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THE STRUCTURE, FUNCTION, AND ASSEMBLY OF THE LIGHT-HARVESTING ANTENNA OF PHOTOSYNTHETIC PURPLE BACTERIA

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1. INTRODUCTION

The photosynthetic apparatus of photosynthetic purple bacteria such as *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* contains at least two types of major light-harvesting pigment-protein complexes: B800-850 and B875. Species such as *Rhodospirillum rubrum* only contain the latter.

The B800-850 comlex shows absorption at 800 nm, due to BChl 800 and at 850 nm due to BChl 850. It contains about one carotenoid per two BChl molecules.

The B875 complex shows absorption at 875 nm due to BChl 875 and contains one carotenoid per BChl molecule (B875 of *Rps. sphaeroides*).

The B800-850 comlex is found at the periphery of the light-harvesting antenna. B875 forms a central network that interconnects several reaction centers (the "lake model" for energy transfer). The dominating direction of excitation energy transfer is from carotenoid to BChl 800, BChl 850 or BChl 875 and then among the different BChl's finally to BChl 875.

Once in the B875 network, the excitation is transferred among a large number of identical BChl 875 molecules, a process which

can be described as a random walk that the excitation performs on the B875 network, until a reaction center is reached where a charge separation may be initiated.

Three concepts are of fundamental importance for the understanding of this process. Firstly, the excitation may be lost during the transfer process. Secondly, upon arrival at the reaction center, the excitation is not necessarily trapped; it may escape from the trap and resume the hopping process. Thirdly, if an excitation finds a closed or inactive trap, it may escape and, due to the fact that the B875 network connects several reaction centers, try elsewhere.

The emissions of BChl 800, BChl 850, and BChl 875 can be observed at all temperatures and these can be used to study the losses that occur in each pool of antenna molecules separately. In addition absorption and linear- and circular-dichroism spectra are available.

Using lithium dodecyl sulfate polyacrylamide gel electrophoresis (LDS-PAGE), a separation of the two antenna complexes in a relatively intact state is obrained[1,2]. In addition several "intermediate" complexes are separated that contain various ratios of BChl 800 and BChl 850.

The amplitude of BChl 800 absorption in all these complexes is rather low, but incubation with LDAO restores this completely.

In this paper I will shortly deal with the spectroscopic properties of these preparations, discuss the transfer and trapping of excitations in the intact system and, finally, make a few comments about the mechanism of association of these comlexes to a fuctioning antenna.

2. THE B800-850 COMPLEX

Figure 1 shows a model for the pigment organization in the B800-850 comlex based on a combination of spectroscopic and biochemical

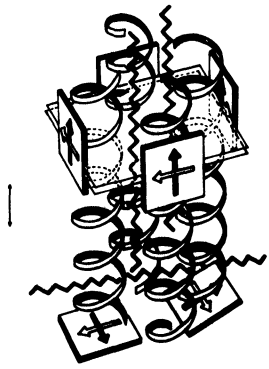

FIGURE 1 Schematic model of the B800-850 complex. The upper square boxes are the porphyrin heads of BChl 850, the lower ones of BChl 800. Open arrows: Q_Y transitions, solid arrows: Q_X transitions. Zig-zag lines are carotenoids (spheroidene) and the spirals represent the α-helical sections of the constituent peptides. The plane of the membrane is horisontal; the vertical bar represents 5 Å. From ref. 6, with permission.

data[3-6]. A minimum unit of B800-850 must contain at least 4 BChl 850, 2 BChl 800, and 3 carotenoid molecules. These pigment molecules are non-covalently bound to two idendical pairs of proteins (α and β), each of which contains a central hydrophobic stretch of amino acids, that is supposed to the α-helical and transmembrane[7,8]. Each apoprotein contains a central histidine at about the same position, which may be the binding site for BChl 850. Two of the four subunits (β) contain a second histidine at about 20-25 Å, measured along the α-helix, from the first, at the hydrophobic/hydrophylic interface. These may be the binding sites for the two BChl 800's.

The BChl 850's have their porphyrin heads arranged in such a way that the Q_Y's are all parallel and the Q_X's are all perpendicular to the membrane plane. In Figure 1 the four BChl 850 Q_Y's

form a circularly degenerate oscillator to account for the observed fluorescence polarization.

If each of the four porphyrin heads shows a small displacement along the normal of the membrane plane, the four BChl 850 Q_Y's may form a left-handed helix, and this induces sufficient rotational strength to account for the observed circular dichroism.

