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Antenna organization of *Rhodopseudomonas acidophila*: a study of the excitation migration

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Transfer of excitation energy was studied in three different cultures of the purple bacterium *Rhodopseudomonas acidophila*, which all contained the core antenna complex B880, but differed with respect to the type of peripheral antenna complex, which was either B800–820, B800–850, or both. Contrary to that of B800–850, the fluorescence emitted by B800–820 did not increase upon light-induced oxidation of P-870. Singlet-singlet annihilation measurements showed that in cells containing only B880 and B800–820 energy transfer can take place within a domain of 50 ± 20 bacteriochlorophyll (BChl) molecules, which number equals that of BChls per reaction center. In cells containing B800–850 energy transfer can take place over at least 200 BChls of B880. Our results are explained by a model where small clusters of B880, the size of one photosynthetic unit, are separated from each other by a more or less continuous array of B800–820 or B800–850 complexes. B800–820, but not B800–850, acts as an energy barrier between different B880 units.

Introduction

In general, energy transfer from photosynthetic antenna pigments to the reaction centers, where the charge separation is induced, takes place with a very high efficiency [1]. The organization of the antenna therefore appears to be optimized for energy transfer. Although much is known about the structure of the reaction centers, similar information about the antenna systems is as yet very incomplete.

The antennae of purple bacteria are ideal model systems for studying energy transfer, since they are spectrally well-defined and have a simpler organization than those of plants. Moreover, the antenna properties can readily be manipulated by changing the growth conditions [2,3] or by genetic engineering [4,5].

This communication presents a study of the fluorescence properties of *Rhodopseudomonas acidophila* grown in three different ways so as to alter its antenna composition; one culture contained two types of 'peripheral' antenna complex, the other two contained only one. Fluorescence spectra were measured in the presence of 'open' and 'closed' reaction centers, where P-870 was either in the reduced or the oxidized state, respectively, and fluorescence quenching by singlet-singlet annihilation induced by intense laser flashes was studied. The latter phenomenon occurs upon double excitation of a BChl molecule by energy transfer from neighboring BChls. A plot of the fluorescence yield versus pulse intensity can be analyzed by means of equations derived by Paillotin et al. [6], to obtain information about the number of connected antenna BChls, called the domain size, and about the rate of excitation migration within the antenna [7,8]. The latter information is contained in the value of a parameter r, which is defined as twice the ratio of the averaged rate of mono-excitation decay and the rate of annihilation per pair of excitations. A main assumption

Abbreviations: BChl, bacteriochlorophyll; B800–820, B800–850, light harvesting complexes with absorption bands near 800 nm and 820 nm and 800 and 850 nm, respectively; B880, light harvesting complex absorbing near 890 nm in *Rps. acidophila*; HL, LL, LT, high light intensity, low light intensity and low temperature, respectively; P-870, primary electron donor.

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underlying the equations is that the excitation distribution is always random, implying that the domain is structurally homogeneous. In order to obtain information about, e.g., the rate of energy transfer between neighboring BChls an explicit description of the domain together with random walk theory should be used [7].

Analysis of the annihilation measurements was complicated by the presence of different spectral components in the antenna of *Rps. acidophila*, and a quantitative interpretation in terms of domain sizes was not possible for all antenna combinations studied. Nevertheless, our results, together with the spectral measurements with open and closed reaction centers, provided sufficient information to draw a general model of the structural arrangement of the antenna complexes in the photosynthetic membrane. This model describes the antenna as consisting of small B880 units, separated from each other by a more or less continuous array of either B800–820 or B800–850 units.

Materials and Methods

Rhodopseudomonas acidophila, strains 7750 and 7050 were grown anaerobically in the light in Pfennig's medium [9] with succinate as the main carbon source. Three different culture conditions were used. The first culture, 7750 LT (low temperature) was grown at 22°C at a light intensity of approx. 10 W m^{-2} from incandescent lamps; 7750 HL (high light intensity) was grown at 30°C and 10 W m^{-2}; 7050 LL (low light intensity) at 30°C and at 0.65 W m^{-2}. Membrane fragments were prepared as described in Ref. 3. They were suspended in a buffer containing 20 mM Tris (pH 8.0) with 65% glycerol (v/v) added to prevent freezing when stored at −20°C and to reduce light scattering.

