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Energy migration in *Rhodobacter sphaeroides* mutants altered by mutagenesis of the peripheral LH2 complex or by removal of the core LH1 complex

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Abstract

The photosynthetic apparatus of the purple bacterium *Rhodobacter sphaeroides* is organised so that light energy absorbed by the peripheral antenna (LH2) complexes migrates towards the core (LH1) complex, before being trapped by the reaction centre (RC). This migration and trapping process has been studied in mutants where the energy levels of the LH2 BChls have been raised by mutagenesis of the C-terminal aromatic residues (Fowler, G.J.S., Visschers, R.W., Grief, G.G., Van Grondelle, R. and Hunter, C.N. (1992) Nature 355, 848–850), and in a mutant which lacks the core complex. In the former case, the alterations to the LH2 complexes did not prevent efficient energy transfer to the LH1-RC complex, but fluorescence emission spectra indicated that the equilibrium of energy within the system was affected so that back transfer from the LH1-RC core is minimised. This mimics the situation found in some other bacteria such as *Rhodopseudomonas acidophila* and *Rps. cryptolactis*. In the mutant lacking LH1, energy is transferred from LH2 directly to the RC, despite the absence of the core antenna. Energy transfer efficiencies for carotenoids and LH2 to LH1 were measured for the blue-shifted LH2 mutants, and were found to be high (70%) in each case. These data, together with measurements of excitation annihilation as a function of incident excitation energy, were used to estimate the domain sizes for energy transfer in these mutants. In the LH2 mutants, domains of about 50 to 170 core BChls were found, depending on the type of mutation. One effect of the removal of LH1 appears to be the reorganisation of the peripheral LH2 antenna to form domains of at least 250 BChls.

Keywords: Light harvesting; Energy transfer; Fluorescence; Domain size; Reaction center; (*Rb. sphaeroides*)

1. Introduction

The photosynthetic apparatus of *Rhodobacter sphaeroides* consists of an interconnecting series of light harvesting (LH) and reaction centre (RC) complexes [1]. These are organised so that energy migrates from the periphery, where the LH2 complexes are thought to lie, to the core where RCs are surrounded by LH1 complexes [2]. Both LH1 and LH2 complexes consist of α and β subunits, each of which is a small intrinsic membrane protein which spans the membrane once, and which binds at least one molecule of bacteriochlorophyll (BChl). Recent X-ray crystallographic data show that the LH2 complex is formed by the association of 9 α subunits in an inner ring, then 18 BChls and 9 β subunits round the perimeter; there are 9 more BChls located towards the cytoplasmic face of the complex [3]. The migration of excitations towards the RC is facilitated by fine-tuning the absorbance properties of the LH complexes; thus, the BChls in the LH2 complex absorb near 800 and 850 nm, whereas the BChls of the LH1 complex lie at a lower energy, absorbing near 875 nm. The precise way in which LH complexes achieve this variation in absorbance properties is unclear, but at least one effect is exerted by the LH protein, through variations

Abbreviations: BChl, bacteriochlorophyll; B800-820, B800-840, B800-850, antenna complexes with Q_y-band absorption maxima near 800 nm and 820, 838 or 850 nm, respectively; B875, antenna complex with Q_y-band absorption maximum near 875 nm; LH1, core antenna (B875); LH2, peripheral antenna; RC, reaction centre.

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in the hydrogen-bonding of the 2-acetyl carbonyls of the BChls. This was demonstrated by Fowler et al. [4,5], who constructed site-directed mutants at positions 44 and 45 on the α subunit, which are normally both Tyr residues in *Rb. sphaeroides*. The mutants YY → FY and YY → FL absorb at 800–838 and 800–826 nm, respectively, and these blue shifts were subsequently correlated with altered hydrogen-bonding patterns, using resonance Raman spectroscopy [5,6]. The energy transfer properties of these mutants have been studied extensively with respect to the internal dynamics of transfer between BChl 800 and the other pigments [7,8], but less attention has been given to the interaction of these blue-shifted LH2 complexes with the rest of the antenna system. The mutagenesis system used for the work described above is well suited to such a comparison, since the expression of the *puc*BA genes encoding the β and α subunits of the LH2 complex [9,10] is directed by a plasmid which can be introduced into either a 'null' $LH2^-LH1^-RC^-$ background (strain DD13, [11]) or a background which contains LH1 and RC complexes (strain DBC1, [12]). Thus, alterations in LH2 complexes can be examined free from interference from other complexes, or in a strain where the interactions between LH2 and the rest of the system can be studied.

Another way in which the photosynthetic unit can be altered is by genetically removing the LH1 antenna, creating an LH2 + RC mutant (strain DPF2(pRKEH2), [11]). This mutant grows photosynthetically, albeit at a reduced rate, in comparison with the wild-type (8 and 4 h doubling times, respectively) but significantly faster than mutant RCO1 which contains only RCs (12 h). This physiological experiment, together with a subsequent time-resolved study which demonstrated that the overall trapping time in DPF2(pRKEH2) is 55 ± 5 ps compared to 60 ± 5 ps for the wild-type [13], suggest that LH2 can interact directly with the RC in a productive way. However there has been no research on this mutant directed at estimating the size of the energy transfer domain connected to the RC.

