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Isolation and characterisation of the different B800-850 light-harvesting complexes from low- and high-light grown cells of *Rhodopseudomonas palustris*, strain 2.1.6

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Two spectrally different types of B800-850 complex have been isolated from high- and low-light-grown cells of *Rps. palustris*. These complexes have distinct pigment and polypeptide composition. The high-light complex has a bacteriochlorophyll : carotenoid ratio of 2 : 1, while the low-light complex has a bacteriochlorophyll : carotenoid ratio of 3 : 1. The efficiency of carotenoid to bacteriochlorophyll singlet-singlet energy transfer in both types of complex is similar.

Introduction

The light-harvesting complexes from purple photosynthetic bacteria represent the simplest intramembrane light-harvesting system [1]. Combined with relatively straight forward procedures for their isolation and purification, this makes them ideal as an experimental system in which to investigate the structural and functional principles of light-harvesting.

A range of antenna complexes have now been isolated and characterised from several different species of purple bacteria [2]. They all appear to be constructed on a common structural principle. Typically they are aggregates of two small, hydrophobic apoproteins (the α and β apoproteins [3]). These apoproteins have a central membrane spanning, α-helical region [4,5] with polar N- and C-termini exposed at either membrane surface. The pigments, bacteriochlorophyll and carotenoids, are non-covalently bound to these apoproteins. In the case of the B800-850 complex from *Rhodopseudomonas acidophila*, for example, the intact, native structure is an $\alpha_6\beta_6$ oligomer [6]. However, the exact number of pigments present, their type and the resulting position of the NIR bacteriochlorophyll absorption bands depends very much upon which particular antenna complex is being studied.

All species of purple bacteria so far studied [7] have a 'core' complex which consists of a reaction centre surrounded by a well-defined, stoichiometric ring of LH1 complexes. Most species then also contain additional, more peripherally arranged antenna complexes (LH2) [2,8], whose amount with respect to the reaction centre content can be quite variable. In well studied cases such as *Rhodobacter sphaeroides* or *Rhodobacter capsulatus* a single type of LH2 complex is present. In others, such as *Rhodopseudomonas palustris* [9], the type of LH2 complex synthesised can vary depending upon the growth conditions. These extra LH2 antenna-types have received very little attention. In this present report we describe the isolation and initial characterisation of the two types of LH2 complex from *Rps. palustris*. More detailed structural and spectroscopic studies will be presented subsequently.

Methods

Cells of *Rps. palustris* strain 2.1.6 were grown photoheterotrophically with succinate as the carbon source at either 10 $W \cdot m^{-2}$ (high light) or at 0.2 $W \cdot m^{-2}$ (low light) at 30 °C. The cells were harvested during late log phase. This was after about 20 h growth in the high-light case and after about 120 h growth in the low-light case. Growth curves were determined by monitoring the absorbance of the cultures at 650 nm.

The harvested cells were either used immediately or stored at −20 °C until required. The cells were broken by a single passage through a French pressure cell at 154 MPa in the presence of a little DNAase and $MgCl_2$. The broken membranes were then sedimented by centrifugation at 12000 × *g* for 15 min, followed by a

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further centrifugation of the supernatant at 180 000 × *g* for 1 h. *Rps. palustris* has intracytoplasmic lamellae rather than chromatophores and so photosynthetic membranes are present in the pellets from both spins. Both pellets were combined and resuspended in 20 mM Tris-HCl (pH 8.0) and their concentration adjusted to give an absorption maximum of 50 cm^{-1} at the most intense NIR bacteriochlorophyll absorption band. The antenna complexes were isolated from the membranes by a modification of the method of Firsow and Drews [10]. The membranes were solubilised by the addition of 1% (v/v) lauryldimethylamine *N*-oxide (LDAO). After stirring in the dark, at room temperature, for 20 min any unsolubilised material was removed by centrifugation at 12 000 × *g* for 15 min. Any cell wall material that contaminates the membranes in the low-speed pellet are removed in this step. The supernatant was diluted with 20 mM Tris-HCl (pH 8.0) to reduce the LDAO concentration to 0.2% (v/v), and then layered onto a sucrose step gradient. The gradient consisted of 6 ml steps of 0.6, 0.4, 0.3 and 0.2 M sucrose prepared in 20 mM Tris-HCl (pH 8.0), containing 0.2% (v/v) LDAO. The gradients were then centrifuged at 180 000 × *g* for 16 h at 4°C. Two pigmented bands were obtained [7,10]. The upper band contained the LH2 complexes and the lower band the RC-antenna 'core' complexes [7,10]. The two bands were collected from the sucrose gradient, concentrated by anion-exchange chromatography on DE-52 cellulose and further purified by a second sucrose gradient centrifugation.

