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ENERGY TRANSFER BETWEEN THE CAROTENOID AND THE BACTERIOCHLOROPHYLL WITHIN THE *B*-800—850 LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEX OF *RHODOPSEUDOMONAS SPHAEROIDES*

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Summary

Energy transfer between carotenoid and bacteriochlorophyll has been studied in isolated *B*-800—850 antenna pigment-protein complexes from different strains of *Rhodopseudomonas sphaeroides* which contain different types of carotenoid. Singlet-singlet energy transfer from the carotenoid to the bacteriochlorophyll is efficient (75—100%) and is rather insensitive to carotenoid type, over the range of carotenoids tested. The yield of carotenoid triplets is low (2—15%) but this arises from a low yield of bacteriochlorophyll triplet formation rather than from an inefficient triplet-triplet exchange reaction. The rate of the triplet-triplet exchange reaction between the bacteriochlorophyll and the carotenoid is fast ($K_{tt} \geqslant 1.4 \cdot 10^8\ s^{-1}$) and also relatively independent of the type of carotenoid present.

Introduction

It is generally accepted that carotenoids serve two major functions in photosynthesis. They have a protective role, preventing the chlorophylls from sensitizing the harmful 'photodynamic reaction' in the presence of light and oxygen [1]; and they also act as accessory light-harvesting pigments, absorbing light at wavelengths where the chlorophylls do not absorb and transferring the light

Abbreviations: BChl, bacteriochlorophyll; Car, carotenoid.

energy to the chlorophylls, so increasing the range of wavelengths over which light may drive photosynthesis [2,3]. The accessory pigment role reflects singlet-singlet energy transfer from the carotenoid to the bacteriochlorophyll and it has been suggested that the protective role reflects triplet-triplet energy transfer from the bacteriochlorophyll to the carotenoid [4].

Goedheer [3] studied the efficiency of singlet-singlet energy transfer from carotenoids to bacteriochlorophyll in whole cells from three different species of photosynthetic bacteria. He found that the efficiency varied, being 90% in *Rhodopseudomonas sphaeroides*, 50% in *Chromatium vinosum* and 30% in *Rhodospirillum rubrum*.

Since these early experiments it has become clear that the majority of the carotenoids within the membrane of purple photosynthetic bacteria are bound, together with bacteriochlorophyll, into discrete, well-defined light-harvesting pigment-protein complexes [5,6]. There are therefore two distinct ways in which the variation in the energy transfer efficiency described by Goedheer [3] might arise. Firstly, the efficiency could depend upon the type of carotenoid present (the carotenoid content of the three species that Goedheer studied was different [7]). A difference in carotenoid type could lead to a variation in the efficiency of energy transfer either through a change in the overlap integral of carotenoid (donor) and the bacteriochlorophyll (acceptor) or through a change in the lifetime of the first excited singlet state of the carotenoid [8]. Secondly, efficiency of transfer might depend strongly upon the structure of pigment-protein complex. The three species Goedheer [3] studied contain different types of light-harvesting complexes [6]. In this case the efficiency of carotenoid to bacteriochlorophyll energy transfer will depend both upon the distance between these molecules and the relative orientation of their respective transition moments which are responsible for the transfer [8]. These factors will be determined by the pigment-protein interactions.

Despite Goedheer's study [3] on the singlet-singlet energy transfer the way in which the efficiency of the triplet-triplet energy transfer from the bacteriochlorophyll to the carotenoid depends upon carotenoid type, has yet to be determined. This is largely because it has proved impossible to saturate the carotenoid triplet formation in intact photosynthetic membranes [9].

Boucher et al. [10] have studied how the efficiency of energy transfer from the carotenoid to the bacteriochlorophyll in reaction centres from *Rhs. rubrum* depends upon the carotenoid type present. They were able to reconstitute carotenoids with reaction centres from the carotenoidless mutant G9. In each case, the carotenoid bound to the same single binding site and took up a *cis*-conformation. The efficiency of the energy transfer was very dependant upon the type of carotenoid added back. They also noted that there was no correlation between which carotenoids were efficient at singlet-singlet energy transfer and which were efficient photoprotectors.