The spectrofluorimeter used for the experiments was described elsewhere [7]. The wavelength of the exciting laser flash was 532 nm with a flash duration of approx. 25 ps. By means of a variable neutral density filter the energy incident on the sample could be adjusted between 10^{-5} and 5 mJ/cm^2. Fluorescence emission spectra were measured with the same apparatus, with excitation by either the laser flash or by a low intensity xenon flash. In the latter case the light was filtered by means of a combination of filters yielding a band with a width of 40 nm, centered at 515 nm. The monochromator for detecting the fluorescence was set at a bandwidth of 1.5 nm for the measurement of fluorescence spectra, and of 4 nm when annihilation at a fixed wavelength was measured. Both the excitation energy and the relative fluorescence were measured with photodiodes (RCA-30810). The absorbance of the samples was kept low (less than 0.05 at 532 nm) in order to achieve a homogeneous light distribution within the sample and to limit the degree of self absorption of fluorescence. Absorption and fluorescence excitation spectra were measured with the apparatus described in Ref. 10.

Changes of the redox state of the reaction center were brought about by continuous background light filtered by a Schott BG-38 filter. All measurements were performed at room temperature.

Results and interpretation

Absorption spectra

Absorption spectra of the three different cultures used in our experiments are shown in Fig. 1. They clearly demonstrate the different pigment composition of the three cultures. Culture 7750 LT contained B880 and B800–820, but no B800–850, 7750 HL contained B880 and B800–850, but no B800–820, while 7050 LL contained all three antenna complexes. In culture 7750 LT the presence of B880 is reflected by an absorption band at 890 nm, in agreement with earlier measurements [3,11]. In the other two cultures this band is only visible as a weak shoulder on the much higher band at 860 nm, due to the presence of B800–850. The spectra also reflect the different carotenoid composition of the two strains under these conditions; 7050 contains rhodopinal and rhodopinal glucoside, whereas 7750 contains mainly rhodopin and rhodopin glucoside [12].

Fluorescence spectra obtained at low excitation intensity

Fluorescence emission spectra for culture 7750 LT are shown in Fig. 2. The fluorescence spectra were recorded in the presence of continuous background illumination (solid line) or in the absence of back-

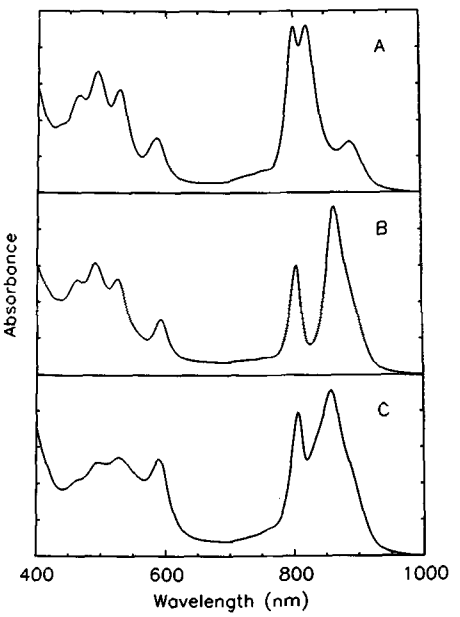

Fig. 1. Absorption spectra of *Rps. acidophila* membranes of the cultures 7750 LT (A), 7750 HL (B) and 7050 LL (C).

