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Carotenoids in photosynthesis

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Carotenoids are usually considered to perform two major functions in photosynthesis. They serve as accessory light harvesting pigments, extending the range of wavelengths over which light can drive photosynthesis, and they act to protect the chlorophyllous pigments from the harmful photodestructive reaction which occurs in the presence of oxygen.

Drawing upon recent work with photosynthetic bacteria, evidence is presented as to how the carotenoids are organized within both portions of the photosynthetic unit (the light harvesting antenna and the reaction centre) and how they discharge both their functions. The accessory pigment rôle is a singlet–singlet energy transfer from the carotenoid to the bacteriochlorophyll, while the protective rôle is a triplet–triplet energy transfer from the bacteriochlorophyll to the carotenoid.

Introduction

Carotenoids, probably because they are so intensely coloured, have been the subject of extensive chemical analysis (see, for example, Isler *et al.* 1971). There is, however, much less information as to how the carotenoids are organized within the photosynthetic apparatus and how this enables them to accomplish their presumed two major functions; namely acting as accessory light-harvesting pigments (see, for example, Goedheer 1959) and protecting the chlorophyllous pigments from sensitizing the harmful 'photodynamic reaction', which occurs in the presence of light and oxygen (Krinsky 1968). This paper reviews some of the current ideas of how the carotenoids are organized and how they are thought to discharge their two main functions. Since most of the recent work in this field has been carried out upon photosynthetic bacteria; the examples discussed here will be drawn from this area. It is hoped, however, that the major conclusions will also be relevant to oxygen-evolving photosynthetic systems.

Carotenoids are located within the photosynthetic unit and function in the 'primary reactions' which occur between the absorption of light and the 'trapping' of that light energy in the primary photochemical redox reaction. The photosynthetic unit may be divided into two parts, the light-harvesting antenna and the reaction centre. The organization and function of the carotenoids are discussed separately for each part of the photosynthetic unit.

The organization of carotenoids within the light-harvesting antenna

The photosynthetic unit of a typical purple non-sulphur photosynthetic bacterium such as *Rhodopseudomonas sphaeroides* consists of about 100 bacteriochlorophyll molecules and about 30–40 molecules of carotenoid per reaction centre (Aagard & Sistrom 1972; Cogdell *et al.* 1976). If the photosynthetic membrane is treated with detergent, for example with LDAO (lauryl dimethyl-amine-*N*-oxide), then the photosynthetic unit may be disrupted into its two

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components each of which may be independently separated and purified (Clayton & Clayton 1972; Reiss-Husson & Jolchine 1972).

The antenna pigments exist as complexes together with small hydrophobic proteins and several types can be distinguished by their differing absorption spectra in the 800–900 nm region. *Rps. sphaeroides* strain 2.4.1 contains two types of antenna pigment–protein complexes called B (Bulk) 870 and B800–850 (where the numbers refer to their absorption maxima in the near infrared (Thornber *et al.* 1978)). So far detailed information is only available for the B800–850 complex (Austin 1976; Cogdell & Crofts 1978).

TABLE 1. THE RELATIVE CONCENTRATIONS OF BACTERIOCHLOROPHYLL AND CAROTENOID IN THE B800–850 ANTENNA COMPLEXES AND THE PARENT CHROMATOPHORES FROM WHICH THEY WERE DERIVED

strain of Rps. sphaeroides	A_{850}	[bacteriochlorophyll] μM	[carotenoid] μM	ratio, bacterio-chlorophyll:carotenoid
2.4.1 chromatophores	63	540	319	1.69
2.4.1 complex	0.84	7.2	2.46	2.92
Ga chromatophores	26.5	246	98.5	2.52
Ga complex	5.75	47.2	15.35	3.07
G1C chromatophores	85	772	296	2.6
G1C complex	4.72	33.4	11.5	2.91