In the present study we set out to determine the effect of carotenoid composition on the efficiency of energy transfer between the carotenoid and the bacteriochlorophyll (both forward singlet-singlet and reverse triplet-triplet) in an isolated light-harvesting pigment-protein complex.

Carotenoid containing strains of *Rps. sphaeroides* have two types of light-harvesting pigment-protein complex [6] one of which (the so-called *B*-800–

850 complex) is rather easy to isolate and purify. This complex contains bacteriochlorophyll and carotenoid in the ratio of 3 : 1 [11].

Unlike the study of Boucher et al. [10] we have been able to vary the type of carotenoid inserted into the same *B*-800—850 antenna complex by isolating complexes from strains of *Rps. sphaeroides* with different carotenoid content, by aerating anaerobically grown wild-type cells or by growing wild-type cells semi-aerobically [11,14].

The pigments are bound to two types of small, hydrophobic polypeptides [12]. The carotenoid is all-*trans* [13] and is bound to a specific site [11] such that its long axis makes a defined angle with respect to the fluorescent bacteriochlorophylls (Vermeglio, A. and Cogdell, R.J., unpublished data).

Materials and Methods

Rps. sphaeroides strains 2.4.1 (wild-type), Ga and G1C (two green mutants) were grown anaerobically in the light with succinate as the sole carbon source. The cells were harvested, disrupted by passage through a French pressure cell and chromatophores isolated by differential centrifugation [15]. The *B*-800—850 complexes were isolated and purified following detergent treatment of chromatophores by a modification of the procedure of Clayton and Clayton [11,15].

The carotenoid composition of strain 2.4.1 was varied by growing the cells anaerobically in the light and then vigorously aerating them for 24 h prior to harvesting and preparing the *B*-800—850 complex.

In addition strain 2.4.1 was also grown semi-aerobically in an illuminated shaking incubator. In this case a photosynthetically grown culture was used to innoculate 400 ml of growth medium in 2-l conical flasks. The flasks were shaken at a low rate (70 rev./min) and illuminated by three 100 watt tungsten bulbs. Under these conditions the cells were very red and contained more than 98% spheroidenone (see below).

The carotenoid composition of the isolated complexes was determined as described by Cogdell et al. [16] and their absorption spectra were recorded on an Unicam SP8000 recording spectrophotometer. The calculation of the efficiency of energy transfer from the carotenoid to the bacteriochlorophyll depends on accurate data from the absorption spectra. It was hard to obtain completely non-scattering samples so differences in efficiency of the order of 2—5% are probably not significant.

The fluorescence excitation spectrum of each of the complexes was determined in a simple home-made fluorimeter. Excitation was provided by passing light from a 150 watt quartz-iodine bulb through a monochromator (High Radiance M300, Applied Photophysics Ltd., London). The fluorescence was collected at right angles from the direction of excitation, passed through a Schott RG715 cut-off filter and a Balzer's 858 nm interference filter and detected by an EMI 9659QB photomultiplier. The signal was amplified, filtered and displayed on a pen recorder. The intensity of the exciting light was determined with a United Detector Technology, Model 40X, Opto-meter (Santa Monica, CA) light meter, placed at the cuvette position.

The fluorescence emission spectra were recorded in a similar apparatus. How-

ever, in this case the exciting light was filtered with a combination of 5 mm Corning 4.97, 2 mm Schott BG18, and a Balzer's Calflex C filter giving broad-band blue light, and the fluorescence was passed through an RG715 cut-off filter and the monochromator prior to detection. The emission spectra were corrected for the photomultiplier sensitivity but not for the transmission characteristics of the monochromator.

The flash photolysis experiments were carried out in the apparatus described by McVie et al. [18] with an automatic back-off facility [19]. Excitation at 694 nm was provided by a Q-switched ruby laser. The intensity of each laser pulse was measured by a photodiode and recorded on a System 2000 laser energy meter (J.K. Lasers, Rugby, U.K.). The intensity of the laser flash was reduced, where required, by a set of calibrated copper sulphate solutions. In order to overcome the problem of photodestruction of the sample due to multiple excitation a 1 cm × 1 mm × 1 mm flow cell was used; the sample in the cuvette was changed after each exciting flash. Before each experiment the sample was thoroughly de-oxygenated by bubbling with nitrogen for 10 min. Thereafter the system was kept closed and flow through the cuvette was driven by the pressure of gas from the nitrogen cylinder.

