Singlet Energy Transfer from Carotenoids to Bacteriochlorophylls

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Chapter 18

Singlet Energy Transfer from Carotenoids to Bacteriochlorophylls

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Summary

The close association of carotenoids and bacteriochlorophylls in anoxygenic photosynthetic bacteria facilitates energy transfer processes between these molecules. A better understanding of the association between these two groups of pigments has been derived from significant, recent advances in: (1) The biochemical methodology for the separation and purification of pigment-protein complexes from photosynthetic bacteria; (2) The chemical procedures for the extraction, identification, and reconstitution of carotenoids into these complexes; (3) The analytical techniques for the chromatographic separation and purification of the carotenoid pigments; (4) The steady-state and time-resolved absorption and fluorescence spectroscopic techniques for exploring the nature of the excited states of carotenoids; and (5) Our understanding of the theoretical framework in which the photochemical properties of carotenoids may be cast. The present chapter will discuss these advances and attempt to provide a connection between the unique electronic structure of carotenoids and the manner in which they function as antenna pigments in photosynthesis.

I. Introduction

The abundance of carotenoids in photosynthetic preparations bears witness to the essential roles these pigments play in photosynthesis. Carotenoids are capable of at least three types of photochemical reactions in the photosynthetic apparatus: (1) Quenching of chlorophyll triplet states; (2) Quenching of chlorophyll singlet states; and (3) Singlet energy transfer from carotenoid to chlorophyll. The first two of these processes provide mechanisms by which carotenoids protect photosynthetic systems from singlet oxygen (generated by chlorophyll triplet states) and the harmful effects of excess light energy. We shall focus on singlet energy transfer from carotenoids, paying particular attention to prokaryotic photosynthetic bacteria. Recent advances in this area have come from systematic studies of the energies and decay rates of carotenoid excited states and measurements of the efficiencies of carotenoid-to-chlorophyll singlet energy transfer in vivo. In this chapter we shall explore the relationships between carotenoid electronic structure and the efficiency of energy transfer.

II. The Electronic Structure of Carotenoid Excited States

The photochemistry of carotenoids can be traced to the unique electronic properties of their conjugated π-electron frameworks (Zechmeister, 1962; Kohler 1993). Most carotenoids contain C_{40} carbon skeletons, corresponding to eight isoprene units. The number of conjugated C=C bonds in naturally occurring carotenoids ranges from 3 (e. g. phytoene) to 13 (e. g. spirilloxanthin) with 9–11 conjugated double bonds being typical for many of the carotenoids involved in photosynthesis (neurosporene, spheroidene, β-carotene, etc.). In addition, carotenoids with up to 19 double bonds have been obtained synthetically (Karrer and Eugster, 1951). The energies and dynamics of polyene excited states (the lowest energy excited singlet (S_1) and triplet (T_1) states are of particular importance in understanding energy transfer) are sensitive to the length of conjugation. The connection between biological function and the carotenoid structure is a topic of considerable interest.

Our current understanding of the low energy electronic states of carotenoids, in large part, is based on experimental and theoretical studies of shorter model systems (Hudson et al., 1982 and 1984; Kohler, 1991). Experimental studies of simple polyenes have exploited their relatively high fluorescence yields and their ability to be incorporated into low temperature n-alkane mixed crystals (Granville et al., 1979; D'Amico et al., 1980; Snyder et al., 1985; Simpson et al., 1987; Kohler et al., 1988). These crystals provide single, well-defined polyene conformations and homogeneous distributions of solvent/solute interactions resulting in vibronically-resolved absorption, fluorescence, and fluorescence excitation spectra. Such spectra lead to precise assignments of electronic and vibrational states and accurate determinations of electronic energies. Since energy transfer from carotenoids originates from vibrationally relaxed, electronically excited states, the 'zero-point' levels of these states are of particular relevance in understanding carotenoid/chlorophyll interactions. The high resolution experiments recently have been extended to model polyenes in molecular beams (Leopold et al., 1984; Heimbrook et al., 1984; Buma et al., 1990, 1991, 1992; Bouwman et al., 1990; Petek et al., 1991, 1992, 1993), resulting in an unprecedented view of the electronic and vibrational states of cold, isolated molecules. Although similar experiments are not yet feasible for carotenoids, these high-resolution results are critical for interpreting the typically broad optical spectra obtained from carotenoid solutions.

It is important to emphasize the profound importance of fluorescence spectroscopy in detecting and understanding the nature of the lowest energy S_1 states of polyenes/carotenoids. The high fluorescence quantum yields of intermediate length polyenes (e.g., octatetraene has $\phi_f \sim 0.6$ in low temperature glasses and mixed crystals (Gavin et al., 1978) has been a major factor in establishing these molecules as prototypical, linearly conjugated molecules. In contrast, shorter polyenes (e.g., unsubstituted dienes and trienes) and longer polyenes (e.g., β-carotene, spheroidene, and many other carotenoids of biological relevance) have fluorescence yields of 10^{-4} or less (Petek et al., 1992; Bondarev et al., 1988 and 1989; Gillbro et al., 1989; Cosgrove et al., 1990). The extremely low fluorescence yields of these compounds have presented significant barriers to the detection of their lowest excited singlet states (S_1).

