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Low-lying electronic states of carotenoids

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Four all-*trans* carotenoids, spheroidene, 3,4-dihydrospheroidene, 3,4,5,6-tetrahydrospheroidene, and 3,4,7,8-tetrahydrospheroi-

from 10 to seven carbon–carbon double bonds, exhibits a systematic crossover from $S_0 \rightarrow S_2$ ($1^1B_u \rightarrow 1^1A_g$) to $S_0 \rightarrow S_1$

Introduction

Carotenoids enhance the light-capturing ability of

ity between donor and acceptor, which promotes orbital overlap. Which of these mechanisms best describes energy transfer in the antenna complexes in

involved in the transfer process. In both cases the rate

The Förster (or coulombic) mechanism of energy transfer is described by through-space interactions between the donor and acceptor electronic transition

In contrast, the Dexter (or exchange) mechanism involves the simultaneous exchange of two electrons between

condensed phases, the strong, symmetry-allowed, S_0 S_2 absorption typically is accompanied by a 'Stokes-

clearly show a trend toward increasing S_2-S_1 energy separation with increasing conjugation.

Materials and Methods

Cells of the *Rhodobacter sphaeroides* bacteria were

natant was yellow and the pellet was tan. After reduc-

alumina (Sigma A-9003) column. Spheroidene was ob-

obtained by a similar extraction procedure and purified

Petroleum ether solutions of each carotenoid were

synthesis and purification of 3,4,5,6-tetrahydro-

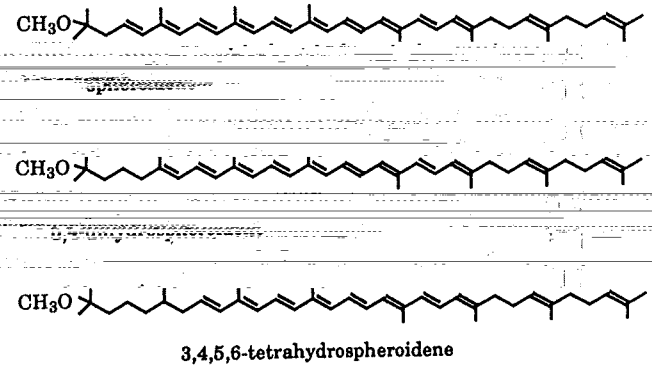
energy transfer, are rather uncertain.

The present work focuses on the electronic energies

drospheroidene and 3,4,7,8-tetrahydrospheroidene

synthetic bacteria. 3,4,5,6-Tetrahydrospheroidene and

having eight and seven conjugated π-electron double bonds, respectively. An important feature of this work has been the development of HPLC techniques which allow the study of isomerically pure samples. The all-trans isomers of these four molecules, which are struc-



3,4,5,6-tetrahydrospheroidene

using a C_{18} reversed-phase column (200 × 4.6 mm) and guard column (both containing Lichrosorb RP-18, 5 μm particles). Samples were eluted at a flow rate of 2.0 ml/min with a mobile phase programmed as follows: 0–4 min, isocratic, methanol/*n*-hexane (95/5 v/v), 4–6 min, linear gradient to methanol/water (95/5 v/v), 6–10 min, linear gradient to methanol/water (98/2 v/v). Chromatograms initially were monitored at

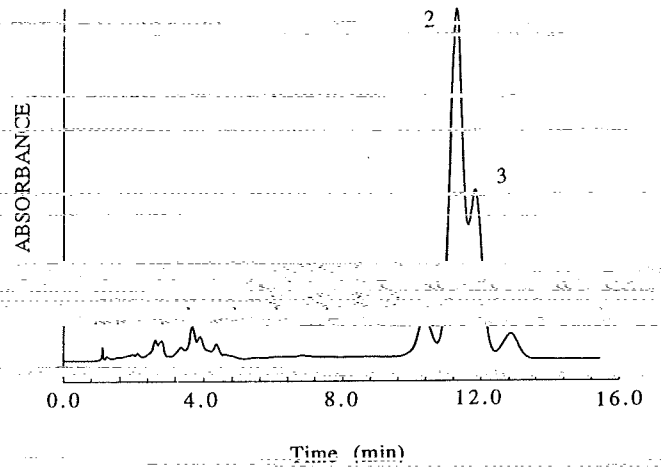


Fig. 2. HPLC of spheroidene sample. The mobile and stationary

mum absorbance. The diode-array detector also allowed the recording of complete spectra (190–600 nm) of individual components which was helpful in assigning components as *cis* and *trans* spheroidene isomers. Individual peaks were collected in quartz cuvettes and fluorescence and fluorescence excitation spectra taken immediately. Reinjection of solutions following fluores-

