Fluorescence Polarization and Low-Temperature Absorption Spectroscopy of a Subunit Form of Light-Harvesting Complex I from Purple Photosynthetic Bacteria†

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Received July 5, 1990; Revised Manuscript Received March 13, 1991

ABSTRACT: Measurements of polarized fluorescence and CD were made on light-harvesting complex 1 and a subunit form of this complex from *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, and *Rhodobacter capsulatus*. The subunit form of LH1, characterized by a near-infrared absorbance band at approximately 820 nm, was obtained by titration of carotenoid-depleted LH1 complexes with the detergent *n*-octyl β-D-glucopyranoside as reported by Miller et al. (1987) [Miller J. F., Hinchigeri, S. B., Parkes-Loach, P. S., Callahan, P. M., Sprinkle, J. R., & Loach, P. A. (1987) *Biochemistry 26*, 5055-5062]. Fluorescence polarization and CD measurements at 77 K suggest that this subunit form must consist of an interacting bacteriochlorophyll *a* dimer in all three bacterial species. A small, local decrease in the polarization of the fluorescence is observed upon excitation at the blue side of the absorption band of the B820 subunit. This decrease is ascribed to the presence of a high-energy exciton component, perpendicular to the main low-energy exciton component. From the extent of the depolarization, we estimate the oscillator strength of the high-energy component to be at most 3% of the main absorption band. The optical properties of B820 are best explained by a Bchl *a* dimer that has a parallel or antiparallel configuration with an angle between the Q_y transition dipoles not larger than 33°. The importance of this structure is emphasized by the results showing that core antennas from three different purple bacteria have a similar structure. Upon association of the B820 subunit by removing the detergent, a B873(reassoc) complex is formed with optical properties nearly identical with the LH1 antenna complex. The fluorescence polarization gives no indications for a different structure of B820(recon), a complex formed by reconstituting the bacteriochlorophyll *a* with only the β-polypeptide from light-harvesting complex 1 from *Rhodospirillum rubrum*.

Photosynthetic purple bacteria have an efficient mechanism to trap light energy. The pigments that constitute the photosynthetic appratus are associated with proteins in antenna complexes (Clayton & Clayton, 1972; Cogdell, 1978; Zuber, 1986a,b). These complexes collect light energy and transfer it to the photochemical reaction center. Antenna complexes and reaction centers reside in specialized domains of the cytoplasmic membrane (Kiley & Kaplan, 1988). The antenna complexes of photosynthetic purple bacteria can be divided into two major types: a core complex (LH1)[1] located near the photochemical reaction center and a peripheral complex (LH2) associated with the core antenna (Aagaard & Sistrom, 1972). *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* possess both types of light-harvesting complexes. *Rhodospirillum rubrum* only has an LH1-type light-harvesting complex. LH1 antenna complexes are produced in a fixed stoichiometry to the photochemical reaction center (Aagaard & Sistrom, 1972; Cogdell et al., 1982). In LH1 antenna complexes, the Bchl *a* (NIR) absorbance maximum is observed around 880 nm.

The absorption of light energy by the pigments in the antenna complexes results in excited states which are rapidly transferred between antenna pigments until the photochemical reaction center is reached where charge separation can take place. The mechanism of transfer of excited states between the antenna complexes has been extensively studied [see for a review, van Grondelle (1985)]. The observed high efficiency

† This research was supported by EEC Research Grant SC1 0004-C (R.v.G.), by the Dutch Foundation for Biophysics (R.v.G.), by U.S. Public Health Service Grant GM11741 (P.A.L.), and by National Science Foundation Grant DMB-877997 (P.A.L.).

