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PIGMENT ORGANIZATION OF THE B800-850 ANTENNA COMPLEX OF *RHODOPSEUDOMONAS SPHAEROIDES*

HERMAN J.M. KRAMER [a], RIENK VAN GRONDELLE [b], C. NEIL HUNTER [c], WILLEM H.J. WESTERHUIS [a] and JAN AMESZ [a]

[a] *Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden,* [b] *Department of Biophysics, Physics Laboratory of the Free University, De Boelelaan 1081, 1081 HV Amsterdam (The Netherlands) and* [c] *Department of Biochemistry, University of Bristol, Medical School, University Walk, Bristol BS8 1TD (U.K.)*

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The B800–850 antenna complex of *Rhodopseudomonas sphaeroides* was studied by comparing the spectral properties of several different types of complexes, isolated from chromatophores by means of the detergents lithium dodecyl sulfate (LDS) or lauryl dimethylamine *N*-oxide (LDAO). Fluorescence polarization spectra of the BChl 800 emission at 4 K indicated that rapid energy transfer between at least two BChl 800 molecules occurs with a rate constant of energy transfer $k_{ET} > 3 \cdot 10^{12}$ s^{-1}. The maximal dipole-dipole distance between the two BChl 800 molecules was calculated to be 18–19 Å. The porphyrin rings of the BChl 800 molecules are oriented parallel to each other, while their Q_y transition moments are mutually perpendicular. The energy-transfer efficiency from carotenoid to bacteriochlorophyll measured in different complexes showed that two functionally different carotenoids are present associated with, respectively, BChl 800 and BChl 850. Fluorescence polarization and linear dichroism spectra revealed that these carotenoids have different absorption spectra and a different orientation with respect to the membrane. The carotenoid associated with BChl 800 absorbs some nanometers more to the red and its orientation is approximately parallel to the membrane, while the carotenoid associated with BChl 850 is oriented more or less perpendicular to the membrane. The fluorescence polarization of BChl 850 was the same for the different complexes. This indicates that the observed polarization of the fluorescence is determined by the smallest complex obtained which contains 8–10 BChl 850 molecules. The B800-850 complex isolated with LDAO thus must consist of a highly ordered array of smaller structures. On basis of these results a minimal model is proposed for the basic unit consisting of four BChl 850 and two BChl 800 and three carotenoid molecules.

Introduction

The light-harvesting pigments of the bacterial photosynthetic membrane are contained in pigment-protein complexes [1,2]. The major light-harvesting pigment-protein complex of *Rhodopseudomonas sphaeroides* is B800-850. In the intact membrane it is organized in large 'lakes' which surround the network of B875 antennae, that connect the reaction centers [3–5]. The B800-850 complex can be isolated from the membrane by various procedures and its properties have been characterized rather extensively [6–12]. The complex isolated by means of the detergent LDAO (B800-

Abbreviations: BChl, bacteriochlorophyll; B800-850 and B875, antenna complexes identified by their near-infrared absorption maxima; BChl 800 and BChl 850, bacteriochlorophyll molecules with their near-infrared absorption maximum; LDS, lithium dodecyl sulfate; LDAO, lauryldimethylamine *N*-oxide; LD, linear dichroism; CD, circular dichroism.

850/LDAO) [7] is though to consist of a basic unit of 3 BChl molecules (1 BChl 800 and 2 BChl 850 and possibly one carotenoid per pair of polypeptides [4,13]. Alternatively, when isolated by means of LDS, the complex (B800-850/LDS) contains almost only BChl 850 and little BChl 800 [9,12]. The size of the B800-850/LDS complex was found to be much smaller than that of the B800-850/LDAO complex [14]. It has been shown that the BChl 800 absorption band can be reconstituted by dialyzing the B800-850/LDS complex against LDAO [10].

The organization of the pigment molecules within the complex has been studied by measurement of the energy-transfer efficiency [11–15], singlet-singlet-annihilation [14], fluorescence polarization [16–18], linear dichroism [18,19] and circular dichroism [8,18]. It has been found that the total efficiency of energy transfer from carotenoid to BChl is high (over 90%). The transfer probability from carotenoid directly to BChl 850 was found to be twice as large as that directly to BChl 800. Measurement of the fluorescence yield of BChl 800 at low temperature indicated a BChl 800-BChl 850 dipole-dipole distance of less than about 21 Å [11]. Measurement of fluorescence polarization and LD measurements of the B800-850/LDAO complex have yielded detailed information about the orientation of the transition moments of the pigment molecules [16,17,19]. On the basis of these results Breton et al. [17] constructed a model, according to which two weakly coupled Q_y transition moments of BChl 850 are almost perpendicular to each other, while the Q_x transition moments are more or less perpendicular to the plane formed by the two BChl 850 Q_y transition moments. The porphyrin ring of BChl 800 was proposed to lie almost parallel, while the carotenoid was though to be more or less perpendicular to this plane [17].

