Biochimica et Biophysica Acta 933 (1988) 132–140
Elsevier

BBA 42750

The organization of the photosynthetic apparatus of *Rhodobacter sphaeroides*: studies of antenna mutants using singlet–singlet quenching

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(Received 7 December 1987)

Key words: Energy transfer; Pigment organization; (*Rb. sphaeroides*)

The organization of the light-harvesting system of the purple bacterium *Rhodobacter sphaeroides* was studied by use of mutants containing only one of the two light-harvesting pigment-protein complexes present in the wild type: B875 (LH1) and B800–850 (LH2). These mutants thus enabled us to study for the first time the properties of these complexes separately in their native environment. By measuring the yield of fluorescence caused by 35 ps, 532 nm flashes of varying energy density, the number of bacteriochlorophyll (BChl) molecules which constitute a domain for energy transfer was obtained as well as the rate constants for energy transfer. It appears that LH1 consists of clusters of about 100 BChl molecules, which, at room temperature, form large assemblies for energy transfer. This aggregation may be mediated by the presence of reaction centres. The LH2 complex also shows a strong tendency to aggregate to form large domains, although a fundamental structural unit of 30 connected $BChl_{850}$ and 15 $BChl_{800}$ molecules appears to exist. We also present evidence that in these mutants the LH1 and LH2 complexes are spectrally inhomogeneous and each contain an additional, long-wavelength-absorbing BChl.

Introduction

In photosynthetic systems each reaction centre is surrounded by a number of accessory pigment molecules which absorb light and transfer their excitation energy to the reaction centre. This causes an increase of the effective absorption cross section of the reaction centre, resulting in an enhanced efficiency of energy metabolism. Such an assembly of a reaction centre and its associated 'antenna' is called a photosynthetic unit. Since the early work of Vredenberg and Duysens [1] it has been known that in purple bacteria several photosynthetic units are connected to each other, so that excitations generated in one photosynthetic unit can migrate to more than one reaction centre. An antenna structure in which several reaction centres are embedded in a matrix of pigment molecules is commonly called the matrix of 'lake' model [2]. The ensemble of antenna pigments between which energy transfer is possible is defined as the 'domain' for energy transfer.

In the photosynthetic purple bacterium *Rhodobacter sphaeroides* several types of antenna molecules are present. The reaction centres are associated with the so-called LH1 or B875 complex, named after the wavelength of maximum

Abbreviations: $BChl_{800}$, $BChl_{850}$, $BChl_{875}$, $BChl_{896}$, bacteriochlorophylls absorbing at the wavelength indicated.

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absorption of its bacteriochlorophyll component. It has been suggested that this complex contains an additional pigment, $BChl_{896}$ [3,4], which may be involved in energy transfer from LH1 to the reaction centre [5,6]. In addition, a second type of complex is present with absorption maxima near 800 and 850 nm which is designated B800–850 or LH2. LH1 and LH2 both contain carotenoids. Models have been proposed in which LH1 interconnects several reaction centres, while LH2 forms large aggregates that surround the LH1-reaction centre assemblies [7,8].

One way to study the structure of photosynthetic antenna systems and energy transfer is by measurement of the fluorescence yield induced by picosecond laser flashes of varying intensity. High-intensity flashes create more than one excitation in a single domain. Collision of two excitation then results in the annihilation of one of them [9,10], causing a decrease of the fluorescence yield. Paillotin et al. [11] derived a theoretical relation between the fluorescence yield and the number of excitations generated in the domain. An important parameter in this relation is the ratio r of the mono-excitation decay rate constant (reflecting fluorescence and other antenna losses, and trapping in reaction centres) and the bi-excitation decay rate constant (reflecting annihilation of one of two colliding excitations). When the fluorescence yield is plotted as a function of the excitation density, r determines the slope of the quenching curves, as well as the degree of quenching caused by a given number of excitations per domain. For instance, for $r = 0$, i.e., with very efficient annihilation, an average excitation density of one per domain causes a quenching of 37%, while for $r = 2$ (less efficient annihilation) only 15% quenching occurs. In addition, higher r-values result in a weaker slope of the quenching curves.