Total pigment extracts of the concentrated antenna preparations were prepared by extraction with 7 : 2 (v/v) acetone/methanol. The samples were re-extracted until the supernatant was completely colourless. Care was taken to keep the extracts, as far as possible, in the dark. The bacteriochlorophyll *a* : carotenoid ratios were determined directly from the absorption spectra of the pooled extracts. The concentration of the bacteriochlorophyll *a* was determined from the absorbance at 772 nm, using a millimolar extinction coefficient of 76 cm^{-1} [11], since carotenoids have no absorbance at this wavelength. The composition of the carotenoids present in the extracts and their concentration were determined as described previously [12]. The identity of the carotenoids was confirmed by mass spectrometry.

The efficiency of the singlet-singlet energy transfer from the carotenoid to the bacteriochlorophyll *a* in the antenna complexes was determined in a home-built fluorescence excitation spectrophotometer as previously described [13].

The polypeptide composition of the antenna complexes was determined by SDS polyacrylamide gradient gel electrophoresis [14]. Gradients of 6–25% acrylamide were used. The antenna apoproteins were extracted from freeze-dried whole cells with 1 : 1 (v/v) chloroform/methanol in the presence of 100 mM ammonium acetate and 10 mM dithiothreitol. The extract was filtered to remove any insoluble material and antenna apoprotein fractions were separated by molecular sieve chromatography on Sephadex LH 60 in the same solvent but without the dithiothreitol. The volume of this fraction was reduced by rotary evaporation, to just before the point where the apoproteins started to precipitate. The extract was then resolved into its constituent apoprotein components by FPLC on a C_8 pro RPC 15 μm reversed-phase column (Pharmacia). The column was developed in a linear gradient of 30–100% (v/v) acetonitrile in water containing 0.1% (v/v) trifluoroacetic acid (TFA).

Results

Fig. 1 shows typical electron micrographs of cells of *Rps. palustris* grown at high and low light intensities. In the low light grown cells the number of intracytoplasmic lamellae is markedly increased. This reflects the increase in the photosynthetic apparatus that occurs in purple bacteria as a typical response to lowering the light-intensity at which the cells are grown [8,15].

The NIR absorption spectra of high and low light grown cells are shown in Fig. 2. The high-light-grown cells have an NIR absorption spectrum rather similar to that seen in *Rb. sphaeroides* or *Rb. capsulatus*, with strong absorption maxima at about 800 nm and about 850 nm. The 850 band is the most intense and is rather asymmetric at its red end, indicating the presence of the B875 (LH1) complex. The shape of the NIR absorption spectrum is markedly different in the low-light-grown cells. Now the 800 nm absorption band is clearly the most intense, while the 850 nm absorption band is much weaker and broader.

Membranes from high- and low-light-grown cells were fractionated into their constituent antenna complexes by solubilisation with LDAO and separation by sucrose gradient centrifugation. Fig. 3 shows the absorption spectra of the different bands obtained from the sucrose gradient centrifugation. Fig. 3A is the spectrum of the bottom band. Since the spectra of the bottom band was identical for both high- and low-light-grown cells, only one spectrum is shown. This spectrum shows the presence of the B875 complex (LH1) and reaction centres (the small peaks at 800 nm and 760 nm). We have previously shown that this complex has identical composition in both these cases [7]. The absorption spectra of the top bands from the gradient are shown in Fig. 3B and C. Quite clearly the type of LH2 antenna complex which is synthesised is controlled by the light intensity [9]. At high light a standard B800-850 complex of the *Rb. sphaeroides* type is synthesised. Both the 800 and 850 nm absorption bands are prominent, with the 850 one the most intense. While at low light the 800 nm