The intense nearly conservative CD around 850 nm has been interpreted to indicate relatively strong exciton coupling between the Q_y transition moments of the two BChl 850 molecules [8,19]. This point was not taken into account in the model used by Breton et al. Moreover, the model is based on the hypothesis that the fluorescence properties of the B800-850/LDAO complex are determined only by the smallest unit which was thought to contain three BChl molecules. However, the B800-850/LDAO complex is extremely large [14] and efficient energy transfer occurs among many BChl 850 molecules. This implies that other configurations for BChl 850, e.g., a random configuration, should also be considered as long as the Q_y transition moments are supposed to be approximately in the same or in parallel planes.

This work reports a study of the optical properties of B800-850/LDAO, of the much smaller B800-850/LDS complex and of the reconstituted B800-850/LDS-LDAO complexes. The results show that the basic unit contains at least six BChl and three carotenoid molecules. Two of the carotenoids are associated with BChl 850; the third one with BChl 800. The complexes in detergent are highly regular aggregates of the basic unit. Part of these results has been reported in preliminary form elsewhere [20].

Material and Methods

Rhodopseudomonas sphaeroides strain 2.4.1 was grown as described elsewhere [21]. Chromatophores were prepared by means of a French press at $3 \cdot 10^5$ Pa or by 10 min sonication at 0°C. The B800-850/LDAO complex was prepared using the method of Clayton and Clayton [7]. The preparation was done in the dark at 4°C to avoid the formation of BChl *a* emitting at 790 nm. The B800-850/LDS complex and 'intermediary' complexes of B800-850 and B875 were obtained by polyacrylamide gel electrophoresis of chromatophores solubilized by LDS using the method of Broglie et al. [9]. The complexes were removed from the gel by homogenization of the gel slices in a solution containing 50 mM Tris buffer (pH 7.5).

The B800-850/LDS complexes obtained in this way showed only a weak BChl 800 absorption band. The ratio of the absorption bands at 800 and 850 nm measured at room temperature varied somewhat from preparation to preparation, but was always lower than 0.25. This ratio could be reduced even further by dialyzing the complex with 0.1% LDS. Reconstitution of the BChl 800 absorption band was obtained by dialyzing the B800-850/LDS complex against 0.1% LDAO [10]. The reconstituted complex will be called B800-

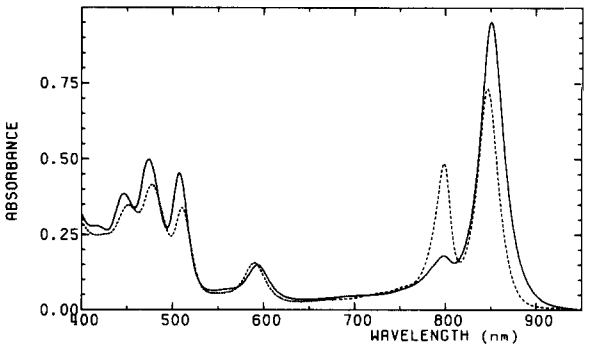

Fig. 1. Absorption spectra of the B800-850/LDS (———) and of the B800-850/LDS-LDAO (- - - - - -) of *Rhodopseudomonas sphaeroides* at room temperature. The two spectra are not exactly comparable, because some dilution of the sample occurred upon dialysis against LDAO.

850/LDS-LDAO.

Low-temperature emission, excitation, absorption, fluorescence polarization and LD spectra were obtained with a single beam spectrophotometer described elsewhere [22]. CD spectra were recorded with an apparatus briefly described in Ref. 23. To keep the samples clear upon cooling, 0.5 M sucrose and 50% glycerol was added. For the LD spectra the complexes were suspended in a polyacrylamide gel. Unless otherwise stated, the preparations were oriented in a plane by pressing the gel in one direction [24].

Results and Interpretation

Absorption and emission spectra

Fig. 1 (solid line) shows the absorption spectrum of the B800-850/LDS complex. As noted earlier [9,10,12] the BChl 800 absorption band has almost disappeared in this complex by the LDS treatment. Exposure of the B800-850/LDS complex to LDAO (0.1%) resulted in complete recovery of the BChl 800 absorption band (Fig. 1, broken line). Together with the reappearance of the BChl 800 band the BChl 850 band narrowed, and shifted to shorter wavelength, as has been noted before [10].

The spectral changes accompanying the reconstitution of the BChl 800 band upon exposure of B800-850/LDS to LDAO are seen more clearly in the absorption spectra of the complexes recorded at 4 K, given in Fig. 2. Again a blue shift and narrowing of the BChl 850 band is observed. In the visible part of the spectrum an absorption increase at 588 nm is observed due to the reappearance of the Q_x band of BChl 800 and a loss of intensity and red shift of the carotenoid absorption bands. The weak band at 893 nm is probably due to some residual B875. This small amount of BChl 875, which was always observed if the complex was prepared from LDS-treated chromatophores, was destroyed by LDAO incubation. The emission spectrum of the B800-850/LDS complex recorded at 4 K (Fig. 2) showed a main emission band at 884 nm with an appreciable shoulder at 901 nm due to emission of the small B75 fraction. The emission spectrum of the B800-850/LDS-LDAO complex consisted of a single band peaking at 873 nm. The absorption and emission spectra of the B800-850/LDAO complex (not shown) were identical to those of B800-850/LDS-LDAO.