The work reported here uses the measurement of fluorescence yield induced by excitation at different intensities. High intensity pulses create more than one excitation in a single domain, and their coincident arrival at an individual BChl molecule results in the annihilation of one of the excitations, and a decrease in the relative fluorescence yield. Quantitative treatment of this kind of experiment relies on earlier work by Paillotin et al. [14] and Den Hollander et al. [15], and has provided useful insights into the functional size of purified LH complexes, as well as the sizes of domains for energy transfer in wild-type and LH2-only and LH1-only strains of *Rb. sphaeroides* [16–18]. The results presented here show that the modifications at positions 44 and 45 on the LH2 α subunit, producing spectrally altered LH2 complexes, affect the energy migration within the photosynthetic system. Energy transfer from the peripheral antenna complexes to the core-RC complex still occurs with high efficiency, but back transfer from the core to LH2 is impeded. In this respect these mutants provide an interesting comparison with two species which are naturally capable of synthesising either B800-850 or B800-820 LH2 complexes, *Rhodopseudomonas acidophila* [19] and *Rps. cryptolactis* [20], where it was noticed that the presence of a B800-820 complex strongly reduced back transfer to the peripheral antenna. The connection between these experiments and the present study is reinforced by the similarity between the amino acid sequence of the α subunit of the mutant B800-820 antenna of *Rb. sphaeroides* and that of the LH2 α subunit of *Rps. acidophila* strain 7050 [4]. However, the arrangement of the antenna complexes in the mutant YY → FL, as reflected by the presence of larger domains for energy transfer between the core BChls appears to be different from that in *Rps. acidophila* and *Rps. cryptolactis*.

2. Materials and methods

The mutants of *Rb. sphaeroides* used were DBC1(WTLH2), DBC1(LH2YY → FY), DBC1(LH2YY → FL) and DPF2(pRKEH2). The nomenclature YY refers to the pair of tyrosines at positions 44 and 45 in the α subunit of LH2. According to the nomenclature for the primary sequences of LH complex subunits proposed recently [6], these residues are at positions +13 and +14 towards the C-terminus, in relation to the histidine ligand for the BChls 850 at position 0. The construction of strain DBC1 and its complemented derivatives is described elsewhere [4,21]. In short, the *puc*BA genes, encoding the LH2 subunits [9,10], were deleted from the chromosome and replaced by a kanamycin resistance cassette resulting in a $LH2^-LH1^+RC^+$ recipient strain (DBC1). Site-directed mutagenesis was used to change Tyr_{+13} to Phe and Tyr_{+14} to Leu in the α subunit of LH2 [4]. Subsequently, either the wild-type or the altered genes were expressed on a plasmid in the $LH2^-$ recipient strain DBC1, resulting in three mutants, each with the native LH1-RC core complex, but with either the wild-type LH2 complex (DBC1-(WTLH2)), or LH2 complexes carrying single (DBC1(LH2YY → FY)) or double (DBC1(LH2YY → FL)) mutations [4].

Strain DPF2(pRKEH2), which has wild-type LH2 and RC complexes but lacks the core LH1 complex, was constructed as described in detail previously [11,13]. Briefly, the deletion strain DPF2 [11,21], in which the *puf*BALMX operon is replaced by a kanamycin resistance cassette and which lacks the RC and LH1 complexes, was complemented by a plasmid (pRKEH2) bearing the *puf*-LMX genes but lacking the *puf*BA genes that encode the LH1 complex.

Membranes for all mutants were prepared from cells grown semi-aerobically in the dark in $M22^+$ medium [22]. Following disruption in a French press, membranes were purified on a 15%–40% (w/w) discontinuous sucrose

gradient and, for spectroscopy, were suspended in a buffer containing 10 mM Tris (pH 8.0). For the fluorescence measurements glucose, glucose oxidase and catalase were added as oxygen scavengers. The sample was purged with N_2 before each measurement. Fluorescence emission spectra were measured at room temperature using the spectrofluorimeter described elsewhere [23]. The sample was excited either by a frequency-doubled Nd:YAG laser with a maximum pulse energy of about 5 mJ/cm^2 at 532 nm and a pulse width of approx. 25 ps, or by a low intensity xenon flash (pulse width 10 μs) with filters providing a bandwidth of 40 nm, centered at 520 nm. Continuous background illumination, filtered by a Schott BG-18 filter, was sometimes used in order to keep the primary electron donor (P) in the oxidised state (P^+). Detection of the time integrated fluorescence yield was achieved by means of a monochromator with a resolution set at 2 or 4 nm for fluorescence emission and annihilation measurements, respectively. The time-integrated fluorescence yield as well as the light intensity of the exciting laser flash were measured by means of an RCA-30810 photodiode. The apparatus used for measuring absorption and fluorescence excitation spectra was described elsewhere [24].

Absorption difference measurements were performed with the apparatus described in Ref. [25]. A Q-switched frequency-doubled Nd:YAG laser, providing saturating flashes at 532 nm with a pulse duration of 15 ns (fwhm), was used for actinic illumination. A tunable Optical Parametric Oscillator (Surelite I, continuum) was used for measuring the excitation spectrum.

3. Results and interpretation

3.1. Absorption spectra

The room temperature absorption spectra of membranes prepared from the four strains are shown in Fig. 1. For DBC1(WTLH2), containing wild-type LH2 genes, the presence of the B800-850 complex is demonstrated by the absorption bands at 801 nm and 850 nm (Fig. 1A). The B875 complex is manifested as a broadening of the red side of the dominant 850 nm band. This strain can be described as a pseudo-wild-type. In comparison, the absorption spectrum of DBC1(LH2YY → FY) shows a blue shift of the BChl 850 absorption band of approximately 13 nm (Fig. 1B). The shoulder at 875 nm is ascribed to the presence of the LH1 complex. For DBC1(LH2YY → FL), the BChl 850 absorption band has shifted further to 820 nm and the presence of LH1 is demonstrated by a now much more clearly resolved band at 875 nm (Fig. 1C). Similar results were reported for these mutant LH2 complexes expressed in strain DD13, which acts as an $LH2^-LH1^-RC^-$ background, resulting in a mutant that contains the LH2 complex as the sole pigment-protein complex [4]. However, a decrease of the long wavelength

