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SIZE AND STRUCTURE OF ANTENNA COMPLEXES OF PHOTOSYNTHETIC BACTERIA AS STUDIED BY SINGLET-SINGLET QUENCHING OF THE BACTERIOCHLOROPHYLL FLUORESCENCE YIELD

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We have measured the singlet-singlet quenching of the bacteriochlorophyll (BChl) fluorescence yield as a function of excitation intensity in a number of antenna complexes isolated from photosynthetic bacteria. Our results show that the lithium dodecyl sulfate (LDS)-B875, LDS-B800 – 850 and lauryldimethylamine *N*-oxide complexes of *Rhodopseudomonas sphaeroides* contain 8, greater than 25 and greater than 600 BChl *a* molecules, respectively. The size of the *Rhodopspirillum rubrum* B880 complex is greater than 70 BChl *a* and that of the water-soluble BChl *a* complex from *Prosthecochloris aestuarii* about 20–25 BChl *a*. These results are discussed in relation to current models of the arrangement of antenna complexes within the photosynthetic membranes.

Introduction

The light-harvesting apparatus of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* is known to be composed of two species of pigment-protein complexes. On the basis of the results of Gogdell and Crofts [1], the predominant antenna pigment-protein complex, the B800-850 complex, appears to consist of a minimal unit of two B850 BChl molecules, one B800 BChl molecule and one carotenoid per two polypeptides, each having an approximate molecular mass of 9 kDa [2] The second type of antenna pigment-protein complex, the B875 complex, is thought to consist of two BChl and two carotenoid molecules per minimal unit of two polypeptides [3].

These antenna complexes can be isolated using a variety of detergents. Clayton and Clayton [4] isolated the B800-850 complex using the detergent lauryldimethylamine *N*-oxide; LDS-polyacrylamide gel electrophoresis yields both the B800-850 and B875 complexes, which have apparent molecular masses of 227 and 74 kDa, respectively (Hunter, C.N , Pennoyer, J D and Niederman, R A , unpublished observations). In addition, this method permits the isolation of several complexes of intermediate size and mixed composition with B800-850 and B875 BChl in variable ratios.

The light-harvesting apparatus of *Rhodospirillum rubrum* consists solely of a B880 antenna complex which can be isolated with Triton or lauryldimethylamine *N*-oxide and contains two BChl and two carotenoid molecules per minimal

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Abbreviations BChl, bacteriochlorophyll, B800-850, B875, B880, attenna complexes identified by their near-infrared absorbance maxima, LDS, lithium dodecyl sulfate

unit (see Ref. 5 for a review). The aggregation state of B880 in vivo is unknown, although it is likely to be smaller than the B800-850 antenna complex of *Rps. sphaeroides* [6].

The water-soluble BChl *a* complex *Prosthecochloris aestuarii* consists of a trimer of a 50 kDa protein [7] Each subunit holds seven BChl *a* molecules at an average distance of 12 Å; the closest distance between two BChl *a* molecules on different subunits is about 24 Å [8].

In this work we use the technique of singlet-singlet annihilation to determine the functional size of these antenna complexes. The technique measures the number of connected BChl molecules in a complex; this unit in the following will also be called a 'domain' [9]. The phenomenon of singlet-singlet annihilation is described by the equation:

$$BChl^* + BChl^* \xrightarrow{\gamma_2} BChl^* + BChl \qquad (1)$$

If a pair of excitations is present in the domain, a certain probability exists that at least one of them is annihilated upon collision. This probability depends on the ratio of the mono-excitation decay rate, γ_1, which includes losses due to fluorescence, triplet formation, etc., and the bi-excitation rate constant of annihilation, γ_2 [10] The parameter γ_2 depends on the size of the domain, the efficiency of annihilation upon collision (usually assumed to be close to 100%) and the rate of energy transfer in the domain [11].

