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Carotenoids as accessory light-harvesting pigments

*Richard J. Cogdell, **Tomas Gillbro, **Per Ola Andersson,
***R.S.H. Liu and ***A.E. Asato.

*Department of Botany, University of Glasgow, Glasgow, G12 8QQ, UK,
**Department of Physical Chemistry, University of Umeå, S-901 87
Umeå, Sweden and ***Department of Chemistry, 2545 The Mall,
University of Hawaii, Honolulu, HI 96822, USA

Abstract - One of the main functions of carotenoids in photosynthesis is to act as accessory light-harvesting molecules. This paper reviews the current status of research on the mechanism of this singlet-singlet energy transfer process. Two excited singlet states S_1 and S_2 are described. Based on fluorescence measurements the energy levels and lifetimes of these two states have been determined for a range of carotenoids. The role of these two states in this energy transfer process is discussed.

The two major functions of carotenoids in photosynthesis, that is accessory light-harvesting and photoprotection, reflect the photophysical properties of their excited singlet and triplet states, respectively (refs. 1,2). This short review will focus on the light-harvesting role of carotenoids and is therefore concerned with their excited singlet states.

In the light-harvesting process light absorbed by the carotenoids is rapidly and efficiently transferred to the chlorophylls, making it available for photosynthesis. This energy transfer is a singlet-singlet exchange reaction. The two main issues that will be discussed in this review are (a) which excited singlet state of carotenoids is involved in this reaction and (b) what is the mechanism of the singlet-singlet energy transfer process? However, before the light-harvesting reactions are examined in detail it is useful to set the scene with some general remarks about carotenoids and their location within the photosynthetic apparatus, which will form the background for what follows.

Carotenoids and polyenes possess two low-lying electronic excited singlet states (refs. 3,4). These are the 1^1B_u (S_2) and the 2^1A_g (S_1) states, and they are responsible for many of the spectroscopic and functional properties of the carotenoids. The strong absorption in the near UV or the visible region of the spectrum that is characteristic of carotenoids represents the 'allowed' transition for the 1^1A_g (ground state) to the 1^1B_u state. The energy of this transition decreases as the number of conjugated double bonds in the carotenoid increases. This effect is illustrated in Table 1 for a series of bacterial carotenoids.

TABLE 1. The dependence of the energy level of the 1^1B_u energy level of carotenoids on the degree of conjugation (n).

Carotenoid	'n'	λmax (nm) in petroleum ether
phytoene	3	285
phytofluene	5	348
ζ-carotene	7	400
neurosporene	9	439
spheroidene	10	455
spirilloxanthin	13	499

It is important to remember this property of carotenoids since this effect of varying the degree of conjugation, through a series of structurally related carotenoids, upon their photophysical properties has been used repeatedly in the experiments described below. The 2^1A_g state is lower lying than the 1^1B_u state but, because of symmetry considerations, is a 'forbidden' state. It has been studied in detail in short polyenes (refs. 3,4,5). Evidence will be presented below which indicates that this S_1 state is important in the singlet-singlet energy transfer process which occurs in light-harvesting. The other important fact that needs to be appreciated in order to understand how carotenoids function in photosynthesis is that they are, in general, not 'free,' floating around within the lipid interior of the photosynthetic membrane, but are non-covalently bound to specific pigment-protein complexes (refs. 1,2). For example, in the purple bacterium Rhodobacter sphaeroides the major, variable antenna complex is composed of six $\alpha\beta$ pairs of apoproteins together with 18 molecules of bacteriochlorophyll a and 9 molecules of carotenoid (ref. 1). The apoproteins (integral membrane proteins) form a 'scaffolding' on which the pigments, including the carotenoids, are organised into a solid-state system, where the structure is optimal for efficient energy transfer. This energy transfer occurs at undiminished efficiency even at 4°K (ref. 6). Very few, if any, of the photochemical functions of carotenoids in photosynthesis would occur if they were not packaged, together with the chlorophylls, in these well defined pigment-protein complexes.