The two BChl 800 molecules have their porphyrin heads almost in the plane of the membrane with the Q_X's and Q_Y's mutually perpendicular. The BChl 800 Q_X's may be tilted out of the membrane plane but at most 25°. The distance between the centers of the two BChl 800 porphyrin heads must be less than 19 Å. From the observed BChl 800 fluorescence yield at low temperatures the BChl 800 - BChl 850 dipole-dipole distance may be estimated to be smaller than 21 Å, if transfer to only one BChl 850 takes place, or, less than 24 Å if two equivalent BChl 850's are involved. The latter value appears to give the best agreement with the structural data.

The carotenoid content of the B800-850 comlex is heterogeneous. About one-third of the carotenoids transfers its excitation exclusively to BChl 800, while the remaining two thirds transfer their excitation energy to BChl 850. The former carotenoid is oriented more or less parallel, the latter perpendicular to the membrane plane. The BChl 800 carotenoid shows a red shifted absorption.

At room temperature energy transfer between the BChl 800's and BChl 850's is fast enough to establish a thermal equilibrium between the excitation densities on both types of BChl molecules. At low temperatures (4 K) the rate of energy transfer from BChl 800 to BChl 850 will be about $3 \cdot 10^{12}$ s^{-1} to account for the observed losses. The low temperature spectroscopic data do not give any indication for a further heterogeneity of the BChl 800 absorption band.

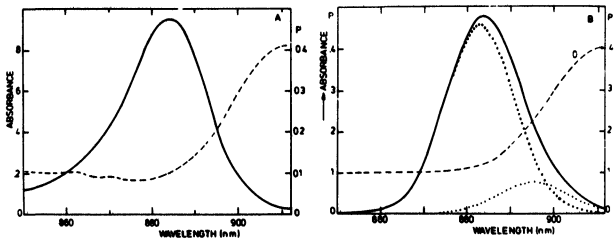

FIGURE 2 Measured (A) and simulated (B) absorption (———) and fluorescence polarization (- - - - -) spectra of chromatophores of Rps. sphaeroides R26. The dashed curves in B represent the major, λ_{max} = 883 nm, and minor, λ_{max} = 896 nm, BChl species present in the B875 antenna. The spectra were recorded at 4 K. From ref. 11, with permission.

3. THE B875 COMPLEX

The minimum unit of B875 probably contains about 6 BChl molecules[9]. At room temperature the polarization of the fluorescence increases somewhat upon excitation in the red wing of the long wavelength absorption band[10,11]. This effect is dramatically enhanced at low temperatures.

In intact chromatophores after cooling below 77 K similar effects are observed. (See Figure 2.) However, at room temperature the polarization of the emission is constant ($p \simeq 0.12$) upon excitation over the whole long wavelength absorption band[11]. To explain this observation it was proposed that each B875 hexamer contains one special BChl molecule, BChl 896, that at low temperatures is responsible for all the emission. At room temperature a thermal equilibrium will exist between the excitation densities on BChl 875 and BChl 896 and this will lead to a decrease in the observed fluorescence polarization in isolated complexes, due to strongly overlapping emission bands. In the intact membrane, at room temperarute energy transfer between different BChl 896 molecules will occur, which leads to the complete absence of these effects.

We note that the proposed BChl 896 appears similar to the "minor form" BChl introduced by Borisov c.s.[12,13]. However, the properties that these authors claim for their special antenna appear in disagreement with those of BChl 896 as discussed above. From these data no evidence can be obtained that supports the hypothesis that BChl 896 forms a special antenna that connects the bulk BChl 875 with the reaction center.

4. TRAPPING, LOSS, AND ANNIHILATION OF EXCITATIONS IN PHOTOSYNTHETIC SYSTEMS

In the intact photosynthetic system the BChl 875 molecules form a network (more or less homogeneous) that connects several reaction centers. At moderate and high picosecond pulse intensities ($\gtrsim 10^3$ photons/cm^2 per pulse) more than one excited BChl 875 molecule may be generated in this network, or domain, and due to extensive excitation transfer two excitations may collide and annihilate at least one of the pair according to the following scheme.

$$\text{BChl}^* + \text{BChl}^* \xrightarrow{k_2} \text{BChl}^* + \text{BChl}$$

The "rate constant" k_2 includes the hopping of both excitations among the BChl 875 molecules and the actual probability of annihilation upon collision. The annihilation competes with the trapping and loss processes at moderate and high pulse intensity and it can be shown that a study of the yield of trapping and losses allows for a determination of the various rate constants involved in the process[14,15]:
the rate of energy transfer between nearest neighbors, k_h, the rate of trapping by active and inactive reaction centers, k_t^o and k_t^c respectively and the rate of annihilation upon collision, k_a. Moreover estimates about the number of connected photosynthetic units (the "domain size") can be obtained.

