

Chapter 18

Singlet Energy Transfer from Carotenoids to Bacteriochlorophylls

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Summary

The present chapter discusses recent developments in the understanding of the energy transfer processes between these molecules. A better understanding of the association between the two molecules is needed to address the following questions: (1) The biological role of the carotenoids in the generation and purification of pigment protein complexes from photosynthetic bacteria; (2) The chemical processes for the synthesis, modification, and transformation of carotenoids into these complexes; (3) The spectroscopic techniques for the study of these complexes; (4) The development of steady-state and time-resolved absorption and fluorescence spectroscopic techniques for exploring the nature of the excited states of these pigments; (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 appearance of carotenoids in filamentous cyanobacteria and the essential roles these pigments play in photosynthesis. Carotenoids are capable of at least three types of photochemical reactions in the photosynthetic apparatus: (1)

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 (or, possibly, chlorophyll triplet states)

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

II. The Electronic Structure of Carotenoid Excited States

isolated molecules. Although similar experiments

the unique electronic properties of their conjugated π -electron frameworks (Zechmeister, 1962; Kohler 1973) in most carotenoids contain C_{11} carotenoid skeletons corresponding to eight isoprene units. The number

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 10 double bonds have been obtained synthetically (Nandi and Lugstol, 1987). The energies and lifetimes of the lowest excited singlet (S_1) and triplet (T_1) states are of

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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 animal polyenes have exploited their relatively high fluorescence yields and their ability to be incorporated

(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

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 systems in molecular beams (Leonard et al., 1984; Heimbrook et al., 1984; Buma et al., 1990, 1991, 1992; Baumman et al., 1990; Patel et al., 1991, 1992, 1993), resulting in an unprecedented view of the electronic and vibrational states of cold

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 measurements in detecting 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., 1979)) has been a major factor in establishing these molecules as

precisely, mostly conjugated molecules, and trienes) and longer polyenes (e.g., β -carotene,

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

The $S_1 \rightarrow S_0$ and $S_0 \rightarrow S_1$ transitions have been

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, $S_0 \rightarrow S_2$ absorptions and the $S_1 \rightarrow S_0$ 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 presence of a symmetry-forbidden $2^1A_g \rightarrow 1^1A_g$ transition which lies below the strong, symmetry-allowed $1^1A_g \rightarrow 1^1B_u$ absorption. The Stokes-shifts thus provide accurate measures of the $1^1B_u \rightarrow 2^1A_g$ energy

verified by high-level calculations on model systems

(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. The idealized symmetry labels in discussing the underlying energy levels of carotenoids.

Extending the optical studies of model systems to carotenoids has been hampered by their broad, almost structureless spectra and extremely low fluorescence yields. Early reports of fluorescence from β -carotene indicated an emission origin coinciding with the

(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

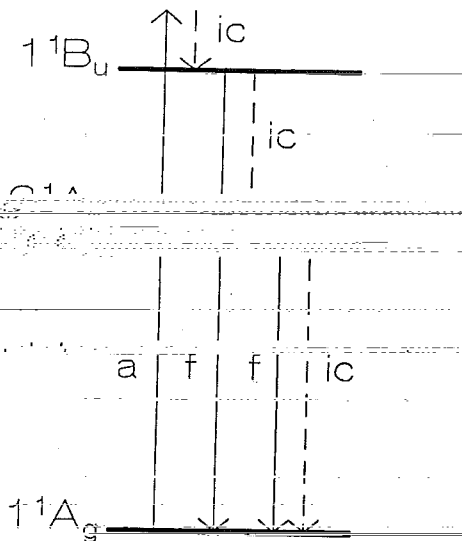


Fig. 1 Schematic representation of the energy levels and transitions.