Fluorescence and fluorescence excitation spectra

solvent were corrected for by subtraction of a solvent blank taken under identical conditions. Fluorescence

obtained by recording the spectrum of a standard lamp

likely are various *cis* spheroidene isomers with the

try of the all-*trans* isomer. Spectra with the strongest

isomers, the HPLC procedures resolve many other

Results

from 475 to 481 nm for the four components. The most

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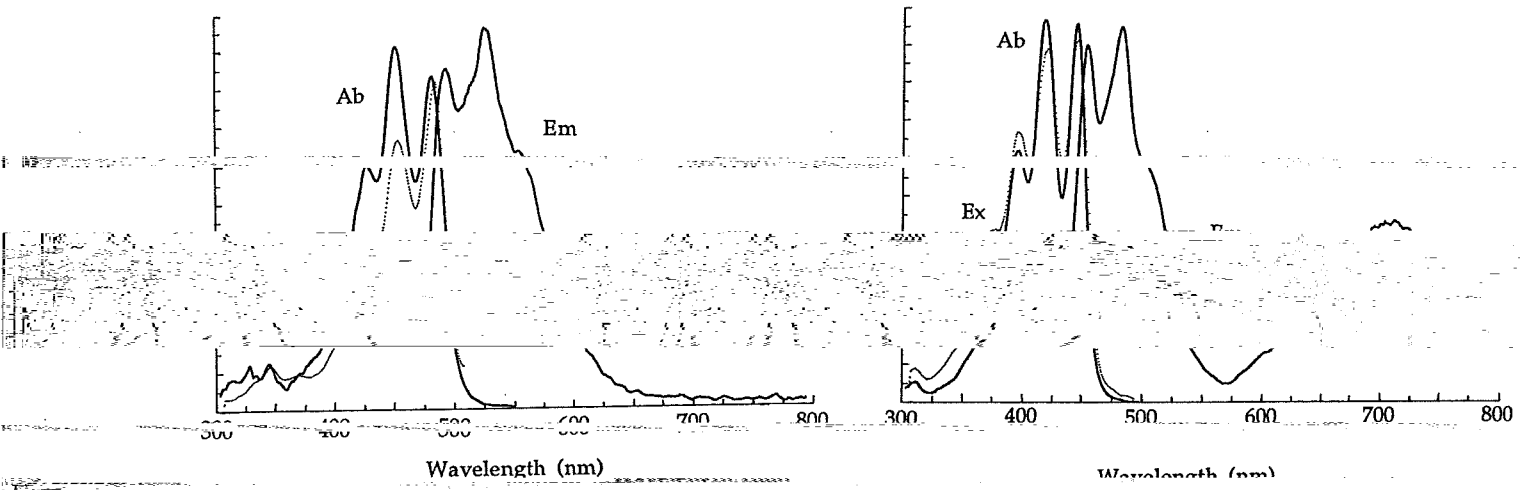
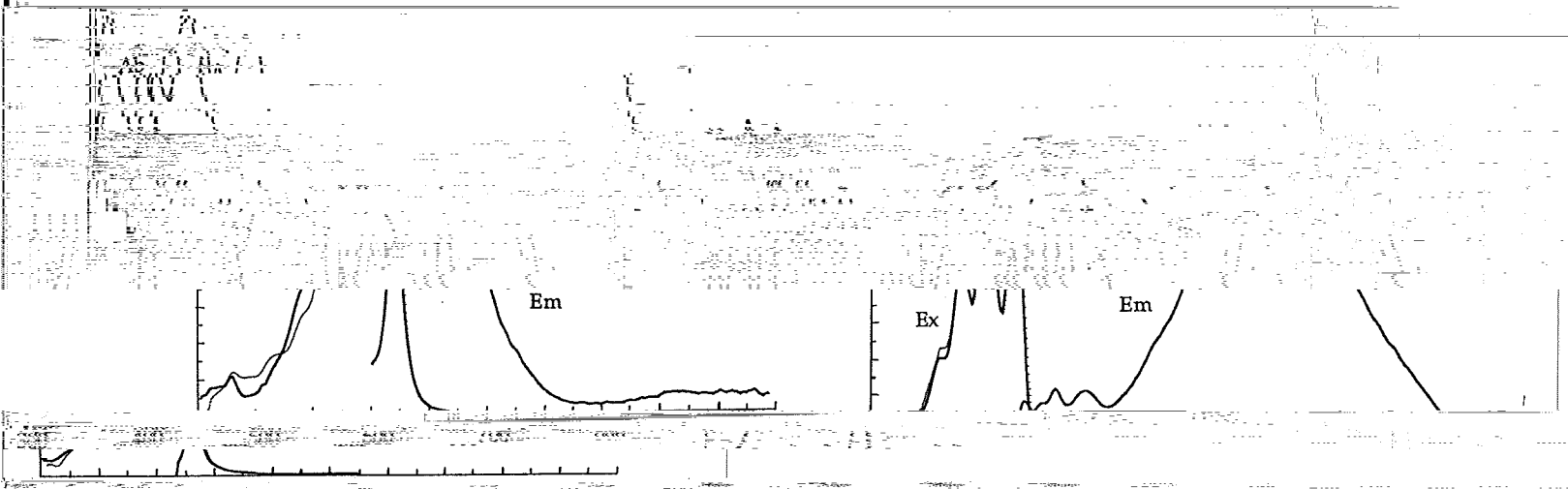
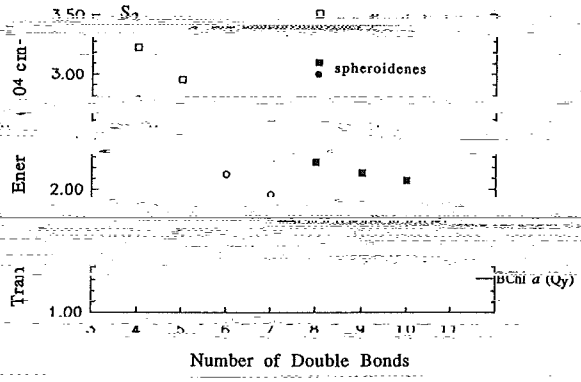