In general, the fluorescence yield ϕ, as a function of the average number y, of excitations created per domain, is given by an expression derived by Paillotin et al. [10]:

$$\phi(y) = \phi(0) r \sum_{k=0}^{\infty} (-1)^k \frac{y^k}{r(r+1)\ \ (r+k)} \frac{1}{(k+1)} \qquad (2)$$

where $r = 2\gamma_1/\gamma_2$ and $\phi(0)$ is the fluorescence yield at low excitation densities. We will consider two limiting cases. The first occurs if the probability of annihilating all excitations but one present in the domain is close to 100%, which can be expected if the domain is relatively small and the energy transfer between the pigments efficient ($r \ll 1$) Then, ϕ can be calculated by the following equation of Paillotin et al. [10]:

$$\phi(y) = \phi(0) \frac{1 - e^{-y}}{y} \qquad (3)$$

The size of the domain can be found directly at the point on the annihilation curve where on average one excitation per domain is created.

The second case arises if the probability of annihilating one of a pair of excitations present in a domain is very small ($r \gg 1$). This occurs if either the domain is relatively large or the energy transfer between the pigments inefficient. In this case, ϕ is given by an equation originally suggested by Swenberg et al. [10,12]:

$$\phi(y) = \frac{\phi(0) r}{y} ln\left[1 + \frac{y}{r}\right] \qquad (4)$$

The size of the domain is obtained only if r is known. However, usually a minimum size of the domain, assuming a minimum value of r, can be estimated.

Intermediate cases arise if $r \simeq 1$. The shape of the annihilation curve directly gives the value of r using Eqn. 2 which then allows calculation of the domain size [10].

Materials and Methods

B800-850 and B875 complexes were prepared from the intracytoplasmic membrane of photosynthetically grown cells of *Rps sphaeroides* by LDS-polyacrylamide gel electrophoresis as described previously [3]. The B800-850 complex was alternatively prepared using lauryldimethylamine *N*-oxide according to the method of Clayton and Clayton [4]. The B880 complex of *R rubrum* wild type was prepared with lauryldimethylamine *N*-oxide according to the method described by Cogdell and Thornber [13]. The BChl *a* protein of *P aestuarii* was prepared by the method of Olson [7]

Measurements were conducted on detergent-solubilized pigment-protein complexes, the exception being the LDS-B875 complex in which case gel slices were used. Emission spectra of the various preparations were recorded on a spectrofluorimeter. The total fluorescence after an intense picosecond flash was measured using a home-built laser spectrofluorimeter. The excitation pulse was generated by a mode-locked Nd-YAG laser, amplified and frequency doubled to 532 nm, giving a final pulse length of 30 ps and maximum energy of 6 mJ. The emitted fluorescence passed

TABLE I

First column ratio of the absorbances at the excitation wavelength and the main BChl peak Second column ratio of excitation and absorption spectra at 532 nm for fluorescence emitted by the main bacteriochlorophyll species (see Fig 1)

Preparation	$A_{532nm}/A_{BChl\ peak}$	Transfer efficiency
LDS-B875	0 3	0 6
LDS-B800-850	0 07	0 7
Lauryldimethylamine *N*-oxide-B880	0 4	0 35
Lauryldimethylamine *N*-oxide B800-850	0 04	0 95
P aestuarri BChl *a* antenna complex	0 01	1 00