For the photodestruction experiments strong white light from a 150 watt, 24 volt quartz iodine bulb was focused onto the reaction cuvette (900 $W \cdot m^{-2}$). The light was filtered through 5 cm of water. The absorption spectra of the complexes were recorded at various times during the irradiation on the Unicam SP8000 spectrophotometer.

Results and Discussion

1. Determination of the emission spectra of the isolated B-800—850 complexes

Fig. 1A shows the emission spectrum of the *B*-800—850 complex from strain G1C. Similar spectra were obtained for the *B*-800—850 complexes isolated from the other strains. The emission band is centred at 855 nm and shows only a very small emission at 780 nm, which is thought to arise from so-called 'free' bacteriochlorophyll which absorbs around 770 nm. The emission spectra from the isolated *B*-800—850 complexes also show a minor band at about 800 nm. Presumably this reflects some fluorescence from the 800 nm absorption band of the *B*-800—850 complex and shows that energy transfer from the 800 nm absorption band to the 850 nm band within the complex is not 100% efficient [20]. Compared with the fluorescence emission spectrum of intact chromatophores (data not shown, and see Ref. 21) the longer wavelength emission which presumably arises from *B*-870 is absent, as would be expected.

If however the isolated, diluted complex in the cuvette is incubated in 2% lauryldimethylamine-*N*-oxide over a period of about 1 hour the 780 nm emission band grows (Fig. 1B). During this time the absorbance of the *B*-800—850 complex decreases at 800 and 850 nm and increases at 770 nm. The excitation spectra reported in the next section, were recorded with low detergent concentrations (<0.3% lauryldimethylamine-*N*-oxide) and only the fluorescence from the 855 nm emission band was collected (see Materials and Methods).

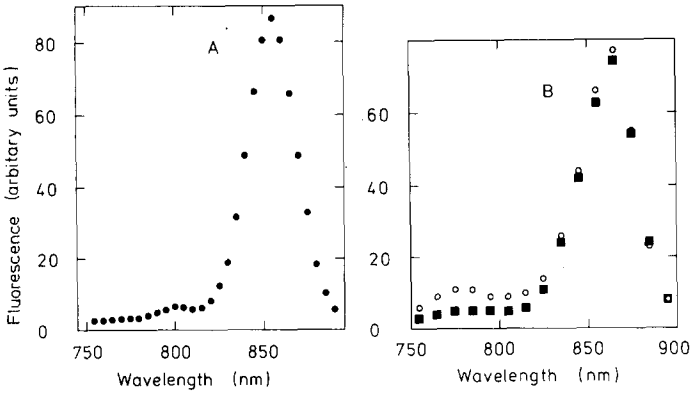

Fig. 1. The emission spectra of the *B*-800—850 light-harvesting pigment-protein complex from *Rps. sphaeroides* G1C ± 1% LDAO. (A) The *B*-800—850 complex was suspended in 20 mM Tris-HCl (pH 8.0), A_{850} = 0.5. Excitation was provided by a quartz iodine bulb filtered with 5 mm of Corning 4-97, 2 mm of Schott BG 18 and a Balzer's Calflex C (broad band blue light). The intensity of excitation was 90 W · m^{-2}. The fluorescence was passed through an RG715 cut off filter and a monochromator (entrance slits 2.5 mm, exit slits 2 nm) and then detected by an EMI photomultiplier. (B) The *B*-800—850 complex was suspended in 20 mM Tris-HCl (pH 8.0), A_{850} = 0.33. Experimental conditions as in (A) except the monochromator exit slit was 10 nm (the wider slits do not resolve the emission band at 800 nm). ■, Spectrum recorded in the presence of buffer. ○, Spectrum recorded 1 h after the addition of 1% lauryldimethylamine-*N*-oxide. The size of the emission spectrum in the presence of lauryldimethylamine-*N*-oxide is larger than in the absence of added detergent even though some of the complex is denatured, because addition of 1% lauryldimethylamine-*N*-oxide causes an increase in fluorescence yield of the complex.