TABLE 2. THE CAROTENOID COMPOSITION OF THE B800–850 ANTENNA COMPLEXES TOGETHER WITH THAT OF THEIR PARENT CHROMATOPHORES

sample	composition (%)	carotenoid
2.4.1 chromatophores	0.06	neurosporene
	92	spheroidene
	7.94	spheroidenone
2.4.1 antenna complex	0.06	neurosporene
	91	spheroidene
	8.94	spheroidenone
Ga chromatophores	56	neurosporene
	13.4	chloroxanthin
	30.6	unknown
Ga antenna complex	60	neurosporene
	13.5	chloroxanthin
	26.5	unknown
G1C chromatophores	100	neurosporene
G1C antenna complex	100	neurosporene

The pigment content of the B800–850 antenna pigment–protein complex from three different strains of *Rps. sphaeroides* is presented in tables 1 and 2. Two major points are clear: there is a strict stoichiometric relation between the bacteriochlorophylls and the carotenoids (3 bacterio-chlorophylls per carotenoid) and secondly the carotenoid content of the complex shows no evidence of selectivity but rather only reflects the carotenoid content of the parent chromato-phore membrane. Figure 1 shows two typical scans of SDS–polyacrylamide electrophoretic gels of the complex. When the complex is denatured by boiling, the pigments fall off and run at the the ion front and a polypeptide of molecular mass $8-10 \times 10^3$ is observed (figure 1*a*). An undenatured sample run in the presence of 0.1 % LDAO and 1 % SDS retains its pigments and

usually runs as one band of molecular mass 50–60×10^3 (figure 1b). Austin (1976) has recently described a careful analysis of the B800–850 complex from *Rps. sphaeroides* strain 2.4.1 and concludes that the minimal functional unit of this complex is three molecules of bacterio-chlorophyll and two molecules of protein (each of molec. mass *ca.* 10000). To this must be added the single molecule of carotenoid. However, it is likely *in vivo* that the complex exists as aggregates of this minimal unit of molecular mass greater than 100000 (Austin 1976). The aggregation state of the complex can be altered by varying the ratio LDAO:SDS (Clayton & Clayton 1972).
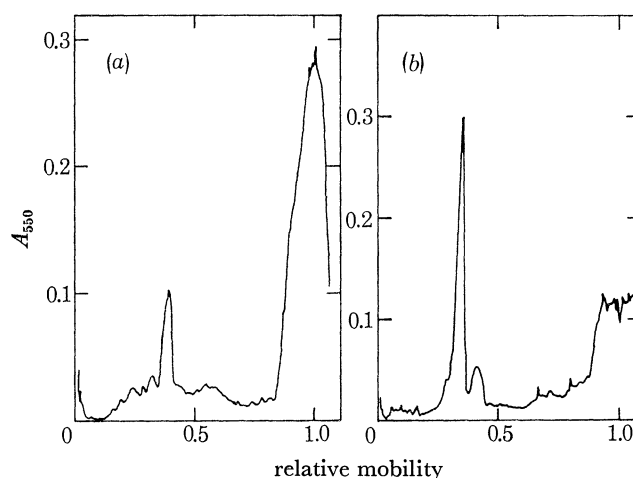

FIGURE 1. SDS polyacrylamide gel electrophoretograms of the B800–850 antenna complex from *Rps. sphaeroides* 2.4.1. The gels were stained with Coomassie blue and scanned in a Gilford 240 spectrophotometer at 550 nm. (*a*) The sample was denatured by boiling in 1 % SDS before application; (*b*) the sample was undenatured and run in 0.1 % LDAO and 1 % SDS.

How does the structure of the isolated B800–850 complex compare with that of the complex *in vivo*, within the photosynthetic membrane? Two lines of evidence suggest that the basic structure of the complex has been little altered by extraction from the membrane. The absorption spectrum of the isolated complex is not significantly different from that of the whole photosynthetic membrane (figure 2) and secondly the circular dichroism (c.d.) spectrum of the complex is again almost the same as that of the intact membrane (figure 3) (Cogdell & Crofts 1978). When the carotenoids are isolated from the complex they show no c.d. spectrum. The large c.d. spectrum of the carotenoids when bound to the complex results from the asymmetry induced by the carotenoid–protein interaction. The fact that this interaction is not significantly altered in the isolated B800–850 complex is strong evidence in favour of the isolated complex retaining its native structure.