THE PROBLEM

Carotenoids have been clearly demonstrated to be efficient accessory light-harvesting pigments for nearly thirty-five years (ref. 7) and it is interesting, therefore, to ask why, after all this time, the detailed molecular mechanism of this process has still not been unravelled. In the past, carotenoids had been thought to be non-fluorescent (ref. 1). This meant that their excited-state lifetime must be extremely short. If this was correct then the time during which energy transfer could occur before the excited state was lost, by way of other fast, competing processes, must also be very short. How then could the singlet-singlet energy transfer process be so efficient? The real problem in this field, until quite recently, was having anything to measure that would allow the mechanism of this process to be investigated. Over the past five years or so this situation has changed dramatically. Carotenoid fluorescence has been clearly demonstrated (for example, see ref. 8-10) and kinetic experiments with sufficient time resolution (psec and fsec) to monitor directly the energy transfer process have begun (refs. 11-14).

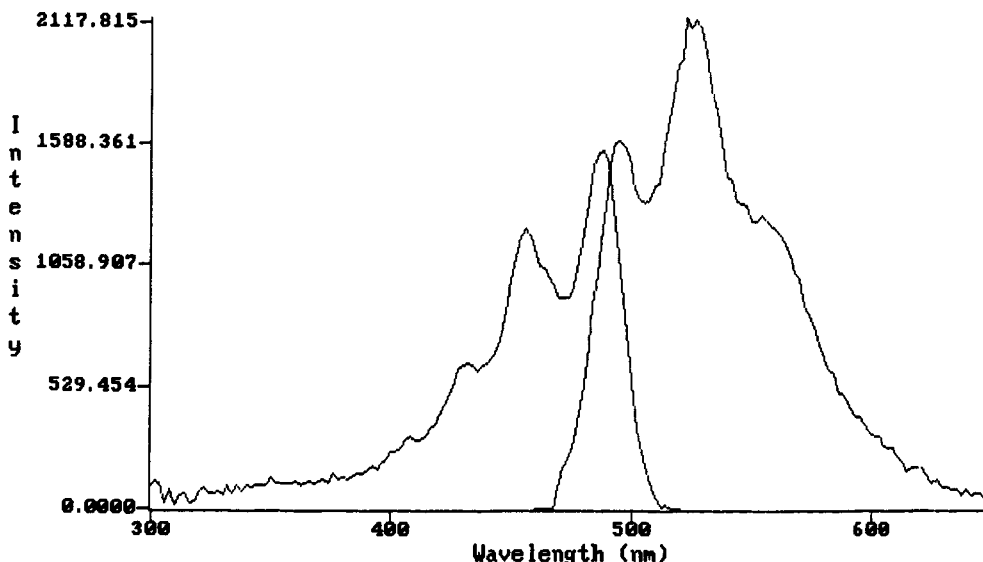

Fig. 1. Fluorescence excitation (left) and emission (right) spectra of spheroidene in n-hexane. For the excitation spectrum the emission was recorded at 530 nm, while for the emission spectrum the sample was excited at 453 nm.

CAROTENOID FLUORESCENCE

The excited-state lifetimes of the S_1 and S_2 states of carotenoids are essential pieces of information when possible mechanisms of carotenoid-to-chlorophyll singlet-singlet energy transfer are being considered. One way to approach this problem is to look for fluorescence from these singlet states. Recently a weak fluorescence has been detected from a range of carotenoids with eight or more double bands (refs. 8-10, 15, 16). The yields of this emission are typically of the order of 10^{-4} and it clearly arises from the S_2 state. Figure 1 shows excitation and emission spectra for the fluorescence from the S_2 state of the bacterial carotenoid, spheroidene (ref. 17). When this fluorescence is recorded from a set of carotenoids with an increasing degree of conjugation (n) from n = 9 to n = 13 the expected relationship between 'n' and the peak wavelength of the emission is seen (ref. 15,16). The fluorescence yield (ϕ_f) of the emission from S_2 is an important piece of information since the yield is directly related the lifetime (τ) of S_2 by the following equation.

$$\phi_f = \frac{\tau}{\tau_{nr}}$$ where τ_{nr} is the natural radiative lifetime of S_2

τ_{nr} can be estimated by integrating the absorption profile of S_2 and has been calculated to be 10^{-9}s (ref. 17). When this value is used together with ϕf of 10^{-4} the actual lifetimes for S_2 can be determined to be of the order of 100-200 fsec. This is very short if the S_2 state were to be the only prospective energy donor for the light-harvesting role of carotenoids.