2. Determination of the fluorescence excitation spectrum of the isolated B-800—850 complexes

The absorption spectra and the fluorescence excitation spectra for each complex were recorded in the 400—620 nm region. A typical result is shown in Fig. 2 for the *B*-800—850 complex from strain G1C. The absorption and fluorescence excitation spectra were normalised in the 590 nm bacteriochlorophyll

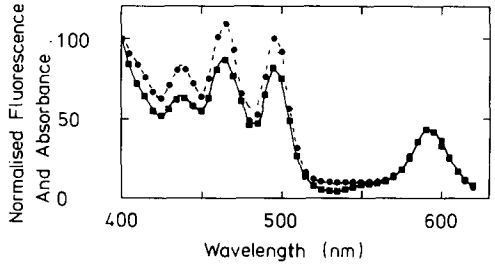

Fig. 2. The normalised absorption and fluorescence excitation spectra of the *B*-800—850 light-harvesting complex from *Rps. sphaeroides* G1C. ■, Fluorescence excitation spectrum. The *B*-800—850 complex was suspended in 20 mM Tris-HCl (pH 8.0), A_{590} = 0.12. The excitation was provided from a quartz iodine bulb and passed through a monochromator, entrance slits 0.5 mm, exit slits 2 nm. The fluorescence was detected after being passed through a Balzer's 858 nm interference filter. •, The normalised absorption spectrum of the *B*-800—850 complex recorded on an Unicam SP8000 recording spectrophotometer. A more concentrated sample was used in order to obtain this spectrum with a greater degree of accuracy. The sample used has an *A* at 590 nm of 0.292. The two spectra were normalised at 590 nm. Energy transfer efficiences were calculated not from the absorption spectrum, but from the fractional absorption spectrum.

TABLE I

THE EFFICIENCY OF CAROTENOID TO BACTERIOCHLOROPHYLL ENERGY TRANSFER IN THE FIVE *B*-800—850 PIGMENT-PROTEIN COMPLEXES STUDIED TOGETHER WITH THEIR RESPECTIVE CAROTENOID COMPOSITION

Type of *B*-800—850 complex	Energy transfer efficiency integrated over the carotenoid fractional absorption bands (%)	Carotenoid composition	
Anaerobic 2.4.1 *	100	spheroidene	92%
		spheroidenone	8%
Anaerobic-aerated 2.4.1. *	95	spheroidene	70%
		spheroidenone	30%
Semi-aerobic 2.4.1 **	76	spheroidene	2%
		spheroidenone	98%
G1C	95	neurosporene	100%
GA	94	neurosporene	60%
		methoxyneurosporene	14%
		chloroxanthin	26%

* Solving simultaneously for energy transfer efficiency of spheroidene for the anaerobic 2.4.1 and anaerobic -aerated 2.4.1 complex gives values of 100% (spheroidene) and 75% (spheroidenone).

** With this complex the 590 nm bacteriochlorophyll absorption band overlaps with absorption from spheroidenone, so the fluorescence excitation spectrum and fractional absorption spectrum were normalised in the Soret band of the bacteriochlorophyll.

absorption band. The excitation spectrum shows that light absorbed by the carotenoid (in this case neursporene [22]) is rather efficient in sensitising the bacteriochlorophyll fluorescence. The yield of carotenoid to bacteriochlorophyll energy transfer was calculated by dividing the excitation spectrum by the normalised fractional absorption spectrum. Table I shows the relative energy transfer efficiencies for each complex studied, together with their respective carotenoid compositions. Essentially the same results were obtained with several different preparations of each of the *B*-800—850 complexes. At the short wavelength end of the carotenoid absorption band there is some underlying absorption due to the Soret band of the bacteriochlorophyll, especially in the case of the complexes from strains G1C and GA. However, when the relative efficiency of energy transfer from carotenoid to bacteriochlorophyll was calculated at each of the three peaks in the carotenoid absorption spectrum, no significant changes in efficiency were found, so that the problem of the contaminating bacteriochlorophyll absorption was neglected.