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[1] Abbreviations: Bchl *a*, bacteriochlorophyll *a*; OG, *n*-octyl β-D-glucopyranoside; B896, intrinsic component of light-harvesting complex I absorbing at 896 nm, causing an increase in polarization in the red wing of the excitation spectrum of the LH1 Q_y band; B873(reassoc), light-harvesting complex 1 of *Rhodospirillum rubrum* obtained by reassociating the B820 OG-solubilized subunit form by dilution and chilling; B873(recon), light-harvesting complex 1 reconstituted from B820(recon); B825, subunit form of light-harvesting complex 1 from *Rhodobacter sphaeroides* obtained by OG titration of light-harvesting complex 1; B820, subunit form of light-harvesting complex 1 from *Rhodospirrilum rubrum* obtained by OG titration of light-harvesting complex 1; B820-(recon), subunit form of light-harvesting complex 1 obtained by reconstituting Bchl *a* and purified α- and β-polypeptides purified from *Rhodospirillum rubrum*; B816, subunit form of light-harvesting complex 1 from *Rhodobacter capsulatus* obtained by OG titration of light-harvesting complex 1; 777(dissoc), 777-nm-absorbing material formed by titrating B820 with 4-5% OG; LH1, light-harvesting complex 1, the core complex; LH2, light-harvesting complex 2, an accessory complex; CD, circular dichroism; OD, optical density; NIR, near-infrared. Throughout the text, we use B873(reassoc) to refer to the complex that is obtained by reassociating the B820 subunit form of LH1 by dilution and chilling into a high molecular weight complex close to identical with the original carotenoid LH1 from *Rs. rubrum*. This means that all the results shown for this complex are obtained with preparations that have at some stage during preparation been dissociated into a B820 subunit form. The question of whether the B873(reassoc) is identical with the carotenoid-free LH1 complex is further addressed in the text.

0006-2960/91/0430-5734$02.50/0

of this energy transfer implies that a specific association of the antenna complexes must exist as the proposed mechanism (Förster energy transfer) is highly dependent on the distance and angle between the chromophores (Förster, 1948).

The constituents of antenna complexes are known from biochemical studies since antenna complexes can be easily isolated and purified from the photosynthetic membrane (Clayton & Clayton, 1972; Picorel et al., 1983). Both LH1 and LH2 complexes consist of two small polypeptides designated α and β which occur in a 1:1 ratio (Brunisholz et al., 1984a,b). Both polypeptides contain a single hydrophobic stretch of amino acids that spans the membrane as an α-helix. The amino acid sequences of both polypeptides are highly conserved among a number of species of purple bacteria (Zuber, 1986b). The polypeptides bind, in the case of LH1 from *R. rubrum*, two Bchl *a* molecules and carotenoid molecule (spirilloxanthin for *Rs. rubrum*, sphaeroidene for *Rb. sphaeroides*) (Cogdell et al., 1982).

The minimal unit of LH1, composed of one α- and one β-polypeptide plus two Bchl *a* molecules, cannot explain the observed optical properties of isolated light-harvesting complexes (Kramer et al., 1984a). To account for the very rapid depolarization of excited states observed after exciting antenna pigments with linear polarized light, it is generally assumed that the minimal unit in the complex is a circular degenerate dimer of $\alpha\beta$ (Kramer et al., 1984a) or a circular degenerate trimer of $\alpha\beta$ (Hunter et al., 1989). Singlet–singlet annihilation measurements on the isolated LH1 complex from *Rb. sphaeroides* indicated 6–8 interacting Bchl *a* molecules in a minimum unit (van Grondelle et al., 1983). In vivo, the LH1 antenna was found to contain at least 100–140 energetically coupled Bchl *a* molecules (Bakker et al., 1983; Vos et al., 1986; Deinum et al., 1989). The LH1 antenna displays spectral inhomogeneity (Sundström et al., 1986a,b). A small fraction of the bacteriochlorophyll absorption constitutes the so-called B896. The origin of this spectral inhomogeneity is still debated, but it is thought to concentrate the excited states in the vicinity of the reaction center (Sundström & van Grondelle, 1990; Bergström et al., 1989).

Interactions with adjacent protein residues or between pigment molecules shift the NIR absorbance maximum of the Bchl *a* molecules from approximately 770 nm in organic solvents to, e.g., 881 nm in *Rs. rubrum* LH1 in vivo. The main cause of this red shift has been ascribed to exciton interactions between neighboring Bchl *a* molecules (Scherz & Parson, 1984a,b; Loach et al., 1985), to interactions between the Bchl *a* pigments and charged amino acid residues (Eccles & Honig, 1983), and to interactions between Bchl *a* and aromatic groups in the protein (Pearlstein, 1988). These phenomena have not been fully characterized and are still subject of much debate (Kramer et al., 1984a; Scherz & Parson, 1986; Brunisholz & Zuber, 1988). Exciton calculations have been applied to find specific configurations of Bchl *a* molecules that give rise to red-shifted absorbance and observed CD spectra of light-harvesting pigment–protein complexes. It was concluded that strong interactions between two Bchl *a* molecules give rise to the NIR absorbance shift, while weak coupling between a number of these Bchl *a* dimers was presumed to explain the observed CD (Scherz & Parson, 1986).