Experimentally, the excitation density generated by a flash can be calculated in principle from the energy density of this flash and the absorbance of the sample at the wavelength of excitation. A comparison of the measured quenching curve with a set of computer-generated curves then yields the value of r, and together with the concentration of the pigment involved, the size of the domain, N_D [8]. Subsequent application of the random walk model of Den Hollander et al. [12] allows a calculation of the rate constants for energy transfer between two antenna molecules (k_h) or for trapping in a reaction centre (k_t). Application of these techniques to *Rb. sphaeroides* has shown that at room temperature the domains consist of several hundred BChl molecules, confirming the existence of large assemblies for energy transfer [8,13]. Upon cooling to 4 K the domains become considerably smaller, suggesting a partitioning of these assemblies into much smaller domains.

Although the properties of the isolated, detergent-solubilized complexes have been extensively investigated (see, e.g., Ref. 14 for a review), it was not possible until now to study LH1 and LH2 separately in vivo. Recently, mutants of *Rb. sphaeroides* have been isolated that contain only one of the two antenna complexes. Use of these mutants enabled us to study the properties and structural arrangement of these complexes in situ, free from the possibility of detergent-induced artifacts. Three different mutants were used: (1) mutant NF57, which contains neither LH1 nor reaction centres, but only LH2; (2) M21, which only contains LH1 and reaction centres and (3) M2192 which contains only LH1, but no reaction centres or LH2. All three mutants contain carotenoids. Their spectral characterization will be described elsewhere [15].

The results presented here indicate that the LH1-reaction centre 'core' consists of 100 bacteriochlorophylls and four reaction centres; these 'cores' can aggregate to form larger domains for energy transfer at room temperature. In the absence of reaction centres, this aggregation does not occur. LH2 exists as large aggregates of clusters containing about 30 $BChl_{850}$ and 15 $BChl_{800}$ molecules. We also present evidence indicating that both antenna complexes are spectrally inhomogeneous.

Materials and Methods

Mutants NF57 and M21 were isolated following chemical mutagenesis of *Rb. sphaeroides* NCIB 8253 with *N*-nitrosoguanidine. Some of the properties of these mutants are described elsewhere [15–17] as well as the construction of mutant M2192 [17]. Membranes were prepared by sonication of the cells, followed by purification of the

crude cell extract on a sucrose step gradient (15% and 40% w/w sucrose). After 4 h of centrifugation at 100 000 × g membranes were collected from the interface. For all experiments the membranes were suspended in 10 mM Tris (pH 8.0) and 5 mM $MgCl_2$, while 0.5 M sucrose and 55% (v/v) glycerol were added to prevent crystallization upon cooling. The absorbance of the sample did not exceed 0.1 at the wavelength of excitation to ensure a homogeneous light distribution within the sample.

For measurements of annihilation curves the samples were excited with 35 ps, 532 nm flashes, the energy density of which could be varied from 0.5 μJ/cm^2 to 5 mJ/cm^2. The curves were recorded with the spectrofluorimeter described in Refs. 8 and 13. For measurements of emission spectra the samples were either excited with 35 ps, 532 nm flashes of 2.5–3 mJ/cm^2 or with 20 μs xenon flashes at 522 nm with an energy density of 100 μJ/cm^2. The detection bandwidth was 7.5 nm for annihilation experiments, unless otherwise indicated, and 1.5 nm for emission spectra. Further details of the experimental procedure are given in Ref. 8.

For a calculation of domain sizes the theory of Paillotin et al. [11] was applied, while we used the formalism of Den Hollander et al. [12] to calculate rate constants for energy transfer, k_h, and for trapping, k_t (see also Ref. 8).

Results

Annihilation quenching

Fig. 1 shows the time-integrated fluorescence yield of membranes from mutant NF57, which contains only LH2. The yield is plotted as a function of the energy density of the 35 ps, 532 nm exciting flashes. The experiments were performed both at room temperature and at 4 K. Van Dorssen et al. [15] observed that the low temperature emission spectrum of NF57 is composed of at least two bands, suggesting the existence of two spectrally different species of BChl *a* ($BChl_{850}$ and $BChl_{870}$). Therefore, we detected the fluorescence at 4 K at both the blue and the red side of the emission maximum (880 and 910 nm, respectively). The room-temperature fluorescence detected at 870 nm may be assumed to originate mainly from $BChl_{850}$. It can be seen that in all cases a strong decrease in fluorescence yield occurred when the energy of the exciting flash was increased. This is attributed to the annihilation of singlet excited states of BChl *a* that migrate through the antenna. At 4 K a higher energy density was needed to produce the same quenching as at room temperature, and the curve became steeper. This indicates that the size of the domain (i.e., the number of BChl *a* molecules between which energy transfer is possible) decreased considerably upon cooling to 4 K.

