}$  retinoids. Typical structures of important members of both of these groups together with some analogues are given in Fig. 1.

# Carbonyl - containing Hydrocarbons



# Hydrocarbons

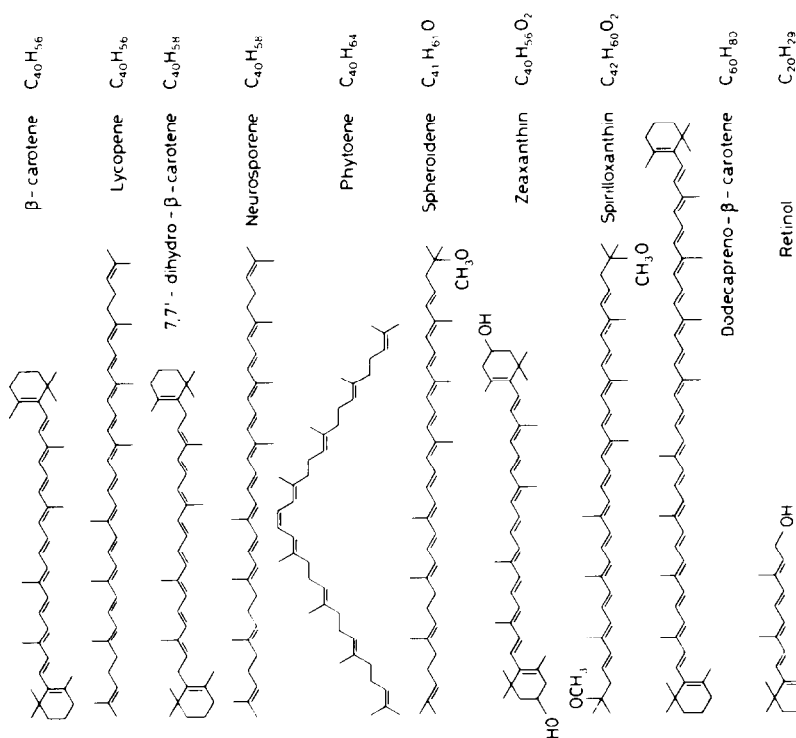


Fig. 1. Carotenoid structures.

The C<sub>40</sub> carotenoids, such as  $\beta$ -carotene, and the related bacteriophotosynthetic pigments, such as neurosporene, spheroidene and spirilloxanthin, have important roles in photosynthesis. In particular, they protect the photosynthetic system from photodynamic (photo-oxidative) damage and, in addition, they are accessory light-harvesting pigments in the antenna system of the photosynthetic organism. In the first of these roles they quench chlorophyll triplet states and could quench any singlet oxygen formed. In the second role they absorb light in the wavelength regions where the chlorophylls and bacteriochlorophylls absorb only weakly and transfer this energy to the reaction centre(s).

A number of workers (*e.g.* ref. 1) have suggested other roles associated with redox processes in photosynthesis, and carotenoid radical cations have been reported in the photosystem II reaction centre [2, 3]. However, the normal electron transfer was blocked in these studies. In addition, it is important to note that many studies of bacterial reaction centres have used carotenoid-less mutants of *Rhodobacter sphaeroides* and therefore carotenoids cannot be essential for the pivotal electron transfer reactions in bacterial reaction centres. Somewhat related are the elegant observations of Gust and Moore and coworkers (see, for example, ref. 4) in which triads and tetrads involving carotenoids-porphyrin(s)-quinones have been shown to undergo multistep electron and energy transfer leading to the production of the carotenoid moiety as a radical ion. This is not an *in vivo* situation and it is not suggested that this observation, of the carotene radical, is related to the normal photosynthetic processes. However, mimicking of both the photoprotective and antenna functions of carotenoids has been achieved in these models.

Photoisomerization of carotenoids, and particularly the 11-cis to all-trans isomerization of the C<sub>20</sub> retinoid, retinal, is crucial to the primary processes of vision. In the visual pigments, such as rhodopsin, the retinal is in the 11-cis, 12-s-trans isomeric form and is bound to a protein (opsin). It is the conformational changes caused in the protein by the photoisomerization of the retinal that initiates the early stages of vision.