Abbreviations: Rb. – Rhodobacter; Rp. – Rhodopseudomonas

This turns out to be a critical issue, since the lowest energy, $S_0 \to S_1$ absorptions in polyenes are extremely weak and difficult to detect on the tails of the $S_0 \to S_2$ absorptions responsible for the intense colors of the carotenoid family.

The $S_1 \to S_0$ and $S_0 \to S_1$ transitions have been directly detected in polyene/n-alkane mixed crystals using fluorescence and fluorescence excitation techniques, and it is to these experiments that we owe much of our current understanding of the nature of the S_1 states in linearly conjugated systems. The most characteristic signature of these spectra is the large (~2000–7000 cm⁻¹) shift in energy ('Stokes-shift') between spectral origins (0-0) of the strongly allowed, $S_0 \to S_2$ absorptions and the $S_1 \to S_0$ emissions. In addition, the S_1 states exhibit anomalously long fluorescence lifetimes (Hudson et al., 1972, 1973, 1982 and 1984). These features can be explained by the following ordering of singlet electronic energies: $S_0\,(1^1A_g) < S_1\,(2^1A_g) < S_2\,(1^1B_u)$. See Fig. 1. The fluorescence thus corresponds to a symmetry-forbidden, $2^1A_g \to 1^1A_g$ transition which lies below the strong, symmetry-allowed $1^1A_g \to 1^1B_u$ absorption. The Stokes-shifts thus provide accurate measures of the $1^1B_u \to 2^1A_g$ energy differences. The above energy scheme has been verified by high-level calculations on model systems (Schulten et al., 1972 and 1976; Ohmine et al., 1978; Tavan et al., 1979 and 1986; Orlandi et al., 1991). Theory shows that simple molecular orbital models (e.g., Hückel theory which incorrectly predicts a $1^1A_g < 1^1B_u < 2^1A_g$ ordering for the lowest electronic states) do not adequately treat the effects of electron-electron repulsion of the π electrons in the polyene chains. Though the u and g symmetry labels (and the concepts of strictly 'allowed' or 'forbidden' transitions) apply only to polyenes with centers of symmetry (e.g., β-carotene), all polyenes seem to exhibit the same electronic energy orderings and similar relative transition strengths. We shall retain the idealized symmetry labels in discussing the low lying energy levels of carotenoids.

Extending the optical studies of model systems to carotenoids has been hampered by their broad, almost featureless spectra and extremely low fluorescence yields. Early reports of fluorescence from β-carotene indicated an emission origin coinciding with the origin of the strongly allowed, $1^1A_g \to 1^1B_u$ absorption (Cherry et al., 1968; van Riel et al., 1983; Haley and Koningstein, 1983; Watanabe et al., 1986; Bondarev et al., 1988, 1989). It is important to note that van
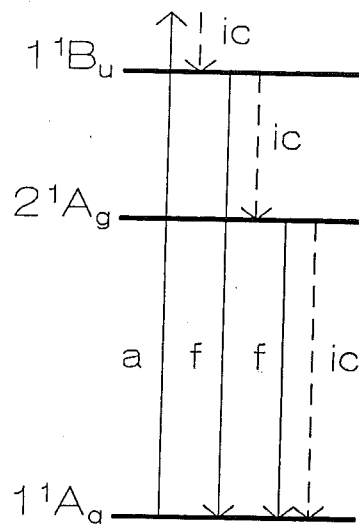

Fig. 1. Schematic representation of the ordering of excited state energies and the intramolecular photochemical processes (a, absorption; f, fluorescence; and ic, internal conversion) carotenoids and polyenes can undergo. The states are labeled by the idealized group theoretical representations in the point group C_{2h}.

Riel et al. (1983) and Bondarev et al. (1988) obtained fluorescence excitation spectra of the weak emission, thus correcting a fundamental shortcoming of the reports of β-carotene fluorescence by Cherry et al. (1968), Haley and Koningstein (1983) and Watanabe et al. (1986). The good agreement between the $1^1A_g \to 1^1B_u$ absorption and the fluorescence excitation spectra proved the existence of β-carotene fluorescence. Later work of Gillbro and Cogdell (1989) and Cosgrove et al. (1990) confirmed the presence of $1^1B_u \to 1^1A_g\,(S_2 \to S_0)$ emissions in β-carotene and related carotenols. The detailed studies of shorter polyenes allows us to identify the emission spectra of β-carotene and other long carotenoids as being due to $S_2 \to S_0$ fluorescence. $S_1 \to S_0\,(2^1A_g \to 1^1A_g)$ emission has not yet been detected in β-carotene, spheroidene, or other carotenoids with more than nine conjugated double bonds, leaving the location of their 2^1A_g states subject to indirect detection.