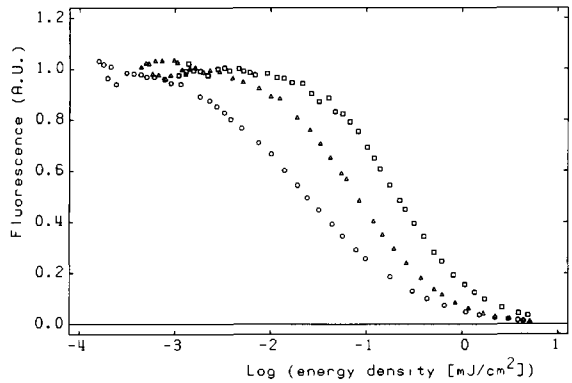

Fig. 1. Time-integrated normalized fluorescence yield induced by 35 ps, 532 nm flashes of varying intensity in membranes of mutant NF57 of *Rhodobacter sphaeroides*. For clarity of presentation the averages of ten successive data points are plotted. ○, Room temperature, detected at 870 nm; □, 4 K, detected at 880 nm; △, 4 K, detected at 910 nm. A.U., arbitrary units.

Comparing the curves for $BChl_{850}$ (circles and squares) with computer-generated curves for different values of the parameter r yielded the r-values ($r = 1$ at 4 K and $r \geqslant 2$ at 298 K), as well as the energies at which on the average one excitation per domain was generated. Together with the absorbance of the sample at 532 nm and the energy-transfer efficiency from pigments absorbing at 532 nm to BChl *a* (65%; Ref. 15), they yielded the number of excited $BChl_{850}$ molecules generated by the flash. The total number of BChl *a* molecules was calculated from the absorbance of the sample at the wavelength of maximum absorption assuming a specific extinction coefficient of 140 $mM^{-1} \cdot cm^{-1}$ [18]. The ratio of these numbers directly yields the size of the domain N_D. In this way we calculated $N_D \geqslant 365$ $BChl_{850}$ molecules at room temperature and $N_D = 30$ $BChls_{850}$ at 4 K. The value of 30 connected $BChl_{850}$ molecules at 4 K agrees well with results obtained

before in wild-type *Rb. sphaeroides* [8] and in isolated LH2 complexes solubilized in lithium dodecyl sulfate [19] and appears to reflect a fundamental structural unit of this complex. At 4 K no energy transfer is possible between these units, but the energy barriers between the units are apparently low enough to allow excitations to migrate efficiently from one unit to another at room temperature. This has also been observed in the LH1 complex of *Rhodospirillum rubrum* [8].

The fluorescence emitted at 910 nm at 4 K (triangles), mainly due to $BChl_{870}$, showed a stronger quenching than that emitted at 880 nm. With $r = 1$ the quenching curve yielded a domain of 85 connected $BChl_{(850+870)}$ molecules. However, a more realistic calculation may be obtained by considering the annihilation in $BChl_{870}$ separately. Since $BChl_{870}$ constitutes only about 15% of the amount of $BChl_{850}$ [15], the emission detected at 910 nm derives mainly from excitations generated in $BChl_{850}$ which have subsequently been transferred to $BChl_{870}$. Back transfer to $BChl_{850}$ is impossible at 4 K. Therefore, the fluorescence yield of $BChl_{870}$ should reflect annihilation in both $BChl_{850}$ and $BChl_{870}$. If we neglect direct absorption by $BChl_{870}$ and assume 100% efficiency of energy transfer from $BChl_{850}$ to $BChl_{870}$, a correction for the effect of annihilation in $BChl_{850}$ can be made, as discussed in Ref. 20 by using the annihilation curve for $BChl_{850}$ as a measure for the number of excitations generated in $BChl_{870}$. With $r = 1$, this yielded a domain of eight connected $BChl_{870}$ molecules.

Fig. 2A shows room-temperature annihilation curves of membranes of mutant M21, which contains LH1 and reaction centres, but no LH2. The reaction centres were either open before the flash (open circles) or closed by background illumination (closed circles). In both cases a steady decrease of the fluorescence yield is observed with increasing flash intensity. The curve with closed reaction centres could be fitted with $r \geqslant 2$, and from the position where on the average one excitation per domain was generated a domain size of the least 330 $BChl_{875}$ molecules was calculated. Since in this mutant about 40 $BChl_{875}$ molecules are present per reaction centre, this means that at least eight reaction centres are connected by the LH1 complex.