The functioning of the carotenoids within the light-harvesting antenna

Unfortunately as yet there are no data available on the functioning of the carotenoid within isolated antenna pigment–protein complexes, so the results discussed in this section refer to experiments carried out on the intact photosynthetic membrane.
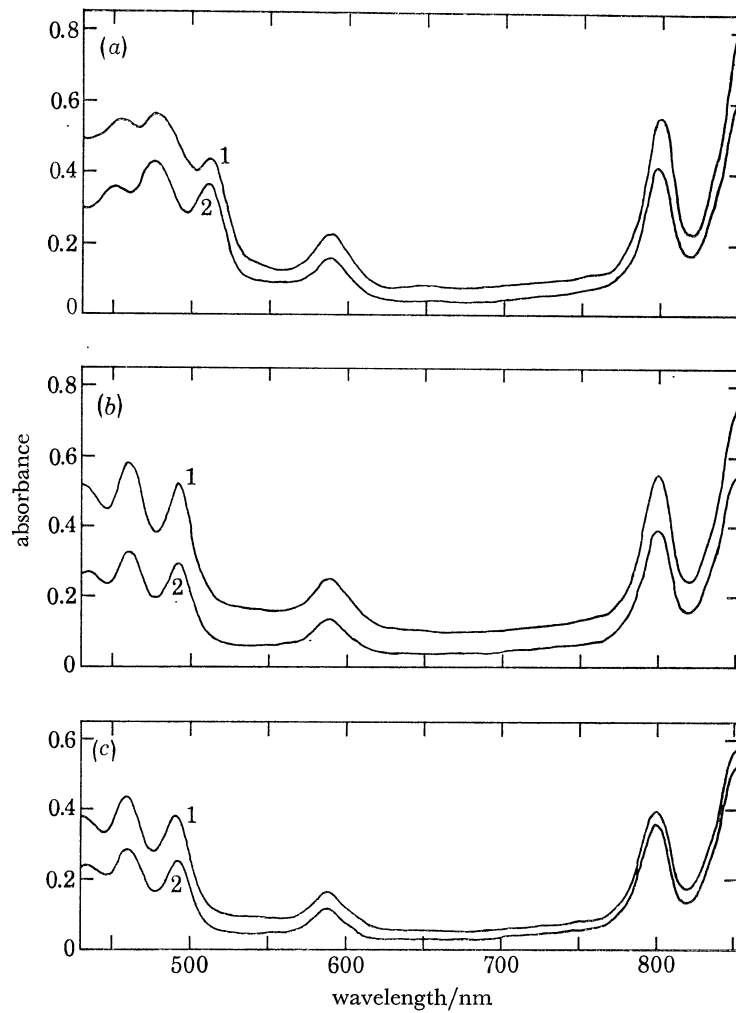
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FIGURE 2. The absorption spectra of the B800–850 antenna complexes from three strains of *Rps. sphaeroides* together with the absorption spectra of their parent chromatophores. (*a*) Strain 2.4.1: (1) B800–850 complex; (2) chromatophores. (*b*) Strain G.1.C: (1) chromatophores; (2) B800–850 complex. (*c*) Strain Ga: (1) chromatophores; (2) B800–850 complex.
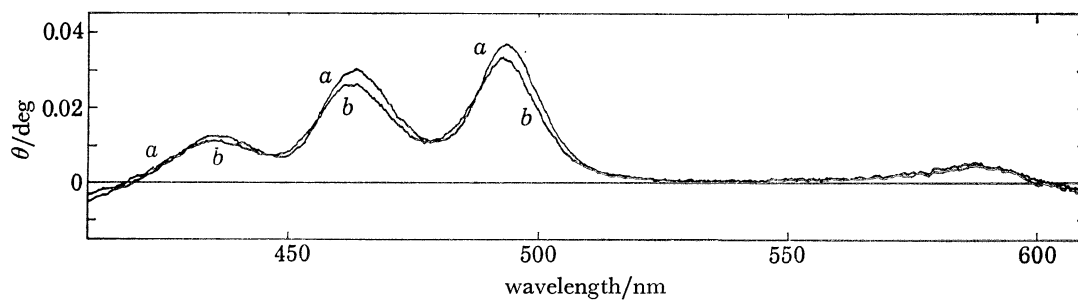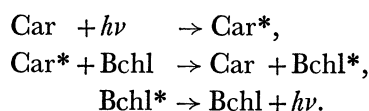