The isolation of a subunit form of the core antenna complex of *Rhodospirillum rubrum* was first reported by Loach et al. (1985). After extraction of the carotenoids from the chromatophores, and titration of the purified complexes with *n*-octyl β-D-glucopyranoside (OG), a reversible dissociation of the core antenna was observed. This dissociation is accompanied by a shift in the absorbance maximum from 873 to 820 nm. At higher concentrations of OG (>1%), a further shift of the absorbance maximum to 777 nm takes place. Gel filtration experiments showed that the molecular weight of the detergent-solubilized B820 form was approximately 55K. If it is assumed that an equal weight of OG is bound, this result suggests that B820 is an $(\alpha\beta)_2BChl_4$ complex. The B820 subunits could be reassociated into B873 either by dilution, by chilling, or by removal of the OG from the sample by dialysis. The molecular weight of the B873 particle exceeds 100K. It was further shown (Parkes-Loach et al., 1988) that the B873 complex could be reconstituted from its purified components: Bchl *a* and the α- and β-polypeptides. The binding sites for Bchl *a* have been probed by using Bchl *a* analogues (Parkes-Loach et al., 1990).

Spectroscopic characterization of this B820 complex was initiated by measuring its absorbance and CD properties at room temperature, its fluorescence lifetime, and its resonance Raman spectroscopy (Chang et al., 1990a). Recently, it was found that similar subunits forms could be obtained from the core antenna of other species of purple bacteria, i.e., *Rb. sphaeroides* (Chang et al., 1990b) and *Rb. capsulatus* (Heller & Loach, 1990). Similar preparation from *Rs. rubrum* have also been prepared by other groups (Ghosh et al., 1988a,b).

In this paper, we present data from low-temperature polarized fluorescence spectroscopy on the different subunits of LH1. Our goal in this study has been to investigate the properties of the subunit form of LH1, by using a combination of spectroscopic techniques. It has been previously shown that the study of subunit forms of light-harvesting complexes, obtained by reversible dissociation, yields information about the delicate structure–function relations in antenna complexes (Clayton & Clayton, 1981; Kramer et al., 1984a; Chadwick et al., 1987; Ghosh et al., 1988a,b; Miller et al., 1987; Parkes-Loach et al., 1988, 1990; Chang et al., 1990a,b). By the combination of several spectroscopic techniques (absorbance and CD), a better understanding of the specific organization of the Bchl *a* pigments in the different subunit forms is reached.

Materials and Methods

n-Octyl β-D-glucopyranoside and Bchl *a* were obtained from Sigma Chemical Co. Glycerol (>99.5% pure) was purchased from BDH Ltd.

Rs. rubrum cells were grown and chromatophores prepared from them (Loach et al., 1963). The B820 subunit form of LH1 from *Rs. rubrum* (wild-type S1) was isolated from chromatophores as described by Miller et al. (1987). The B825 subunit forms from LH1 from *Rb. sphaeroides* (wild-type 2.4.1 and mutant strain puc 705-BA) and *Rb. capsulatus* (wild-type and mutant strain MW 442) were isolated by using similar methods (Chang et al., 1990b; Heller & Loach, 1990). Samples for low-temperature spectroscopy were prepared in 50 mM phosphate buffer, pH 7.8, containing 50–55% (volume/volume) glycerol and varying amounts of OG. Reassociated samples were obtained by dilution followed by overnight storage of the B820 samples at 4 °C. The presence of glycerol in the medium retarded the dissociation of the B873 into B820 subunits and shifted the equilibrium concentration. In general, 40–50% more OG was required to stabilize the B820 as compared to samples without glycerol (Miller et al., 1987). Bchl *a* samples were prepared by dissolving lyophilized Bchl *a* in acetone and adding small amounts of this solution to phosphate buffer containing 5% OG (Parkes-Loach et al., 1988).