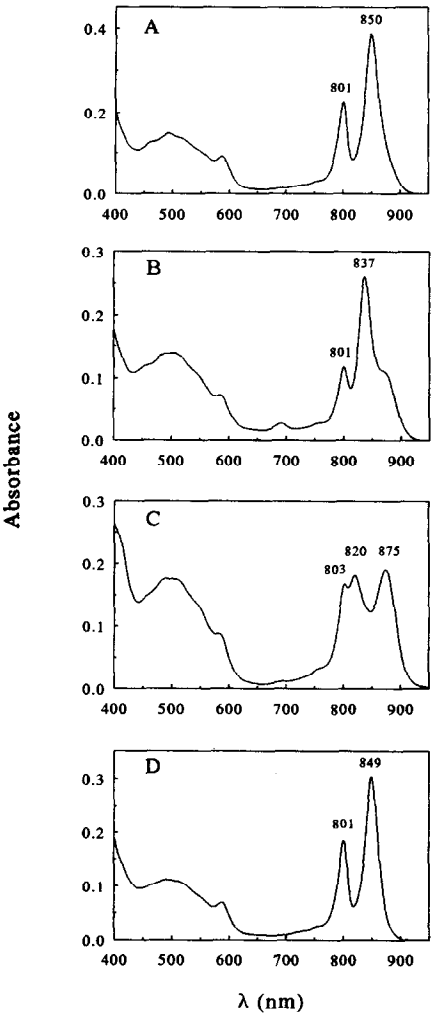

Fig. 1. Absorption spectra of membranes from *Rb. sphaeroides*, strains DBC1(WTLH2) (A), DBC1(LH2YY → FY) (B), DBC1(LH2YY → FL) (C) and DPF2(pRKEH2) (D), measured at room temperature.

band of LH2 (BChl 840) with respect to that of BChl 800, as observed at 77K [4], did not occur at room temperature (Fig. 1). For DBC1(LH2YY → FL) a decrease of the BChl 820 absorption and an increase of the B875 absorption relative to the BChl 800 absorption, as observed at 77K [4], was observed. The bands near 460, 490, 510 and 550 nm are ascribed to carotenoid absorption, in this case to a mixture of spheroidene and spheroidenone.

The absorption spectrum of DPF2(pRKEH2) is shown in Fig. 1D. The absorption bands at 801 and 849 nm are due to the presence of the LH2 antenna. The absence of LH1 is demonstrated by a loss of the broadening on the red side of the BChl 850 absorption band observed in spectra of the pseudo wild type (Fig. 1A).

3.2. Energy transfer from the core antenna to the peripheral antenna

Fig. 2 shows the fluorescence emission spectra of the three DBC1 mutants obtained with a weak xenon flash

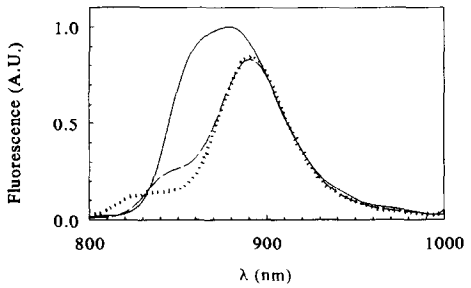

Fig. 2. Fluorescence emission spectra of membranes of DBC1(WTLH2) (solid line), DBC1(LH2YY → FY) (dashed line) and DBC1(LH2YY → FL) (dotted line). The spectra were normalised at 920 nm. The samples were excited with a low-intensity xenon flash and the primary donor was kept in the oxidised state by means of continuous background illumination.

centered at 520 nm, which excites the antenna carotenoids and thus elicits fluorescence from both LH1 and LH2. The RCs were kept in the oxidised state by means of continuous background illumination (see Methods). The spectra were normalised to the fluorescence emitted from the LH1 antenna complex in the region above 900 nm. The fluorescence originating from the peripheral antenna was much more abundant in membranes with the wild-type LH2 complex (solid line) than in the ones with LH2 containing the replaced tyrosine residues. This higher level of LH2 fluorescence in the pseudo wild type was too large to be accounted for by differences in the ratio of LH2 to the RC/LH1 core between the strains, and could indicate that back transfer of energy from the core to the peripheral antenna in the mutated strains is thermodynamically more difficult than in the pseudo wild type. This might very well be the case, since for *Rhodopseudomonas acidophila* [19] and *Rps. cryptolactis* [20], which contain B800-820, it has been observed that back transfer of energy from the core antenna to B800-820 complexes is severely restricted. This was confirmed by the variable fluorescence measurements shown in Fig. 3. Photooxidation of the primary donor caused an increase of the fluorescence by a factor of about 1.7 for the pseudo wild type and 2.3 for the single and double mutants (measured at the fluorescence maximum). In the case of the pseudo-wild-type the increase of the peripheral fluorescence was almost proportional to that of the core, due to back transfer of excitation energy from LH1 (Fig. 3A). In marked contrast, there was hardly any increase in fluorescence arising from LH2 in the 820 nm region of DBC1(LH2YY → FL). This indicates that, as in *Rps. acidophila* [19] and *Rps. cryptolactis* [20], back transfer of energy from the B875 complex to the mutated LH2 complex in DBC1(LH2YY → FL) does not occur.

3.3. Energy transfer from the peripheral antenna to the reaction centre

Fig. 4 shows the absorption difference spectrum (light minus dark) of the LH1-minus strain DPF2(pRKEH2)

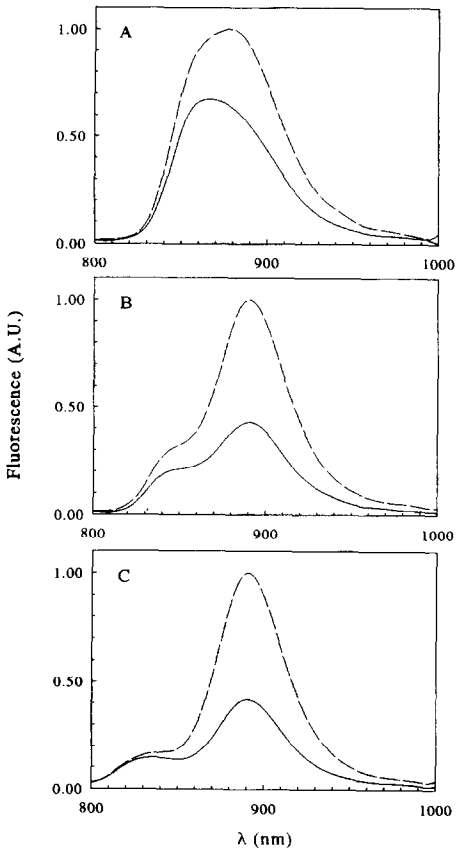

Fig. 3. Fluorescence emission spectra of membranes of DBC1(WTLH2) (A), DBC1(LH2YY → FY) (B) and DBC1(LH2YY → FL) (C), obtained with a low intensity xenon flash, with the primary donor in reduced state (solid lines) and in the oxidised state (dashed lines).