tation spectra are in good agreement, as expected for these well purified samples. There are some traces of contaminants that compete with spectroscopic probes



400 nm, however, the fluorescence spectrum is independent of the wavelength of excitation, as expected for emission from a single all-trans spheroidene species.

The spectra of 3,4-dihydrospheroidene ('methoxyspheroidene' however, there are indications in the 700–800 nm region of a weak, longer wavelength emission, indicating $S_1 \rightarrow S_0$ fluorescence ($2^1A_g^- \rightarrow 1^1A_g^-$) in this molecule. It is important to note that 3,4-dihydrospheroidene (nine conjugated double bonds) is the longest carotenoid for which the low energy $2^1A_g^-$ state has been observed. The presence of $S_1 \rightarrow S_0$ emission in methoxyneurosporene is supported by comparing its fluorescence spectrum with those of its the less-conjugated homologs 3,4,5,6-tetrahydrospheroidene (Fig. 6) and 3,4,7,8-tetrahydrospheroidene (Fig. 7). Excitation of the latter compound gives almost exclusively emissions ($S_2 \rightarrow S_0$) of 3,4,5,6-tetrahydro-



energies for α,ω-dimethylpolyenes and spheroidenes as a function of

bands) observed for $S_0 \rightarrow S_2$ absorption in room temperature

nm) and bacteriochlorophyll *a* ($Q_x = 573$ nm, $Q_y = 769$ nm) transi-

(and their second derivatives) with spectra of analo-

almost identical trends noted in the two series further

The $1^1B_u^+$ (S_2) and the $2^1A_g^-$ (S_1) states of

polyenes with the same extent of conjugation (Fig. 8).

lished) and Ref. 16.

(T. Mercy and R.L. Christensen, unpublished data). It [illegible] ... in this molecule the 1^1B_u state is shifted dramatically to the red, but the 2^1A_g state is virtually unshifted relative to the corre- [illegible] model trienes. These effects carry over to the spheroidenes and result in smaller S_2-S_1 energy differences than [illegible] less substituted polyenes with the same length [illegible] of conjugation.

[illegible] $S_1 \to S_0$ to $S_2 \to S_0$ emission [illegible] polyenes has been attributed to the increase in the S_2-S_1 energy difference with increasing polyene length [14,17]. The resulting decrease in the rate of $S_2 \to S_1$ internal conversion following the well-known "energy [illegible] internal conversion rates apparently become suffi- [illegible] to allow $S_2 \to S_0$ fluorescence to compete [illegible] The [illegible] model has been applied to several aromatic compounds that violate Kasha's Rule (Kasha's Rule states that large molecules in condensed phase fluoresce from their lowest excited singlet states) and appears to give a quantitative account of $S_2 \to S_1$ internal conversion in these systems. For linear polyenes, on the other hand, the rather abrupt changeover from $S_1 \to S_0$ to $S_2 \to S_0$ [illegible] [17]. This suggests that at least some of the parameters [illegible] changes in molecular geometry, vibronic [illegible] further highlighted by comparing the excited state dy- namics of β-carotene and [illegible] β-carotene [4] [illegible] S_2-S_1 energy differences [illegible] element for detectable emission from S_2 in both are- [illegible] of the 2^1A_g state of spheroidene [illegible] the polyenes are of fundamental importance in under- [illegible] in the B800-850 light-harvesting complex of [illegible]