FIGURE 3. The circular dichroism spectra of the B800–850 complex of *Rps. sphaeroides* G1C and its parent chromatophores. The concentration of the chromatophores and the B800–850 complex was adjusted to an optical density of *ca*. 1.0 at 500 nm. (*a*) B800–850 complex; (*b*) chromatophores.

Figure 4 shows the action spectrum of excitation of bacteriochlorophyll fluorescence in whole cells of *Rps. sphaeroides* (Goedheer 1959). It shows clearly that light absorbed by the carotenoids can be used to sensitize the bacteriochlorophyll fluorescence. This is singlet–singlet energy transfer from the carotenoid to the bacteriochlorophyll, i.e.

$$\text{Car} + h\nu \rightarrow \text{Car}^*,$$
$$\text{Car}^* + \text{Bchl} \rightarrow \text{Car} + \text{Bchl}^*,$$
$$\text{Bchl}^* \rightarrow \text{Bchl} + h\nu.$$

This is the light harvesting rôle of the antenna carotenoid, making light available for photosynthesis over a wider spectral range. The efficiency of this carotenoid to bacteriochlorophyll energy transfer process varies from species to species, e.g. 90 % in *Rps. sphaeroides* and 30 % in *R. rubrum* (Goedheer 1959). The reason for these differences remains to be explained, but they could derive either from the variation in carotenoid type or the organization of the carotenoid within the antenna complex concerned.
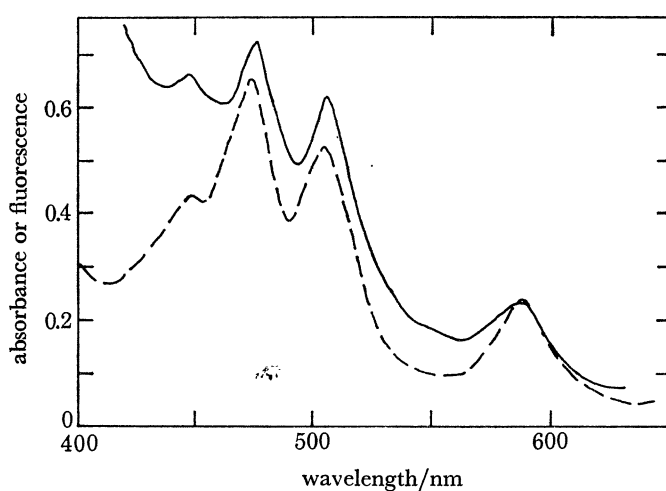

FIGURE 4. The absorption (——) and fluorescence excitation (- - -) spectra of whole cells of *Rps. sphaeroides*.
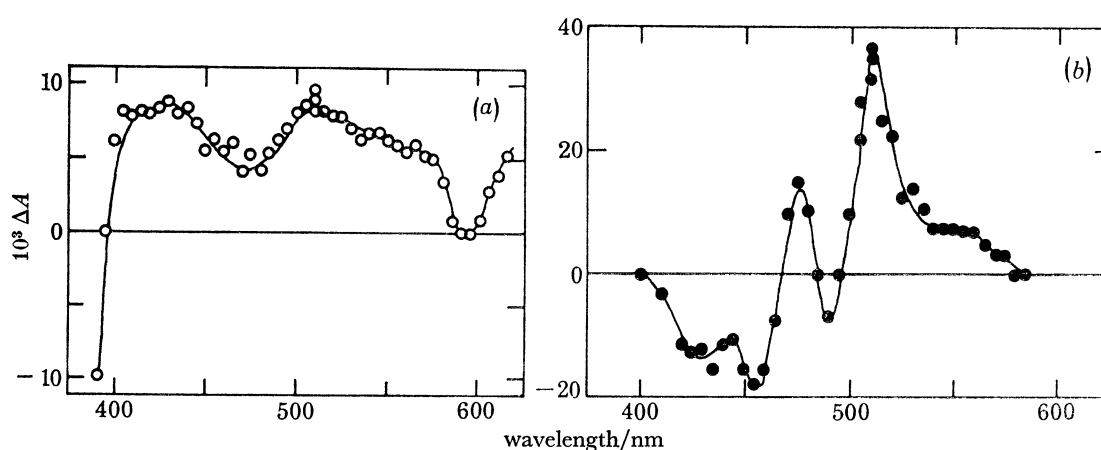