Energy transfer from carotenoid to bacteriochlorophyll

The excitation spectra of the BChl 850 emission for B800-850/LDS and B800-850/LDS-LDAO recorded at 4 K are given in Fig. 3. From these spectra, the energy-transfer efficiency from carotenoid to BChl 850 was calculated by comparing the ratios of the amplitudes of the 515 nm carotenoid band and the Q_x transition of BChl at 590 nm in the absorption and excitation spectra. To avoid errors due to scattering, the amplitude of the Q_x band was taken from the difference in ab-

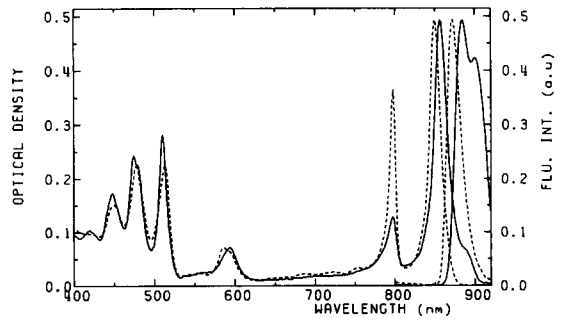

Fig. 2. Absorption spectra of the B800-850/LDS (———) and the B800-850/LDS-LDAO (- - - - -) complex recorded at 4 K. The spectra were normalized at their long-wavelength maxima. The corresponding emission spectra (normalized) upon excitation at 590 nm are also shown.

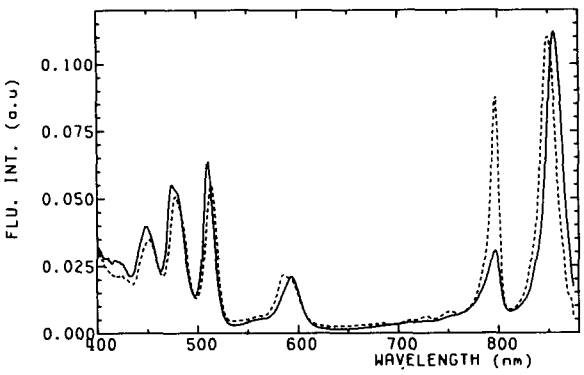

Fig. 3. Excitation spectra of the B800-850/LDS (———) and the B800-850/LDS-LDAO (- - - - - -) complexes recorded at 4 K. The detection wavelengths were 875 and 880 nm, respectively.

sorbance at the peak wavelength near 590 nm and the minimum at 630 nm. The energy transfer efficiency from carotenoid to BChl 850 was found to be between 70 and 75% for the B800-850/LDS complex, both at 4 K and at room temperature. Since there was some residual BChl 800 in the preparation, this value agrees well with the number calculated for the efficiency of direct energy transfer from carotenoid to BChl 850 in the B800-850/LDAO complex [11]. The efficiency of energy transfer increased to 90–100%, when the complex was exposed to LDAO. The latter value is identical to that found for the B800-850 complex directly prepared in LDAO [11]. The efficiency of energy transfer from BChl 800 to BChl 850 was also close to 100% both in the B800-850/LDAO and in the reconstituted complex. These results therefore support the notion [11] that there are two pools of carotenoid. One of these, comprising one third of the total pool transfers its energy to BChl 800, the remaining two thirds directly to BChl 850. Upon dislocation of BChl 800 from its proper binding site by LDS [10] the carotenoid associated with BChl 800 is unable to transfer excitation energy to BChl 850.

Fluorescence polarization

The polarization of BChl 850 fluorescence was measured for the B800-850/LDS, B800-850/LDS-LDAO and B800-850/LDAO complexes (Table I). Data are also given for an 'intermediary' complex B800-850/B875 (see Materials and Methods) which showed a ratio of the absorbance at 850 and 875 nm of 1.1. The size of the intermediary complex was determined both by singlet-singlet annihilation (R. van Grondelle, C.N. Hunter, J.G.C. Bakker and H.J.M. Kramer, unpublished data) and by comparison of its rate of migration on the gel with that of the B875 and B800-850 complexes [9,12]. By both methods we found that the B800-850/B875 contains 8–10 BChl 850 molecules. The degree of polarization of the BChl 850 emission upon excitation in the BChl 850 and BChl 800 Q_y absorption bands was 0.14 ± 0.01 for all complexes studied. This value was constant across the absorption band in agreement with that found by Breton at al. [17] for B800-

TABLE I

p-VALUES AT VARIOUS WAVELENGTHS AND TEMPERATURES

The standard errors are ±0.02 for B850 emission and ±0.03 for B800 emission. The properties of the 'intermediate' complex B800-950/B875/LDS-LDAO are described in the text.