However, if the fluorescence is recorded for carotenoids with a degree of conjugation of less than 8 double bonds then an emission is seen with a much larger Stokes shift and with a significantly higher yield than that typical of an emission from S_2. This emission has been shown to come from S_1 (ref. 15-17). It is illustrated in Fig. 2 for phytofluene which has five conjugated double bands. In this Figure a small emission can be seen, coming from S_2 (small Stokes shift, low yield) followed by the major emission band coming from S_1 (large Stokes shift, high yield). The yield of the S_1 emission is 0.05 ± 0.01 which is equivalent to a lifetime of ~ 2nsec for S_1.

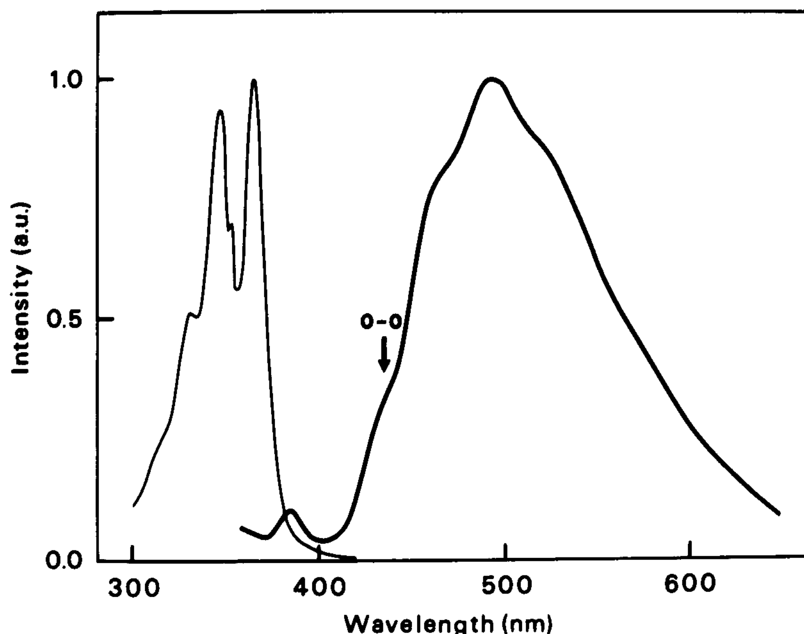

Fig. 2. Fluorescence excitation (left) and emission (right, bold) spectra of phytofluene in n-hexane. The excitation and emission wavelengths were 346 nm and 520 nm respectively.

Fluorescence measurements have now been used to determine both the energy of the S_1 and S_2 states of carotenoids and their lifetimes, as a function of the degree of conjugation (refs. 15-17). The results are illustrated in Figs. 3 and 4.

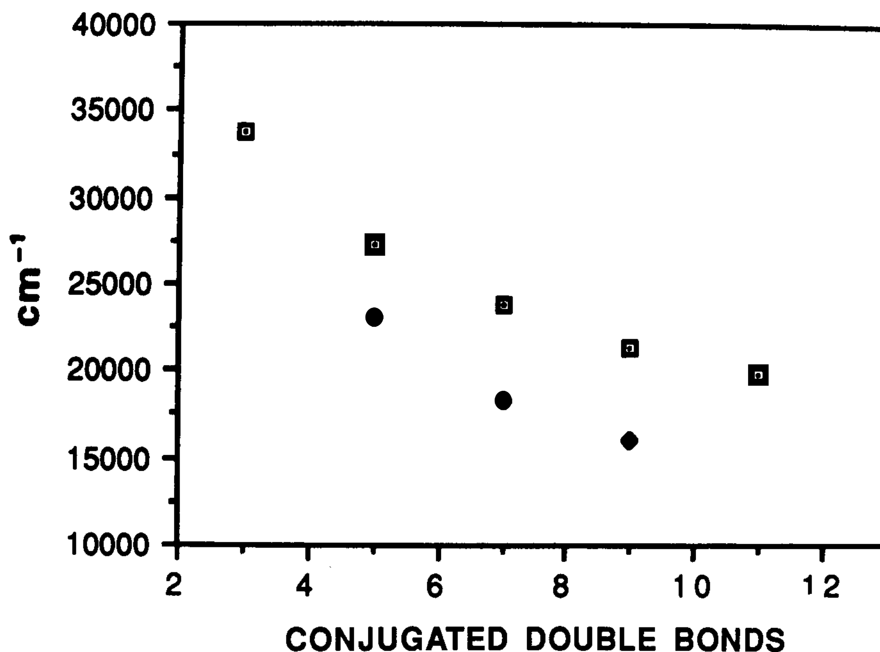

Fig. 3. A plot of the energy of the 0 - 0 transitions for the S_1 ◆ and S_2 ▣ states in the phytoene to lycopene series of carotenoids, recorded in n-hexane.