compared to that of chromatophores prepared from the *Rb. sphaeroides* wild type. *ortho*-Phenanthroline (25 mM) was added to the samples in order to inhibit electron transfer between the RC acceptor quinones Q_A and Q_B and to stimulate recombination of the radical pair state $P^+Q_A^-$ [26]. The spectra were obtained with saturating laser flashes and were normalised to the maximal absorbance of the LH2 complexes for both strains. The two difference spec-

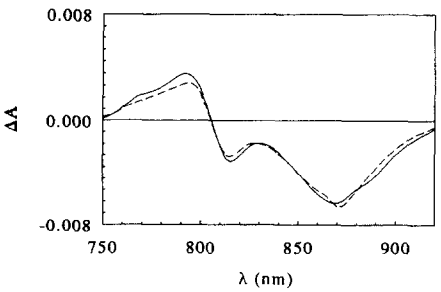

Fig. 4. Absorbance difference spectrum of DPF2(pRKEH2) (solid line) and chromatophores from *Rb. sphaeroides* wild type (dashed line) induced by saturating laser flashes (532 nm) in the presence of *ortho*-phenanthroline (25 mM). The spectra were normalised to the absorbance at 849 nm and 853 nm, respectively.

tra were identical with respect to shape and amplitude, indicating that the number of RCs relative to the number of BChls in LH2 is the same in DPF2(pRKEH2) and *Rb. sphaeroides* wild type.

In order to confirm that excitations are transferred efficiently from the antenna to the RC in the LH1-deficient mutant, as indicated by the time-resolved measurements conducted by Hess et al. [13], we performed an experiment in which we scanned the excitation wavelength over the antenna and RC absorption bands (750–900 nm) with non saturating excitation flashes and detected the resulting absorbance change near the maximal bleaching of the RC primary donor at 880 nm (data not shown). The relative absorbance change per incident photon was calculated. The resulting excitation spectrum resembled the absorption spectrum of the LH2 antenna and showed no significant contribution by the RC bacteriopheophytin band near 760 nm. We therefore conclude that excitations absorbed by the LH2 antenna are indeed transferred to the RCs to bring about charge separation in the absence of LH1. Although we did not quantify the efficiency of this energy transfer, it is clear that photo-oxidation of the primary donor occurs mainly as a result of excitations absorbed by the antenna rather than by direct absorption by the RC constituents.

3.4. A comparison of fluorescence emission spectra at low and high excitation densities

Non-linear processes such as singlet-singlet annihilation play an important role as quenchers of excess excitation energy at high excitation densities [14,15], with the extent of annihilation being strongly dependent on the specific structure of the antenna. Because of the energy gradient present in the bacterial antenna system, rapid excitation energy transfer from the peripheral antenna to the core antenna will result in a higher extent of annihilation in the latter complex. Therefore, the shape of the fluorescence emission spectra obtained at high and low excitation densities reflects the spectral and structural differences between core and peripheral antennae in different strains. In Fig. 5 the fluorescence emission spectra obtained with a high intensity laser pulse are compared to those obtained with a weak xenon flash. For the purpose of comparing the relative levels of fluorescence from LH1 and LH2 the spectra were normalised in the region above 900 nm, where fluorescence arises from LH1 only. Subtraction of the fluorescence spectra yielded bands at 856, 846 and 831 nm for the pseudo wild type, the single mutant DBC1-(LH2YY → FY) and the double mutant DBC1(LH2YY → FL), respectively. These wavelengths correspond to emission from the peripheral LH2 antenna. The fact that the emission from LH1 is lowered relative to that emitted from LH2 at high intensities indicates that, as expected, the extent of annihilation in the core antenna exceeds that in the peripheral antenna in all of the strains.

The spectra for DPF2(pRKEH2) are shown in Fig. 5D.

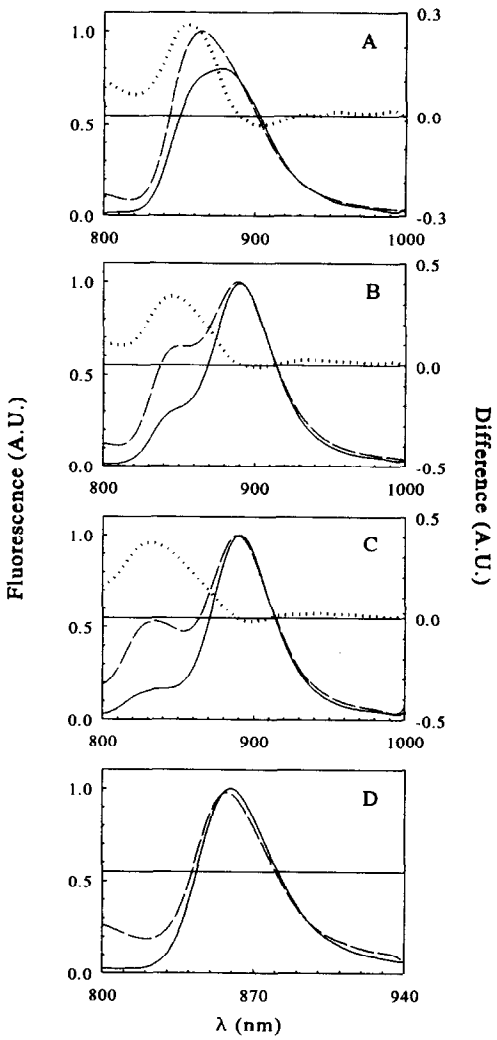

Fig. 5. Fluorescence emission spectra of membranes of DBC1(WTLH2) (A), DBC1(LH2YY → FY) (B), DBC1(LH2YY → FL) (C) and DPF2(pRKEH2) (D), obtained with a low intensity xenon flash (solid lines) and with a high intensity laser flash (dashed lines). The spectra were normalised in the region near 900 nm. The dotted lines represent the difference of the two spectra. The reaction centres were kept in the oxidised state by means of background illumination.