Complex	λ_{det}	*T* (K)	Excitation wavelength (nm):	*p*					
				515	580	588	600	800	850
B800-850/LDS	865	300		−0.09	−0.11		−0.22	0.14	0.14
B800-850/LDS-LDAO	865	300		−0.075	−0.05			0.14	0.14
B800-850/LDAO	865	300		−0.7	−0.03		−0.20	0.14	0.14
B800-850/B875/LDS	865	300							0.13
B800-850/LDS	880	4						0.15	0.14
B800-850/LDS-LDAO	875	4						0.15	0.14
B800-850/LDAO	875	4			+0.01		−0.20	0.14	0.13
B800-850/LDAO	806	4		+0.10		+0.07			

850/LDAO and for the BChl 860 light-harvesting complex of *Rps. sphaeroides* strain R-26. These results indicate that the orientation of the BChl 850 Q_y transition moments are circularly degenerate within a plane [16,17,25] and that the Q_y transitions of BChl 800 are parallel to this plane. Moreover, the degree of polarization was independent of temperature. Apparently, neither the number of connected BChl 850 molecules in the complexes, which varied from 8–10 BChl 850 molecules in the B800-850/B875 complex to about 30 in B800-850/LDS and several hundreds of BChl 850 molecules in the B800-850/LDS-LDAO or B800-850/LDAO complexes [14], nor a lowering of the temperature which strongly decreased the rate of energy transfer between the BChl 850 molecules [21,26], affects the degree of depolarization of fluorescence. This shows that the depolarization of the fluorescence is fully determined by the smallest obtainable structure and that the B800-850 complexes consist of a highly ordered arrangement of these structures.

Polarization spectra in the visible region of the BChl 850 emission are given in Fig. 4. The Q_x band of BChl 850 has a rather strong negative polarization ($p = -0.19$ for the B800-850/LDS-LDAO and $p = -0.22$ for the B800-850/LDS complex) around 600 nm. For the B800-850/LDS-LDAO complex the polarization increases at shorter wavelength to a value of +0.01 at 580 nm, due to the presence of the Q_x band of BChl 800, which band is lacking in the B800-850/LDS complex. This in agreement with the results of Breton et al. [17].

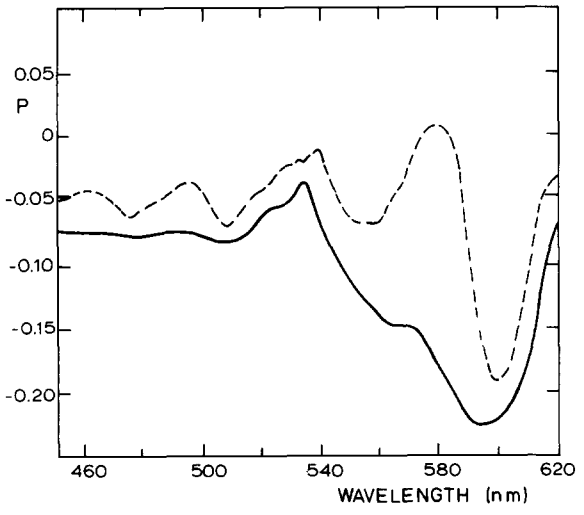

Fig. 4. Fluorescence polarization spectra of the B800-850/LDS (———) and of B800-850/LDS-LDAO (- - - - - -) recorded at 4 K. The detection wavelength was 880 nm.

In Fig. 5, the polarized fluorescence spectra of BChl 800 at 4 K upon excitation in the Q_x band are shown. Although the emission yield was low [11], the spectra clearly show a positive polarization. Values obtained for different preparations varied between 0.04 and 0.10 (Table I). Because at this temperature no back transfer from BChl 850 to BChl 800 is possible [11,21], the positive polarization upon excitation in the Q_x band can only be explained by assuming direct energy transfer between at least two BChl 800 molecules. The simplest model then is one in which two BChl 800 molecules have approximately perpendicular orientation of their Q_y transitions and approximately parallel porphyrin rings. In order to obtain sufficient depolarization, the rate of excitation transfer between the two BChl 800 molecules must significantly exceed that from BChl 800 to BChl 850, which was determined to be $3 \cdot 10^{11}$ s^{-1} at 4 K (see Ref. 11).

Using the well-known Förster equation for dipole-dipole energy transfer:

$$k_{ET} = k_F \left(\frac{R_0}{R}\right)^6$$

we calculated the maximum distance R between the two BChl 800 molecules. k_{ET}, the rate constant for energy transfer between the two BChl 800 molecules, was assumed to be equal to $3 \cdot 10^{12}$ s^{-1} or more, i.e., at least ten times the rate of energy transfer from BChl 800 to BChl 850. From the absorption and emission spectra at 4 K we calculated $R_0 \geqslant 114$ Å, taking the orientation factor $\kappa^2 = 2.25$, the maximal value for two perpendicular transition dipoles. With a rate constant for fluorescence $k_F = 6 \cdot 10^7$ s^{-1} [11] this yielded a maximal dipole-dipole distance between the two BChl 800 molecules of 18–19 Å.