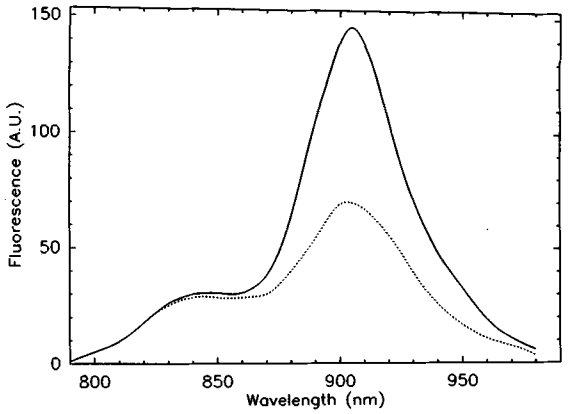

Fig. 2. Fluorescence spectra of *Rps. acidophila* membranes culture 7750 LT obtained with a low intensity xenon flash with P-870 reduced (dotted line) and with P-870 oxidized (solid line) by means of continuous background illumination. A.U., arbitrary units.

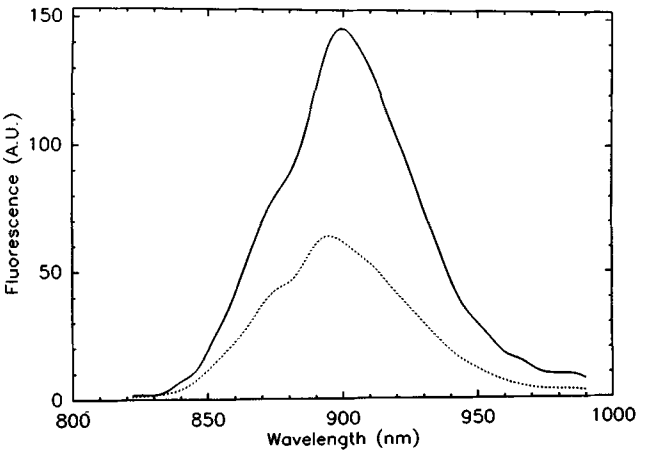

Fig. 3. Fluorescence spectra of culture 7750 HL obtained with a low intensity xenon flash with P-870 reduced (dotted line) and with P-870 oxidized (solid line).

ground illumination (dotted line). Absorbance difference measurements showed that the increase in fluorescence yield caused by the background illumination can be ascribed to photo-oxidation of P-870. It was checked by means of varying the light intensity that P-870 was essentially completely oxidized. The spectra showed two bands with maxima near 840 nm and 903 nm, originating from fluorescence of B800–820 and B880, respectively. The intensity of the band near 840 nm was independent of the redox state of the reaction centers, in contrast to the band at 903 nm which showed an increase of the fluorescence by a factor of about 2.1 upon oxidation of P-870. These observations agree with the notion that only B880 is directly connected to the reaction center [13]. They also show that 'up-hill' energy transfer from B880 to B800–820 does not occur, since otherwise fluorescence changes would also be observed in the band near 840 nm.

The same measurements were performed on *Rps. acidophila* culture 7750 HL. The fluorescence spectra with P-870 oxidized (solid line) or reduced (dotted line) are shown in Fig. 3. The spectra show a peak at about 899 nm and a weak shoulder at approx. 870 nm, originating from B880 and B800–850, respectively. Contrary to culture 7750 LT, there was an increase of the fluorescence intensity over the entire spectrum upon oxidation of P-870, by a factor which varied from 2.4 to 2.0 between 970 and 870 nm. We thus conclude that the fluorescence of both B800–850 and B880 is affected by the redox state of the reaction center, indicating that excitations can be transferred 'up-hill' from B880 to B800–850. It should be noted that this does not mean that both complexes should show the same degree of fluorescence increase, which would only occur if the energy is equilibrated extremely fast between the two complexes. This condition does not apply as was shown before for *Rhodobacter sphaeroides*, of which the fluorescence spectrum, as well as its increase upon P-870 oxidation [14], is similar to that of culture 7750 HL.

Fig. 4 shows the fluorescence spectrum of culture 7050 LL, which contains all three complexes, B800–820, B800–850 and B880. There is a maximum of fluorescence at 900 nm associated with B880. A shoulder near 870 nm, which was less pronounced than in 7750 HL, may be attributed to B800–850. The increase of fluorescence by about a factor 2.0 was independent of the wavelength. It should be mentioned, however, that in this culture the fluorescence due to B800–850 could not be clearly distinguished from the fluorescence of B880. Apart from this, the fluorescence properties of cultures 7050 LL and 7750 HL were very similar.