As well as their relevance in photosynthesis and vision, the carotenoids have several other roles in photobiology and photomedicine and are of commercial importance as food and drink colorants. From both a medical and photochemical point of view the most important current use of  $\beta$ -carotene (although still somewhat controversial) is to treat the hereditary porphyric disease, erythropoietic protoporphyria (epp). In this use the porphyrin is believed to intercept the protoporphyrin triplet (<sup>3</sup>pp), thus preventing the formation of singlet oxygen (<sup>1</sup>O<sub>2</sub><sup>\*</sup>) which is known to be biologically (and chemically) very damaging



In addition, if some singlet oxygen is formed from  $^3\text{pp}$ , *i.e.* if process (1) does not totally preclude process (2), then  $\beta$ -carotene can also protect by the following energy transfer (quenching) process



Of course, treatment of epp with carotene is not a cure, but simply ameliorates the acute skin photosensitivity associated with this disease. Interestingly, forms of porphyria involving porphyrins other than pp, for example those involving an excess of uroporphyrin, are not amenable to carotene therapy. This implies the importance of the (close) location of the carotene with respect to the porphyrin in the skin. The protective role of carotenoids in photosynthesis can also be understood in terms of the above reactions, with the energy transfer from the chlorophyll triplet to the carotene and the subsequent harmless deactivation of the carotenoid triplet being most important.

The retinoids are also important to several body functions; indeed, the World Health Organization reports many thousands of new cases of blindness each year in the Third World due to retinol (vitamin A) deficiency. Furthermore, the drugs etretinate and isotretinoin (derivatives of retinoic acid) (Roche Products) are used for a wide range of dermatological problems including psoriasis and eczema keratoticum. Finally, the scavenging effects of carotenoids for a wide range of potentially damaging species ( $^1\text{O}_2^*$ , triplets and radicals) may suggest the therapeutic use of these molecules. Typically, anticancer properties have been claimed for both  $\text{C}_{40}$  and  $\text{C}_{20}$  carotenoids (see refs. 5–11).

## 2. Light absorption and fluorescence

Carotenoids exhibit intense colours, which is one reason for their commercial use as food colorants, and is also, for example, related to the colouring of many bird feathers. Such colours arise because members of the  $\text{C}_{40}$  series exhibit absorption bands in the visible region of the spectrum with high molar absorption coefficients (extinction coefficients)  $\epsilon_s$ . Typically, all-trans  $\beta$ -carotene has an  $\epsilon_s$  value of approximately  $1.5 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 460 nm in organic solvents such as benzene. Those carotenoids which have a *cis* band show somewhat reduced  $\epsilon_s$  values, but an extra band in the UV known as the '*cis*' peak. Thus 15,15'-*cis*- $\beta$ -carotene has  $\epsilon_s \approx 9 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 460 nm with an additional peak (compared with the all-trans isomer) at approximately 340 nm with  $\epsilon_s \approx 5 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ .

As might be expected, the  $\epsilon_s$  value increases and  $\lambda_{\text{max}}$  moves to a longer wavelength as the number of conjugated double bonds increases; typical examples are listed in Table 1.

The initial (intense) absorption of a carotenoid originates from the ground state ( $^1\text{Ag}$ ) to an excited state, termed  $^1\text{Bu}^*$ . It is known [12, 13] that an

TABLE 1

Ground state absorption maxima and  $\epsilon_s$  values as a function of the number  $n$  of conjugated double bonds

Carotenoid	$n$	$\lambda$ (nm)	$\epsilon_s$ (dm <sup>3</sup> mol <sup>-1</sup> cm <sup>-1</sup> )
Retinal	5	380	$3.7 \times 10^4$
7,7' Dihydro- $\beta$ -carotene	8	405	$1.2 \times 10^5$
$\beta$ -Carotene	11	466	$1.6 \times 10^5$
Decapreno- $\beta$ -carotene	15	517	$1.8 \times 10^5$
Dodecapreno- $\beta$ -carotene	19	550	$1.7 \times 10^5$