The relative efficiencies of the carotenoid to bacteriochlorophyll energy transfer are high (in the range of 75—100%). There is little variation of efficiency with carotenoid composition except with spheroidenone. However considering that the peak absorption of spheroidenone is about 50 nm further to the red than that of either neurosporene or chloroxanthin [23] the energy transfer efficiency seems remarkably insensitive to carotenoid composition. The simplest explanation of these results is that the major factor controlling the efficiency of the carotenoid to bacteriochlorophyll energy transfer in these *B*-800—850 complexes is the geometry of the system; that is the topology of

the pigments with respect to each other as determined by the pigment-protein interactions. The importance of these geometric factors is clearly emphasised by the situation where the complexes are partially disorganised by mild denaturation (in the presence of high detergent concentration). Under these conditions most of the bacteriochlorophyll absorption is found at approx. 770 nm and carotenoid does not sensitise the bacteriochlorophyll fluorescence at 780 nm, only that residual emission at 855 nm.

The carotenoid type does, however, have some role to play in determining the efficiency of energy transfer (e.g. spheroidenone vs. spheroidene) although this difference is relatively small.

3. Characterisation of the carotenoid triplet of the isolated B-800—850 antenna complexes

When the isolated antenna complexes are excited with a Q-switched ruby laser flash a strong transient absorbance change is induced which decays in a few microseconds. The difference spectrum of this change, illustrated for strain G1C (Fig. 3), is similar to those previously described for the carotenoid triplet state in intact photosynthetic membranes [9]. The decay kinetics of the change ($t_{1/2}$ = 4.1 μs) shown in Fig. 4a for strain GA are again comparable with those found in intact membranes [9]. The same is true of all the *B*-800—850 complexes studied. The decay of the transient is accelerated in the presence of oxygen, $t_{1/2}$ = 2 μs (Fig. 4b). Assuming the concentration of oxygen in air saturated buffer at 20°C is 280 μM [24] the second order rate constant for the quenching by oxygen is $5.3 \cdot 10^8\ M^{-1} \cdot s^{-1}$. This agrees very closely with the

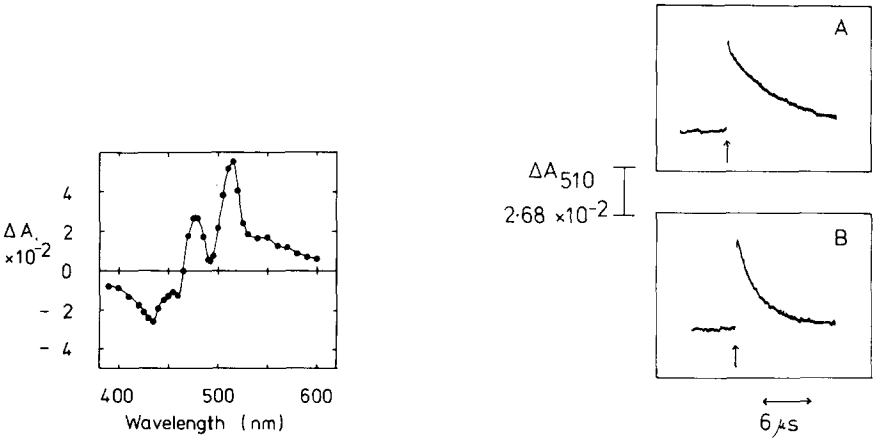

Fig. 3. The difference spectrum for the formation of a carotenoid triplet state of the *B*-800—850 light-harvesting pigment-protein complex from *Rps. sphaeroides* G1C. *B*-800—850 from strain G1C was diluted in 50 mM Tris-HCl (pH 8.0), 0.1% lauryldimethylamine-*N*-oxide to give an *A* at 694 nm of 0.28. Saturation of the absorbance change was tested at 510 nm, and a laser intensity which was just above saturating (laser pulse attenuated by a 33% transmission copper sulphate filter) was used for excitation.