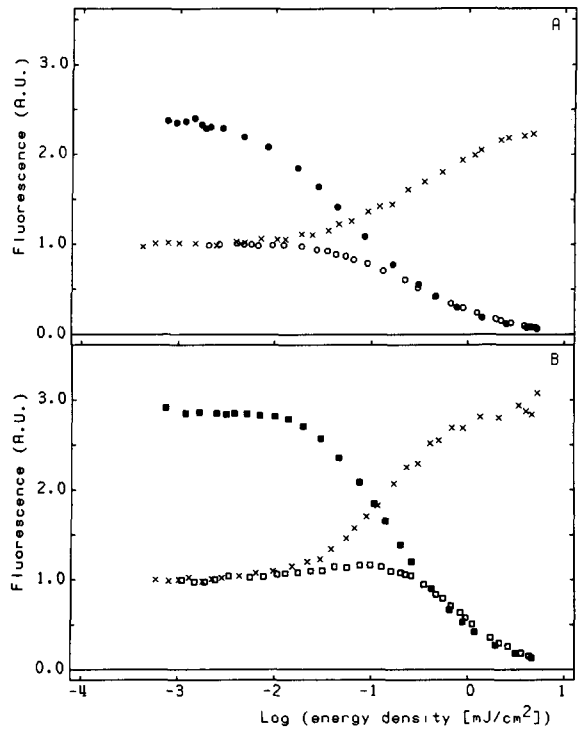

Fig. 2. Annihilation curves of membranes of mutant M21. Open symbols: reaction centres open before arrival of the flash; closed symbols refer to closed reaction centres. In addition, the fluorescence intensity caused by a weak xenon flash following the laser pulse is plotted (×) (see text). (A) Room temperature, detected at 903 nm; (B) 4 K, detected at 910 nm.

The curve with all the reaction centres open before arrival of the exciting laser flash shows a lower fluorescence yield at low flash energy due to the presence of quenching reaction centres, but at high excitation energy the fluorescence yield is approximately the same as with initially closed reaction centres, indicating that at these energies more than 90% of the reaction centres are closed by the actinic flash. The same conclusion was obtained when the fraction of reaction centres closed was measured by a weak probing xenon flash given shortly after the excitation flash (Fig. 2A, crosses). It can be seen that a more than two-fold increase in fluorescence occurs upon increasing laser flash energy, due to closing of reaction centres. At high energy the curve saturates, indicating that most reaction centres are indeed closed by the flash.

Fig. 2B shows the same experiments performed at 4 K. As mentioned in the introduction, there is

evidence that LH1, like LH2 contains a small amount of a long wavelength absorbing pigment, $BChl_{896}$. However, as will be shown below, the effect of quenching on the emission spectrum is less pronounced in this case. It was thus not possible to separate the emissions from the two populations of pigments sufficiently, and the fluorescence was therefore measured in a broad band around the maximum of the emission band. The results obtained in this way can be compared with those obtained earlier with wild-type strains of *Rb. sphaeroides* and *R. rubrum* [8,13]. It can be seen that the curve with initially closed reaction centres (closed squares) is steeper than that obtained at room temperature. An *r*-value of 1 was found in this case, and a domain size $N_D = 100$, corresponding to only 2–3 connected reaction centres. The curve for open reaction centres (open squares) now showed an increase of fluorescence yield between approx. 10^2 and approx. 10^{-1} mJ/cm^{-2}. This effect, which has not been observed before in purple bacteria, may be explained by the competition of two processes. On the one hand the fluorescence yield decreases because of annihilation effects, on the other hand the exciting flash closes reaction centres, giving rise to an increase in fluorescence yield. Den Hollander et al. [12] have shown by theoretical calculations that an increase of fluorescence yield at intermediate energies can arise only if not more than three reaction centres are connected in a single domain. The closing of traps by the laser flash as measured with a weak probing xenon flash is again given (crosses).

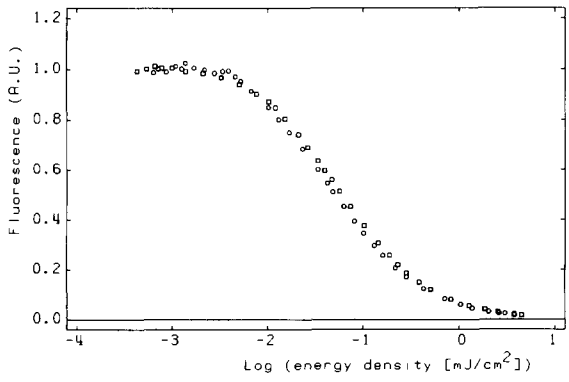

Fig. 3. Annihilation curves of membranes of mutant M2192. ○, Room temperature (895 nm); □, 4 K (909 nm).