Fluorescence spectra obtained at high excitation intensity

Fluorescence spectra of *Rps. acidophila* were also obtained using a high intensity laser flash for excitation and compared with those obtained with xenon flash

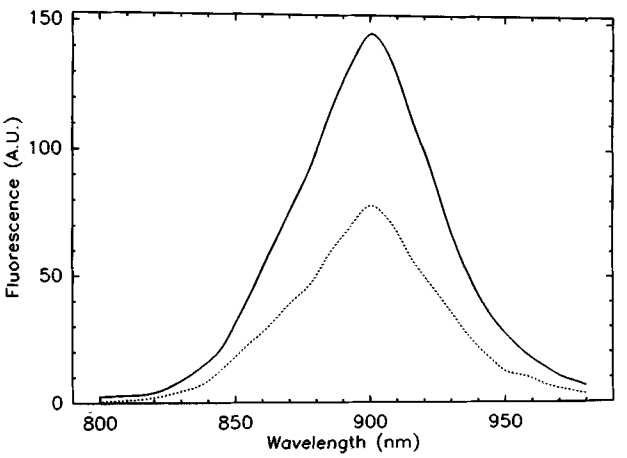

Fig. 4. Fluorescence spectra of culture 7050 LL obtained with a low intensity xenon flash with P-870 reduced (dotted line) and with P-870 oxidized (solid line).

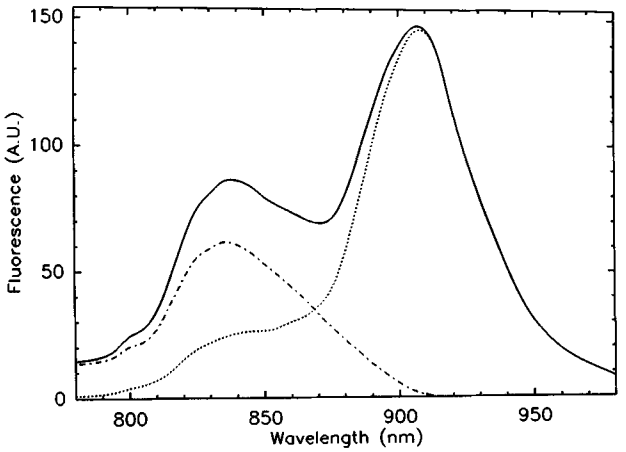

Fig. 5. Relative fluorescence yield as a function of wavelength of *Rps. acidophila* culture 7750 LT with xenon flash excitation (dotted line) and with an intense laser flash of 1 mJ/cm^2 (solid line). The latter spectrum was multiplied by about 40 in order to normalize the spectra at wavelengths longer than 910 nm. The difference of the two spectra is given by the broken line.

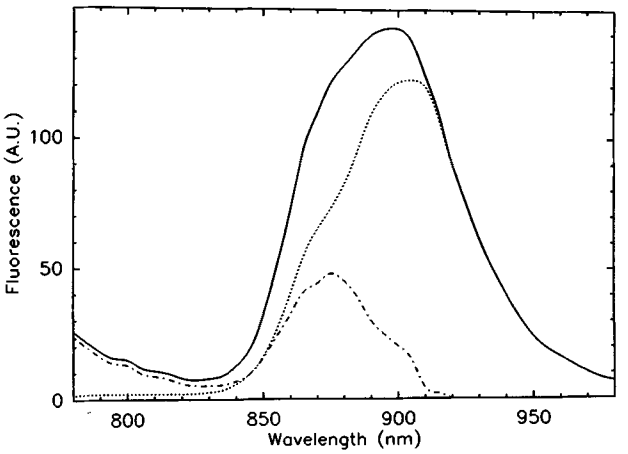

Fig. 6. Fluorescence spectra (normalized) of culture 7750 HL with xenon flash excitation (dotted line) and with an intense laser flash (solid line). The broken line was obtained by subtraction of the two spectra.