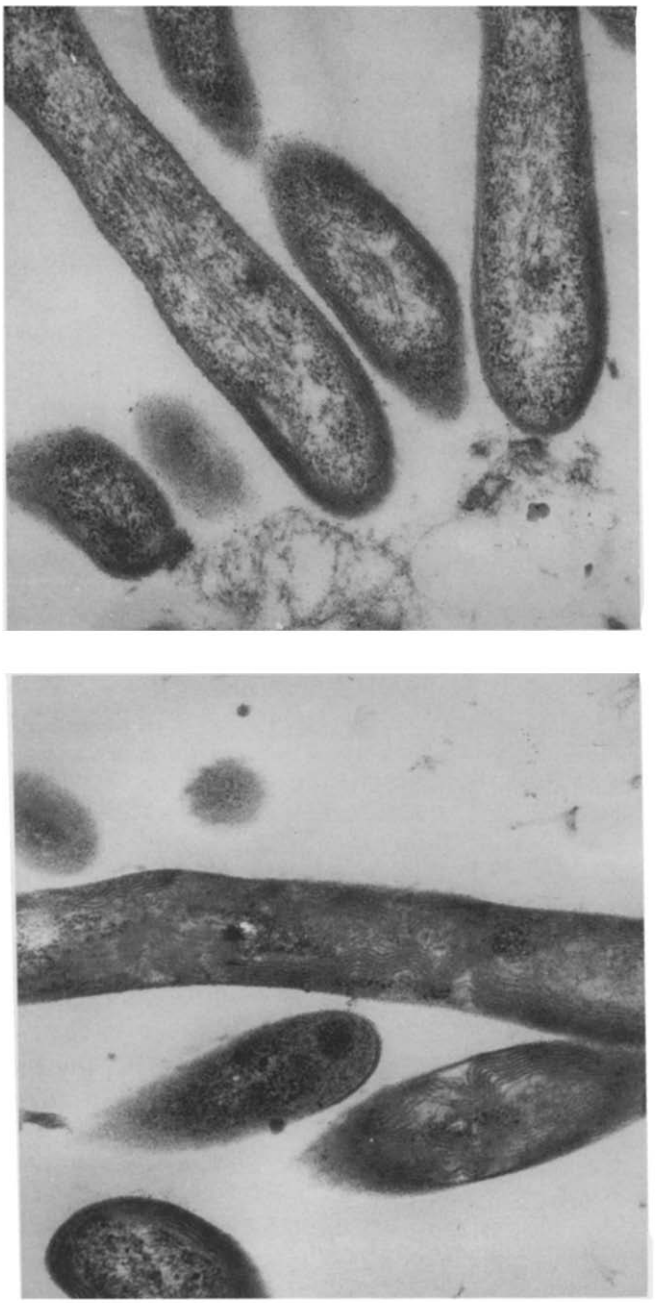

Fig. 1. Electron micrographs of thin sections through cells of *Rps. palustris* grown at high (top) and low (bottom) light-intensities. The magnification is 15000.

band is the most intense and the 850 nm one is small and rather broad.

The polypeptide composition of the two types of LH2 complex was investigated by SDS polyacrylamide gel electrophoresis. Both complexes have low-molecular-weight polypeptides in the 5–7 kDa region. The polypeptide patterns are similar in each case (Fig. 4); however, they are complicated. Most purple bacterial antenna apoproteins are soluble in organic solvents such as 1 : 1 (v/v) chloroform/methanol in the presence of 100 mM ammonium acetate [17]. Following an initial purification of the organic extract by molecular sieve chromatography on Sephadex LH60 the antenna apoproteins can be separated by FPLC on reversed-phase columns [15]. Fig. 5 shows the antenna polypeptide pattern from high- and low-light-grown cells. The two bands on the right of each part of Fig. 5 are the α- and β-apoproteins of the B875 complex. The group of bands to the left of each part of this figure are the B800-850 apoproteins. There is a clear difference in composition in the LH2 region between the high- and low-light membranes. We have used the greater resolution of the FPLC system to isolate the individual apoproteins for sequencing (these results will be presented in a subsequent paper (Brunisholz et al., unpublished results)). However, it is useful to note here that the high-light complex contains two types of α- and β-apoprotein, while the low-light complex contains four types of each apoprotein.

The carotenoid composition of the HL and LL B800-850 complexes was determined in order to be able to calculate average extinction coefficients to use in the bacteriochlorophyll *a* : carotenoid ratio determinations. A typical set of results are presented in Table I.