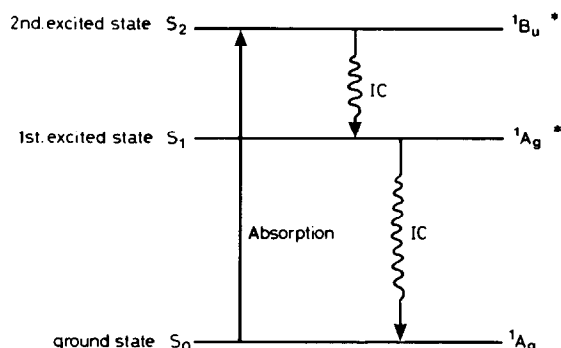


Fig. 2. Light absorption-deactivation scheme for C<sub>40</sub> carotenoids.

additional, lower-lying excited singlet state (<sup>1</sup>Ag\*) also exists, but the transitions involving this state and the ground state are forbidden. Nevertheless, this state is of importance in understanding the very weak fluorescence behaviour of carotenoids (as discussed below) and in understanding some of the photobiological processes involving carotenoids, such as their role as photosynthetic accessory pigments in antenna complexes.

The C<sub>40</sub> carotenoids are usually regarded as non-fluorescent, although in the last year or so [14, 15] two groups have reported a very weak emission with a fluorescence quantum yield  $\phi_F$  of approximately  $3 \times 10^{-5}$ . Taken with a calculated radiative lifetime  $\tau_r$  of approximately 1 ns, this implies a non-radiative lifetime  $\tau_n$  of the <sup>1</sup>Bu\* state of approximately  $3 \times 10^{-14}$  s ( $\tau_n = \tau_r \phi_F$ ). Gillbro and Cogdell [14] measured the ground state recovery to correspond to a process about 200 times slower than this, which is interpreted in terms of an absorption-deactivation scheme as shown in Fig. 2 in which the excited S<sub>2</sub> state decays by a fast internal conversion (IC) to the forbidden <sup>1</sup>Ag\* (S<sub>1</sub>) state and the kinetics of the recovery of the ground state are mainly determined by the somewhat slower decay of the <sup>1</sup>Ag\* (S<sub>1</sub>) state. The fluorescence emission detected shows only a very small Stokes shift and is presumably due to the <sup>1</sup>Bu\* state (S<sub>2</sub>); no fluorescence has yet been reported at longer wavelengths. Bondarev *et al.* [15] obtained  $\tau_n \approx 10$  ps for the <sup>1</sup>Ag\* state which was independent of solvent viscosity and may well be typical of the

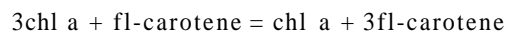
*in vivo* values. This suggests that it is the  $^1\text{Ag}^*$  state which is involved in the antenna energy transfer process in photosynthetic systems and not the much faster decaying  $^1\text{Bu}^*$  state.

For the C20 retinoids such as retinal and retinol the fluorescence has been well established for a number of years. For retinal, both  $n\sim r^*$  and  $\sim r\sim r^*$  excited states can arise and the observation of fluorescence in hydrogen-bonding solvents but not in hydrocarbon solvents has been related to excited state switching with only the  $7\text{rvr}^*$  ( $^1\text{Ag}^*$ ) state giving fluorescence [16].

The fluorescence of retinol is rather different in behaviour to the C40 carotenoids and shows a substantial Stokes shift. This is possibly due to an emission from the  $^1\text{Ag}^*$  state and may well also correlate with the anomalously long  $\text{VF}$  values for this molecule [17, 18].

### 3. The lowest triplet state ( $\text{T}_1$ )

The first reports of the detection of the triplet states of fl-carotene and retinol were given by Truscott and coworkers [19, 20]. These triplet states have since been studied by several groups [21-23]. In general, for the vast majority of carotenoids (but not some carbonyl-containing molecules such as retinal (see below)) the quantum yield of triplet formation  $\phi_T$  is near zero. Thus in the early work the major parameters, such as the triplet-triplet absorption spectra, triplet lifetimes and reactivities, were determined using energy transfer from a triplet sensitizer such as chlorophyll a (chl a) to populate the carotenoid triplet



More recently, the technique of pulse radiolysis [23] has been used to generate the triplet states without the need for a sensitizer [24].