At high excitation energies the emission spectrum was shifted somewhat to shorter wavelengths, indicating that the extent of annihilation is larger among the longer wavelength BChls contributing to the BChl 850 absorbance band. This might be due to a somewhat longer lifetime of the excitations in the red-shifted BChls [27]. Otherwise, the two spectra are similar, as would be expected. The increase of the high intensity emission spectrum at short wavelengths (< 830 nm) might be due to some free BChl *a*, fluorescing at about 780 nm [28], that shows hardly any annihilation.

3.5. Domain sizes

Information about the number of BChls between which energy transfer can occur (domain size) can be obtained by

measuring the fluorescence yield at one specific wavelength as a function of the incident excitation energy. Paillotin et al. [14] derived an expression for the total fluorescence yield as a function of the average number of excitations in one domain for a homogeneous antenna system with the RCs in a fixed state. This expression contains an important parameter r denoting the ratio of the mono-excitation decay rate (the overall rate of decay due to trapping and loss for a single excitation in a domain) and the bi-excitation decay rate (the overall rate of decay due to annihilation for a pair of excitations on a domain). By fitting our experimental data to the curves derived from the model described in Ref. [14], for different values of r, the best value for r and the energy for which there is, on the average, one excitation per domain can be obtained. This provides information about the size of the domain (N_D) and the efficiency of annihilation in relation to the mono-excitation decay processes within that domain.

The efficiency of energy transfer to the core antenna for photons absorbed at 532 nm by the carotenoids of LH2 and LH1, and for photons absorbed by BChl of the peripheral antenna, was measured by comparing fluorescence excitation spectra with absorption ($1 - T$) spectra (Fig. 6). The fluorescence was detected at 910 and 880 nm for the DBC1 mutants and DPF2(pRKEH2), respectively. The energy transfer efficiencies for excitations which are absorbed at 532 nm and then migrate to the BChls of the core antenna were found to be 75% for the pseudo-wild-type DBC1(WTLH2) and DBC1(LH2YY → FL), and 85% for the single mutant DBC1(LH2YY → FY). The efficiency for energy transfer from the peripheral antenna to LH1 was close to 100% for the pseudo-wild-type and the single mutant, and about 85% for the double mutant (estimated at the absorbance maximum of the 'BChl 850' pigments of each of the LH2 complexes). For the LH1-deficient mutant DPF2(pRKEH2), the excitation spectrum coincides with the absorption spectrum over the entire

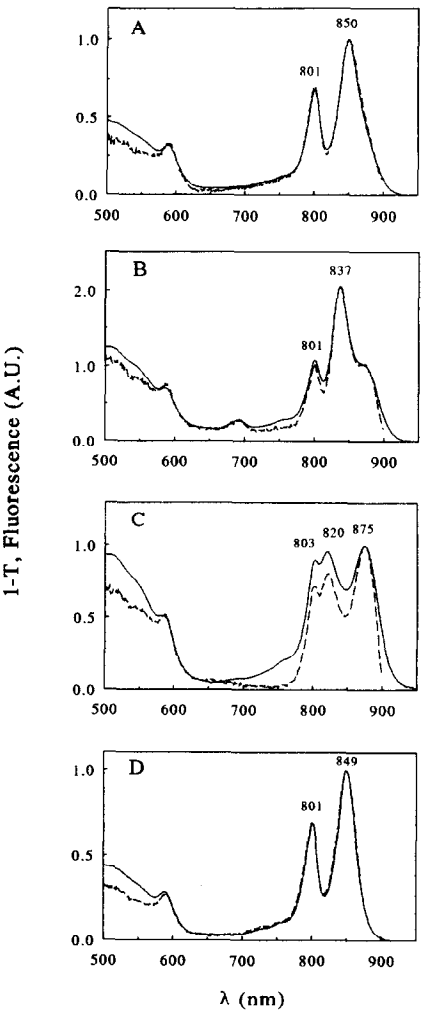

Fig. 6. Fluorescence excitation spectra (dashed lines) and absorption ($1-T$) spectra (solid lines) of membranes of DBC1(WTLH2) (A), DBC1(LH2YY → FY) (B), DBC1(LH2YY → FL) (C) and DPF2(pRKEH2) (D). The fluorescence was recorded at 910 nm and at 880 nm for the DBC1 mutants and DPF2(pRKEH2), respectively.

Table 1
Domain sizes (N_D) in the LH1 antenna

Fraction (%)	DBC1(WTLH2) (Pseudo wild type)		DBC1(LH2YY → FY) (Single mutant)		DBC1(LH2YY → FL) (Double mutant)	
	r	N_D (BChls)	r	N_D (BChls)	r	N_D (BChls)
0	5.0	397	5.0	241	5.0	237
50	5.0	292	5.0	193	5.0	180
55	5.0	279	5.0	188	5.0	176
60	5.0	269	1.6	61	**5.0**	**169**
65	2.0	107	1.3	49	5.0	165
70	1.6	83	**1.3**	**47**	5.0	157
75	1.3	66	1.0	37	5.0	150
80	**1.0**	**50**	1.0	34	5.0	141
85	0.7	36	0.7	25	–	–
90	0.6	29	0.6	21	5.0	119
95	0.25	17	0.5	17	–	–

The data were obtained from analysis of the annihilation curves at 910 nm after correction for annihilation in the peripheral antenna (see text). The correction was calculated for different fractions of excitations reaching B875 via the peripheral antenna at low excitation densities (first column). The bold numbers refer to the curves in Fig. 7

region from 600 to 880 nm, and reveals an efficiency of 70% for energy transfer from the carotenoid absorbing at 532 nm. The excitation energy transfer efficiencies derived from these spectra were used to analyze the annihilation data. BChl concentrations were calculated using extinction coefficients of 140 mM^{-1} cm^{-1} for the core antenna [29] and 184 mM^{-1} cm^{-1} at the Q_y maxima at 820, 837 or 850 nm for the peripheral antenna [30], depending on the mutant. The number of BChl molecules per domain (N_D), was calculated from the intensity at which the average number of excitations per domain equals 1.