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

reports of β -carotene fluorescence by Cherry et al. (1968); Haley and Koningstein (1983) and Watanabe et al. (1986). The good agreement between the $1^1A_g \rightarrow 1^1B_u$ absorption and the fluorescence excitation spectra provided the existence of β -carotene fluorescence. Later work of Gillbro and Cogdell (1989) and Cosgrove et al. (1990) confirmed the presence of $1^1B_u \rightarrow 1^1A_g$ ($S_2 \rightarrow S_0$) emissions in β -carotene and related carotenoids. The detailed studies of shorter polyenes all indicated the presence of fluorescence from β -carotene and other long carotenoids as being due to $S_2 \rightarrow S_0$ fluorescence. $S_1 \rightarrow S_0$ ($2^1A_g \rightarrow 1^1A_g$) emission has not yet been detected in β -carotene, but it is likely that this emission is present in other long polyenes with more than 11 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 Bondarev et al. (1988, 1989) 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) state ~ 3500 cm^{-1} above the S_1 (1^1B_u) 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_1 - S_0 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

carotene (Watanabe, et al., 1987) could not reproduce the theoretical (1977-1979) results. This showed that Raman excitation profiles shared the same limitations as standard absorption measurements in terms of weak $2^1A_g \rightarrow 1^1A_g$ transitions on 10^9 s timescale. The absorption and fluorescence excitation profiles of β -carotene and other carotenoids thus are not reliable based on the extrapolation of trends observed in the $2^1A_g \rightarrow 1^1A_g$ transition energies of shorter, more fluorescent polyenes.

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 absorption spectra 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

of two conjugated double bonds (Christensen and Kohler, 1973; Hemley and Kohler, 1977). Nishimura et al. (1977) illustrated in the comparison of the fluorescence excitation and emission spectra of β -apo-12'-carotenol and hexadecaheptane (Fig. 2), the insufficient resolution in the carotenol spectra to allow the accurate identification of electronic origins. The important feature of Fig. 2 is the almost identical $2^1A_g \rightarrow 1^1A_g$ transition energies of the two heptanes. This illustrates that for β -12'-carotenol the loss in conjugation due to the nonplanarity between the ring and side chains is offset by the stabilizing effects of the isoprenoid structure,

which is known to have a stabilizing effect on 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 a series of eight conjugated double bonds was dominated by 'anti-Kasha' $S_2 \rightarrow S_0$ transitions, which have been observed for β -

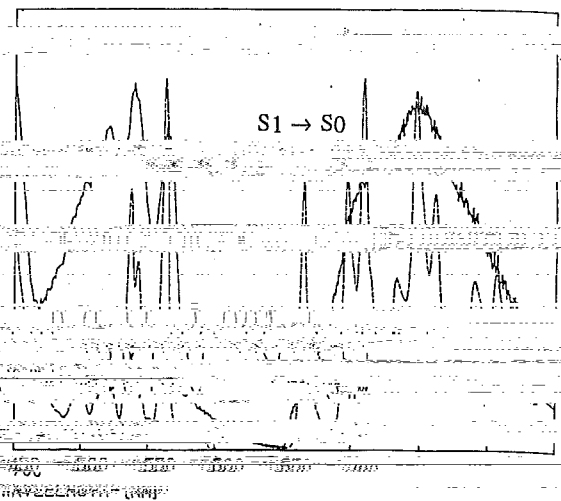


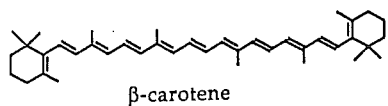
Fig. 2. Comparison of fluorescence and fluorescence excitation spectra of β -12'-carotenol (from Hemley, 1977).

The fluorescence spectrum of β -12'-carotenol (from Hemley, 1977) was obtained by exciting at 413 nm. The excitation spectrum was obtained by monitoring the fluorescence intensity at 650 nm. The fluorescence excitation spectrum was recorded with a monochromator.

carotene (van Riel et al., 1983; Watanabe et al., 1986).

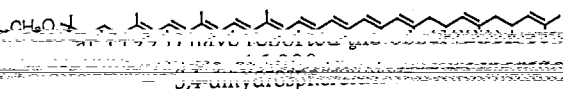
attributed to a larger $S_2 \rightarrow S_0$ energy difference and the resultant decrease in $S_2 \rightarrow S_1$ radiationless decay (following the energy-gap law of Englman and Jortner, 1970). This allows S_2 emission to compete with internal conversion. The lack of S_1 fluorescence prohibits the direct observation of the S_1 state in longer carotenoids. Nevertheless, trends noted in the model polyenes suggested 2^1A_g energies considerably higher than those of the corresponding polyenes (Shashidhar et al., 1977 and 1979; (1977-1979)).