Annihilation curves of membranes of the mutant M2192 are shown in Fig. 3. This mutant contains only LH1 but no LH2 or reaction centres. The experiments were again performed at room temperature (circles) and at 4 K (squares). It can be seen that in this case the two curves almost coincide and that there is no difference in slope (*r*-value). This indicates that the domain sizes must be approximately the same at both temperatures. A calculation yielded $N_D = 150$ (298 K) and $N_D = 125$ (4 K), with $r = 1$ in both cases. We conclude that mutant M2192, which lacks reaction centres, does not assemble several of these units of 125–150 $BChl_{875}$ molecules into a larger domain.

Emission spectra

In the previous section it was shown (Fig. 1) that the annihilation characteristics of mutant NF57 depended on the wavelength at which the fluorescence was detected, and this effect was ascribed to the presence of a minor long-wavelength absorbing BChl, $BChl_{870}$. In order to check this hypothesis, we determined the emission spectrum at different energy densities of excitation.

Fig. 4 shows the 4 K emission spectrum of NF57, induced by either a weak 522 nm, 20 μs

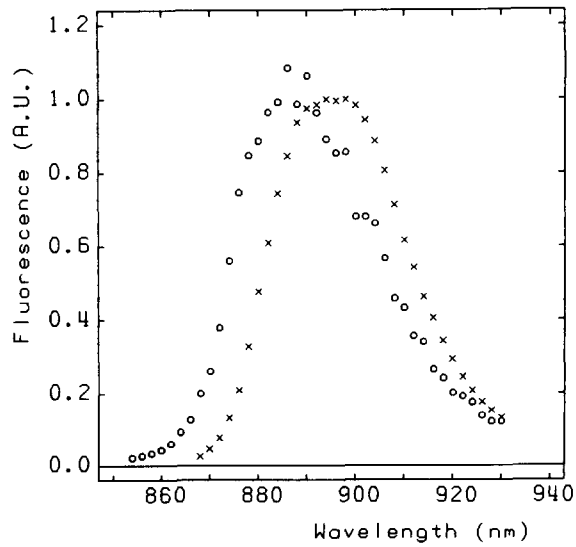

Fig. 4. Emission spectra of membranes of mutant NF57 at 4 K. ○, Induced by a 30 ps, 532 nm laser flash; (×): induced by a weak xenon flash at 519 nm. The spectra are approximately normalized at their emission maximum. They were not corrected for the wavelength response of the apparatus.

flash (crosses) or a 35 ps, 532 nm flash of about 3 mJ/cm^2. At high flash energy, where annihilation has caused the fluorescence yield to drop by more than 90%, the emission spectrum has shifted by 5–7 nm to shorter wavelengths. This is in agreement with the results shown in Fig. 1 and can be explained by the stronger annihilation in $BChl_{870}$, resulting in less $BChl_{870}$ emission relative to $BChl_{850}$ emission and thus in an apparent blue shift of the emission band.

In Fig. 5 we show the high (circles) and low (crosses) excitation energy spectra of membranes of mutant M21 at 4 K. Again we observe a blue shift of the emission upon high energy excitation, as was also observed in *R. rubrum* and *Rb. sphaeroides* wild type [8]. This is most probably caused by the presence of the long-wavelength pigment, $BChl_{896}$, which is supposed to be present in the LH1 antenna of several purple bacteria [3,4], and the contribution of which to the emission spectrum decreases at high excitation densities.

In view of the proposed role of $BChl_{896}$ as a pigment connecting the reaction centres with the main LH1 matrix, we also measured high and low energy emission spectra of mutant M2192. The result is shown in Fig. 6. Although less clear than in mutant M21 it can be observed that the spectra are not identical and that also in the absence of reaction centres the antenna is not homogeneous.