For the DBC1 mutants, annihilation curves were recorded by measuring the relative fluorescence yield from LH1 at 910 nm as a function of the incident energy density of the 532 nm excitation flash (Fig. 7, solid circles). The curves were analyzed with the equation of Paillotin [14] and could all be fitted with $r \geq 5$; the number of connected BChl molecules (N_D) was found to be at least 240. In this first analysis it was assumed that annihilation only occurs in LH1, but this is an over-simplification, as most of the excitations reach the core antenna by energy transfer from the peripheral complexes. As a result, annihilation processes in the peripheral antenna must be taken into account in order to get an more accurate estimate of domain sizes, even though they occur to a lesser extent than in LH1 (Fig. 5). Accordingly, we determined the annihilation curves for fluorescence emitted by the peripheral antenna at 860, 850 and 840 nm for DBC1(WTLH2), DBC1(LH2YY → FY) and DBC1(LH2YY → FL), respectively (Fig. 7, open circles) and applied a correction to the annihilation curves recorded at 910 nm. This correction also took into account the consideration that different fractions of excitations reach the core via the peripheral antenna in the different strains and assumed that energy transfer from the peripheral anenna to LH1 was unidirectional. In order to estimate the fraction of excitations reaching the core via the peripheral antenna in the different strains, we estimated the relative numbers of BChls in the peripheral and core antenna, using the absorption spectra in the Q_y-band region (Fig. 1), and assumed the same ratios for the carotenoids absorbing at 532 nm. From these calculations, the fractions of excitations reaching the core via the peripheral antenna (at low excitation energies) appeared to be at least 80%, 70% and 60% for DBC1(WTLH2), DBC1(LH2YY → FY) and DBC1(LH2YY → FL), respectively.

Following these corrections, the annihilation curves were again analyzed using the equations given in [14]. The results of this analysis are summarised in Table 1. With the assumed transfer of 80% and 70% of the excitations to the core via the peripheral antenna for the pseudo-wild-type and single mutant, respectively, the corrected annihilation curves could be fitted with values of $r = 1$ or $r = 1.3$, resulting in domain sizes of N_D of about 50 BChls. The corrected curves are shown in Fig. 7 (triangles). For DBC1(LH2YY → FL) the value of r showed only a weak

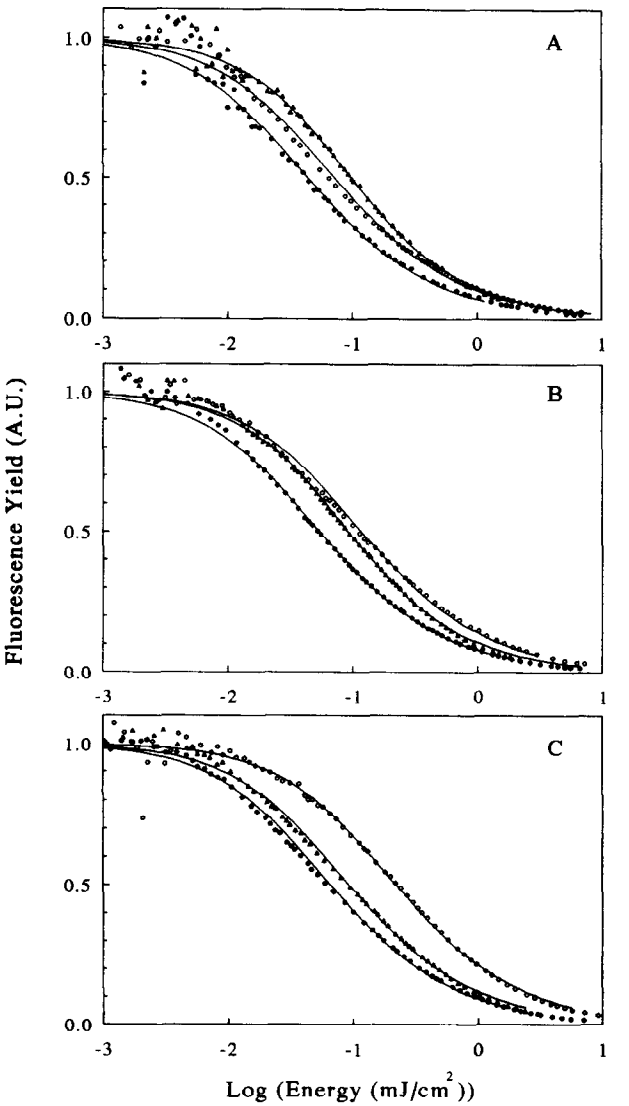

Fig. 7. The relative fluorescence yield from LH1 at 910 nm (solid circles), as a function of the incident energy density of the excitation flash. (A) DBC1(WTLH2); (B) DBC1(LH2YY → FY); (C) DBC1(LH2YY → FL). The open circles describe the relative fluorescence yield from LH2 at 860 nm (A), 850 nm (B) and 840 nm (C). The triangles describe the relative fluorescence yield of LH1 corrected for annihilation in the peripheral antenna (see text), assuming that 80% (A), 70% (B) and 60% (C) of the excitations reach the core via the peripheral antennae at low excitation densities. The data were normalised at low energy densities and fitted with the equation of Paillotin et al. [14] with $r = 5$ (open and closed circles). The corrected data were fitted with $r = 1$ (A), $r = 1.3$ (B) and $r = 5$ (C). The primary donor was kept in the oxidised state by means of continuous background illumination.