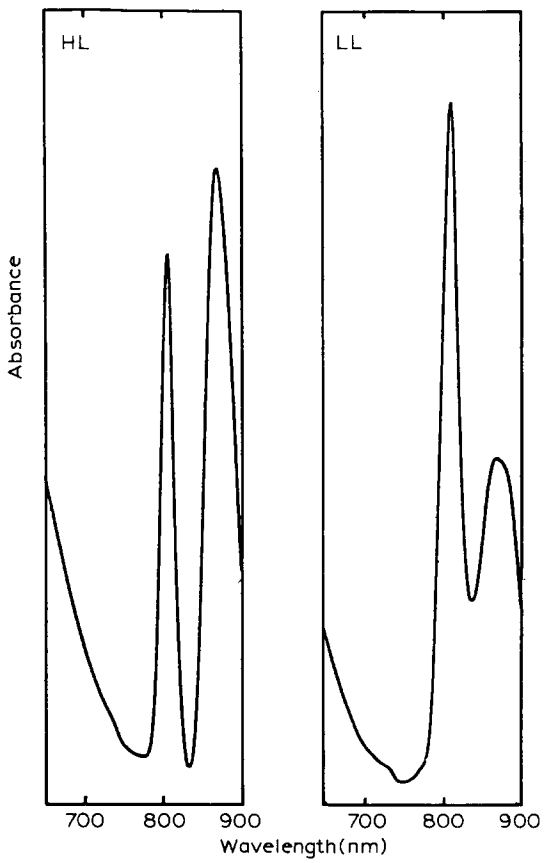

Fig. 2. The NIR absorption spectra of whole cells of *Rps. palustris* grown at high and low light-intensities. These spectra were recorded in a Pye-Unicam SP8-500 spectrophotometer. The concentration of the cells was adjusted so that their absorbance in the NIR was similar.

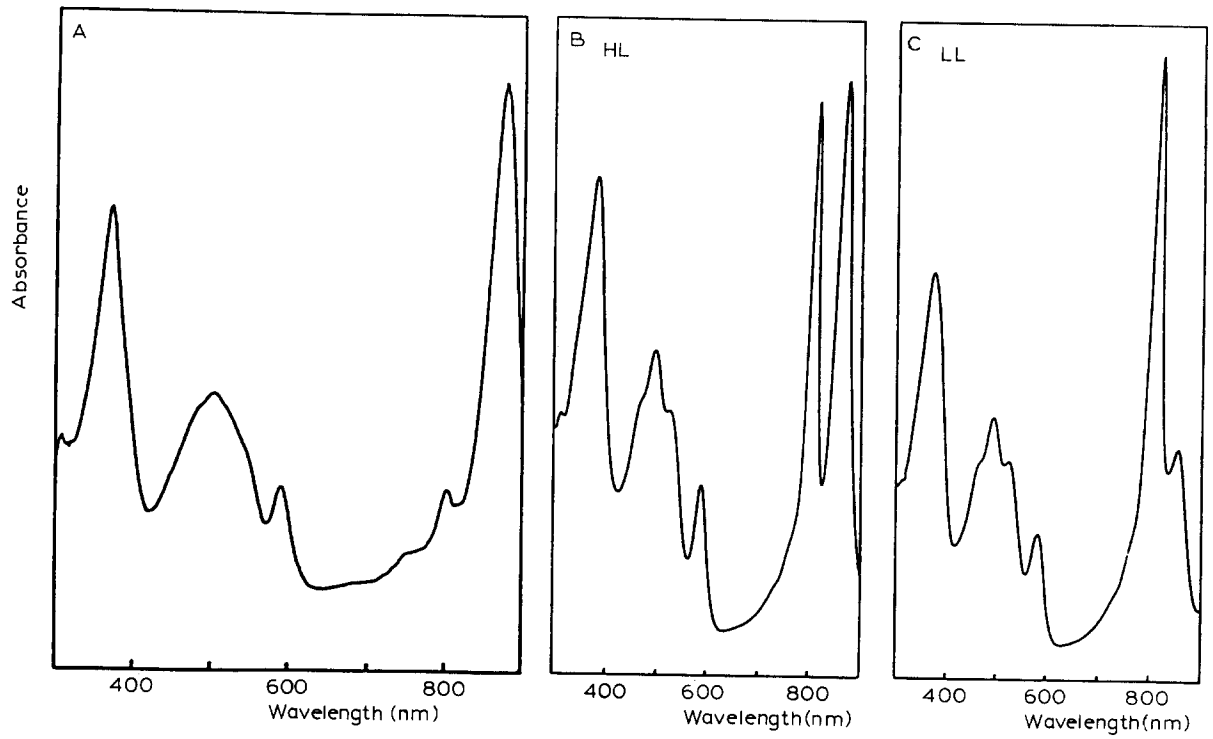

Fig. 3. The absorption spectra of the bands removed from the sucrose-gradient fractionation of the detergent-solubilised membranes from high- and low-light-grown cells. (A) The bottom band from the high light grown cells. This is the RC-B875 complex. The spectrum of this fraction was the same from low light grown cells. (B) The top band from the high-light grown cells. (C) The top band from the low-light grown cells.