excitation. The results of the measurements on culture 7750 LT for the xenon (dotted line) and the laser flash (solid line) are given in Fig. 5. Both spectra were obtained under conditions where P-870 was oxidized. The emission around 840 nm was much more pronounced in the laser flash spectrum than in that of the xenon flash. The difference between the two spectra reflects the different extents of singlet-singlet annihilation in the two pigment complexes, resulting from unequal excitation densities due to rapid energy transfer from B800–820 to B880. Subtracting the two spectra after normalization in the region above 910 nm yielded a band with a maximum at 835 nm which we ascribe to B800–820. However, at the far blue edge of the difference spectrum and of the spectrum obtained with the laser flash, the fluorescence intensity did not approach zero. This is caused by a small amount of 'free' BChl, fluorescing at about 780 nm [15], which hardly shows any singlet-singlet annihilation, and therefore will be strongly enhanced relative to the spectrum obtained with the xenon flash. The same applies to BChl absorbing and fluorescing at about 800 nm, which may be expected to show little annihilation because of rapid energy transfer within B800–820 to BChl absorbing at 820 nm [16].

Measurements on culture 7750 HL are shown in Fig. 6. As with 7750 LT, the quenching was strongest at wavelengths above 910 nm. Subtraction now yielded a band at 875 nm, which may be attributed to B800–850 [11]. Like for culture 7750 LT, this band did not return to zero at the far blue side.

Essentially the same results as those with 7750 HL were obtained with culture 7050 LL (Fig. 7). The spectrum obtained with the laser flash extended somewhat further towards the blue than that of 7750 HL, indicating that in culture 7050 LL some of the fluorescence originates from B800–820.

Annihilation measurements at specific wavelengths

The relative fluorescence yield as a function of the laser flash energy for culture 7750 LT, with B800–820 and B880 only, is shown in Fig. 8. The measurements were performed at 840 and 930 nm, respectively, in the presence of continuous background illumination to keep P-870 oxidized. The measurements at 930 nm may be used to obtain information about the domain size of B880 by applying Eqn. 20 of Paillotin et al. [6]. Generating curves according to that equation for different values of the parameter r and fitting them to the data, we found that at 930 nm the best fit was obtained with $r = 0.7$. Such a small value for r means that the relative efficiency of annihilation per pair of excitations within one domain is high. In general this

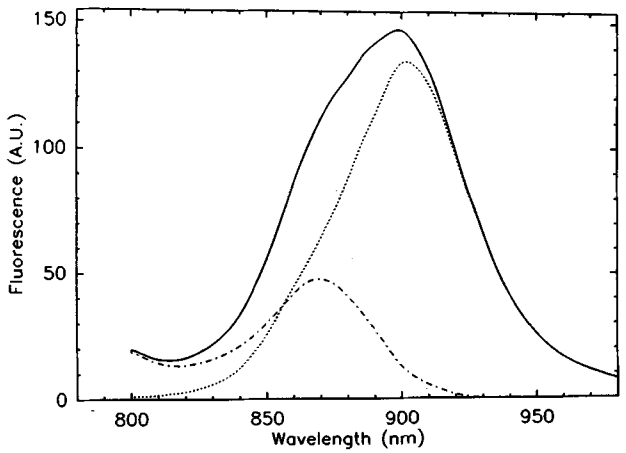

Fig. 7. Fluorescence spectra (normalized) of culture 7050 LL with xenon flash excitation (dotted line) and with an intense laser flash (solid line). The broken line was obtained by subtraction of the two spectra.