In the carotenoid region the polarized excitation spectra at 4 K for BChl 850 emission in B800-850/LDS and in the reconstituted complex were clearly different (Fig. 4). The B800-850/LDS

complex showed a negative polarization ($p = -0.08$) which was nearly constant between 460 and 520 nm. This indicates that the transition dipole of the carotenoid that transfers its excitation energy to BChl 850 makes a fairly large angle with the plane of the Q_y transitions of BChl 850. The spectrum of the reconstituted complex shows the contribution of different pools of carotenoid with different orientations and absorption spectra. In agreement with the results discussed in the previous section we conclude that this spectrum now also reflects the carotenoid pool associated with BChl 800. Apparently, this carotenoid has an orientation approximately perpendicular to the other one and more in the plane of the Q_y transitions of BChl 850. The position of the maxima and minima of the spectrum indicates that it absorbs at somewhat longer wavelengths than the carotenoid associated with BChl 850. Spectra measured at room temperature did not allow a discrimination between the two pools of carotenoid, but the polarization values (see Table I) at least qualitatively support the proposed orientations. The degree of polarization for BChl 800 emission at 4 K was clearly positive at 515 nm (see Table I), which again indicates an approximately parallel orientation of the carotenoid to the porphyrin planes of BChl 800, in agreement with the above conclusion.

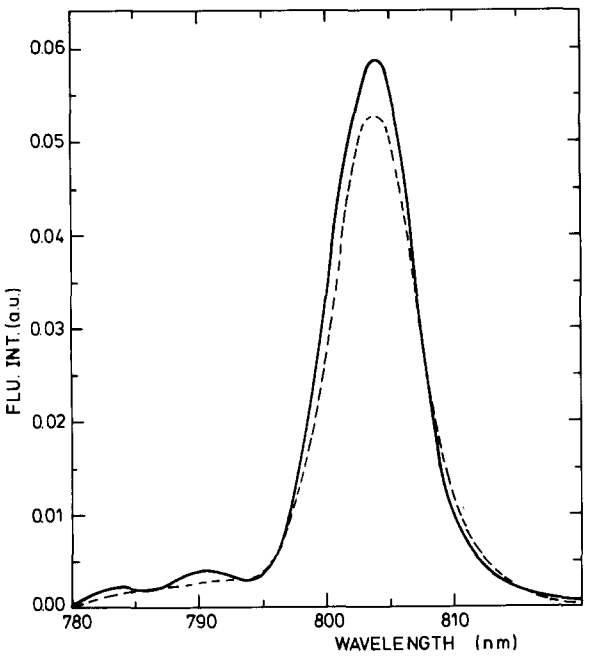

Fig. 5. Polarized fluorescence spectra of the fluorescence emitted by BChl 800 in the B800-850/LDAO complex at 4 K. The analyzing polarizer was either parallel (———) or perpendicular (- - - - - -) to the direction of polarization of the exciting light. The excitation wavelength was 588 nm. A small offset of about 0.02 due to back-ground light [11] was subtracted from both spectra.

Linear dichlorism

The linear dichroism spectra of B800-850/LDAO (Fig. 6) in a pressed gel were in agreement with the conclusions obtained from fluorescence polarization. The Q_y transitions of BChl 800 and BChl 850 showed a strong dichroic signal, the sign of which indicated an orientation approximately parallel to the plane of orientation (i.e., perpendicular to the pressing direction). Comparison with the LD spectra of whole cells and chromatophores, obtained by various orientation techniques [16,17,27,28], indicates that the plane of orientation in our experiments is equivalent to the plane of the membrane. In contrast to the results of Bolt and Sauer [19] who observed an increase in the dichroic ratio from 0.28 to 0.5, we did not observe a variation of the dichroic ratio across the absorption band of BChl 850, not even at 4 K, neither in the B800-850/LDAO (Fig. 6), nor in the B800-850/LDS complex (not shown). In order to obtain the same symmetry of orientation as in the experiments of Bolt and Sauer, who used a stretched

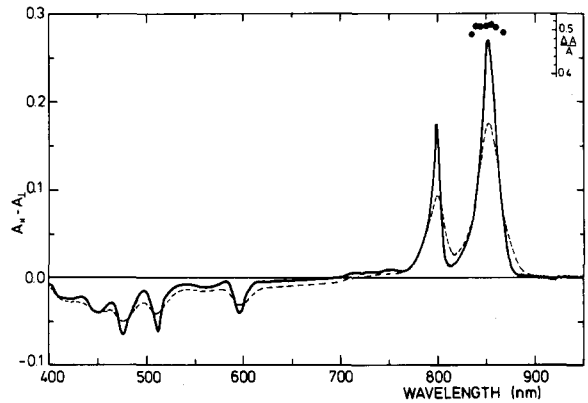

Fig. 6. Linear dichroism spectra of the B800-850/LDAO complex at 300 K (- - - - - -) and at 4 K (———). Values for $(A_{\parallel} - A_{\perp})/A$ of the BChl 850 band are given in the right top corner. $A_{\parallel}$ is defined as the absorbance for light polarized parallel to the plane of orientation (see text).

polyvinyl alcohol film, we also measured the LD spectrum in a gel that was pressed in two perpendicular directions, as described by Vasmel et al. [23]. Again the dichroic rate was found to be constant between 840 and 880 nm.

Comparison of the low temperature LD spectrum with the absorption spectra of Fig. 2 shows that the orientations of the BChl Q_x transitions are different, as earlier observed [17,19]. The Q_x transition of BChl 850 is approximately perpendicular whereas that of BChl 800 is more nearly parallel to the membrane. This indicates that the porphyrin rings of BChl 800 are approximately parallel, those of BChl 850 more or less perpendicular to the membrane.