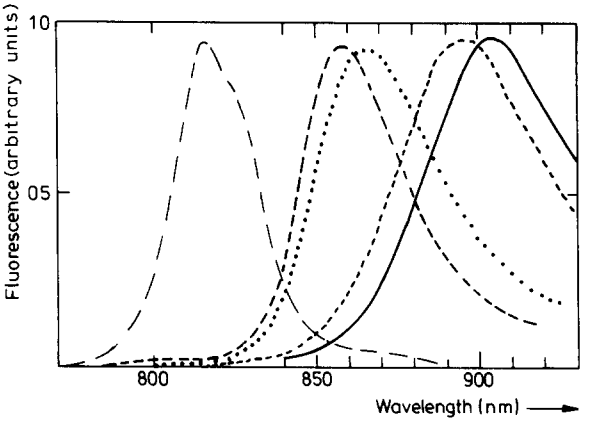

Fig 1 Emission spectra of LDS-B875 (------), LDS-B800-850 ( ) and lauryldimethylamine *N*-oxide-B800-850 (— — —) complexes of *Rps sphaeroides* wild type, B880 of *R rubrum* (———) and the BChl *a* antenna complex of *P aestuarii* (- - -) All spectra were recorded at room temperature and the excitation wavelength was 532 nm The spectra were normalized with respect to their emission maximum

through a monochromator shielded from the 532 nm pulse by a Scott KV-550/2 filter and was detected by a photodiode (RCA C30810) All experiments were performed at room temperature

The number of excitations per BChl generated by the picosecond flash was calculated from the ratio of the absorption coefficients at the excitation wavelength (532 nm) and the main infrared BChl *a* absorption peak and from the efficiency of energy transfer to BChl for excitations absorbed at 532 nm either by carotenoids, when present, or by BChl. These data are summarized in Table I. Values for the energy-transfer efficiency for the antenna complexes from *Rps sphaeroides* were taken from data reported elsewhere [15–17]. For the B880 complex of *R rubrum* the energy-transfer efficiency was determined in a separate experiment (see Results and Discussion). The value for the BChl *a* compelx of *P aestuarii* is assumed to be close to 100%, because this preparation contains no carotenoids

Results and Discussion

The B875 complex of Rps. sphaeroides and the B880 complex of R rubrum

The emission spectra of these antenna complexes are shown in Fig. 1 The LDS-B875 complex of *Rps sphaeroides* emits maximally at 897 nm, the B880 complex of *R. rubrum* at 903 nm For the B880 complex of *R rubrum* the efficiency of energy transfer for excitations absorbed at 532 nm to BChl was found to be rather low (±35%), the value obtained being comparable to that observed for intact cells (31%).

Fig 2 shows the fluorescence yield as a function of the laser pulse intensity for these two antenna complexes, the intensity of the flash has been calibrated in absorbed photons The experimental curve obtained for the LDS-B875 complex of *Rps sphaeroides* (trace B) is in perfect agreement with the curve predicted by Eqn. 3, suggesting a small complex in which annihilation of all but one excitations occurs The intensity at which there is on average one excitation per LDS-B875 complex corresponds to one excitation per eight B875 BChl molecules (at this intensity the fluorescence yield has dropped to 63% of its low-light value). We therefore conclude that the LDS-B875 complex of *Rps sphaeroides* consists of eight connected B875 BChl molecules among which efficient energy transfer takes place This number corresponds to a molecular mass of about 70 kDa, which is not much different from the 74 kDa obtained from electrophoresis data It is noteworthy that, even at the highest pulse intensities, where there are three to four excitations per LDS-B875 complex, no deviation from Eqn. 3 occurs.