Fluorescence excitation and emission spectra (90° detection) and CD spectra were measured by using a home-built spec-

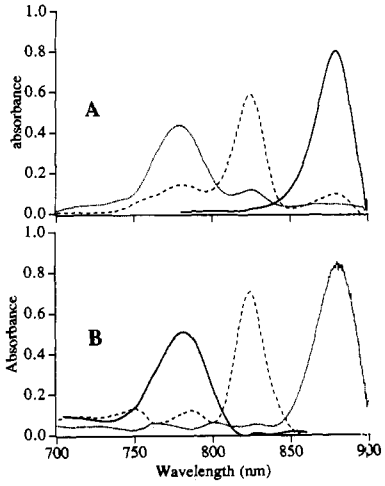

FIGURE 1: (Panel A) Low-temperature (77 K) absorbance spectra of 777(dissoc) (—), B820 (---), and B873(reassoc) (···) from *Rhodospirillum rubrum* LH1 in phosphate buffer, pH 7.5, and 55% glycerol. OG concentration was 0.5%, 1.2%, and 4.5%, respectively. Spectra were measured in 1 × 1 cm acryl cuvettes. (Panel B) Low-temperature (77 K) absorbance spectra of 777(dissoc) (—), B825 (---), and B873 (···) from *Rhodobacter sphaeroides* in 50 mM phosphate buffer, pH 7.5, in 3%, 1.12%, and 0.6 OG, respectively.

trophotometer. Fluorescence spectra were measured by using a halogen tungsten light bulb, a single monochromator for selection of the excitation wavelength, and a double monochromator to detect the emitted fluorescence. The instrument contained a Glan–Thompson polarizer in both detection light paths and a Glan-Taylor polarizer in the excitation light path. For the detection of fluorescence beyond 900 nm, an S1-cathode photomultiplier or a photodiode (EG&G, type HUV4000B) was used. Uncracked glasses at 77 K for fluorescence measurements were obtained in 1 × 1 cm acryl cuvettes (Sarstedt). The absorption of samples was kept below 0.2 cm^{-1} to prevent distortion of spectra by self-absorption. All fluorescence spectra shown have been corrected for the spectral intensity variations of the detector sensitivity and the light source emittance. The degree of polarization of the fluorescence was defined as

$$p(\lambda) = \frac{I_{\parallel}(\lambda) - C(\lambda)I_{\perp}(\lambda)}{I_{\parallel}(\lambda) + C(\lambda)I_{\perp}(\lambda)} \qquad (1)$$

with $p(\lambda)$ = the polarization at wavelength λ, $I_{\parallel}(\lambda)$ = the intensity of fluorescence emission polarized parallel to incident light, $I_{\perp}(\lambda)$ = the intensity of fluorescence emission polarized perpendicular to incident light, and $C(\lambda)$ = the wavelength-dependent correction factor for differences in sensitivity of the detector for parallel and perpendicular polarized light.

For low-temperature measurements, samples were cooled in a liquid nitrogen cryostat (Oxford Instruments Ltd.). Low-temperature CD was measured on samples in special 2-mm cells with a detachable side (Hellma Co.). CD was measured by using a optomodulator (Hinds) and a lock-in amplifier (Ithaco). All data were transferred to a SUN3/SUN4 computer system for further analysis.

RESULTS

Figure 1A shows the low-temperature (77 K) absorption spectra of the three forms of the LH1 antenna complex of *Rs. rubrum*, 777(dissoc), B820, and B873(reassoc), obtained in 4.5%, 1.2%, and 0.5% OG, respectively. The absorption spectrum of the 777(dissoc) species is the broadest of the three spectra and strongly resembles the spectrum of Bchl *a* in detergent micelles. The low-temperature (77 K) absorption