A similar heterogeneity is observed in the carotenoid region. The minima in the LD spectra are at somewhat shorter wavelengths than those in the absorption spectrum. This suggests an approximately perpendicular orientation for the carotenoid associated with BChl 850 (see previous section), while the carotenoid associated with BChl 800 has a more parallel orientation, in agreement with the results obtained in the previous section.

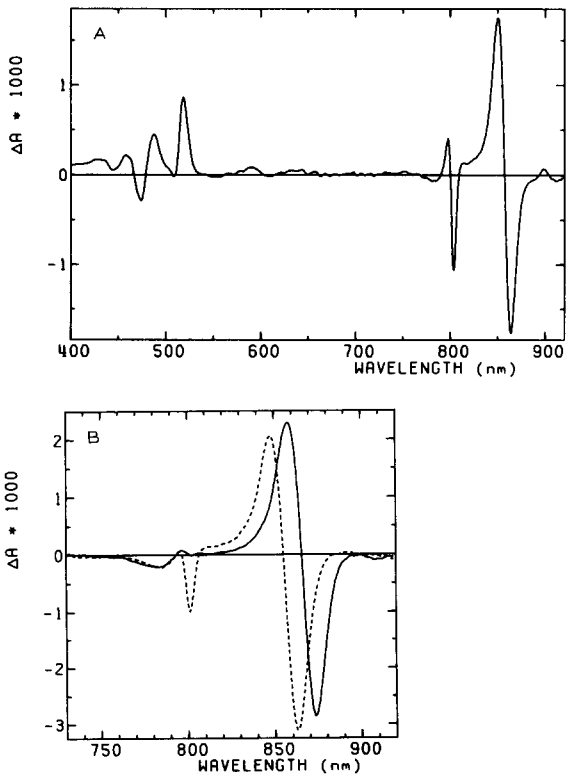

Fig. 7. Circular dichroism spectra of (A) B800-850/LDAO and (B) B800-850/LDS (———) and B800-850/LDS-LDAO (- - - - -) recorded at 77 K. The absorbances of the samples at their maxima near 850 nm were all about 1.

Circular dichroism

In Fig. 7, the CD spectra of B800-850/LDAO (Fig. 7A) and of B800-850/LDS and B800-850/LDS-LDAO complexes (Fig. 7B) are given. The CD of B800-850/LDS is clearly non-conservative over the BChl 850 band indicating that not only exciton interaction between degenerate states but also interactions with higher excited states contribute to the CD in this region [29,30]. The CD of B800-850/LDAO at 77 K is basically the same as reported at room temperature [8,18]. In the BChl 850 band the CD is more conservative, possibly due to a positive non-conservative contribution from BChl 800–BChl 850 interactions. In the 800 nm region, a corresponding negative band appears to be present. The simplest explanation for the remaining bands around 800 nm is that a conservative contribution by BChl 800–BChl 800 exciton interaction is superimposed on this negative band. The CD of B800-850/LDS-LDAO was analogous but not identical to that of the normal LDAO preparation. Apparently, the native configuration of BChl 800 is not totally restored although all other experiments seem to indicate so.

Discussion

The results reported in this work demonstrate that exposure of the B800-850/LDS complex to LDAO not only leads to a full recovery of the BChl 800 absorption band but also restores all the characteristic properties of the original B800-850/LDAO complex with respect to energy transfer and orientation of the pigments. Only the CD seems to indicate a small deviation from the native structure. Because of this almost perfect reconstitution of the complex by the LDAO treatment we conclude that a comparison of the photophysical properties of the preparations is a valid approach to study the pigment organization of the B800-850 complex.

The main conclusions concerning the organization of the pigment molecules in the B800-850

complex that can be obtained from our experiments are the following. (1) The basic unit of the B800-850 complex contains at least 2 BChl 800 and 4 BChl 850 molecules. The dipole-dipole distance between the two BChl 800 molecules is maximally 18–19 Å. They have approximately parallel porphyrin rings and more or less perpendicular Q_y transition moments. (2) The complex contains functionally different carotenoids, associated with BChl 800 and with BChl 850, respectively, in agreement with the earlier observed energy transfer efficiencies between carotenoid and BChl 800 and between carotenoid and BChl 850 [11]. The two carotenoid pools differ in their absorption spectra, the absorption maxima of the carotenoid associated with BChl 800 being located at somewhat longer wavelength, and in their relative orientation with respect to the plane of the membrane. (3) The depolarization of the BChl 850 emission is fully determined by the smallest structure obtained, which consisted of 8–10 BChl 850 molecules. This shows that the B800-850/LDAO and the B800-850/LDS complexes must consist of a highly ordered array of smaller units.

The disappearance of the 800 nm absorption band in the B800-850 complex in the presence of LDS is accompanied by a loss of the interaction with the carotenoid molecule associated to the BChl 800 as witnessed by the impaired energy transfer from this carotenoid, and by the blue shift in the absorption band of the carotenoid. Moreover, the CD spectra show that the interaction with the BChl 850 is lost (see also Ref. 10), which may also reflect the broadening of BChl 850 absorption band at 4 K. At room temperature this broadening is also partly due to the presence of a small amount of BChl 875, the band of which is not resolved in the absorption spectrum.