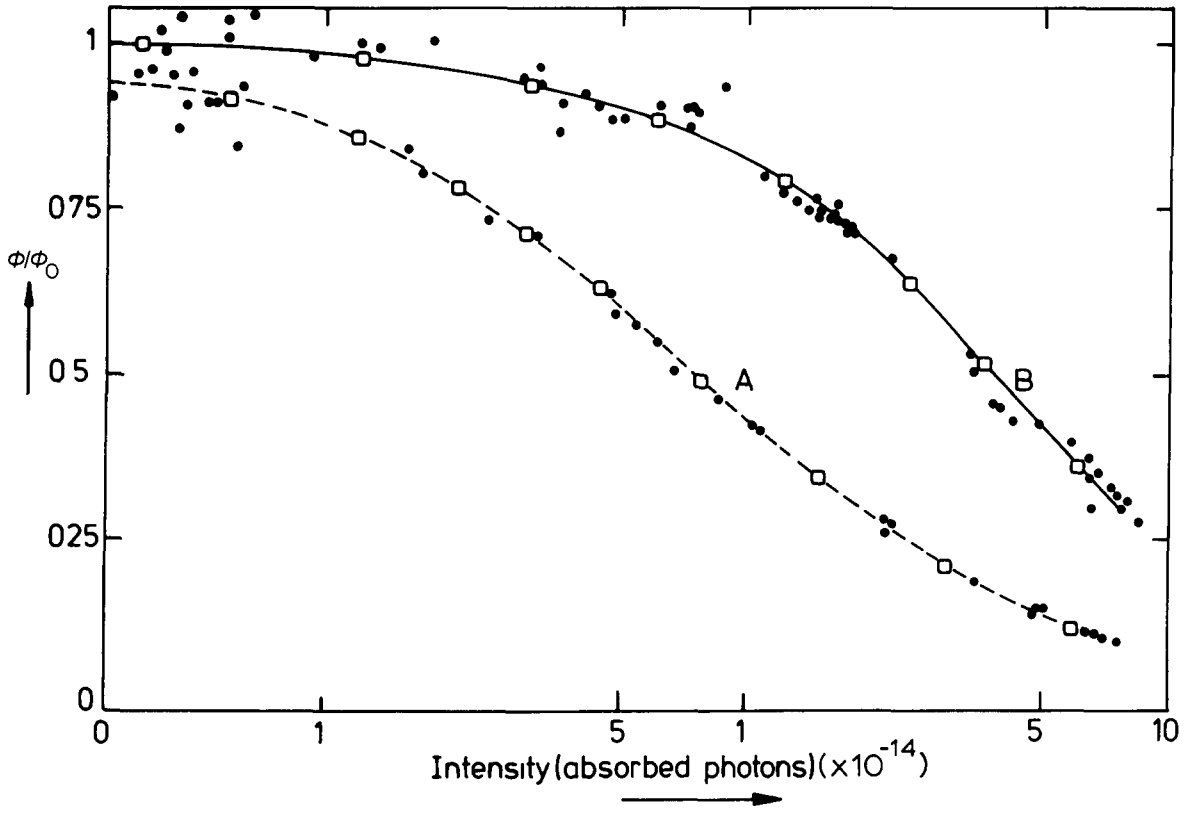

Fig 2 Fluorescence yield as a function of flash intensity for the B880 complex of *R rubrum* (A) and the LDS-B875 complex of *Rps sphaeroides* wild type (B) For both preparations the fluorescence was detected at 900 nm For the LDS-B875 complex ϕ/ϕ_0 was normalized to 1, for the B880 complex of *R rubrum* to 0 94 at low intensities The laser energy is given in absorbed photons The illuminated surface of the cuvette was 0 25 cm^2, the optical path length was 5 mm and the absorbance at 532 nm was 0 1 for both samples The experiment with LDS-B875 (B) was fitted with Eqn 3, that with B880 (A) using Eqn 2 with $r = 0\,5$ The fits are marked in both cases by □-----□

The fluorescence quenching in the B880 complex of *R. rubrum* occurs at somewhat lower flash intensities compared to the LDS-B875 complex of *Rps sphaeroides* (Fig. 2, trace A), which suggests that this antenna complex is larger. The intensity at which at least one excitation per B880 complex occurs corresponds to a minimum complex size of 70 connected B880 BChl molecules. However, the shape of the fluorescence yield vs. intensity curve is not described by Eqn. 3, meaning that the B880 complex is even larger. Using Eqn 2 good fits are obtained for $0.5 \leq r \leq 1$, the fit for $r = 0.5$ is shown in Fig. 2 These values of r suggest that the B880 complex contains between 100 and 140 B880 BChl molecules, among which efficient energy transfer occurs. This indicates that this antenna complex is not a coincidental structure, but that it represents part of the total light-harvesting apparatus of *R rubrum*. In intact chromatophores several of these building blocks may be coupled to reaction centers to form the total pigment matrix of about 800 connected B880 BChl molecules (Bakker, J.G.C and Van Grondelle, R , unpublished observations).