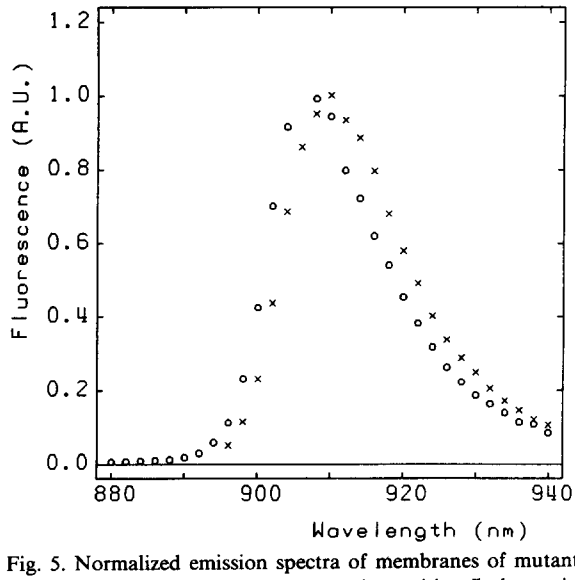

Fig. 5. Normalized emission spectra of membranes of mutant M21 with high (○) and low (×) intensity exciting flashes as in Fig. 4.

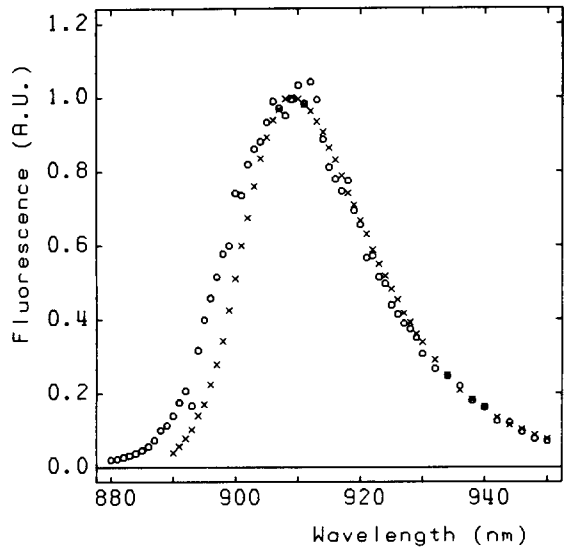

Fig. 6. Normalized emission spectra of membranes of mutant M2192 with high (○) and low (×) intensity exciting flashes as in Fig. 4.

Rates of energy transfer

As mentioned in the introduction, the results from the annihilation experiments can be used to calculate rate constants for energy transfer in the antenna complexes. The parameters, accessible by the random walk model of Den Hollander et al. [12], are the rate constant for energy transfer between neigbouring antenna molecules (k_h) and the rate constant for trapping in a reaction centre (k_t), provided the value of r, the fluorescence yield (ϕ_f), the rate of decay in the absence of reaction centres (k_1) and the number of antenna molecules per reaction centre are known. The rate constant k_1 is the inverse of the lifetime of excited antenna complexes in the absence of reaction centres. For this lifetime a value of 1 ns has been reported, independent of the temperature [21]. This corresponds to $k_1 = 1 \cdot 10^9\ s^{-1}$.

We determined the yields of fluorescence, ϕ_f, of our preparations by comparing their fluorescence yields to that of chromatophores of *R. rubrum* ($\phi_f \simeq 1\%$ if the reaction centres are in the open state [22]). The results are given in Table I.

TABLE I

CHARACTERISTICS OF THE ANTENNA SYSTEMS (LH2 AND LH1, RESPECTIVELY) OF THE THREE MUTANTS STUDIED

The numbers for NF57 at 4 K refer to $BChl_{850}$ as determined from $BChl_{850}$ fluorescence quenching. ϕ_f^o and ϕ_f^c are the fluorescence yields with open and closed reaction centres, respectively, relative to the fluorescence yield of *R. rubrum* chromatophores with open reaction centres (which is about 1% [22]; these data can thus also be interpreted as the fluorescence yields in percents.) The data for NF57 and M2192 are presented in the column for closed reaction centres. The other symbols are explained in the text.

Nutant	Temperature (K)	ϕ_f^o	ϕ_f^c	r	N_D	k_h (s^{-1})	k_t^o (s^{-1})	k_t^c (s^{-1})
NF57	298	–	4.1	$\geqslant 2$	$\geqslant 365$	$4.6 \cdot 10^{11}$	–	–
NF57	4	–	7.8	1	30	$2.4 \cdot 10^{10}$	–	–
M21	298	0.7	1.8	$\geqslant 2$	$\geqslant 330$	$9.6 \cdot 10^{11}$	$1.7 \cdot 10^{12}$	$4.9 \cdot 10^{11}$
M21	4	3.8	11	1	100	$7.2 \cdot 10^{10}$	$7.9 \cdot 10^{11}$	$3.5 \cdot 10^{10}$
M2192	298	–	4.8	1	150	$2.8 \cdot 10^{11}$	–	–
M2192	4	–	11	1	125	$9.5 \cdot 10^{10}$	–	–

For M21 the number of antenna molecules per reaction centre was found to be 40, as determined from the extent of bleaching at 868 nm in saturating continuous light. At room temperature, ϕ_f^c was obtained from the experimentally observed fluorescence increase upon closing of the reaction centres. At 4 K, ϕ_f^c was obtained by measuring the relative fluorescence of a sample that was cooled down with continuous strong illumination. From these numbers, together with the value of r and the domain size N_D, we calculated the rate constants for energy transfer k_h and the rates of trapping k_t. The results are presented in Table I.