An early attempt to locate the low-lying 2^1A_g state in β-carotene was made by Thrash et al. (1977, 1979) using resonance Raman excitation profiles. This work suggested that the 2^1A_g state was ~3500 cm⁻¹ below the 1^1B_u state placing the $S_1\,(2^1A_g)$ states of β-carotene and other carotenoids well above the $S_1\,(Q_y)$ states of most chlorophylls. These energetics have been widely cited in discussions of the antenna function of carotenoids in photosynthesis (Siefer-

mann-Harms, 1985). However, a relatively small S_2-S_1 energy difference for β-carotene was not consistent with trends noted in shorter polyenes (Snyder et al., 1985; Cosgrove et al., 1990). Furthermore, a later investigation of the Raman excitation profiles of β-carotene (Watanabe, et al., 1987) could not reproduce the Thrash et al. (1977 and 1979) results. This showed that Raman excitation profiles shared the same limitations as standard absorption measurements in detecting weak $1^1A_g \rightarrow 2^1A_g$ transitions on top of strong $1^1A_g \rightarrow 1^1B_u$ absorption tails. Location of the 2^1A_g state in β-carotene and other carotenoids thus has relied on the extrapolation of trends observed in the $2^1A_g \rightarrow 1^1A_g$ transition energies of shorter, more fluorescent polyenes.

Cosgrove et al. (1990) carried out a systematic study of the connection between the fluorescence properties of shorter polyenes (relatively strong emissions from S_1 (2^1A_g)) and longer carotenoids (weak emissions from S_2 (1^1B_u)). A series of carotenols with 7–11 conjugated double bonds was purified using HPLC techniques, and absorption, fluorescence, and fluorescence excitation spectra were obtained in 77 K glasses. The shorter, less conjugated members of this series exhibited the Stokes-shifted $S_1 \rightarrow S_0$ emissions of short model polyenes. Optical spectra of the carotenols are significantly broader due to well-understood complications brought about by twisting of the cyclohexenylidene end group (Christensen and Kohler 1973; Hemley and Kohler 1977). Nevertheless, as illustrated in the comparison of the fluorescence excitation and emission spectra of β-apo-12'-carotenol and hexadecaheptaene (Fig. 2), there is sufficient resolution in the carotenol spectra to allow the accurate identification of electronic origins. An important feature of Fig. 2 is the almost identical $2^1A_g \rightarrow 1^1A_g$ transition energies of the two heptaenes. This illustrates that in β-apo-12'-carotenol the loss in conjugation due to the nonplanarity between the ring and side-chain is offset by the stabilizing effects of the isoprenoid structure, making hexadecaheptaene an unexpectedly appropriate model for the more complicated carotenol. (By implication, we might predict that the electronic states of β-carotene will have energies quite similar to those of corresponding states of model polyenes with eleven co-planar double bonds.)

Cosgrove et al. (1990) found that the fluorescence of carotenols with more than eight conjugated double bonds was dominated by 'anti-Kasha' $S_2 \rightarrow S_0$ fluorescence as previously had been observed for β-
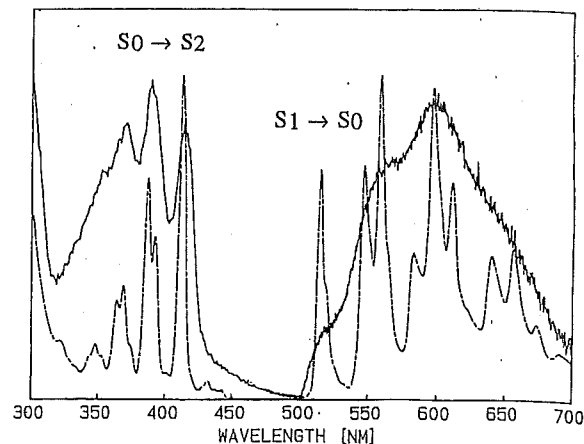

Fig. 2. Comparison of fluorescence and fluorescence excitation spectra of all-trans-2,4,6,8,10,12,14-hexadecaheptaene (dashed lines) and all-trans-β-apo-12'-carotenol (solid lines). The fluorescence spectrum of hexadecaheptaene in *n*-pentadecane at 77 K was obtained by exciting at 414 nm, and the fluorescence excitation spectrum monitored the emission intensity at 558 nm. The fluorescence spectrum of β-apo-12'-carotenol in 77 K EPA was obtained by exciting at 413 nm. The excitation spectrum was obtained by monitoring the fluorescence intensity at 650 nm. This figure from Cosgrove et al. (1990) was reproduced with permission.

carotene (van Riel et al., 1983; Watanabe et al., 1986; Bondarev et al., 1988; Gillbro et al., 1989). The cross-over from $S_1 \rightarrow S_0$ to $S_2 \rightarrow S_0$ emissions was attributed to a larger $S_2 \rightarrow S_1$ energy difference and the resultant decrease in $S_2 \rightarrow S_1$ radiationless decay (following the 'energy-gap law' of Englman and Jortner, 1970). This allows S_2 emission to compete with internal conversion. The lack of S_1 fluorescence prohibits the direct observation of the S_1 state in longer carotenoids. Nevertheless, trends noted in the model polyenes suggested 2^1A_g energies considerably lower than those originally suggested by the work of Thrash et al. (1977 and 1979).