dependence on the fraction of excitations reaching the core via the peripheral antenna. This was partially due to the relatively high energy value at which annihilation effects in the peripheral antenna occur (Fig. 7C, open circles). Analysis of the data obtained at 840 nm for the double mutant indicated that the domain size of the peripheral antenna of DBC1(LH2YY → FL) is relatively small (50–80 BChls) and therefore annihilation effects occur only at

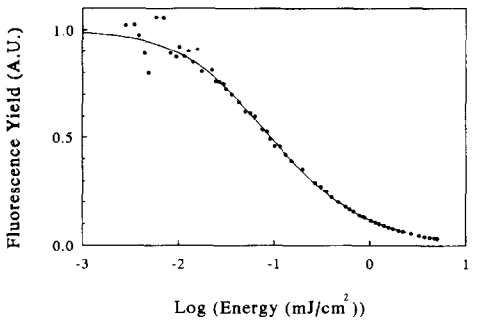

Fig. 8. The relative fluorescence yield from LH2 at 860 nm of membranes of strain DPF2(pRKEH2), as a function of the incident energy density of the excitation flash normalised at low energy densities. The primary donor was kept in the oxidised state by means of continuous background illumination. The data was fitted with the equation of Paillotin et al. [14] with $r = 5$.

relatively high excitation densities. The results obtained for DBC1(LH2YY → FL) differ from those obtained previously with *Rps. acidophila* (culture 7750 LT) [19] in that a small domain size as a result of inefficient back transfer to the peripheral antenna is not observed.

For strain DPF2(pRKEH2), annihilation curves were recorded at 880, 860 (Fig. 8) and 844 nm and were fitted with the equation of Paillotin et al. [14] with $r = 5$, giving rise to estimates of the lower limit of the number of connected BChls of about 260, 370 and 240 respectively. This result is similar to the results obtained with membranes of the LH2-only mutant NF57 by Vos et al. [18].

4. Discussion

Our results, like those published earlier [4,7,8,13] show that, although the mutants used in this study were grown in darkness and under semi-anaerobic conditions, they are fully competent photosynthetically, at least with respect to the primary processes. They show that alteration of the tyrosine residues near the C-terminus of the α subunit of LH2 produces new photosynthetic systems which are not only efficient in terms of energy transfer within the pigments of the LH2 complex [7,8], but also in terms of the wider role of the antenna in directing energy towards the RC. The blue shifts that arise as a consequence of disrupting hydrogen bonds to the BChls 850 [5] have some interesting effects on the way in which excitation energy equilibrates in the intact unit, notably in concentrating excitations in the core complex. This occurs because of the steeper energy gradient created by raising the energy of the BChl 850 pigments in the peripheral antenna. Although wild-type strains of *Rb. sphaeroides* do not synthesise a B800-820 LH2 antenna, some bacterial species do. It is therefore of interest to compare the results reported here with those obtained earlier with *Rps. acidophila* [19] and *Rps. cryptolactis* [20]. Mutant DBC1(LH2YY → FL) resembles *Rps. acidophila* strain 7750 grown at low temperature, and *Rps. cryptolactis* grown at low light intensities [19,20], both of which have a B800-820 peripheral antenna. One advantage of the mutagenesis-based strains used in the present study is that we have been able to examine the single mutant DBC1(LH2YY → FY), which synthesises a B800-840 complex and is spectroscopically intermediate between these strains. The equilibration of excitations within these altered units follows a predictable pattern; inspection of the spectra of the enhanced fluorescence arising from closure of the RC (Fig. 3) shows that the back-transfer of energy from the core antenna to the peripheral antenna in the DBC1 mutants of *Rb. sphaeroides* becomes less extensive when the peripheral antenna is shifted to shorter wavelengths. For the mutant DBC1(LH2YY → FL), the fluorescence originating from the peripheral antenna did not depend on the redox state of the RC primary donor. These observations agree with those obtained with *Rps. acidophila* [19] and *Rps. cryptolactis* [20].

The annihilation measurements with the pseudo-wild-type DBC1(WTLH2) and with the single mutant DBC1(LH2YY → FY) indicate that in both mutants the number of LH1 BChls between which energy transfer can occur is roughly 50. Assuming a ratio of approx. 25–30 LH1 BChls per RC [31], this would be equivalent to two core complexes. This result is very similar to that obtained with *Rps. cryptolactis* [20] where a model was proposed in which each LH1 unit is associated with its own complement of peripheral antenna, but without an extensive interconnecting network as in *Rps. acidophila* [19].

For the double mutant DBC1(LH2YY → FL) the domains for energy transfer within LH1 appear to be much larger and to contain at least five or six core complexes ($N_D \approx 170$ BChls; Table 1). Since energy transfer from LH1 to the peripheral antenna tends not to occur in this mutant, we suggest that a model of several interconnecting LH1/RC cores, as originally proposed by Monger and Parson [32], may apply here. It should be noted that the absorption spectrum (Fig. 1) indicates that the amount of peripheral antenna is relatively small in this mutant, in comparison with LH1. This may favour the formation of interconnecting LH1/RC complexes.