For all three mutants studied the rate constants for energy transfer k_h are between $3 \cdot 10^{11}$ and 10^{12} s^{-1} at room temperature, which is of the same order of magnitude as observed in *R. rubrum* and wild type *Rb. sphaeroides* [8]. Upon cooling to 4 K the rate of energy transfer decreases about 10-fold, which is again comparable to what was observed in the wild-type strains [8] and can be explained by a decrease in Förster overlap integral between the emission and absorption bands.

The rate constants for trapping of an excitation in a reaction centre are higher than observed in wild type *R. rubrum* for example. This is also reflected in the more complete closure of the reaction centres by a high intensity flash (Fig. 2A, cf. Fig. 2 of Ref. 13). We also applied the master equations formulated by Den Hollander et al. [12], which express the relation between the fluorescence yield or the fraction of traps closed and the number of excitations per domain, to the curves measured for M21 at 4 K with open reaction centres. The computer calculations, based on the rate constants presented in Table I, are given in Fig. 7 for 1, 2, 3 or 4 connected reaction centres ($N_D = 40$, 80, 120 or 160, respectively), together with the measured curves. It can be seen that the fraction of traps closed, calculated in this manner, corresponds very well with the experiment. However, a good fit of the annihilation curve was not obtained when a homogeneous system of two or three connected reaction centres is assumed. We must therefore assume that, although the domains contain on the average 2–3 connected reaction centres, at least some of them contain only one.

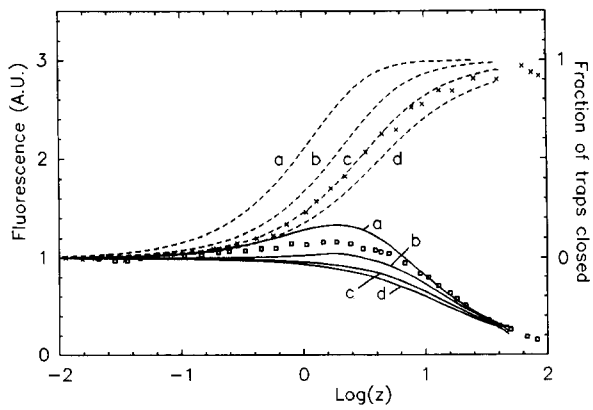

Fig. 7. Plot of computer-generated curves of the fluorescence yield (———) and the fraction of traps closed (- - - - - -) as a function of the number of excitations per domain, z. (a), (b), (c) and (d): 1, 2, 3 and 4 connected photosynthetic units, respectively. □ and ×, the data from Fig. 2B (see text).

Discussion

The organization of the light-harvesting antenna of wild-type *Rb. sphaeroides* has been studied by a variety of spectroscopic techniques [2]. Our earlier work [8] has indicated a structure of the antenna at low temperature (4 K) that comprises a central core of about 100 $BChl_{875}$ molecules containing four reaction centres, surrounded by 4–5 LH2 complexes, each of which is an aggregate of about 30–40 $BChl_{850}$ molecules. Moreover, LH1 contains an additional pigment, $BChl_{896}$, which is supposed to transfer excitations from $BChl_{875}$ to the reaction centre. Several questions, however, remained unanswered: (1) how does the presence of the LH2 antenna influence the aggregation state of the LH1-reaction centre complexes? (2) what is the aggregation state of the LH2 antenna in the absence of LH1 and reaction centres? (3) what is the role of the reaction centre itself?

Although the isolated LH2, LH1 and reaction centre complexes are available, solubilized in detergent, for this work we have chosen to examine the properties of several antenna mutants of *Rb. sphaeroides*. In this way we have obtained information about the properties of the light-harvesting complexes in their native environment that was previously inaccessible.