DeCoster et al. (1992) extended the Cosgrove (1990) study to provide a systematic comparison of the energy levels of a series of α,ω-dimethylpolyenes (for which the vibronic resolution allows accurate measurement of electronic energies) with a series of iso-structural spheroidene analogs varying only in their extent of conjugation (7, 8, 9 and 10 double bonds). See Fig. 3. The energies of the S_2 and S_1 electronic origins are summarized in Fig. 4. The Cosgrove (1990) study points to the following conclusions: (1) The S_2-S_1 energy difference increases with increasing conjugation, (2) The S_2 and S_1 energies
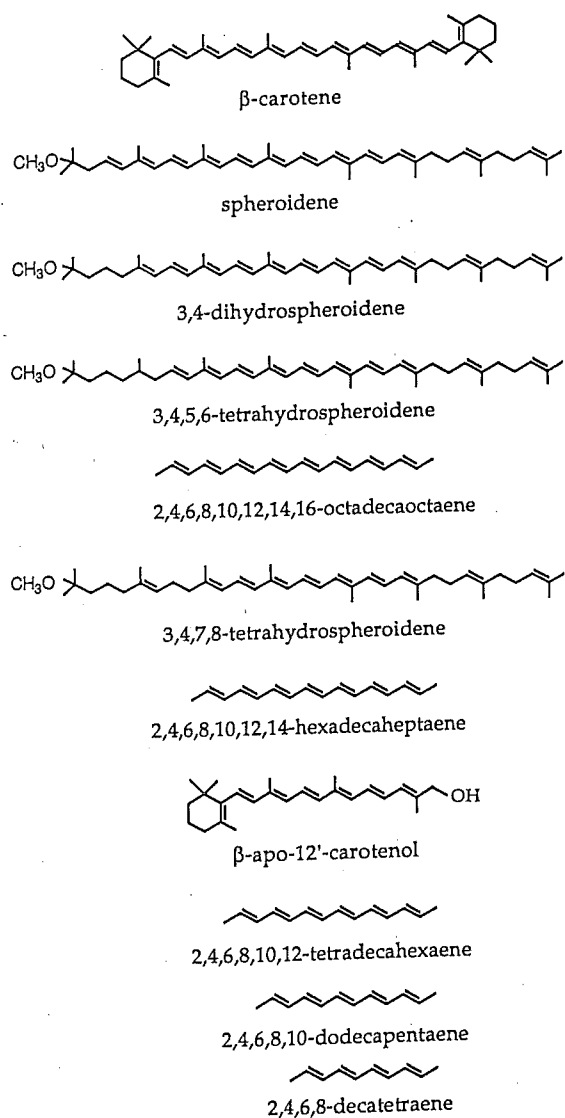
β-carotene

spheroidene

3,4-dihydrospheroidene

3,4,5,6-tetrahydrospheroidene

2,4,6,8,10,12,14,16-octadecaoctaene

3,4,7,8-tetrahydrospheroidene

2,4,6,8,10,12,14-hexadecaheptaene

β-apo-12'-carotenol

2,4,6,8,10,12-tetradecahexaene

2,4,6,8,10-dodecapentaene

2,4,6,8-decatetraene

Fig. 3. The structures of several polyenes and carotenoids discussed in the present work

the possibility that the S_1 state of β-carotene (11 double bonds) lies below the S_1 state (Q_y) of its chlorophyll acceptor.

Recent estimates of the 2^1A_g energies of spheroidene, β-carotene and other long carotenoids appear to span a rather wide range. For example, Watanabe et al. (1993) suggest a 2^1A_g energy for spheroidene of 14,900 cm^{-1} (hexane)/14,600 cm^{-1} (CS$_2$). Mimuro et al. (1993) have reported the observation of the 2^1A_g → 1^1A_g(0-0) transition for neurosporene at ~16,000 cm^{-1}, an energy considerably above the 15,300 cm^{-1} obtained by DeCoster et al. (1992) from fluorescence spectra (Fig. 4). The fluorescence assigned to 2^1A_g → 1^1A_g emission by Watanabe et al. (1993) has almost all of its intensity in a single vibronic band (673 nm) analogous to a porphyrin derivative. This contrasts with the extended vibronic development of typical 2^1A_g → 1^1A_g polyene emissions (e. g. Fig. 2). Analysis of the weak, long wavelength absorption spectrum of neurosporene (Mimuro et al., 1993) probably overestimates the 2^1A_g zero-point energy. Franck-Condon envelopes of polyene 2^1A_g ← 1^1A_g absorptions show a steep monotonic rise in vibronic intensities with increasing energies due to the $(\Delta E)^{-2}$ energy dependence of vibronic mixing between the 2^1A_g and 1^1B_u states (Petek et al., 1991). This is clearly evident in high resolution spectra of model polyenes (Simpson et al., 1987; Kohler et al., 1988). The 2^1A_g ← 1^1A_g(0-0) bands are thus extremely weak compared to higher energy vibronic bands, making them difficult to detect in low resolution experiments.