The absence of detectable $S_1 \to S_0$ fluorescence from long polyenes, such as spheroidene and β-carotene, thwarts the direct detection of the 2^1A_g states in these systems, at least by conventional, one-photon, optical techniques. One, thus, must rely on the extrapolation of the 2^1A_g energies observed for shorter dimeth- ylpolyenes and spheroidene derivatives (Fig. 8). This indicates that the 2^1A_g state of spheroidene (10 double bonds) most likely is higher in energy than the S_1 (Q_y) [illegible] energy transfer in *Rhodobacter sphaeroides* most likely occurs via the spheroidene 2^1A_g state. On the other hand, the data presented in Fig. 8 suggests that the S_1 state of β-carotene (11 double bonds) lies below the S_1 state (Q_y) of its chlorophyll acceptor [illegible] β-carotene indicated that its 2^1A_g [illegible] 17,250 cm⁻¹, 5500 cm⁻¹ below the origin of the 1^1B_u state [25,26]. This places the 2^1A_g state well above the 15,150 cm⁻¹ (660 nm) S_1 state of chlorophyll *a*, an energy scheme that has been widely cited in discussions of energy transfer in green plant photosynthesis [27–29]. However, the early resonance Raman results cannot be repeated [30] and a 17,000 cm⁻¹ 2^1A_g state in β-caro- tene clearly is incompatible with the data summarized in Fig. 8 which indicate a β-carotene 2^1A_g state in the [illegible] (and correspondingly large S_2-S_1 energy differences) [illegible] these systems.

The dominance of $S_1 \to S_0$ emissions [illegible] for mechanisms of light harvesting in photosynthetic antenna complexes. As first pointed out by Snyder et [illegible] well be a necessity given that its S_1 state most likely lies well below the lowest excited singlet state [illegible] photosynthetic bacteria are reduced to 20–50% from [illegible]

fluorescence spectrum of the donor and the absorption

changes associated with the $S_1 \rightarrow S_0$ transition typically

with relatively weak intensity at wavelengths corresponding to transitions between the zero-point levels of the S_0 and S_1 states (0-0 bands). This contrasts with the absorption and emission spectra of chlorophylls, for which a large fraction of the Q_y transition strength

consequence, the overlap between carotenoid $S_1 \rightarrow S_0$

small, even in cases where the S_1 energy of the donor is equal to or slightly greater than the S_1 energy of the acceptor. S_1(carotenoid) to S_1(chlorophyll) energy transfer will be enhanced when the acceptor (0-0) band

nature of the polyene $S_1 \rightarrow S_0$ transition, further requires short-range electron exchange (Dexter transfer) rather than coulombic interactions for transfer involving a carotenoid $^1A_g^-$ state. The high efficiency of

δ). This implicates the carotene S_2 state as the donor state. Although the rate of $S_2 \rightarrow S_1$ internal conversion is reduced by the relatively large S_2–S_1 energy differ-

200–400 fs lifetimes for the S_2 states. How can S_2

the antenna complexes. Strong coulombic interactions

tronic transitions at short distances also may include terms due to weak excitonic interactions [39,47].

states) would further accelerate energy transfer from

[4]. For polyene

Absorption and fluorescence spectroscopy of short model polyenes [5,16], carotenols [17], and spheroidenes have provided a detailed understanding of how the electronic energies of polyene S_2 (1^1B_u) and S_1 (2^1A_g) states depend on conjugated length (Fig. 8). Extrapolation of these results to molecules such as

energies than previously thought. Longer carotenoids also exhibit S_2 fluorescence. This is consistent with their low S_1 energies, relatively large S_2–S_1 energy gaps,

sufficient energy and lifetimes to funnel excitation into the S_1 states of chlorophyll acceptors. For β-carotene/chlorophyll a complexes in green plants, the lower polyene S_1 (2^1A_g) energy makes it unlikely that this

Regardless of the mechanism of singlet energy transfer, it is remarkable that carotenoids function as efficient energy donors in photosynthesis in a wide

through fast, non-radiative decay (internal conversion)

thermal relaxation to carotenoid ground states.

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