Average extinction coefficients were determined to be 142 ± 5 $mM^{-1} \cdot cm^{-1}$ at 475 nm for the HL complex and 151 ± 5 $mM^{-1} \cdot cm^{-1}$ and 470 nm for the low light complex. The wavelength shift in the two extracts is due to the change in the relative amount of lycopene with respect to the amount of rhodovibrin. The data presented in Table I compare well with previous analyses of the carotenoid composition of whole cells of *Rps. palustris* [18].

The bacteriochlorophyll *a*: carotenoid ratios of the two B800-850 complexes were measured for a number of preparations of each of the complexes isolated from several different batches of cells. The average ratio for the HL B800-850 complex was 1.98 ± 0.14: 1 and was the result of ten different determinations on at least three different preparations of the antenna complex. This agrees rather well with the ratio of 2: 1 measured for the analogous B800-850 complex from *Rb. sphaeroides* [12]. The ratio for the LL B800-850 complex was $3.05 \pm 0.$ 1: 1 and was the result of 25 different determinations on at least four different preparations of the antenna complex. It remains to be determined whether this complex contains less carotenoid or extra bacteriochlorophyll.

TABLE I

The carotenoid composition (%) of the B800-850-complex from high-light and low-light-grown cells

The identities of the carotenoids were confirmed by mass spectrometry (data not shown).

Carotenoid type	Low-light complex	High-light complex
Lycopene	25	10
Anhydrorhodovibrin	8	6
Spirilloxanthin	55	57
Rhodopin	6	6
Rhodovibrin	6	20

The efficiency of the singlet-singlet energy transfer from the carotenoid to the bacteriochlorophyll in the complexes was determined by comparing the action spectrum for bacteriochlorophyll fluorescence with the fractional absorption spectrum. A typical example is shown for the LL B800-850 complex in Fig. 6. The results are presented in Table II. The two B800-850 complexes show similar average energy transfer efficiencies of about 38%, while the B875 complex has a rather lower efficiency of about 33%. Interestingly, the energy transfer efficiency is not the same for each of the different vibrational bands in the carotenoid's absorption spectrum (Fig. 6).

Discussion

The results presented here confirm and extend the work of Hayashi et al. [9]. Low-light-grown cells of *Rps. palustris* do synthesise a completely different type of B800-850 complex. The low-light complex and the more

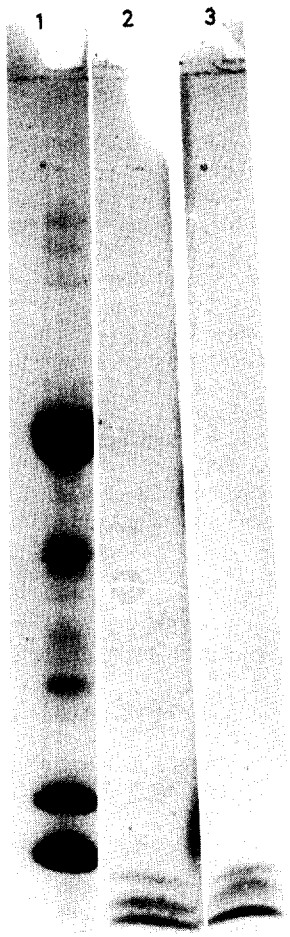

Fig. 4. A comparison of the polypeptide composition of the LH2 complexes from high and low light grown cells. Lane 1, Molecular weight standards bovine serum albumin (68000), alcohol dehydrogenase (41000), myoglobin (17000), and cytochrome *c* (12000). Lane 2, B800-850 complex from low-light-grown cells. Lane 3, B800-850 complex from high-light-grown cells.

usual high-light B800-850 complex have distinct pigment and polypeptide compositions.

The polypeptide compositions of the two complexes are unusual. Recently Tadros [19] has isolated, cloned and sequenced the structural genes for the LH2 apoproteins in *Rps. palustris*. They found four distinct

TABLE II

The efficiencies of the carotenoid to bacteriochlorophyll singlet energy transfer in the isolated antenna complexes from Rps. palustris

0-0 is the redmost vibrational band of the carotenoids, 0-1 the middle and 0-2 the bluemost one. The data shown are ± S.E.