Similar uncertainties in estimates of the 2^1A_g energy of β-carotene are probably of more relevance, given the possibility that this state may not have enough energy to participate in energy transfer to chlorophyll a (S_1 (Q_y) energy of ~15,000 cm^{-1} (Sauer (1975)). For example, Andersson et al. (1992a) have obtained S_1 → S_0 fluorescence spectra from the shorter members of a series of β-carotene analogs. Based on estimates of (0-0) energies of shorter members of this series (unlike the spectra of spheroidenes and model polyenes, the fluorescence spectra of the carotene analogs are not sufficiently resolved to observe electronic origins), these authors estimate a β-carotene 2^1A_g energy of ~ 14,500 cm^{-1}. This is significantly higher than the value predicted from extrapolation of the data presented in Fig. 4 and might permit energy transfer from 2^1Ag. Andersson et al. (1992a) also suggest that the S_2 → S_1 energy gap does not increase with increasing conjugation, a

of the more highly substituted spheroidenes are lower than those of α,ω -dimethylpolyenes with the same conjugation, (3) Spheroidene (like β-carotene) is essentially an S_2 emitter, (4) Of particular interest for this review is the comparison of the extrapolated S_1 energies of spheroidene and β-carotene with the S_1 energies of bacteriochlorophylls and chlorophylls. These extrapolations place the S_1 (2^1A_g) energy of spheroidene at 14,000–14,500 cm^{-1}, above the S_1 levels of bacteriochlorophylls, allowing singlet energy transfer to occur via the spheroidene 1^1A_g state. However, the data presented in Fig. 4 strongly suggest

Fig. 4. $S_0 \to S_1$ ($1^1A_g \to 2^1A_g$) and $S_0 \to S_2$ ($1^1A_g \to 1^1B_u$) transition energies for α,ω-dimethylpolyenes and spheroidenes as a function of conjugated length. Energies of the S_1 states are the electronic origins (0-0 bands) observed for $S_1 \to S_0$ fluorescence in room temperature methanol. Energies of the S_2 states are the electronic origins (0-0 bands) observed for $S_0 \to S_2$ absorption in room temperature methanol. Dimethyl polyene data are from Morey and Christensen (unpublished data) and Kohler et al., (1988). Chlorophyll a (Q_x = 575 nm, Q_y = 660 nm) and bacteriochlorophyll a (Q_x = 573 nm, Q_y = 769 nm) transition energies in diethyl ether at room temperature are taken from Sauer (1975). This figure from DeCoster et al. (1992) was reproduced with permission.

result inconsistent with trends noted both in model polyenes and the spheroidenes. Haley et al. (1992) recently suggested a β-carotene 2^1A_g energy of ~14,200 ± 400 cm⁻¹, based on the *maximum intensity* of the reflectance spectrum in zeolite. However, as discussed above, a broad spectrum does not facilitate the identification of a relatively weak electronic origin which very likely lies on the long wavelength tail of the 700 nm peak. Another intriguing result is the recent observation of a weak absorption background in the inverse Raman (Raman loss) spectrum of canthaxanthin (Jones et al., 1992). This shows evidence for a low lying absorption at ~600–700 nm, though the identification of the (0-0) band of the $2^1A_g \leftarrow 1^1A_g$ transition again is subject to considerable uncertainty. Though the earlier (Thrash et al., 1977, 1979) estimates of the 2^1A_g energy of β-carotene clearly should be abandoned, higher resolution spectroscopic work will be needed to accurately locate the $2^1A_g \leftarrow 1^1A_g$ electronic origins in β-carotene and other long carotenoids.

III. The Dynamics of Carotenoid Excited States

A wide variety of methods have been employed for measuring the excited state dynamics of carotenoids. Indirect methods include ground state depletion

(Dallinger et al., 1981b; Wylie and Koningstein 1984), lifetime broadening of resonance-Raman spectral lines (Haley and Koningstein 1983), time resolved resonance-Raman spectroscopy (Noguchi et al., 1989; Hashimoto and Koyama 1990; Kuki et al., 1990) and emission yield determinations (Shreve et al., 1991a; Gillbro and Cogdell 1989; Cosgrove et al., 1990, Katoh et al., 1991; Mimuro et al., 1991; and Andersson et al., 1991). The direct methods are picosecond (Wasielewski and Kispert 1986; Gillbro and Cogdell 1989) and femtosecond (Shreve et al., 1991b and 1991c, Frank et al., 1993) time resolved transient absorption measurements.