Fig. 4. Q-switched ruby laser flash induced carotenoid triplets. (A) The *B*-800—850 antenna complex from strain GA was suspended in 50 mM Tris-HCl (pH 8.0), 0.1% lauryldimethyl-*N*-oxide. *A* at 694 nm was 0.28. The sample was thoroughly bubbled with nitrogen prior to the laser flash. ↑ notes where the laser fired and an upward deflection was an increase in absorbance. The band width of the amplifier was 5 MHz. (B) Sample as in (A) except that it was bubbled with air before excitation.

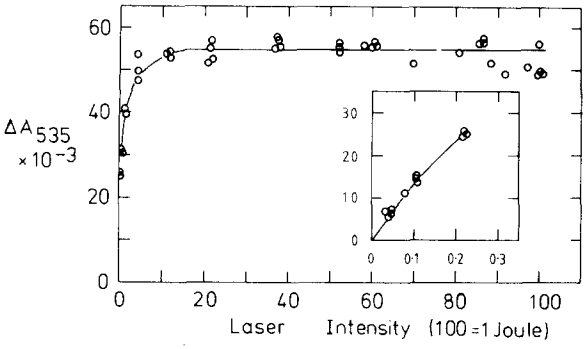

Fig. 5. The variation of the size of the carotenoid triplet with laser intensity. *B*-800—850 from strain 2.4.1 grown anaerobically was diluted in 50 mM Tris-HCl (pH 8.0), 0.1% lauryldimethyl-*N*-oxide to give an *A* at 694 nm of 0.28. When the sample had been thoroughly bubbled with nitrogen the extent of the Q-switched laser induced carotenoid triplet was measured as a function of the intensity of the excitation pulse. The laser intensity was varied with a set of copper sulphate solutions of known *A* at 694 nm. The insert shows the low intensity data on an expanded scale, the axes of the insert are $\Delta A_{535} \times 10^{-3}$ and incident laser intensity, where 100 = 1 Joule. Note that the sample in the cuvette was changed after each laser flash to prevent any artifacts due to photodestruction.

value of the oxygen quenching rate constant obtained by Monger et al. [9] $5 \cdot 10^8\ M^{-1} \cdot s^{-1}$. We therefore conclude that the transient represents the carotenoid triplet state.

As we describe below (Fig. 5), using these isolated pigment-protein complexes it is possible completely to convert all the carotenoid present in the reaction cuvette to its triplet state. So it has been possible directly to determine the extinction coefficients for the triplet state formation. The differential extinction coefficient for the formation of the neurosporene triplet (*B*-800—850 from strain G1C) is 17.9 $mM^{-1} \cdot cm^{-1}$ at 510 nm and for spheroidene (*B*-800—850 from anaerobically grown strain 2.4.1) is 9.5 $mM^{-1} \cdot cm^{-1}$ at 535 nm. These values are rather smaller than that reported for the triplet state of spheroidene (29 $mM^{-1} \cdot cm^{-1}$) generated in cyclohexane [25].

4. Determination of the efficiences of the carotenoid triplet formation in the B-800—850 antenna complexes

Fig. 5. shows a typical light intensity curve for carotenoid triplet formation with a sample of *B*-800—850 from strain 2.4.1 grown anaerobically. It is clear that at the higher laser intensities the carotenoid triplet production has been saturated. The absolute triplet yields were determined by comparing the concentration of carotenoid triplets produced with a weak laser flash with the concentration of triplets formed by the absorption of the same number of quanta by a solution of standard with known triplet extinction and triplet yield [26, 27], i.e.

$$\phi_{CarT} = \phi_{StandT} \cdot \frac{\Delta A_{CarT}}{\Delta A_{StandT}} \cdot \frac{\epsilon_{StandT}}{\epsilon_{CarT}}$$

where ϕ_{CarT} = yield of carotenoid triplet, ϕ_{StandT} = yield of triplet formation of the standard, ΔA the respective flash-induced absorption changes and ϵ the res-

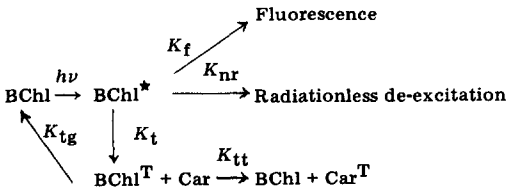

Fig. 6. A model of the photochemical processes leading to the production of the carotenoid triplet state.

pective differential extinction coefficients. We used methylene blue in approx. 0.03 M H_2SO_4 as the standard assuming $\phi_{MB^T} = 0.58$ and $\epsilon_{M_{MB^T}}$ at 375 nm = 6000 cm^{-1} [28].