Our results show that in mutant M21, which contains only the LH1 complex and reaction centres, the light-harvesting system is strongly aggregated at room temperature: at least eight reaction centres are interconnected by the LH1 antenna to form a single domain for energy transfer. An even more extensive aggregation was observed for *R. rubrum*, in which 15–25 reaction centres are interconnected [8,13]. It should be noted, however, that M21, unlike *R. rubrum*, does not form normal invaginations of the intracytoplasmic membrane (Niederman, R.A. and Hunter, C.N., unpublished observations); tubular membranes which are twofold enriched in phospholipid, compared to chromatophores of the wild type, are present. This may 'dilute' the complexes in the membrane and thus prevent the formation of large aggregates.

Upon cooling to 4 K the domain size of M21 decreases significantly to about 100 $BChl_{875}$ molecules, as observed earlier in *R. rubrum* ($N_D \approx 150$ $BChl_{875}$) and wild-type *Rb. sphaeroides* ($N_D \approx 100$ $BChl_{875}$) [8]. The apparent partitioning at 4 K can be explained by assuming that low energy barriers exist within the domains, that can easily be overcome at room temperature, but not at 4 K. Such energy barriers may simply consist of 'gaps' in the domain structure, where the pigment-to-pigment distance is slightly increased.

Mutant M2192, which has only LH1 but no reaction centres, shows much less aggregation at room temperature with a domain size of only about 150 $BChl_{875}$. In this case this parameter does not decrease significantly upon cooling; the calculated value at 4 K is about 125 $BChl_{875}$, similar to that of the wild-type and mutant M21. Apparently, in *Rb. sphaeroides* LH1 is normally organized in units of about 100–150 $BChl_{875}$ molecules, which at room temperature aggregate to larger domains. This organization depends in some way upon the presence of reaction centres. It is possible that in the absence of reaction centres the geometry of the LH1 matrix is altered enough to produce an arrangement unfavourable for energy transfer [4].

Both M21 and M2192 show intensity-dependent emission spectra although this is most evident in M21. The simplest explanation for this observation is that in both mutants LH1 contains the additional long-wavelength antenna pigment, $BChl_{896}$, which is thought to facilitate energy transfer from the main antenna to the reaction centre.

Mutant NF57, which contains only LH2, also displays a strong aggregation of pigments at room temperature, with at least a few hundred $BChl_{850}$ molecules per domain, similar to the LH2 complex solubilized in the detergent lauryldimethylamine *N*-oxide (LDAO) [19]. At 4 K, the domain size of NF57, as judged from the $BChl_{850}$ fluorescence quenching, reduces to about 30 $BChl_{850}$. This size corresponds very well with the number estimated for chromatophores of wild-type *Rb. sphaeroides* [8] and to that reported for LH2 complexes solubilized by means of lithium dodecyl sulfate (LDS) [19]. Apparently, the number of 30 reflects the size of the basic structural unit of LH2. At room temperature, in the membrane, larger aggregates of these units can be formed. It is likely that the above-mentioned 'gaps' between the units

are just the sites where the detergent can most easily disrupt the membrane.

NF57 shows absorption and emission properties [15] that are different from those of the isolated LH2 complex in LDAO [23,24]. It appears that, as for LH1, a minor, long-wavelength absorbing pigment is present [15]. Our results indicate that this pigment, $BChl_{870}$, forms clusters of approx. eight molecules which accept excitation energy from $BChl_{850}$, and which may facilitate energy transfer from $BChl_{850}$ to LH1.

For all three mutants studied the rates of energy transfer between neighbouring antenna molecules are between $3 \cdot 10^{11}$ and $1 \cdot 10^{12}$ s^{-1} at room temperature, decreasing about 10-fold upon cooling to 4 K. This is in agreement with the observations made with the wild-type strains of *Rb. sphaeroides* and *R. rubrum* [8,13], indicating that the pigment–pigment distances within the domains are essentially unchanged in the mutants.

In conclusion, the results obtained with the mutants have confirmed and extended our earlier results with wild-type *Rb. sphaeroides*. The basic building blocks are LH1 complexes, containing 100–150 BChl molecules, and LH2 complexes containing 50 BChl molecules ($BChl_{800}$ + $BChl_{850}$). These basic units form aggregates that act as domains for energy transfer at room temperature. For the aggregation of the LH1 'core' units (but not for LH2) the presence of reaction centres may be required. The pigment organization in each basic unit is not modified in the mutants as compared to the wild type. Special long-wavelength components exist in each complex, possibly involved in directed downhill energy transfer [25].

Acknowledgements

This work was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research (ZWO). CNH was supported by a travel grant from the European Molecular Biology Organization (EMBO), and by the Agricultural and Food Research Council of Great Britain.

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