The LDS-B800-850 and lauryldimethylamine N-oxide-B800-850 complexes of Rps sphaeroides

The emission spectra of these two pigment-protein complexes are also shown in Fig. 1. The absorption spectra of the two complexes are identical to those reported elsewhere [14]. We observe that the emission spectrum for the LDS-B800-850 complex is broadened and shifted to longer wave-

lengths compared to the lauryldimethylamine *N*-oxide-B800-850 complex (maxima at 858 and 866 nm, respectively). This agrees with the observation that the LDS-B800-850 has a relatively stronger absorption at the long-wavelength side of the 850 nm absorption band In the LDS-B800-850 complex the 800 nm absorption peak was depressed, which agrees with the absence of the B800 emission band in the emission spectrum of this antenna complex and more 'free' BChl emission at 780 nm was observed in comparison with the lauryldimethylamine *N*-oxide-B800-850 complex These effects are complementary to those noted by Clayton and Clayton [14].

Fig 3 shows the intensity dependence of the B850 fluorescence yield for the two B800-850 complexes of *Rps sphaeroides*. For the LDS-B800-850 complex the annihilation starts only at relatively high intensity, indicative of a small complex. The intensity at which the fluorescence yield is reduced to 63% of its low-light value indicates that the LDS-B800-850 complex in solution contains at least 30 connected BChl *a* molecules. The fluorescence yield of the LDS-B800-850 as a function of flash intensity is best fitted using again the expression by Paillotin et al. (Eqn 2) with $1\,5 \leq r \leq 2$ (Fig. 3 shows the fit for $r = 2$), from which a complex size between 70 and 100 connected BChl *a* molecules can be calculated.

Shiozawa et al. [18] obtained a minimum molecular mass for the B800-850 complex of *R capsulata* of approx 180 kDa, which corresponds to a minimum number of 30 BChl per complex, assuming three BChl *a* molecules per two 9 kDa polypeptides The 227 kDa obtained from electrophoresis data for the LDS-B800-850 complex would give about 35 BChl *a* per complex The fluorescence quenching experiment suggests that in solution two to three of these 30 BChl complexes form larger aggregates, in which the excitations may be transferred from one 30-BChl unit to another, but not very efficiently to explain the large value of *r*

The fluorescence quenching of the lauryldimethylamine *N*-oxide-B800-850 complex starts at very low intensities, indicative of a large number of connected BChl In this antenna complex the intensity dependence of the fluorescence yield is best described by Eqn. 4. The 37% decrease in the fluorescence yield is obtained at an intensity approx. 20-fold lower than that for the LDS-B800-850 complex, which suggest an antenna complex of at least 600 connected BChl *a* molecules. Similar results were obtained for the lauryldimethylamine