To do so we must make a simplified model for the B875 network.

In the following it is assumed that the domain may be represented by a square lattice. Each domain contains λ reaction centers, that are distributed regularly. The number of antenna molecules per reaction center is N and energy transfer takes place to nearest neighbors only. The reaction center occupies only a single lattice point and the rates of excitation transfer to and from the reaction center are assumed to be equal.

We then calculate the probabilities of trapping by an open, f_t^o, or a closed trap, f_t^c, for a single excitation:

$$f_t^{o,c} = [N(1-z)[G_N(o;z) + \eta_t^{o,c}/(1-\eta_t^{o,c})]]^{-1}\Big|_{z=1-\varepsilon}$$

where $\varepsilon = k_\ell/(k_\ell + 4k_h)$ and $1-\eta_t^{o,c} = k_t^{o,c}/(4k_h + k_\ell + k_t^{o,c})$ with k_ℓ the rate of loss processes, including fluorescence, for each antenna molecule $G_N(o;z)$ is the Greens function of the lattice representing the photosynthetic unit.

Very similarly we fing for the probability of annihilation, f_a, of one excitation of a pair of excitations in a domain, in the absence of trapping:

$$f_a = [N_D(1-z)[G_{N_D}(o,z) + \eta_a/(1-\eta_a)]]^{-1}\Big|_{z=1-\varepsilon}$$

where N_D is the number of pigment molecules per domain ($N_D = \lambda N$) and η_a is the probability that the excitations will escape annihilation upon collision:

$$1-\eta_a = k_a/(2k_\ell + 8k_h + k_a)$$

G_{N_D} is the Greens function of the lattice representing the domain. We note the occurance of N_D in the expression for f_a,

Using these probabilities $f_t^{o,c}$ and f_a we can define a set of <u>effective</u> rate constants for trapping by open/closed traps, $k_t^{o,c}$, and for annihilation, k_2:

$$k_t^{o,c} = k_\ell \cdot f_t^{o,c}/(1 - f_t^{o,c})$$

$$k_2 = k_\ell \cdot 2f_a/(1 - f_a)$$

We will be able to describe the competition between annihilation and trapping after one additional assumption. This concerns the fact that even if all the reaction centers in a domain are open before the pulse, a fraction of them will become closed during the pulse. For the description of trapping in such a mixture of open and closed traps no exact theory is available and therefore we make the following approximations: if a fraction x of the traps in a domain is closed, the rate of trapping in such a domain is given by:

$$k_1(x) = (1-x)k_1^o + xk_1^c$$

It is possible to show that although this expression is not exact, it is nevertheless a good approximation[15].

To take into account the fluctuations occurring in each domain and moreover the fact that there are many domains in a photosynthetic system, we use a Pauli-Master equation[14-16]. This leads to an expression for the total probability of loss per excitation, $U_n(z)$, and the total fraction of traps closed, $V_n(z)$, where n is the average number of open reaction centers in a domain before the pulse is given ($o \leqslant n \leqslant \lambda$) and z is the average number of excitations genereated per domain.

Figure 3 shows an experiment with chromatophores of the purple bacterium R. rubrum[15]. The excitation wavelength was 532 nm and the pulse width about 30 ps. The fluorescence was detected at 900 nm. The fluorescence yield vs pulse intensity was measured with either all the traps initially closed (state $P875^+$) or open. Moreover the fluorescence yield induced by a weak Xenon flash, fired about 1 ms after the picosecond laser pulse was measured and in principle this yields the fraction of traps closed by the intense laser pulse.

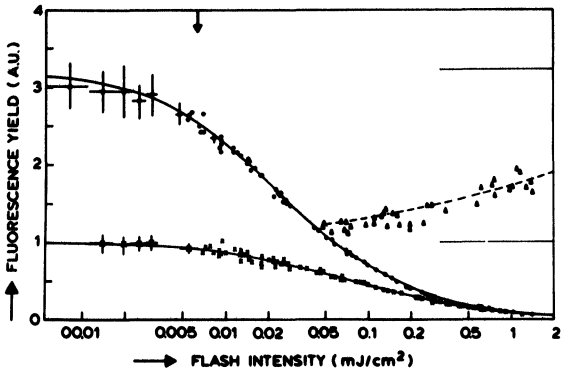

FIGURE 3 Fluorescence yield as a function of the pulse intensity in R. rubrum chromatophores, with all reaction centers in the closed state by continuous background illumination; x, with all the reaction centers open before the pulse. The fluorescence detected with a weak xenon flash 1 ms after the laser pulse in the case where the reaction centers are initially all open is shown by the open triangles (△). The arrow indicates the intensity of the laser flash where there is on the average on excitation per domain. From ref. 15, with permission.