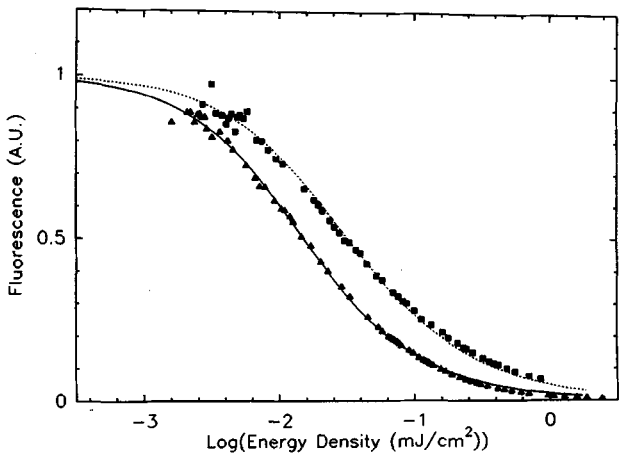

Fig. 8. The relative yield of fluorescence plotted as a function of the incident energy density of the excitation flash for *Rps. acidophila* culture 7750 LT. ■, detected at 840 nm; ▲, detected at 930 nm. The curves are normalized at low energy densities. The data at 930 nm are fitted with Eqn. 20 from Ref. 6 with $r = 0.7$. The dotted curve gives the best fit for 840 nm with $r = 2$ (see text).

implies a small domain size, in contrast with previously performed measurements on purple bacteria [7,8]. The domain size can be calculated when the number of excitations per flash in B880 is known [8].

Fig. 9 shows the excitation spectrum for B880 fluorescence. Comparison with the absorption spectrum shows that the efficiency of energy transfer for quanta absorbed by carotenoid at 532 nm is $45 \pm 5\%$. Using this number, we obtained a domain size of 80 ± 20 BChls in B880, by applying the method and assumptions given in Ref. 8. The true domain size, however, must be smaller than about 80 BChls, because annihilation in B800–820 was neglected in the calculation. Most of the excitations are not directly transferred to B880 but first to B800–820 and only subsequently to

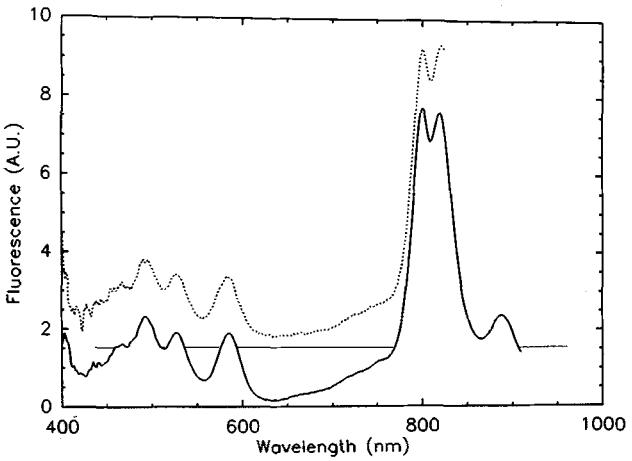

Fig. 9. Fluorescence excitation spectra of culture 7750 LT. The fluorescence was recorded at 930 nm (solid line) and at 840 nm (dotted line), respectively. For clarity, the measurements at 840 nm are shifted upwards. The absorbance of the sample at 800 nm was 0.08.

B880. For a proper analysis of the curve recorded at 930 nm we now have to correct the measurements for annihilation in B800–820, which is given by the curve recorded at 840 nm. This operation requires knowledge about the amount of B800–820 present relative to B880, their relative absorptions at 532 nm and the efficiencies for energy transfer from carotenoid to the BChls in the two complexes.

The efficiency for energy transfer from B800–820 to B880 was more than 90% as can be seen from a comparison of the spectrum of Fig. 9 (solid line) with the absorption spectrum (Fig. 1). From the excitation spectrum of B800–820 fluorescence (Fig. 9) it follows that the efficiency for energy transfer from carotenoid to BChl within this complex is $45 \pm 5\%$. Taking into account that most of the absorption at 532 nm may be ascribed to B800–820, this number agrees with the overall efficiency for energy transfer from carotenoid to B880, mentioned above. Approximately the same efficiency was observed in the isolated B800–820 complex (data not shown). It is significantly lower than that obtained for isolated B800–820 from strain 7050 (70%; see Ref. 11), presumably because of the different type of carotenoid present. From the absorption spectrum in the Q_y-band region we estimated that the ratio between the number of BChls absorbing at 820 and those at 890 nm is 3.0 ± 0.5. If we assume that the same ratio applies to the absorption at 532 nm, it follows that, at low intensity, $75 \pm 10\%$ of the excitations arrive in B880 via B800–820. Using these values we corrected the measurements at 930 nm by the method discussed in Ref. 17 and found an r-value of 0.3 ± 0.2 and a domain size of 50 ± 20 BChls for B880. In spite of the uncertainty involved in this calculation, the result indicates that B880 forms small units, the size of which equals the number of BChls per reaction center as determined by Dawkins et al. [18].