DeGroot et al. (1992) extended the Cosgrove (1990) study to provide a systematic comparison of the energy levels of a series of β -carotenoids (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



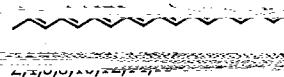
the possibility that the S_1 state of β -carotene (11 double bonds) lies below the S_1 state (Q_y) of its chlorophyll acceptor.

Recent estimates of the 2^1A_g energies of spheruloid-like structures and other long carotenoids appear to span a rather wide range. For example, Watanabe (1992) estimates the 2^1A_g energy of β -carotene to be $14,900 \text{ cm}^{-1}$ (hexane)/ $14,600 \text{ cm}^{-1}$ (CS_2). Mimuro et al. (1993) estimate the 2^1A_g energy of β -carotene to be $15,300 \text{ cm}^{-1}$.

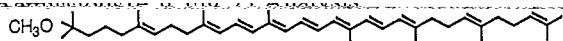


Watanabe et al. (1992) estimate the 2^1A_g energy of spheroidene to be $15,300 \text{ cm}^{-1}$, an energy considerably above the $15,300 \text{ cm}^{-1}$ 2^1A_g energy of β -carotene.

3,4,5,6-tetrahydro-spheroidene

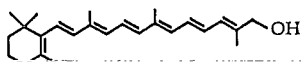


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2,4,6,8,10,12-tetradecaheptaene



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β-apo-12-carotenol

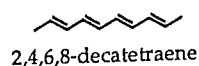
2,4,6,8,10,12-tetradecaheptaene

Mimuro et al. (1993) estimate the 2^1A_g energy of spheroidene to be $15,300 \text{ cm}^{-1}$, an energy considerably above the $15,300 \text{ cm}^{-1}$ 2^1A_g energy of β -carotene.

β-apo-12-carotenol

2,4,6,8,10-dodecapentaene

Mimuro et al. (1993) estimate the 2^1A_g energy of spheroidene to be $15,300 \text{ cm}^{-1}$, an energy considerably above the $15,300 \text{ cm}^{-1}$ 2^1A_g energy of β -carotene.



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β-apo-12-carotenol

discussed in the present work

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of the more highly substituted spheroidene than those of α,ω -dimethylpolyenes with the same conjugation, (3) Spheroidene (like β -carotene) is essentially an S_2 emitter, (4) β -carotene

$S_1 \rightarrow S_0$ fluorescence spectra from the shorter β -carotene analogs. Based on

for this review is the comparison of the extrapolated S_1 energies of spheroidene and β -carotene with the S_1 energies of β -carotene and chlorophyll

estimates of (0,0) energies of shorter members of this series (with the spectra of spheroidene and β -carotene, the fluorescence spectra of the β -carotene analogs are not sufficiently resolved to

these extrapolations place the S_1 (2^1A_g) energy of β -carotene at $15,300 \text{ cm}^{-1}$.

observe electronic origin), β -carotene 2^1A_g energy of $\sim 14,500 \text{ cm}^{-1}$. This is

levels of bacteriochlorophyll, allowing singlet energy transfer to occur via the spheroidene 1^1A_g state.

is $\sim 1000 \text{ cm}^{-1}$ below the S_1 energy of chlorophyll acceptor.