The fits shown in Figure 3 were done for $\lambda \simeq 14\text{-}17$, $N_D \simeq 700\text{-}800$, $k_\ell = 5 \cdot 10^8\ s^{-1}$, $k_1^o = 9.5 \cdot 10^9\ s^{-1}$.

It is calculated from the random walk expressions that these values correspond to

$$k_h > 10^{12}\ s^{-1}$$

$$k_t^o = (4\text{-}6) \cdot 10^{11}\ s^{-1}$$

$$k_t^c = 1.4 \cdot 10^{11}\ s^{-1}$$

$$k_a > 5 \cdot 10^{12}\ s^{-1}$$

If $k_a \gg k_h$ is assumed, or perfect annihilation upon a collision of two excitations, it follows that: $k_h = (1\text{-}2) \cdot 10^{12}\ s^{-1}$.

Using the Förster expression for excitation energy transfer between two neighboring antenna molecules, it is found that the

"average" BChl 875 - BChl 875 dipole-dipole distance is about 13-15 Å. It may be noted that these numbers are in close agreement with those given be Freiberg et al. (see these proceedings) from an analysis of the fluorescence lifetimes.

We finally remark that very similar results were obtained with chromatophores of Rhodopseudomonas capsulata.

5. THE ASSEMBLY OF THE LIGHT-HARVESTING ANTENNA OF RHODOPSEUDOMONAS SPHAEROIDES

Aerobically grown cells of Rps. sphaeroides will develop a photosynthetic light-harvesting system if the oxygen tension is low enough. This enables one to study the membrane bound pigment-protein complex over a wide concentration range compared to e.g. membrane area. Moreover, using sucrose density centrifugation, a pigmented band can be separated (UPB) that is probably a precursor of a true chromatophore[17,18]. Excitation annihilation and fluorescence emission spectra were studied in a number of these preparations at different stages of development[19]. The largest contrast was found between 0hr UPB/0hr chromatophores (0hr reflects the fact that these membranes were isolated almost immediately after interrupting the O_2-flow) and 40hr chromatophores.

In all preparations energy transfer from all the pigments to BChl 875 was good, although the 0hr UPB had an increased BChl 850 fluorescence level[19,20]. The fluorescence yield versus pulse intensity curves for the 0hr preparations were shifted to about 6-fold higher intensities (see Figure 4) implying the existence of much smaller domains, approximately 100-200 BChl 875 molecules (3-6 connected reaction centers). The 40hr preparation gave a domain size of about 1000-2000 connected BChl molecules. Intermediate preparations gave intermediate results. In general UPB fractions have smaller domains than chromatophores.

Thus, even at the extremely early stages of development,

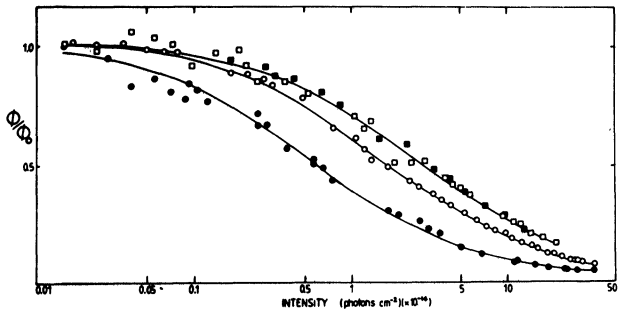

FIGURE 4 Fluorescence yield as a function of flash intensity for membranes isolated from Rps. sphaeroides. The excitation flash was a 30 ps, 532 nm pulse from a frequency doubled, modelocked Nd^{3+}-YAG laser. The fluorescence of all preparations was detected at 900 nm. The absorbance for all samples at 532 nm was 0.1. Φ/Φ_o was normalized to 1.0 at low intensities. The symbols for the curves are: □ 0hr UPB; ■ 0hr ICM; ○ 21hr UPB; ● 40hr ICM. From ref. 19, with permission.

several reaction centers are connected. There is about a five to ten-fold increase in domain size, and an apparent increase in the energy transfer efficiency during development.

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