A. Dynamics of S_1 States

The lifetimes of the 2^1A_g states of a range of carotenoids have now been investigated. Using picosecond transient absorption spectroscopy, S_1 lifetimes of 8.4 ± 0.6 ps for β-carotene, 5.2 ± 0.6 ps for canthaxanthin and 25.4 ± 0.2 ps β-8'-apocarotenal in toluene were found (Wasielewski and Kispert, 1986). Wasielewski et al. (1989) also measured the temperature dependence of the lowest excited singlet state lifetime of all-trans-β-carotene and fully deuterated all-trans-β-carotene in 3-methylpentane. In this work the authors reported an 8.1 ± 0.5 ps 2^1A_g lifetime for all-trans-β-carotene and a 10.5 ± 0.6 ps 2^1A_g lifetime for fully deuterated all-trans-β-carotene.

The weak dependence of the 2^1A_g lifetime on isotopic substitution suggests that conformational changes in the C-H bending and stretching modes are not primary factors in the decay of the 2^1A_g state of carotenoids to the ground state. It was postulated that changes in the frequencies of the C-C stretching modes along the carbon backbone of the carotenoid provide the appropriate accepting modes for nonradiative decay of the 2^1A_g state.

Shreve et al. (1991a,b) used femtosecond ground state depletion/recovery techniques to record an 11 ps lifetime of the 2^1A_g state of β-carotene in CS_2. These authors extended their studies to carotenoids extracted from photosynthetic bacteria and found a 9.1 ps lifetime of the S_1 state of spheroidene in cyclohexane. Noguchi et al. (1989), Hashimoto and Koyama (1990) and Kuki et al. (1990) used picosecond time-resolved resonance Raman spectroscopy to examine the 2^1A_g states of β-carotene and spheroidene and concluded that the lifetime of the 2^1A_g states of these molecules must be at least 10 ps. Thus, several workers have converged on the range 10 ± 2 ps for the lifetimes of the 2^1A_g states of both β-carotene and spheroidene. (See also below.) In addition, Gillbro and Cogdell (1989) used picosecond ground state depletion/recovery experiments and measured the lifetime of the 2^1A_g state of spheroidenone, a carbonyl-containing derivative of spheroidene, to be 15 ps in CS_2.

Monitoring time-resolved fluorescence from the 2^1A_g state is another manner in which one may probe the S_1 dynamics of shorter carotenoids. The time-resolved fluorescence decay profile of fucoxanthin ($n = 8$) in CS_2 displayed a major kinetic component of 41 ps, while that of β-8'-apocarotenal in the same solvent was 16 ps (Katoh et al., 1991). Andersson et al. (1992) measured a 2.0 ns lifetime for emission from the 2^1A_g state of a β-carotene analog having 5 double bonds.

In an attempt to understand the controlling features of nonradiative decay of the 2^1A_g state of carotenoids, Frank et al. (1993) analyzed a series of four *all-trans*-carotenoids: 3,4,7,8-tetrahydrospheroidene, 3,4,5,6-tetrahydrospheroidene, 3,4-dihydrospheroidene, and spheroidene (Fig. 3). These molecules have π-electron conjugations that systematically increase from 7 to 10 carbon-carbon double bonds. Otherwise, they are structurally identical. The $S_1 \rightarrow S_n$ absorptions decayed with single-exponential kinetics revealing S_1 lifetimes of $\tau = 407 \pm 23$ ps for 3,4,7,8-tetrahydrospheroidene, 85 ± 5 ps for 3,4,5,6-

tetrahydrospheroidene, 25.4 ± 0.9 ps for 3,4-dihydrospheroidene, and 8.7 ± 0.1 ps for spheroidene in petroleum ether. The data were analyzed in terms of the energy gap law for radiationless transitions (Englman and Jortner, 1970).

$$k_{ic} = A \cdot \exp(-\gamma \Delta E / \hbar \omega_M) \qquad (1)$$

where k_{ic} is the internal conversion rate constant, in this case very well approximated by $1/\tau$ because k_{ic} for polyenes is much larger than the S_1 radiative rate constant, $k_f \sim 10^6$ to $10^7\,s^{-1}$. A is a pre-exponential factor that depends on the S_1-S_0 energy difference, ΔE,

$$A = \frac{C^2 \sqrt{2\pi}}{\hbar \sqrt{\hbar \omega_M \Delta E}} \qquad (2)$$

For the four spheroidenes A can be safely approximated as a constant. $\hbar \omega_M$ is the energy of the high frequency 'acceptor' modes (presumably C=C stretches of ~ 1600 cm^{-1}), and γ can be related to the relative displacement of the potential surfaces in the two electronic states. The $S_1 \rightarrow S_0$ transition energies deduced for 3,4,5,6-tetrahydrospheroidene, 3,4,7,8-tetrahydrospheroidene and 3,4-dihydrospheroidene compounds from fluorescence experiments (DeCoster et al., 1992) were 18,400 cm^{-1}, 16,700 cm^{-1} and 15,300 cm^{-1}, respectively. The $S_1 \rightarrow S_0$ transition energy for spheroidene has not previously been assigned. A fit of the energy gap law expression to these data yielded a 2^1A_g (S_1) state energy value of 14,100 cm^{-1} for spheroidene, in good agreement with the value determined by extrapolation from fluorescence studies ($\sim 14,000$ cm^{-1}).