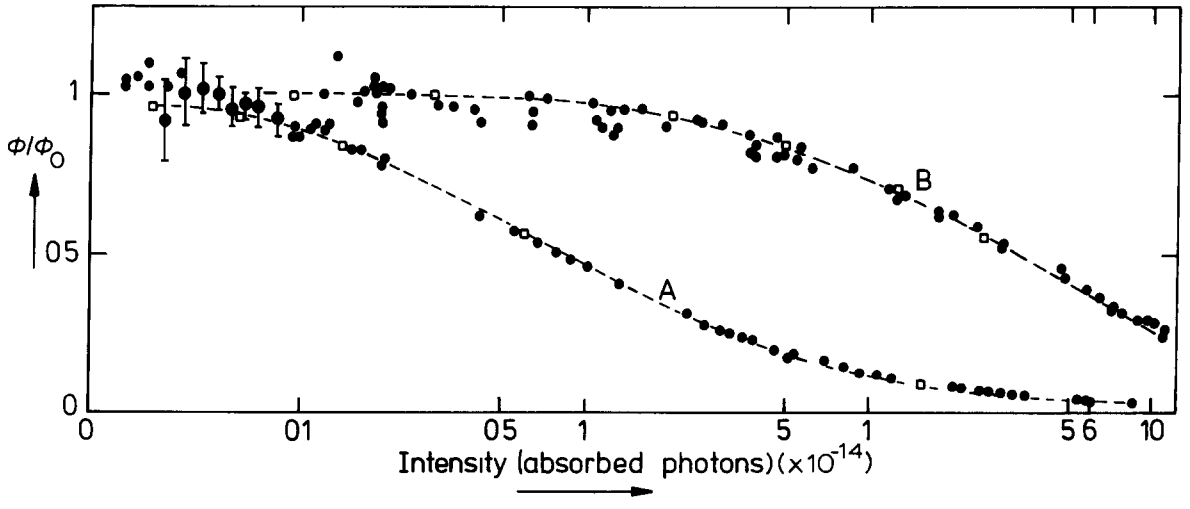

Fig 3 Fluorescence yield as a function of flash intensity for lauryldimethylamine *N*-oxide-B800-850 (A) and LDS-B800-850 (B) of *Rps sphaeroides* wild type The fluorescence was detected at 860 nm, the fluorescence yields are normalized with respect to the low-intensity values The absorbance at 532 nm was 0 1 for both samples The other conditions were as described in the legend of Fig 2 The experiment with LDS-B800-850 was fitted using Eqn 2 with $r = 2$, the one with lauryldimethylamine *N*-oxide-B800-850 with Eqn 4 The fits are marked in both cases by □------□

N-oxide-B800-850 complex of *Rps capsulata*. The size of the lauryldimethylamine *N*-oxide-B800-850 complex in solution obtained from these fluorescence quenching experiments seems rather large However, using a Sepharose CL-4B (Pharmacia Fine Chemicals AB) column the lauryldimethylamine *N*-oxide-B800-850 complex, described in this work, was found in the void volume of this column, indicating a molecular mass of at least 10^4 kDa or a minimum number of 700 BChl molecules per complex We do not know if an antenna complex of this size occurs in the membrane. Because the fluorescence quenching experiment suggests that relatively efficient energy transfer occurs among these 600 or more connected BChl molecules a coincidental aggregation seems unlikely. The finding that the shape of the fluorescence quenching curve in B800-850-rich membranes prepared from *Rps sphaeroides* [19] was very similar to that for the lauryldimethylamine *N*-oxide-B800-850 complex supports this idea.

The BChl a complex of P aestuarii

The emission spectrum of the water-soluble BChl *a* complex of *P. aestuarii* (Fig. 1) shows the typical broad peak centered around 815 nm, in agreement with earlier observations [20] Fig. 4 shows the fluorescence quenching curve obtained for this complex Only at the highest pulse intensities a 20–30% quenching of the emission observed. This is due mainly to the very low absorption of this complex at 532 nm (approx. 1% of the 810 nm absorption, see Table I). Assuming that the fluorescence yield as a function of the pulse intensity is described by Eqn. 3, the highest intensity gives about 0.5 excitation per complex. This yields a size of this antenna complex of 24 ± 4 BChl *a*, which is in good agreement with earlier determina-