The triplet yields were found to be quite variable, from 0.02 to 0.15. However this variation did not depend upon the carotenoid type, rather it varied depending on the amount of detergent present with the sample. Those samples which had been stored with a high detergent to bacteriochlorophyll ratio gave the higher triplet yields while those samples which had been stored with low detergent to bacteriochlorophyll ratios gave the lower triplet yields.

The lack of effect of carotenoid type on the yield of carotenoid triplet formation reveals several interesting features of the photochemistry of these isolated antenna complexes. Consider the processes involved in production of the carotenoid triplet (Fig. 6).

The laser light is absorbed by the bacteriochlorophyll and the carotenoid triplet is generated by a triplet-triplet exchange reaction with bacteriochlorophyll triplets [9]. If the laser induced change with the G1C *B*-800—850 is monitored at 465 nm, where there is no change due to the formation of Car^T (see Fig. 3), then a transient which decays in less than 100 ns is seen. We presume that this is the bacteriochlorophyll triplet state. The yield of $BChl^T$ (ϕ_{BChl^T}) is $K_t/(K_f + K_{nr} + K_t)$ while the yield of Car^T (ϕ_{Car^T}) is $(K_{tt}/K_{tg} + K_{tt}) \times \phi_{BChl^T}$). Previous studies with chromatophores from *Rps. sphaeroides* [9] showed that in the absence of carotenoids K_{tg} is small, approx. 10^5 s^{-1}, while K_{tt} (in the presence of carotenoids) is large, approx. $(0.5—1.0) \cdot 10^8$ sec^{-1}. It is clear from these rates that even if K_{tt} varied, depending upon the carotenoid type, by a factor of 10^2 the yield of Car^T would still remain essentially the same. We have estimated the rise time of carotenoid triplet formation (i.e. K_{tt}) for each type of complex and it is extremely rapid, $t_{1/2} \leqslant 50$ ns. These rise times were not sufficiently longer than the width of the exciting flash and so accurate data upon these rise times will require a shorter exciting flash and a system with a faster response time. However, estimated rise times agree well with those previously reported for whole chromatophores by Monger et al. [9].

It is clear from Fig. 6 that ϕ_{Car^T} will depend strongly upon ϕ_{BChl^T}. We have described above, that depending upon the type and concentration of detergent present, the yield of fluorescence of the *B*-800—850 antenna complexes can vary several fold. Heathcoate and Clayton [29] describe similar effects of detergent upon the fluorescence yield of the *B*-800—850 antenna complex from *Rps. sphaeroides*. The conditions which lead to higher fluorescence yields (high detergent to bacteriochlorophyll ratios) are the conditions which give the higher yields of carotenoid triplets. A higher fluorescence yield means that the

excited singlet state of the bacteriochlorophyll has a longer lifetime and this will in turn allow more intersystem crossing to the triplet. More bacteriochlorophyll triplets then yield more carotenoid triplets.

The adjustment of the samples to a new detergent concentration is a very slow process and takes hours to occur. However, on a sample of *B*-800—850 from strain G1C which had been stored in low lauryldimethylamine-*N*-oxide (< 0.05% v/v, detergent: bacteriochlorophyll; 0.2 : 1) we tested the effect of increasing the lauryldimethyl-*N*-oxide concentration on both the fluorescence yield and carotenoid triplet yield. Increasing the lauryldimethylamine-*N*-oxide concentration to give a ratio of detergent: bacteriochlorophyll of 85 : 1 increased the fluorescence yield by 49% and the carotenoid triplet yield by 52%, both measured after a 1.5-h incubation with the detergent.