The triplet-triplet absorption of all-trans retinal can be generated without a sensitizer and consequently this excited state was first reported more than 25 years ago [25]. In general, for all the carotenoids studied the same trends can be observed in the wavelength maxima  $\text{Ar}_{\sim}$  of the triplet absorption as discussed above for the ground state absorption (increasing molar absorption coefficient and a red (bathochromic) shift with an increasing number of conjugated double bonds ( $n$ )). Typical examples of this wavelength shift are 315 nm, 450 nm, 480 nm, 515 nm and 635 nm for phytoene ( $n=3$ ), retinal ( $n=6$ ), 7,7'-dihydro-fl-carotene ( $n=8$ ), fl-carotene ( $n=11$ ) and dodecapreno-fl-carotene ( $n=19$ ).

The triplet yields of many isomers of retinal, some related molecules such as protonated and unprotonated Schiff bases of retinal, various carbonyl-containing analogues (C13, C15, C17, Cae, Ce4, Cao and C40) and various cis and di-cis isomers of C $\sim$ 5 aldehyde and C1a ketone [26-28] have been discussed from 1975 to the present day; these have given a description of the relative positions of the  $n\sim\sim^*$  and  $\sim r\sim\sim^*$  singlet and triplet levels in the polyenals and polyenones and thus account well for the triplet properties of molecules such as retinal.

The lifetime of the lowest triplet state of carotenoids usually lies in the range  $10 \pm 5 \mu\text{s}$ , which is rather short for hydrocarbons. Furthermore, it must be realized that the spectroscopic (or vertical) triplet state formed initially undergoes extremely fast relaxation to a twisted triplet in which the central double bond is believed to be at  $90^\circ$  to its original configuration. This has been demonstrated [29] for  $\beta$ -ionone (a short-chain carbonyl-containing polyene), for which the relaxed or twisted form is reduced in energy by approximately  $65 \text{ kJ mol}^{-1}$  compared with the vertical triplet energy. Interestingly, the triplet (relaxed) lifetimes of these short-chain polyenes are much shorter (approximately 150 ns) than for the  $\text{C}_{20}$  and  $\text{C}_{40}$  carotenoids.

#### 4. Photoisomerization

While it is generally accepted that photoisomerization to a mixture of isomers (mainly all-trans) takes place via the lowest triplet state there has been much debate as to whether isomerization also takes place via the excited singlet state. Certainly, for the  $\text{C}_{40}$  carotenoids it seems that no isomerization can be detected from the singlet state, whereas for retinal, the excited singlet, a vibrationally excited triplet and a relaxed triplet have been invoked to account for the variation in photoisomerization with wavelength. The major component of the mixture of isomers produced is always the all-trans form. Thus in a recent study, Jensen *et al.* [30] have obtained a quantum yield of isomerization of the all-trans isomer of retinal to other isomers of  $0.15 \pm 0.05$ , whereas the quantum yield of 11-cis to the all-trans isomer is approximately unity.

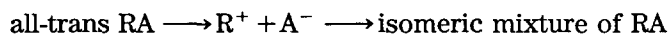
Time-resolved resonance Raman (T3R) spectroscopy has been used to study the photoisomerization of both groups of carotenoids [31–33]. Several isomers of  $\beta$ -carotene have been studied with virtually no difference in the T3R spectra of the lowest triplet state, which may well imply that a common triplet state (in the relaxed form) exists for all the isomers. Thus these results indicate that each cis configuration transforms mainly to the all-trans form via a common relaxed triplet. A consequence of this description is that the 9-cis and 13-cis molecules undergo two rearrangement steps following the initial (spectroscopic) triplet production. Some recent T3R studies have involved all-trans  $\beta$ -apo-carotenal [34] and the all-trans, 7-cis, 9-cis, 13-cis and 15-cis isomers of  $\beta$ -carotene [35]. The latter work has shown that only the all-trans and 15-cis isomers produce an identical triplet, with the other isomers producing different triplet species of their own. With regard to the initial and final ground state isomers, relaxation is observed to the original isomer for the all-trans, 7-cis and 9-cis isomers, whereas isomerization to the all-trans isomer is observed for the 13-cis and 15-cis isomers, *i.e.* a similar process to the 11-cis to all-trans isomerization noted above [30].