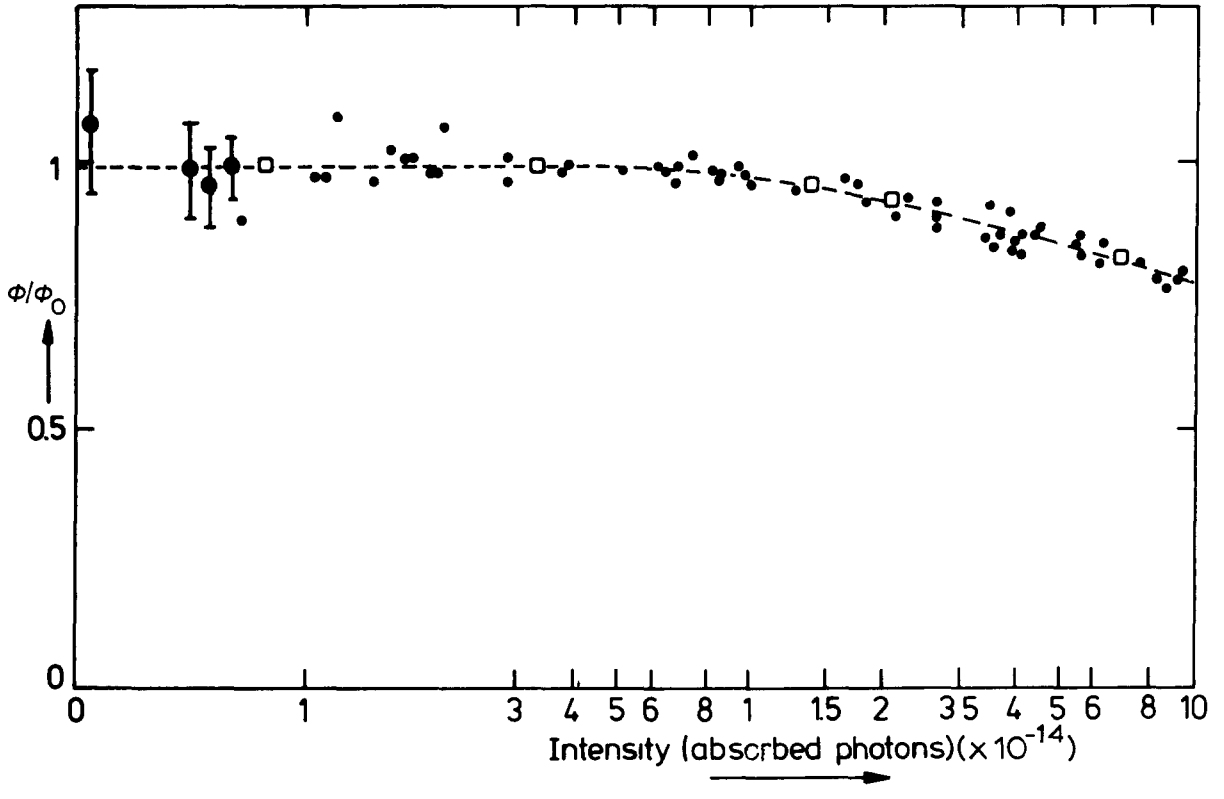

Fig 4 Fluorescence yield as a function of flash intensity for the BChl *a* complex of *P aestuarii* The fluorescence was detected at 815 nm The absorbance at 532 nm was 0 1 Other conditions were as described in the legend to Fig 2 The fluorescence yield is fitted with Eqn 3 (□-----□)

tions [7]. Our results suggest that the three subunits of this complex form a highly organized structure such that efficient energy transfer among all the BChl *a* molecules of the complex occurs. This agrees, of course, with the proposed positions of the BChl *a* molecules within the trimer [8].

Conclusions

To conclude, we may say that the measurement of the fluorescence yield after an intense picosecond flash allows a relatively accurate estimate of the functional size of the various antenna complexes used in this study. Even the size of the small LDS-B875 complex could be obtained In our opinion these experiments suggest the following in vivo structure of the light-harvesting antenna of a photosynthetic bacterium like *Rps sphaeroides*. The large lauryldimethylamine *N*-oxide-B800-850 complex may represent all or a large part of the 'lake' of B800-850 [20]. This is in turn composed of units of 25–30 BChl *a* molecules, since this is the smallest obtainable homogeneous form of this light-harvesting pigment-protein complex [3] This lake is arranged peripherally around much smaller units of the B875 light-harvesting antenna complex [21], 100–140 BChl *a* each, which would then consist of 12–18 units of B875 complexes (eight BChl *a* per complex), approximately four of which could surround and interconnect each reaction center

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