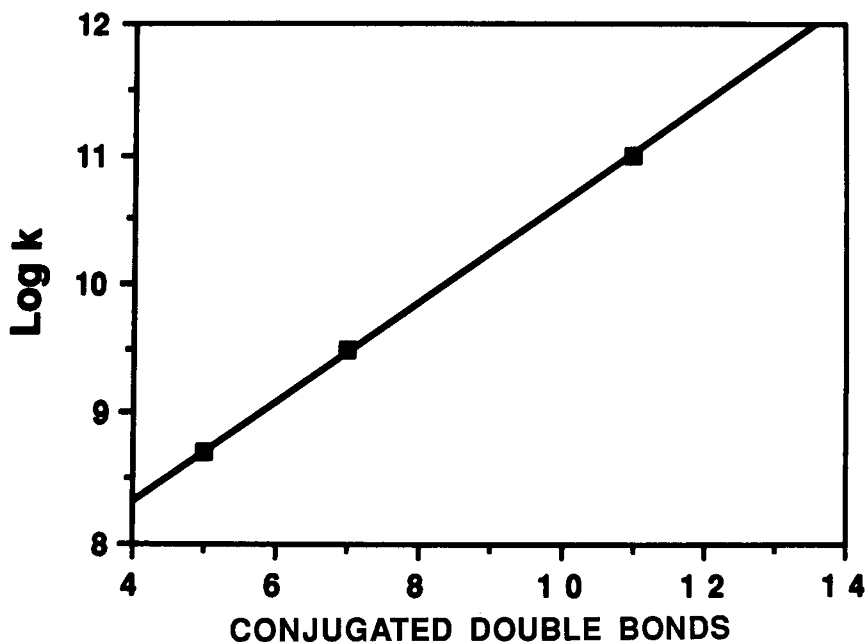

Fig. 4. The logarithm of the S_1 decay rate, k (s^{-1}), as a function of the degree of conjugation of a series of 'mini-carotenoids.'

THE ENERGETICS AND KINETICS OF THE ENERGY TRANSFER PROCESS

Figure 3 shows the energy levels of the S_1 and S_2 states of a series of bacterial carotenoids. The energy of the lowest excited singlet state of Bchl*a* is 12,500 cm^{-1} (850nm) in a typical antenna complex. This is close to the extrapolated value for the S_1 state of spheroidene (n = 10). Clearly, efficient singlet-singlet energy transfer requires that the energy of the donor carotenoid's singlet state is equal to or above that of the acceptor Bchl*a*'s singlet state. When n = 13, i.e. with spirilloxanthin, the level of the S_1 state would be lower than that of the lowest excited singlet state of Bchl*a* and this may explain why *in vivo* the efficiency of energy transfer from this carotenoid is less than 50% (ref. 1). Since the lifetime of the S_1 state is longer than that of the S_2 state, this would immediately make a singlet-singlet energy transfer from S_1, rather than S_2, more favourable, because it would potentially allow more time for that transfer to occur. What now has to be examined directly are the kinetics of the formation and decay of these two singlet states for carotenoids, both *in vitro* and *in vivo*, in an antenna complex.

The lifetime of the S_1 state of isolated carotenoids *in vitro* has been probed directly on the psec timescale by both resonance Raman spectroscopy and by flash photolysis (refs. 11, 18, 19). It is clear from these experiments that, for a range of carotenoids with between 9 and 13 conjugated double bonds the S_1 state lasts for a few picoseconds. If the timescale of the flash photolysis experiments is extended into the fsec range then transients can be seen due to S_2 and the interconversion between S_2 and S_1. The simplest interpretation of the experiment illustrated in Fig. 5 is that initially following excitation S_2 is formed. Then in about 200 fsec S_2 decays into S_1. Finally, S_1 decays back to the ground state in 7-8 psec. Similar results have been obtained with spheroidene by Shreve *et al* (ref. 14).