A particularly interesting picosecond T3R study of  $\beta$ -carotene [36] has reported the resonance Raman spectrum of the  $^1\text{Ag}^*$  state. The spectrum is

in excellent agreement with the predictions of previous polyene studies [37]. Furthermore, the work has emphasized the extremely high efficiency with which the  $\beta$ -carotene system converts absorbed energy from the  $^1\text{Bu}^*$  to the  $^1\text{Ag}^*$  state.

Studies of bacterial photosynthetic carotenoids [38] by T3R spectroscopy have given the  $\pi$  electron distribution along the carotenoid C—C backbone; this is estimated to be less regular in the triplet state than in the ground state, but not to the extent suggested by previous theoretical calculations [39].

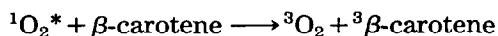
Finally, an additional route to photoisomerization of some retinoids arises via a photodissociation process; thus for retinyl acetate (RA)



where the classical carbenium ion ( $\text{R}^+$ ) and acetate ion can recombine to an isomeric mixture. In view of this type of monophotonic photodissociation process, topical use may well be expected to lead to some skin photosensitivity.

## 5. The lowest excited triplet energy level

The triplet energy level of the  $\text{C}_{40}$  carotenoids is not well established. Extrapolation of some early oxygen ( $\text{S}_0 \longrightarrow \text{T}_1$ ) perturbation studies leads to an estimate of approximately  $75 \text{ kJ mol}^{-1}$ , but the extrapolation is substantial. Certainly, it is established [40] that exchange energy transfer from singlet oxygen to  $\beta$ -carotene to produce triplet  $\beta$ -carotene is an efficient process



with a second-order rate constant ( $1.4 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  in many hydrocarbon solvents) of about half the diffusion-controlled value, possibly suggesting that the triplet energy level of  $\beta$ -carotene is approximately iso-energetic with  $^1\text{O}_2^*$  ( $92 \text{ kJ mol}^{-1}$ ). In a recent study [41], in which the temperature dependence of the quenching of  $^1\text{O}_2^*$  by  $\beta$ -carotene was studied, the results were interpreted in terms of a  $\beta$ -carotene triplet level somewhat above that of  $^1\text{O}_2^*$ . In contrast, Conn and MacLean [42] have measured the quenching of the triplet state of silicon naphthalocyanine (triplet level, approximately  $90 \text{ kJ mol}^{-1}$ ) by  $\beta$ -carotene and interpret their results in terms of the  $\beta$ -carotene triplet level being somewhat below that of  $^1\text{O}_2^*$ . It is possible that the extensive twisting of the conjugated polyenes during the energy transfer process makes such methods unsatisfactory for measuring triplet levels and some more direct measurement, such as an extension of the oxygen perturbation technique to the near-IR region, would be worthwhile. Very recently Lous and Hoff [43] have used magneto-optical difference spectroscopy to study the reaction centres of *Rhodobacter sphaeroides* 2.4.1. which contains 15–15'-mono-*cis*-spheroidene and *Rhodospirillum rubrum* S1 which contains 15,15'-spirilloxanthin. Using a curve-fitting routine based on a  $^3\text{bacteriochlorophyll} \rightleftharpoons ^3\text{carotenoid}$  equilibrium, these workers obtained

triplet levels of  $85 \text{ kJ mol}^{-1}$  and  $92 \text{ kJ mol}^{-1}$  for spheroidene and spirilloxanthin respectively. This is the opposite of the order expected on the basis of the number of conjugated double bonds (10 for spheroidene and 13 for spirilloxanthin).

Unlike the  $C_{40}$  carotenoids, the lowest triplet levels of the retinoids are reasonably well established with values of approximately  $155 \text{ kJ mol}^{-1}$  and  $160 \text{ kJ mol}^{-1}$  for retinol and retinal respectively (obtained by the energy transfer technique [44]).