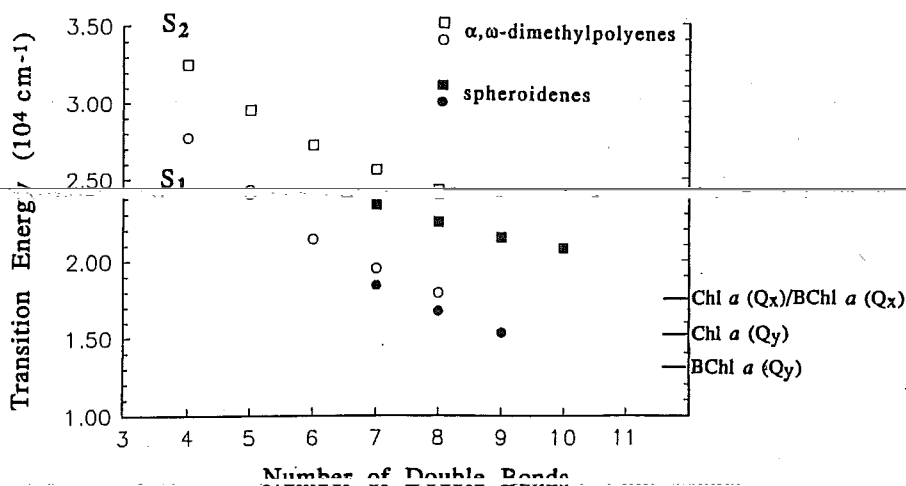


Fig. 4. S₁ (11A_g ← 11A_g) and S₂ (11A_g ← 11B_g) transition energies for α,ω -dimethylpolyenes and spheroidenes. Energies of the S₂ states are the electronic origins (0-0 bands) observed for S₂ → S₀ fluorescence in room temperature methanol. Energies of the S₁ states are the electronic origins (0-0 bands) observed for S₂ → S₁ absorption in room temperature methanol. Dimethylpolyene data are from Masuda and Christie (1981) and Masuda et al. (1982). Spheroidene data are taken from Thrash et al. (1977). The ground-state absorption spectra were performed 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 $\sim 14,200 \pm 400 \text{ cm}^{-1}$ 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 $\sim 600\text{--}700 \text{ nm}$, 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, 1978) assignment of the electronic origin of the 2^1A_g state

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

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 (Nanachi et al., 1980; Hashimoto and Kovama 1990; Kuki et al., 1990) and emission yield determinations (Shreve et al., 1991; Gillbro and Coddell 1989; Coggins 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 Coddell 1989) and femtosecond (Shreve et al., 1991b and 1991c; Frank et al., 1993) time resolved transient absorption measurements.

A. Dynamics of S₁ States

The S_1 state dynamics of carotenoids has been investigated using picosecond transient absorption spectroscopy. S_1 lifetimes of $8.4 \pm 0.6 \text{ ps}$ for β -carotene, $5.2 \pm 0.6 \text{ ps}$ for canthaxanthin and $25.4 \pm 0.2 \text{ ps}$ for β -zeaxanthin in toluene were found (Wasielewski and Kispert, 1986). Wasielewski et al. (1989) also measured the state lifetime of all-trans- β -carotene and fully deuterated all-trans- β -carotene in 2-methylpropane. The S_1 lifetimes for all-trans- β -carotene and 1,9,9-d₁₀-all-trans- β -carotene were found to be $10.5 \pm 0.6 \text{ ps}$ and $10.5 \pm 0.6 \text{ ps}$, respectively. The 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

10-15 ps for the lifetimes of the 2^1A_g states of β -carotene and spheroidene. (See also below.) In addition, Gilbro and Cogger (1993) have previously measured the lifetime of the 2^1A_g state of spheroidene, to be 15 ps in CS_2 .

Mentioned above, nonradiative 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 (Kotab 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 a similar systematic series of β -carotene and β -carotene analogs of nonradiative decay of the 2^1A_g state of carotenoids (Andersson et al. 1992): 2,4,7,8-tetrahydro-spheroidene, 3,4,5,6-tetrahydro-spheroidene, 3,4-dihydro-spheroidene, 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 $S_1 \rightarrow S_0$ absorptions decayed with single-exponential kinetics revealing S_1 lifetimes of $\tau = 407 \pm 23$ ps for 3,4,7,8-tetrahydro-spheroidene, 95 ± 5 ps for 3,4,5,6-

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

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, $k_r \sim 10^6$ to 10^7 s $^{-1}$. A is a pre-exponential factor that depends on the S_1 - S_0 energy difference,

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

For the four spheroidenes A can be safely approximated as a function of the energy of the donor frequency 'acceptor' modes (presumably C=C stretching or C-H bending) and the relative displacement of the potential surfaces in the two electronic states. The $S_1 \rightarrow S_0$ transition energies determined for 2,4,5,6-tetrahydro-spheroidene, 3,4,7,8-tetrahydro-spheroidene and 3,4-dihydro-spheroidene 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) in this study analogs of β -carotene possessing 5, 7, 9, and 11 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 $1/\Delta E$ functionality in the pre-exponential term (Eq. (2)) must be considered in treating the data.