In vivo, as yet, the results are not entirely clear. On the psec timescale two groups (refs. 11, 18) have shown that the decay of the bleaching of the carotenoid's ground state matches the rise time of the bleaching of the Bchl*a* (i.e. arrival of the energy of the Bchl*a*). This occurs on the psec time range and therefore implicates the S_1 state of the carotenoid. However, on the fsec time scale evidence has been presented that some of the energy may be transferred from S_2 (refs. 13,14). Using the B800-850 complex from *Rhodobacter sphaeroides*, Shreve *et al* (ref. 14) proposed a

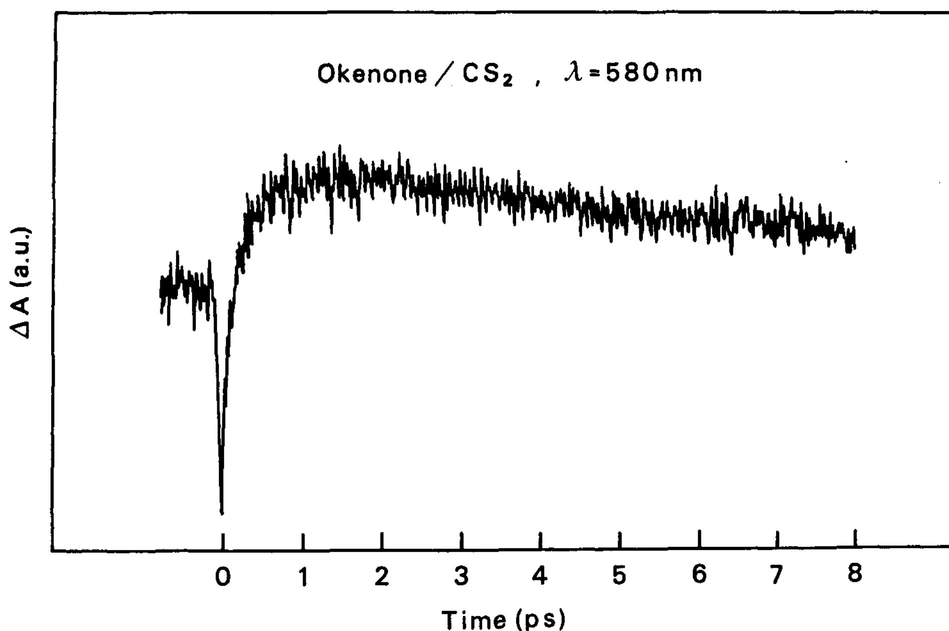

Fig. 5. The kinetics of okenone in CS_2 excited and measured at 580 nm. The excitation at 580 nm was provided by a 70 fsec laser pulse.

kinetic model based upon their fsec studies that involved energy transfer to the Bchla from both S_1 and S_2. Clearly now what is needed is more fsec studies, especially where different carotenoids with different degrees of conjugation can be bound into the same pigment-protein complex, for example, as done by Frank et al (ref. 20).

POSSIBLE MECHANISMS

Two basic physical mechanisms have been frequently discussed to explain the process of singlet-singlet energy transfer from carotenoids to chlorophylls. These are the Förster 'weak interaction' mechanism, which is basically resonance transfer and typically occurs over distances of 10-20Å, and the Dexter 'electron exchange' mechanism, which requires direct contact of the π-π bond electron shells. It is not very useful at this stage to engage in a detailed analysis of which of these two, or indeed other mechanisms, best explains the available data. What is urgently needed is structural information. Until a high resolution picture is available for an antenna complex where the relative positions of the carotenoid and chlorophyll molecules are visualised it will be almost impossible to distinguish between these possible physical mechanisms. In this regard it is hopeful that three research groups now have three-dimensional crystals of bacterial antenna complexes which diffract X-rays to beyond 3A (for example see ref. 21). It may be that by the time of the next Carotenoid Symposium one or more of these structures will be solved, the 'black box' will be opened up and all its internal details revealed.

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