FIGURE 5. Laser flash induced difference spectra of the metastable triplet states induced in chromatophores from two strains of *Rps. sphaeroides*. (a) Strain R26 (carotenoidless); (b) strain Ga (carotenoid containing). Chromatophores, with their primary electron acceptor chemically reduced, were excited by a strong Q-switched ruby laser pulse (600–900 nE absorbed cm^{-2}).

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It is now well documented that the presence of carotenoids protects the bacteriochlorophyll from the destructive 'photodynamic reaction' (see, for example, Griffith *et al.* 1955), but how is this protective function accomplished?

If chromatophores from the carotenoidless strain of *Rps. sphaeroides* R26 are illuminated with short supersaturating flashes then a metastable state is seen, which decays on the microsecond time scale (Monger *et al.* 1976; Monger & Parson 1977). From the lifetime of this state and its difference spectrum (figure 5a) it has been concluded that it is the triplet state of the antenna bacteriochlorophyll. In the absence of oxygen this state decays with a half-time of about 70 μs. The decay half-time is accelerated by the presence of oxygen and yields a second order quenching constant of about 8×10^7 M^{-1} s^{-1}. It is thought that it is the triplet of the antenna bacteriochlorophyll which is the sensitizer for the harmful 'photodynamic reaction'. In carotenoid-containing strains, which are excited with short supersaturating flashes a new metastable state is seen (Monger *et al.* 1976; Kung & DeVault 1976; Renger & Wolff 1977). This state decays with a half-time of 2–8 μs, depending on the strain used.

It has been concluded from the variation of the difference spectrum of this state with the carotenoid content (figure 5b) and from its lifetime that it is the triplet state of the antenna carotenoid. Because carotenoids in solution are not directly excited to their triplet state (Bennasson *et al.* 1975) and because this antenna carotenoid triplet state is also generated if the exciting flash is only absorbed by the bacteriochlorophyll, it has been concluded that antenna carotenoid triplets arise from triplet–triplet energy transfer from bacteriochlorophyll to the carotenoids, i.e.

$$Bchl + h\nu \to Bchl^*,$$
$$Bchl^* \to Bchl^T,$$
$$Bchl^T + Car \to Bchl + Car^T.$$
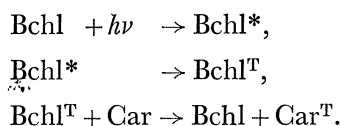
The half-rise time of Car^T formation is *ca.* 20 ns. This means that the quenching of the bacteriochlorophyll triplet state by the carotenoid is about 1000 times faster than its reaction with oxygen. Hence the carotenoid is protective.