Contrary to the measurements at 930 nm, those at 840 nm could not be simulated by any value of r, because the slope of the experimental curve was smaller than of any theoretical curve. Fig. 8 (dotted line) shows a 'best fit' with $r = 2$; curves for higher r-values are very similar [6]. We will show elsewhere [19] that this effect may occur when the lifetime of the excitations is comparable to the length of the laser pulse. A short lifetime of the excitations in B800–820 originates from rapid energy transfer from this complex to B880. It may be as short as 10 ps as estimated by comparing the fluorescence yield of both complexes and assuming that the lifetime of excitations in B880 is less than 200 ps [20] when P-870 is oxidized.

Results of measurements on culture 7750 HL, with B800–850 and B880 only, are shown in Fig. 10. The measurements were performed at 860 and 930 nm, respectively, with closed reaction centers. For the fluorescence recorded at 860 nm, no proper fit could be

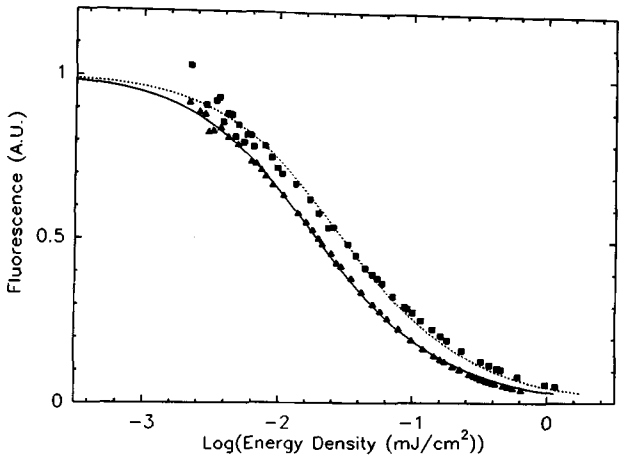

Fig. 10. The relative yield of fluorescence plotted as a function of the incident energy density of the excitation flash for culture 7750 HL. ■, detected at 860 nm; ▲, detected at 930 nm. The data at 930 nm are fitted with Eqn. 20 from Ref. 6 with $r = 2$. The dotted curve gives the best fit for 860 nm with $r = 2$ (see text).

obtained due to the small slope of the curve, presumably for the same reason as given for culture 7750 LT. Moreover, the fluorescence recorded at 860 nm contains an unknown amount of fluorescence emitted by B880, as is clear from Fig. 6. The measurements performed at 930 nm could be fitted with $r \geqq 2$. Although at this wavelength most of annihilation will take place in B880, the effects caused by energy transfer from B880 to B800–850 cannot be neglected for this culture. Therefore, the results cannot be analyzed properly by the equation of Paillotin et al. [6], which is only valid for spectrally homogeneous systems. If, however, the inhomogeneity is disregarded, it can be calculated that the number of connected BChls in B880 is at least about 200, using an overall efficiency of 35–40% for the energy transfer from carotenoid to B880, as determined from the fluorescence excitation spectrum (not shown). This indicates that the domain size may be comparable to the ones previously determined for *Rb. sphaeroides* and *Rb. capsulatus* [7,8].