In a similar fashion, Andersson and Gillbro (1992b) studied a systematic series of β-carotene analogs. In this study, analogs of β-carotene possessing 5, 7, 9, 11 (β-carotene), 15 and 19 conjugated double bonds were analyzed by femtosecond transient absorption experiments. The S_1 decay rates were found to increase with the number of carbon-carbon double bonds in qualitative agreement with the energy gap law. The variation in the 2^1A_g energies for this series of carotenoids was so extensive, however, that the full energy dependence including the explicit $1/\sqrt{\Delta E}$ functionality in the pre-exponential term (Eq. (2)) must be considered in treating the data.

B. Dynamics of S_2 States

The lifetimes of the 1^1B_u states of a range of carotenoids have been directly measured. Shreve et al. (1991a,b) used femtosecond transient absorption measurements and determined the room temperature lifetimes of the 1^1B_u states of β-carotene and spheroidene to be 200–250 fs and 340 fs, respectively. The lifetimes varied slightly depending upon the solvent. This group also found that the 1^1B_u state decayed into a second excited state which had a lifetime of 11 ps for β-carotene and 9.1 ps for spheroidene. This second state is in all probability the 2^1A_g state.

C. Connection Between Direct and Indirect Measurements of S_1 and S_2 Dynamics

The fluorescence yield of any state can be expressed as:

$$\Phi_f = \frac{\tau}{\tau_r} \qquad (3)$$

where τ is the lifetime and τ_r is the natural radiative lifetime of the state (Strickler and Berg, 1962). The natural radiative lifetime of the S_2 (1^1B_u) state (but not the S_1 (2^1A_g) state) of a carotenoid can be estimated by integrating its absorption profile. This procedure yields a value of about 1 ns for the S_2 radiative lifetime of β-carotene (Gillbro and Cogdell 1989; Shreve et al., 1991a). Fluorescence quantum yields lead to lifetimes of ~200 fs for the 1^1B_u states of β-carotene and spheroidene. This number agrees very well with the direct kinetic measurements of Shreve et al. (1991a,b).

The yield of S_1 emission from the previously described β-carotene analog having 5 carbon-carbon double bonds was $7 \pm 3 \times 10^{-3}$ (Andersson et al., 1992). This value, combined with the 2.0 ns lifetime of this state, yields a natural radiative lifetime for the 2^1A_g state of 300 ns If similar natural radiative lifetimes are assumed for the β-carotene analog with 7 carbon-carbon double bonds and for β-carotene (11 carbon-carbon double bonds), then fluorescence yield measurements indicate lifetimes of their 2^1A_g states of ~240 ps and 3-10 ps, respectively. These values are in good agreement with the lifetimes determined by direct time-resolved absorption measurements and ground state recovery experiments (Andersson and Gillbro, 1992).

IV. Implications for Energy Transfer

The efficiency, ε, of energy transfer between two molecules is defined by

$$\varepsilon = \frac{k_{ET}}{k_{ET} + \sum_i k_i} \qquad (4)$$

where k_{ET} is the rate constant for energy transfer and k_i represents any one of several alternative excited state decay pathways. Energy transfer from carotenoids to chlorophylls can easily be demonstrated by comparing the absorption and chlorophyll fluorescence excitation profiles from antenna pigment-protein complexes isolated from photosynthetic bacteria or algae. Normalization of the two spectral profiles at a specific wavelength (e. g. the maximum of the bacteriochlorophyll Q_x transition near 600 nm in the B800–850 complex from photosynthetic bacteria) allows the calculation of ε. Efficiencies measured in this manner range from greater than 90% for spheroidene in the B800–850-complex from *Rhodobacter (Rb.) sphaeroides* wild type strain 2.4.1 (Cogdell et al., 1981; Noguchi et al., 1990; van Grondelle et al., 1982) to ~ 25% for the carotenoids, spirilloxanthin, rhodopin, and lycopene in the B880 complex from *Rhodopseudomonas (Rp.) acidophila* (Angerhofer et al., 1986). In general, the longer chain carotenoids show lower efficiencies of energy transfer to chlorophylls (Frank and Cogdell, 1993).