Organization of the carotenoid within the reaction centre

In the last 5 years there has been a great leap forward in our understanding of the primary photochemical redox reaction of bacterial photosynthesis (see Parson & Cogdell 1975). These advances stem entirely from the availability of isolated, purified reactions centres devoid of antenna pigments. A reaction centre from *Rps. sphaeroides* consists of three polypeptides (molec. masses 21, 24 and 29×10^3), four bacteriochlorophylls, two bacteriophaeophytins (where the central Mg^{2+} is replaced by H^+), one or two ubiquinones, one ferrous iron atom and, in reaction centres from carotenoid-containing strains, one molecule of a specific carotenoid (Okamura *et al.* 1974 and Straley *et al.* 1973). Table 3 shows the carotenoid content of reaction centres prepared from two different strains of *Rps. sphaeroides* (Cogdell *et al.* 1975). This table also shows the carotenoid content of the parent chromatophores, and, in contrast to the B800–850 light-harvesting antenna complexes, the reaction centres show a large degree of specificity with respect to which carotenoid is bound to the reaction centre. This specific carotenoid binding to the reaction centre produces a strong induced c.d. spectrum (figure 6). Boucher *et al.* (1977) have recently studied the carotenoid–reaction centre interaction by using

carotenoidless reaction centres from *Rhodospirillum rubrum*. When they added carotenoids to these reaction centres, about 1 mol of carotenoid was bound per mol of reaction centre. Boucher *et al.* (1977) took a difference spectrum of the reaction centres with and without bound carotenoid (figure 7) and from this concluded that the carotenoid was in a central mono*cis* configuration. Resonance Raman spectroscopy of carotenoid containing reaction centres from *Rps. sphaeroides* also suggested a *cis*-conformation for the reaction centre carotenoid (Lutz *et al.* 1976, 1978).

TABLE 3. ANALYSIS OF THE CAROTENOID CONTENT OF REACTION CENTRES OF
RPS. SPHAEROIDES STRAINS 2.4.1 AND Ga

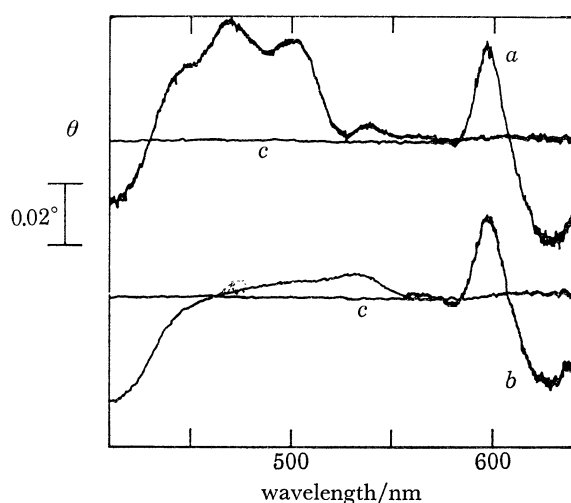| strain	molecules of carotenoid per reaction centre	composition of carotenoid types (%)
2.4.1	1.24	spheroidene 99 spheroidenone 1
Ga	1.06	chloroxanthin 94 neurosporene 6

FIGURE 6. The c.d. spectrum of photochemical reaction centres from *Rps. sphaeroides* in the visible region. (*a*) Strain 2.4.1 (carotenoid containing); (*b*) strain R26 (carotenoidless); (*c*) baseline.

It has also proved possible to locate, within limits, where within the reaction centre the carotenoid molecule must lie. If reaction centres from *Rps. sphaeroides* strain 2.4.1 (in the presence of 0.01 % LDAO) are treated with 0.1 % SDS they dissociate into a monomer and a dimer of the two lighter subunits (Okamura *et al.* 1974). All the pigments including the carotenoid remain on the dimer, which also retains its photochemical activity. The primary photochemical reaction is the oxidation of a special pair of reaction centre bacteriochlorophylls (P) and the reduction of the primary electron acceptor (Fe–UQ), i.e.

$$P(Fe\text{–}UQ) \xrightarrow{h\nu} P^+(Fe\text{-}UQ)^-.$$
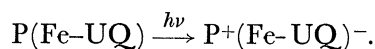
This then creates two fixed charges within the low dielectric milieu of the reaction centre, setting up a local electric field inside the reaction centre. Some pigments when subjected to electric fields undergo electrochromic shifts in their absorption spectra (Labhart 1967). This