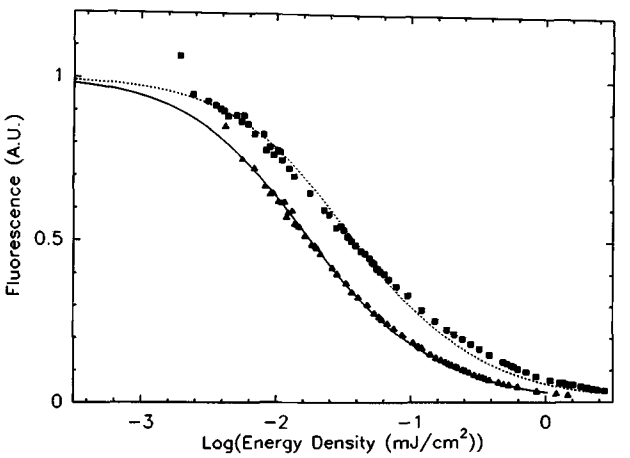

Fig. 11. The relative yield of fluorescence plotted as a function of the incident energy density of the excitation flash for culture 7050 LL. ■, detected at 860 nm; ▲, detected at 930 nm. The data at 930 nm are fitted with Eqn. 20 from Ref. 6 with $r = 2$. The dotted curve gives the best fit for 860 nm with $r = 2$ (see text).

Annihilation measurements for culture 7050 LL at 860 and 930 nm are shown in Fig. 11. The results were similar to those obtained with 7750 HL. Those at 860 nm could again not be fitted to the equation of Paillotin et al., because the slope of the experimental curve is too small. The curve at 930 nm could be fitted with $r \geqq 2$.

Discussion

Our results show that the patterns of energy migration in *Rps. acidophila* are strongly affected by the types of peripheral antenna complexes present in the membrane. From the fluorescence measurements as a function of the redox state of P-870 we concluded that excitations, once arrived in B880, can be transferred back to B800–850, but not to B800–820. The latter effect is likely to be caused by the large energy gap between B880 and B800–820 (0.11 eV), and correspondingly small overlap integral in the Förster equation. The reverse process, energy transfer from B800–820 to B880, turned out to be quite fast and efficient, in spite of the smaller overlap integral than for B800–850. It may be noted, however, that even for the more extreme case of energy transfer from B806 to B870 in *Erythrobacter* sp. OCh 114 rapid and efficient energy transfer was reported [21,22].

The annihilation measurements revealed that the domain sizes of B880 are small in culture 7750 LT where only B800–820 is present, while in the other systems the excitation transfer extends over larger distances. It may be noted here that the domain size as determined for culture 7750 LT seems smaller than the units determined previously at low temperature for

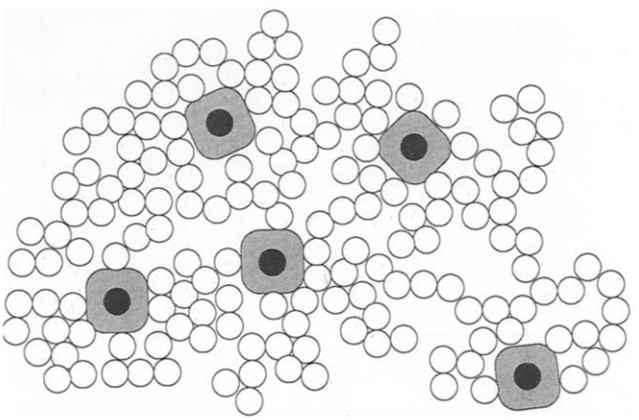

Fig. 12. Model of the photosynthetic antenna of *Rps. acidophila*. The peripheral antennae (B800–820 and/or B800–850, depending on the culture) are indicated by the open circles, the core antenna (B880) by the grey rounded boxes and the reaction centers by the black circles.

Rhodospirillum rubrum [17], which has no peripheral antenna.

The model of the antenna given in Fig. 12 explains all our results. In this model B880 forms small complexes, the size of the photosynthetic unit, containing one reaction center. These units are separated by either B800–820 or B800–850. Our model differs from that proposed by Monger and Parson for *Rb. sphaeroides*, which consists of 'channels' of B880 connecting the reaction centers [23]. Our results show that, at least for culture 7750 LT, the latter model does not apply. Our model also explains the results obtained with the other cultures, since B800–850 is not an efficient energy barrier. Therefore, B800–850 fluorescence will depend on the redox state of P-870 and larger domain sizes for B880 will be observed.

Acknowledgements

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