The presence of a larger initial population of bacteriochlorophyll triplets can be demonstrated by making use of the 'photodynamic effect'.

5. The effect of illuminating the B-800—850 antenna complexes with strong white light in the presence of oxygen

Pigment-protein complexes which contain bacteriochlorophyll, but which lack carotenoids, are rapidly photodestroyed by irradiation with strong light in the presence of oxygen. This is the 'photodynamic effect' and it is believed to be due to the harmful production of such products as singlet oxygen [30,31]. The singlet oxygen is thought to be generated by a triplet-triplet exchange reaction between the bacteriochlorophyll triplet and triplet oxygen [4] i.e.

$$BChl^{T} + {}^{3}O_2 \rightarrow BChl^{s0} + {}^{1}O_2({}^{1}\Delta g).$$

The presence of carotenoids in the pigment-protein complexes provides a great deal of protection by quenching the lifetime of the bacteriochlorophyll triplet by about 10^3 [9]. However, even in the presence of carotenoids there is still a slow photodestruction of the bacteriochlorophyll (Fig. 7).

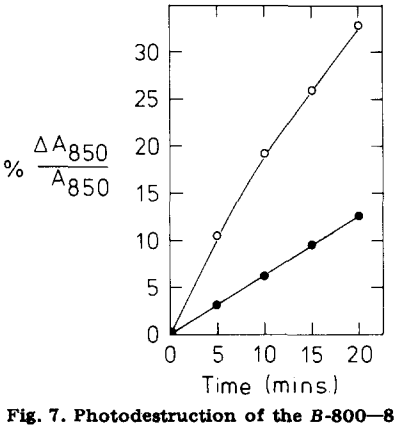

Fig. 7. Photodestruction of the *B*-800—850 antenna complex. Two samples of *B*-800—850 antenna complex from strain G1C were diluted in 50 mM Tris-HCl (pH 8.0), 0.1% lauryldimethylamine-*N*-oxide to give an *A* at 850 nm of between 0.5 and 0.6. The carotenoid triplet yields of both samples had been determined previously: ○———○, $\phi_{Car}T$ = 6.7%; ●———●, $\phi_{Car}T$ = 1.9%. Strong white light from 150 watt, 24 volt quartz iodine bulb was filtered through 5 cm of water and focused into the cuvette. The irreversible photobleaching at 850 nm was recorded as a function of time of illumination.

We have examined the rate of photodestruction with samples of the *B*-800—850 complexes with both high and low carotenoid triplet yields. Typical results for two samples of *B*-800—850 from strain G1C are shown in Fig. 7. Independent of the carotenoid type present in the complexes those with the higher carotenoid triplet yields are more sensitive to the photodestruction than those with the lower carotenoid triplet yields.

If it is accepted that this photodestruction is indeed sensitised by bacteriochlorophyll triplets then a higher rate of photodestruction implies a higher steady state population of bacteriochlorophyll triplets. These results then provide evidence that the variation in the carotenoid triplet yield is indeed due to a change in the yield of bacteriochlorophyll triplet formation.

It is interesting to point out that even though the absorption spectrum of these *B*-800—850 antenna complexes shows no significant changes with detergent concentration (until they are denatured) there are nonetheless subtle changes in their photochemical reactions taking place. Depending on the detergent: bacteriochlorophyll ratio the yields of bacteriochlorophyll fluorescence and intersystem crossing to the triplet manifold show marked variation. The reason for this variation is not clear although it may depend on the degree of aggregation of the complexes. Heathcoate and Clayton [29] estimated that the fluorescence yield of the *B*-800—850 antenna complexes varied between a few percent and about 50%. Taking our maximum carotenoid triplet yield of about 15% then this implies the yield of bacteriochlorophyll triplets is also 15%. Therefore 35% or more of the singlet excited bacteriochlorophylls must decay by radiationless processes.

In summary, it appears that unlike the case with reaction centres from *Rhs. rubrum* [10] energy transfer between the carotenoid and the bacteriochlorophylls in these *B*-800—850 pigment-protein complexes, both at the singlet and triplet energy levels, is rather independent of the carotenoid type present and appears to be controlled mainly by the pigment-protein interactions.

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