As stated above, our results lead to a model for a basic unit, consisting of at least four BChl 850, two BChl 800 and three carotenoid molecules. All the Q_y transition moments of both BChl 800 and 850 are in approximately parallel planes, which according to the LD work are more or less parallel to the membrane. In order to account for the observed value of approximately 0.07 for the fluorescence polarization of BChl 800 upon excitation in the Q_x band of BChl 800, we have to assume an angle of less than 24° between the Q_x transition moments of the BChl 800 molecules and the plane of the Q_y transitions. The Q_x transition moment of the BChl 850 molecules are perpendicular to this plane. The carotenoid molecules associated with BChl 850 are predominantly perpendicular and the carotenoid associated with BChl 800 is approximately parallel to the membrane.

A model that incorporates these features is shown in Fig. 8. It also takes into account the results of Zuber et al. [31] who recently determined the primary structure of the polypeptides of the B800-850 complex. The two subunits, LHC I and II, both contain a homologous hydrophobic stretch, which is presumably α-helical [32] and contain a histidine as the most probable binding site for BChl [33]. The binding site of BChl 800 is probably at the transition of the central α-helical section and the more polar region at the N-terminal side of LHC I, where a second histidine molecule is present. The first histidines are about 16 amino acid residues from the polar region yielding a distance of 24 Å, in reasonable agreement with the proposed distance of 21 Å between BChl 850 and BChl 800, calculated from the energy transfer efficiency [11].

The extent of exciton splitting of the 850 nm band cannot be determined exactly from the CD spectrum, but its shape poses an upper limit of about 200 cm^{-1}. This implies a rotational strength of at least one Debye-magneton. If it is assumed for the sake of simplicity that the CD spectrum arises solely from the interaction of pairs of BChl 850 molecules and that the Q_y transitions are mutually perpendicular our model agrees with the increased CD signal if a vertical displacement for one of the molecules of a pair is assumed. Calculation based on the equations given by Pearlstein [34] gave a displacement of about 1 Å, well within the limits implied in the model. The model also explains the absence of an increase of the linear dichroism across the 850 nm band. The absence of a variation of the fluorescence polarization across the absorption band may be explained by a decay of exciton coherence on the time-scale of fluorescence [35].

The model shown in Fig. 8 contains the minimum number of pigment molecules needed to explain the spectroscopic data presently available.

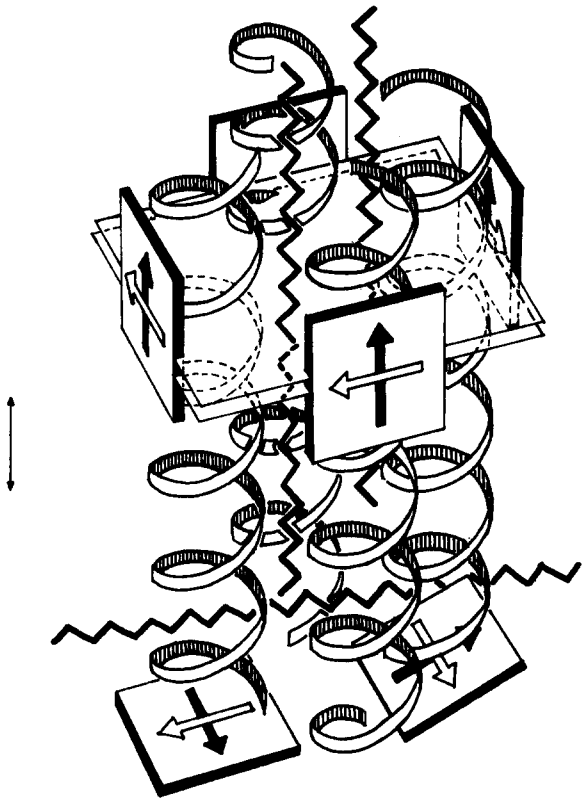

Fig. 8. Schematic picture of the proposed model for the B800-850 antenna complex. The basic unit consists of four BChl 850 molecules (upper boxes), 2 BChl 800 molecules (lower boxes), three carotenoids (zigzag lines) and two proteins, each consisting of two subunits. The helixes symbolize the α-helix regions, which are supposed to be transmembrane. The Q_y transitions (open arrows) of two of the BChl 850 molecules (left front and right back) are in the same plane, while the Q_y transition of the remaining BChl 850 molecules are in a parallel plane, which is vertically displaced by about 1 Å. The Q_x transition moments (solid black arrows) of the BChl 850 are perpendicular to these planes. The Q_y transitions of the BChl 800 molecules are both in a plane parallel to BChl 850 Q_y transitions, while the Q_x molecules are tilted out of this plane at an angle smaller than 24°. The bar represents 5 Å.

Larger subunits cannot be excluded, however. In order to fit the basic unit as depicted in Fig. 8 into larger aggregates, one must assume the presence of lipids between the units in order to prevent strong interaction between BChl 850 molecules of different units. The presence of lipids in the B800-850/LDAO complex has indeed been reported [36] and even appears necessary for the stability of the complex [37]. Finally it should be noted that in our model we assume a ratio of 2:1 for the number of BChl *a* and carotenoid molecules, in disagreement with the ratio of 3:1 found earlier [4,13]. However, recent pigment extraction experiments (Ref. 36 and C.N. Hunter and R.A. Niederman, unpublished data) strongly support the ratio of 2:1.