The measurements on the LH1-minus mutant DPF2(pRKEH2) confirm and extend those obtained earlier using time-resolved spectroscopy [13], which showed that energy is efficiently transferred from LH2 to the RC, despite the absence of LH1. The excitation spectrum for oxidation of the primary donor leads to the conclusion that excitations absorbed by the antenna are transferred to the RCs to bring about charge separation. This is consistent with the time-resolved studies of Hess et al. [13]. However, this previous work did not address the question of the size of the photosynthetic unit, i.e., is the number of LH2 BChls per RC, a parameter which could have a significant effect on the overall trapping time. This is addressed in the experiment shown in Fig. 4, which shows that the number

of RCs relative to the number of BChls in LH2 is approximately the same in both DPF2(pRKEH2) and the *Rb. sphaeroides* wild-type strain. Annihilation measurements conducted on membranes from strain DPF2(pRKEH2) show that a functional domain in this mutant contains at least 250 BChls 850 connected for energy transfer. It would appear that the absence of LH1 from this mutant promotes the formation of larger assemblies of LH2, since the domain size of the peripheral antenna of DBC1-(WTLH2) at least is relatively small (50–80 BChls). The recent X-ray crystallographic data [3] show that LH2 exists as rings containing 27 BChls (18 BChl 850 and 9 BChl 800), which would be equivalent to between 2 and 4 rings per core complex in, for example, DBC1(LH2YY → FL), and 5 to 8 rings per reaction centre in DPF2(pRKEH2). Now that the crystallographic structure of LH2 is known in detail, it will be interesting to extend the scope of the mutagenesis and spectroscopic work undertaken here to examine further the way in which LH complexes and RCs are organised to form larger, functional assemblies.

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References

[1] Van Grondelle, R., Dekker, J.P., Gillbro, T. and Sundstrom, V. (1994) Biochim. Biophys. Acta 1187, 1–65.
[2] Hunter, C.N., Olsen, J. and Van Grondelle, R. (1989) Trends Biochem. Sci. 14, 72–76.
[3] McDermott, G., Prince, S.M., Freer, A.A., Hawthornthwaite-Lawless, A.M., Papiz, M.Z., Cogdell, R.J. and Isaacs, N.W. (1995) Nature, in press.
[4] Fowler, G.J.S., Visschers, R.W., Grief, G.G., Van Grondelle, R. and Hunter, C.N. (1992) Nature 355, 848–850.
[5] Fowler, G.J.S., Sockalingum, G.D., Robert, B., Grief, G.G. and Hunter, C.N. (1994) Biochem. J. 299, 695–700.
[6] Olsen, J.D. and Hunter, C.N. (1994) Photochem. Photobiol. 60, 521–535.
[7] Van der Laan, H., de Caro, C., Schmidt, T., Visschers, R.W., Van Grondelle, R., Fowler, G.J.S., Hunter, C.N. and Völker, S. (1993) Chem. Phys. Lett. 212, 569–580.
[8] Hess, S., Visscher, K.J., Sundstrom, V., Fowler, G.J.S. and Hunter, C.N. (1994) Biochemistry 33, 8300–8305.
[9] Kiley, P.J. and Kaplan, S. (1987) J. Bacteriol. 169, 3268–3275.
[10] Ashby, M.K., Coomber, S.A. and Hunter, C.N. (1987) FEBS Lett. 213, 245–248.
[11] Jones, M.R., Fowler, G.J.S., Gibson, L.C.D., Grief, G.G., Olsen, J.D., Crielaard, W. and Hunter, C.N. (1992) Mol. Microbiol. 6, 1173–1184.
[12] Burgess, J.G., Ashby, M.K. and Hunter, C.N. (1989) J. Gen. Microbiol. 135, 1809–1816.
[13] Hess, S., Visscher, K., Ulander, J., Pullerits, T., Jones, M.R., Hunter, C.N. and Sundström, V. (1993) Biochemistry 32, 10314–10322.
[14] Paillotin, G., Swenberg, C.E., Breton, J. and Gaecintov, N.E. (1979) Biophys. J. 25, 513–533.
[15] Den Hollander, W.T.F., Bakker, J.G.C. and Van Grondelle, R. (1983) Biochim. Biophys. Acta 725, 492–507.
[16] Van Grondelle, R., Hunter, C.N., Bakker, J.G.C. and Kramer, H.J.M. (1983) Biochim. Biophys. Acta 723, 30–36.
[17] Vos, M., Van Grondelle, R., Van der Kooij, F.W., Van de Poll, D., Amesz, J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 501–512.
[18] Vos, M., Van Dorssen, R.J., Amesz, J., Van Grondelle, R. and Hunter, C.N. (1987) Biochim. Biophys. Acta 933. 132–140.
[19] Deinum, G. Otte, S.C.M., Gardiner, A.T., Aartsma, T.J., Cogdell, R.J. and Amesz, J. (1991) Biochim. Biophys. Acta 1060, 125–131.
[20] Kramer, H., Deinum, G., Gardiner, A.T., Cogdell, R.J., Aartsma, T.J. and Amesz, J. (1995) Biochim. Biophys. Acta 1231, in press.
[21] Hunter, C.N., McGlynn, P., Ashby, M.K., Burgess, J.G. and Olsen, J.D. (1991) Mol. Microbiol. 5, 2649–2661.
[22] Hunter, C.N. and Turner, G. (1988) J. Gen. Microbiol. 134, 1471–1480.
[23] Bakker, J.G.C., Van Grondelle, R. and Den Hollander, W.F.T. (1983) Biochim. Biophys. Acta 725, 508–518.
[24] Rijgersberg, C.P., Van Grondelle, R. and Amesz, J. (1980) Biochim. Biophys. Acta 592, 53–64.
[25] Kleinherenbrink, F.A.M. (1992) Thesis, University of Leiden.
[26] Halsey, Y.D. and Parson, W.W. (1974) Biochim. Biophys. Acta 347, 404–416.
[27] Timpmann, K., Freiberg, A. and Godik, V.I. (1991) Chem. Phys. Lett. 182, 617–622.
[28] Van Grondelle, R., Kramer, H.J.M. and Rijgersberg, C.P. (1982) Biochim. Biophys. Acta 682, 208–215.
[29] Clayton, R.K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 495–500, The Antioch Press, Yellow Springs.
[30] Clayton, R.K. and Clayton, B.J. (1981) Proc. Natl. Acad. Sci. USA 78, 5383–5587.
[31] Aagaard, J. and Sistrom, W.R. (1972) Photochem. Photobiol., 209–225.
[32] Monger, T.G. and Parson, W.W. (1977) Biochim. Biophys. Acta 460, 393–407.