These steady state experiments are particularly interesting when comparing a systematic series of complexes. For example, Frank et al. (1993) analyzed the B850 complex from the carotenoidless mutant *Rb. sphaeroides* R-26 after it had been reconstituted with the four spheroidene analogs discussed above (Fig. 3). This study attempted to provide a systematic approach to exploring the effect of excited state energies, spectral overlap and excited state lifetimes on the efficiency of carotenoid-to-bacteriochlorophyll singlet energy transfer. The efficiencies of energy transfer for the four reconstituted complexes could be rationalized from changes in spectral overlaps and an increase in the rate of $2^1A_g \rightarrow 1^1A_g$ internal conversion with increasing conjugation. The work suggested that the general trend of lower efficiencies of energy transfer from carotenoids to bacteriochlorophyll for longer chain carotenoids (e. g. spirilloxanthin) may be due to the 2^1A_g states of these

molecules lying below the S_1 state of bacteriochlorophyll. Augmenting these studies with transient dynamics data carried out directly on the isolated pigment-protein complexes would test this hypothesis.

Upon excitation of the B800–850-complex from *Rp. acidophila* strain 7750, Wasielewski et al. (1986b) observed a bleaching of the carotenoid absorption, which was restored with a time constant of 5.6 ± 0.9 ps. The rise-time of a bleaching of a bacteriochlorophyll band at 860 nm occurred in 6.1 ± 0.9 ps indicating the arrival of the carotenoid excitation at the bacteriochlorophyll site. Similar experiments were carried out by Gillbro et al. (1988) on the B800–850-complex from *Rps. acidophila* strain 7050. Energy transfer from the carotenoid to the bacteriochlorophyll took place in 3 ± 1 ps. Femtosecond time-resolved experiments were carried out (Trautman et al., 1990b) on the thylakoid membrane preparations from the diatom *Phaeodactylum tricornutum* and from *Nannochlorpsis sp*. The time for the carotenoid-to-chlorophyll energy transfer in the latter species was found to be $< 240 \pm 40$ fs (*i. e.* instrument limited). Energy transfer in the diatom was bi-exponential with transfer times 500 ± 100 fs and 2.0 ± 0.5 ps and relative amplitudes of 1.7 ± 0.7:1.

Using femtosecond time-resolved techniques, Trautman et al. (1990a) and Shreve et al. (1991b) studied the B800–850 complex isolated from *Rb. sphaeroides* strain 2.4.1. The data were interpreted within the framework of a model whereby the carotenoids transfer energy to both the 800 nm and 850 nm-absorbing bacteriochlorophylls with the majority of the carotenoids present in the complex transferring their energy directly to the 850 nm bacteriochlorophylls in ~300–400 fs. The remainder of the carotenoids transfer their energy via the 800 nm bacteriochlorophylls to the 850 nm-absorbing molecules. The best fit of the dynamics data requires that energy transfer can originate from both the 1^1B_u and the 2^1A_g states of the carotenoid. The suggestion that energy transfer from carotenoids may originate from their 1^1B_u (S_2) states opens up the possibility of S_2 transfer in higher plant systems where S_1 might be too low to participate in energy transfer to chlorophyll *a*.

A fundamental goal of research in this area is to relate the efficiencies and kinetics of carotenoid/chlorophyll energy transfer to the electronic structures and excited state dynamics of the donors and acceptors. Energy transfer between unlike molecules usually is explained using one of two established models. These are the dipole-dipole or coulomb (Förster) mechanism or the electron exchange (Dexter) mechanism. (Förster 1948, 1965; Dexter, 1953; see also Chapter 15 by Struve in this volume). Which of these applies to energy transfer between antenna carotenoids and chlorophylls will depend critically on the answers to the following questions: (1) How do specific molecular features (e. g. distance, geometry, structure, extent of conjugation, stereochemistry, functional groups, etc.) control energy transfer? (2) Which carotenoid excited electronic states (1^1B_u or 2^1A_g) participate in the transfer and how is the overall energy transfer efficiency partitioned between them? (3) Which chlorophyll electronic states (those associated with the Q_x or Q_y transitions or other states) are involved in the transfer?

In principle, which mechanism (coulomb or exchange) operates between carotenoids and chlorophylls can be determined by analyzing how various molecular features affect the efficiency of energy transfer. Singlet energy transfer measurements using steady state and direct dynamics techniques need to be carried out on several different types of complexes including: (1) Well-defined pigment-protein complexes where a range of different carotenoids can be incorporated into a single controlled binding site. As alluded to above, this has recently been provided by Frank et al. (1993), but more work in this area is needed; (2) A variety of antenna complexes whose structures are known and have one carotenoid molecule bound in a single site. In experiments of this sort, the distances between donor and acceptor molecules can be correlated with the measured rates of the energy transfer. 3-dimensional structures of antenna complexes that contain chlorophylls and carotenoids are forthcoming (Cogdell et al., 1985; Papiz et al., 1989; Guthrie et al., 1992). With data on these complexes, a thorough understanding of the factors that control energy transfer between carotenoids and chlorophylls will ultimately be possible.

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