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happens to the reaction centre carotenoid. From studying the reaction centre carotenoid band shift, Cogdell *et al.* (1977) concluded that the carotenoid must lie well towards the P side of the reaction centre.
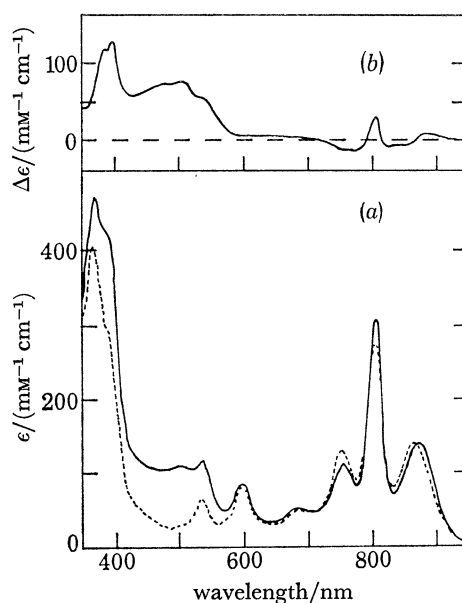

FIGURE 7. The absorption spectra of photochemical reaction centres from a carotenoidless strain of *R. rubrum*, G9, and from a carotenoid-containing strain, S1. (*a*) – – –, Strain G9; ——, strain S1. (*b*) The difference spectrum of the absorption of reaction centres from strain S1 minus the absorption of an equal concentration of reaction centres from strain G9.

THE FUNCTION OF THE REACTION CENTRE CAROTENOID

Obviously since the reaction centre carotenoid is diluted out by the antenna carotenoids by a factor of 30 or 40, statistically its rôle in absorbing incident radiation and transferring it to the reaction centre bacteriochlorophylls must be minimal. However, it is possible to show that singlet–singlet energy transfer from the carotenoid to the bacteriochlorophyll can occur. Figure 8 illustrates that light absorbed by the spheroidene in reaction centres from *Rps. sphaeroides* strain 2.4.1 will excite fluorescence from the reaction centre bacteriochlorophyll (Cogdell *et al.* 1976). Boucher *et al.* (1977), in their reconstitution studies with *R. rubrum* reaction centres, measured the efficiency of energy transfer from the carotenoid to the reaction centre bacteriochlorophyll for four different carotenoids (90 % for spheroidene, 35 % for spheroidenone, 30 % for chloroxanthin and 20 % for spirilloxathin). Since all the carotenoids seemed to be bound to the same site on the reaction centre these authors suggested that the energy transfer efficiency is largely a function of the carotenoid type.

Q-switched laser flash activation of reaction centres in which the primary electron acceptor has been chemically reduced induces the transient formation of reaction centre triplet states. In reaction centres from the carotenoidless mutant of *Rps. sphaeroides* R26 a reaction centre bacteriochlorophyll triplet state is formed and decays at room temperature with a half-time of *ca.* 6 μs (figure 9*a*). In carotenoid-containing reaction centres this bacteriochlorophyll triplet state is quenched by the formation of a carotenoid triplet state (figure 9*b*) (Cogdell *et al.* 1975). The reaction centre carotenoid triplet state is formed in 20–30 ns by triplet–triplet

energy transfer from the reaction centre bacteriochlorophyll triplet (Parson & Monger 1977) and decays at room temperature with a half-time of 2–6 μs. Boucher *et al.* (1977) investigated the effect of the reaction centre carotenoid on the photodynamic reaction with reaction centres from *R. rubrum*. When the carotenoidless reaction centres were subjected to strong illumination in the presence of oxygen they were progressively irreversibly bleached (figure 10).
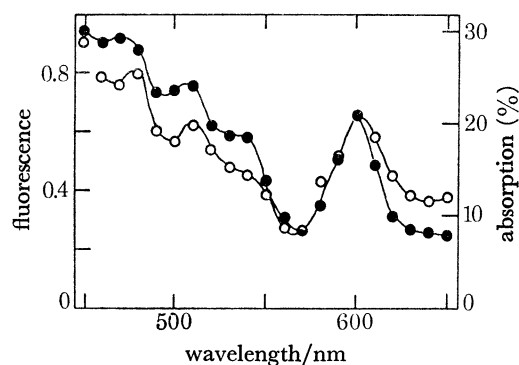