## 6. Singlet oxygen–carotenoid interaction

The early work of Wilkinson and coworkers (see above) has confirmed that  $^1\text{O}_2^*$  reacts with  $\beta$ -carotene by an exchange electron transfer mechanism. These studies have been extended to several other carotenoids [45–48]. Both recent studies [47, 48] have shown that all-trans lycopene quenches  $^1\text{O}_2^*$  somewhat faster than  $\beta$ -carotene, although there is little quantitative agreement between the two sets of data. However, solvent effects and possible aggregation in the mixed ethanol-containing solvent system used by Mascio *et al.* [47] may account for this (benzene was used in ref. 48). The major significance of these findings is associated with the increasing interest in the role of  $\beta$ -carotene as an anticancer agent. Clearly, if lycopene is a more efficient  $^1\text{O}_2^*$  quencher, the strategies for chemoprevention of cancer should be extended from  $\beta$ -carotene to lycopene and possibly other carotenoids. Indeed it has been suggested that some nutrient component other than  $\beta$ -carotene, which is present in green and yellow vegetables, may protect against cancer. Of the other carotenoids studied [48] it has been found that dodecapreno- $\beta$ -carotene quenches singlet oxygen twice as efficiently as  $\beta$ -carotene itself (in toluene). The interaction of oxygen with the triplet state of retinol has been investigated by Smith [49] who showed that three pathways were involved: energy transfer (to give singlet oxygen), charge transfer (enhanced intersystem crossing) and carotenoid isomerization. Overall the charge transfer is most important and the yield of singlet oxygen from reaction of oxygen with the retinol triplet is approximately 0.25.

## 7. Radical ions

The possible role of radical ions in both photosynthesis and vision has often been discussed and such species certainly arise in electron transfer blocked photosynthetic reaction centres. Nevertheless, it is by no means certain that such species arise in the *in vivo* processes. There have been a number of studies of the radical ions of a wide range of polyenes, polyenones and some related systems [22, 23, 39, 50, 51]. In general, the radical anions and cations have similar  $\lambda_{\text{max}}$  values with the radical cation often absorbing at longer wavelengths but with both radicals absorbing at substantially longer

wavelengths than the parent molecule. Typical examples for the parent, radical cation and radical anion respectively are (nanometres in hexane): phytoene, 285, 470, 470; retinoic acid, 360, 590, 510;  $\beta$ -carotene, 450, 1050, 900.

In micellar environments the carotenoid radical characteristics imply a location in the outer mantle of the micelle [22]. In addition, electron transfer reactions between carotenoid radical anions ( $\text{Car}^{\bullet-}$ ) and porphyrins (P) have been shown [52] to be near diffusion controlled, but are very inefficient for  $\text{Car}^{\bullet+}$  except when the porphyrin is chlorophyll *a*. Thus, apart from when this chlorin is involved, the reaction

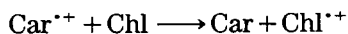


is very efficient and



is not efficient.

The possible relevance of the efficient transfer



to photosynthesis is intriguing.

All of the above studies of the radical ions of carotenoids were based on pulse radiolysis measurements about 10–15 years ago. More recently, using the pulse radiolysis technique Bobrowski and Das [50, 51] have measured the association of radical cations with the parent molecules. These “dimers” are produced with equilibrium constants around  $350 \pm 100 \text{ M}^{-1}$  and are most likely to be formed when the carotenoid terminates with a heteroatom and the solvent is non-polar. These studies suggest that such associative behaviour may be important in the role of these systems as charge mediators in lipid bilayer membranes.

## 8. Conclusions

In view of the possible role of carotenoids as a chemopreventative treatment for cancer and their established role in other areas of medicine, photosynthesis and vision, it is not surprising that photostudies of these systems continue to be of widespread interest. It seems likely that carotenoids not only quench the damaging singlet oxygen species *in vivo* and, more importantly, its precursor, the sensitizer triplet state, but can also protect against damage by a wide range of radical species.

## Acknowledgments

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