B. Dynamics of S_1 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

spheroidene to be 200, 250 fs and 240 fs. 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

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

where τ is the lifetime and σ 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 1980; 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

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

IV. Implications for Energy Transfer

The efficiency ϵ of energy transfer between molecules is defined by

$$k_{ET} + \Sigma k \quad (4)$$

where k_{ET} is the rate constant for energy transfer and Σk is the sum of the rate constants for all other state decay pathways. Energy transfer from carotenoids to chlorophylls can easily be demonstrated by comparing the chlorophyll fluorescence excitation profiles from antenna pigment-protein complexes isolated from photosynthetic bacteria or algae. Normalization of the

maximum of the bacteriochlorophyll fluorescence (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 *Rhodospirillum rubrum* (Rh) *sphaeroides* wild type (Frank et al., 1990; van Grondelle et al., 1982) to ~25% for the carotenoids, spirilloxanthin, rhodopin, and lycopene in the B880 complex from *Rhodospirillum rubrum* (Rh) *sphaeroides* wild type (Frank et al., 1990).

Longer chain carotenoids show lower efficiencies of energy transfer (Frank et al., 1990).

These steady state experiments are particularly interesting when applied to the study of protein-carotenoid complexes. For example, Frank et al. (1992) analyzed the B800 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

suggested that the general trend of lower efficiencies of energy transfer from carotenoids to bacterio-

determined by direct time-resolved absorption measurements and ground state spectroscopy

molecules lying below the S_1 state of bacteriochlorophyll. An alternative, more detailed, hypothesis for the transient dynamics data carried out directly on the isolated pigment-protein complexes would test this hypothesis.

Upon excitation of the B800-850 complex from the ground state, a bleaching of the carotenoid absorption was observed which was restored with a time constant of 3.0 ± 0.9 ns.

The rise-time of the bleaching of the bacteriochlorophyll band at 800 nm occurred in 6.1 ± 0.9 ns.

The arrival of the carotenoid excitation at the bacteriochlorophyll site. Similar experiments were carried out by Gill et al. (1990) and by Frank et al. (1993).

Transfer from the carotenoid to the bacteriochlorophyll took place in 3 ± 1 ns. Femtosecond time-resolved

on the thylakoid membrane preparations from the diatom *Phaeodactylum tricornutum* and from *Nannochloropsis sp.* The time for the carotenoid-to-chlorophyll energy transfer in the latter species was found to be 240 ± 40 fs.

Energy transfer in the diatom was bi-exponential with transfer times 500 ± 100 fs and 2.0 ± 0.5 ps and relative amplitudes of $1.7 \pm 0.7:1$.

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

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 to 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 2^1A_g states. The fact that energy transfer from carotenoids to bacteriochlorophylls 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 structure and excited state dynamics of the donors and

usually is explained using one of two established

(Förster) mechanism or the electron exchange (Dexter) mechanism. (Förster 1948, 1965; Dexter, 1953; see also Chapter 15 by Struve in this volume.)

Which is the primary transfer pathway between antenna carotenoids and bacteriochlorophylls will depend critically on the answers to the following questions: (1) How do specific molecular features (e. g. distance,

extent of conjugation, etc.) affect the energy levels of the donor and acceptor states (1^1B_u or 2^1A_g) participate in the transfer and

the nature of the transition (excited singlet or excited triplet) and the nature of the acceptor state (excited singlet or excited triplet).

exchange) operates between carotenoids and chlorophylls. (2) How do various molecular features affect the efficiency of energy transfer.

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

uncontrolled complexes where 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 known three-dimensional structures of antenna complexes that

(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

be possible.

understanding of energy transfer between carotenoids and chlorophylls will

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