FIGURE 8. The absorption and fluorescence excitation spectra of photochemical reaction centres from *Rps. sphaeroides* 2.4.1. ●, Absorption; o, fluorescence.
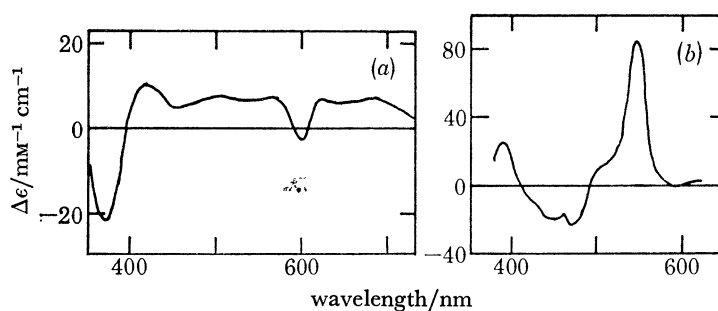

FIGURE 9. The laser flash induced difference spectra of the metastable triplet states in photochemical reaction centres from two strains of *Rps. sphaeroides*: (*a*) strain R26 (carotenoidless); (*b*) strain 2.4.1 (carotenoid-containing). The reaction centres were chemically reduced and excited by a Q-switched laser pulse; (*a*) at 834 nm and (*b*) at 694 nm.
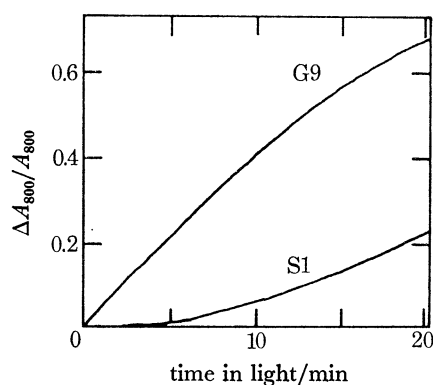

FIGURE 10. The differential photodestruction of P800 in the photoreaction centre from two strains of *R. rubrum*. The time course of the photodestruction of reaction centres from strain G9 (carotenoidless) and S1 (carotenoid containing) under bright white light provided by the IR2, mode of illumination of the Cary 14R spectrophotometer.

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By the use of suitable chemical reporter reactions (for example the bleaching of 1,3-diphenyl-isobenzofuran) they showed that the photodynamic reactions involved both singlet oxygen and superoxide. They suggested that the harmful singlet oxygen was generated by triplet–triplet energy transfer from the reaction centre bacteriochlorophyll triplet. The presence of carotenoid in the reaction centres protected them from photodestruction. If this photodynamic reaction was sensitized by the reaction centre bacteriochlorophyll triplet, then the quenching of the lifetime of that bacteriochlorophyll triplet by the carotenoid by two or three orders of magnitude would easily explain the protective effect (figure 10).

Boucher *et al.* (1977) also showed that the photodynamic reaction continued under conditions where sensitization by the reaction centre bacteriochlorophyll triplet state was impossible. Carotenoids were also protective in this reaction and so they considered two other possible protective reactions of the carotenoid: (*a*) direct quenching of singlet oxygen by the carotenoid promoting the carotenoid to its triplet state; (*b*) carotenoid oxidation (Mathis & Verméglio 1972; Krinsky 1968). Carotenoid oxidation has not been demonstrated in photosynthetic bacteria (apart from the conversion of spheroidene to spheroidenone in cells of *Rps. sphaeroides* or *Rps. capsulata* by molecular oxygen in a *light-independent reaction*) but the cyclic oxidation and reduction of carotenoids (the 'epoxide cycle') has been shown to be part of the mechanism whereby carotenoids protect plants from the 'photodynamic reaction' (Krinsky 1968).

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