Type of complex	% Energy transfer efficiency			
	vibrational band excited			average
	0–2	0–1	0–0	
B875	29 ± 3	33 ± 3	37 ± 3	33
Low-light B800-850	31 ± 5	36 ± 4	45 ± 2	39
High-light B800-850	31 ± 3	36 ± 3	43 ± 3	37

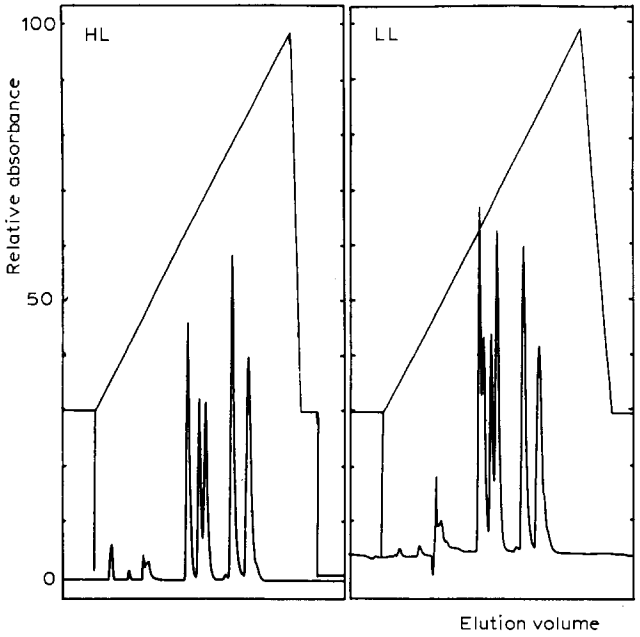

Fig. 5. FPLC chromatograms of the antenna apoprotein extracts from high- and low-light-grown cells. The organic solvent soluble fraction from the Sephadex LH60 column was applied to the FPLC reverse phase column (15 μm, C_8 pro RPC) and developed in a gradient of 30% acetonitrile, 0.1% trifluoroacetic acid, 70% water, to 100% acetonitrile, 0.1% trifluoroacetic acid, over a 30 min. period. The absorbance was monitored at 280 nm and the upper continuous solid line shows the development of the gradient.

$\alpha\beta$ structural genes and were able to isolate and purify some of the apoproteins by HPLC. We have confirmed this multiplicity of apoprotein types by sequencing the antenna apoproteins directly (Brunisholz et al., unpublished results). It appears that in *Rps. palustris* the LH2 antenna complexes are formed by aggregation of more than single types of α- and β-apoproteins.

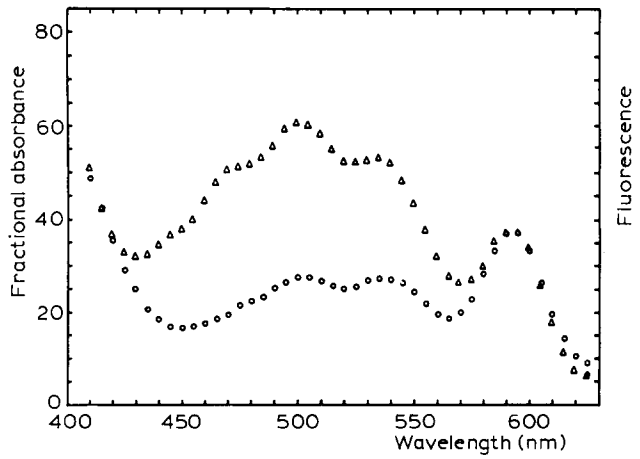

Fig. 6. A comparison of the fractional absorption spectrum with the fluorescence excitation spectrum of the low-light B800-850 complex. △, fractional absorption spectrum; ○, normalised fluorescence excitation spectrum (arbitrary units). The two spectra were normalised at 590 nm in the bacteriochlorophyll absorption band.

The fluorescence excitation spectra of the two B800-850 complexes are interesting because the efficiency of the singlet-singlet energy transfer from the carotenoid to the bacteriochlorophyll is different for each of the absorption bands of the carotenoid. Recently, Gillbro and Cogdell [20] have provided convincing direct experimental evidence that this energy transfer occurs from the carotenoids' $^1Ag^*$ state. Differences in the efficiency of energy transfer depending upon where in the carotenoid's absorption spectrum the excitation occurs is consistent with the involvement of the $^1Ag^*$ state [21]. A full spectral characterisation of the low light B800-850 complex will be presented in a subsequent paper (Van Mourik et al., unpublished results).

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