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References

1 Clayton, R.K. (1981) Photosynthesis. Physical Mechanisms and Chemical Patterns, Cambridge University Press, Cambridge, U.K.
2 Thornber, J.P., Trosper, T.L. and Strouse, C.E. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 133–160, Plenum Press, New York
3 Monger, T.G. and Parson, W.W. (1977) Biochim. Biophys. Acta 460, 393–407
4 Cogdell, R.J. and Thornber J.P. (1980) FEBS Lett. 122, 1–8
5 Niederman, R.A. and Gibson, K.D. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 79–118, Plenum Press, New York
6 Cogdell, R.J. and Thornber, J.P. (1978) in CIBA Foundation, symp. no. 61, Chlorophyll Organization and Energy Transfer in Photosynthesis, pp. 61–79, Excerpta Medica, Amsterdam
7 Clayton, R.K. and Clayton, B.J. (1972) Biochim. Biophys. Acta 283, 492–504
8 Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011–2019
9 Broglie, R.M., Hunter, C.N., Delepelaire, P., Niederman, R.A., Chua, N.-H. and Clayton, R.K. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 86–91
10 Clayton, R.K. and Clayton, B.J. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 5583–5587
11 Van Grondelle, R., Kramer, H.J.M. and Rijgersberg C.P. (1982) Biochim. Biophys. Acta 682, 208–215
12 Hunter, C.N., Niederman, R.A. and Clayton, R.K. (1981) Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 3, pp. 539–545, Balaban International Sciences Services, Philadelphia, PA
13 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409–416
14 Van Grondelle, R., Hunter, C.N., Bakker, J.C.G. and Kramer, H.J.M. (1983) Biochim. Biophys. Acta 723, 30–36
15 Cogdell, R.J., Hipkins, M.F., MacDonald, W. and Truscott, G. (1981) Biochim. Biophys. Acta 634, 191–202
16 Breton, J. and Vermeglio, A. (1982) in Photosynthesis:

Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. 1, pp. 153–194, Academic Press, New York
17 Breton, J., Vermeglio, A. Garrigo, M., and Paillotin, G. (1981) Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 3, pp. 445–459, Balaban International Sciences Services, Philadelphia, PA
18 Bolt, J. and Sauer, K. (1981) Photochem. Photobiol. 34, 653–661
19 Bolt, J. and Sauer, K. (1979) Biochim. Biophys. Acta 546, 54–63
20 Kramer, H.J.M., Van Grondelle, R., Hunter, C.N. and Amesz, J. (1984) Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. 2, pp. 203–206, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
21 Rijgersberg, C.P., Van Grondelle, R. and Amesz, J. (1980) Biochim. Biophys. Acta 592, 53–64
22 Kramer, H.J.M. and Amesz, J. (1982) Biochim. Biophys. Acta 682, 201–207
23 Vasmel, H., Meiburg, R.F., Kramer, H.J.M., De Vos, L.J. and Amesz, J (1983) Biochim. Biophys. Acta 724, 333–339
24 Abdourakhmanov, I.A., Ganago, A.O., Erokhin Y.E., Solov'ev, A.A. and Chuganov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
25 Albrecht, A.C. (1961) J. Mol. Spectr. 6, 84–108
26 Feick, R., Van Grondelle, R., Rijgersberg, C.P. and Drews, G. (1980) Biochim. Biophys. Acta 592, 53–64
27 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500–515
28 Breton, J. (1974) Biochem. Biophys. Res. Comm. 59, 1011–1017
29 Parson, W.W. (1984) Proceedings of the 6th International Congress on Photosynthesis, Brussels, Belgium, in the press
30 Tinoco, I., Jr. (1962) Adv. Chem. Phys. 4, 113–160
31 Zuber, H., Brunisholz, R., Frank, G., Füglistaller, P., Sidler, W. and Theiler, R. (1983) Workshop on Molecular Structure and Function of Light-Harvesting Pigment Protein Complexes and Reaction Centers, ETH Zürich, Abstract nr. II, pp. 6–7
32 Nabedryk, E. and Breton, J. (1981) Biochim. Biophys. Acta 635, 515–524
33 Lutz, M. and Roberts, B. (1983) Workshop on Molecular Structure and Function of Light-Harvesting Pigment Protein Complexes and Reaction Centers, ETH Zürich, Abstract nr. II, p. 11
34 Pearlstein, R.M. (1982) in Photosynthesis. Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. 1, pp. 293–329, Academic Press, New York
35 Rahman, T.S., Knox, R.S. and Kenkre, V.M. (1979) J. Chem. Phys. 44, 197–211
36 Radcliffe, C.W., Pennoyer, J.D., Broglie, R.M. and Niederman, R.A. (1984) Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. 2, pp. 215–220, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
37 Teiler, R., and Zuber, H. (1983) Workshop on Molecular Structure and Function of Light-Harvesting Pigment Protein Complexes and Reaction Centers, ETH Zürich, Abstract nr. II, p. 8