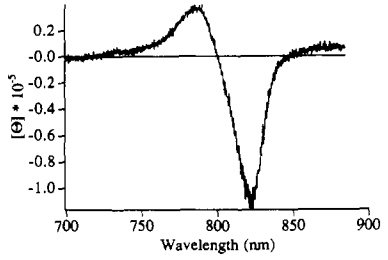

FIGURE 2: Low-temperature (77 K) CD spectrum of B820 from *Rhodospirillum rubrum* in 50 mM phosphate buffer/1.2% OG. The spectrum was recorded on a home-built spectrophotometer, spectral bandwidth 6 nm. The sample was contained in special 2-mm quartz cuvettes with a detachable side. To calculate the molar ellipticity, we used a millimolar absorbance of 70 for the B820 main absorption band.

spectrum of B820 is much narrower than the 777(dissoc) absorption spectrum, and the integrated spectrum of B820 is estimated to be 15% hyperchromic [after correction for the 777(dissoc) contribution]. At liquid nitrogen temperature, the maximal absorbance is shifted to 825 nm. The B820 absorption spectrum in Figure 1A further indicates the presence of some 777(dissoc) and B873(reassoc). The total amount of these species varies from preparation to preparation, but is usually less than 10% of the B820 absorption peak. Upon formation of the B873(reassoc) by decreasing the OG concentration, the absorption maximum shifts further to the red. The absorption spectrum also broadens compared to the B820 absorption spectrum, indicative for the contribution of more spectral forms to this absorption band. The integrated absorption spectrum of B873(reassoc) is 25% hyperchromic compared to B820. The absorption spectrum of the reassociated B873(reassoc) form is close to identical with the absorption spectrum of membranes from the carotenoid-less mutant *Rs. rubrum* G9+ (Ghosh et al., 1988a,b; Loach et al., 1985; Miller et al., 1987), showing that the shift from B873 to B820 induced by adding OG is fully reversed upon lowering the OG concentration.

Low-temperature absorption spectra of 777(dissoc), B825, and B873(reaasoc) from *Rb. sphaeroides* in 3.0%, 1.15%, and 0.55% OG are shown in Figure 1B. The B825 spectrum of *Rb. sphaeroides* is similar to the B820 from *Rs. rubrum*. As for *Rs. rubrum*, the B825 from *Rb. sphaeroides* has a narrower NIR absorption band than 777(dissoc) and is approximately 10–20% hyperchromic at 77 K, after integration and correction for contributions from the different species. The B873(reassoc) spectrum is similar to the spectrum of the LH1-only mutant M2192 from *Rb. sphaeroides* (Hunter et al., 1989) apart from a small blue shift of 7 nm of the strong NIR transition probably caused by the absence of carotenoids. The B873-(reassoc) spectrum is 20% hyperchromic compared to the B825 form at 77 K. The B873(reassoc) band is also broader than the B825 absorption band, further indicating the presence of more spectral forms in this band.

Figure 2 shows the NIR CD spectrum taken at 77 K for B820 from *Rs. rubrum*. The spectrum is essentially identical with the room temperature spectrum (Chang et al., 1990a). the maxima, however, are red-shifted due to sharpening of the absorption bands at low temperature. The shape of this CD spectrum and that of the B873 form are not very different from the room temperature spectra. The absence of additional CD bands at liquid nitrogen temperature rules out the possibility that the signal is composed of two different Bchl *a* molecules with conservative signals. The B820 CD in the NIR region is not conservative with the large negative peak at 825 nm almost coincident with the absorption maximum at 77 K. A

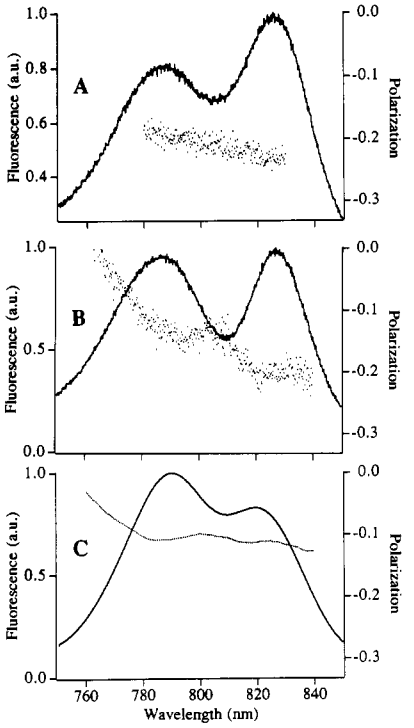

FIGURE 3: (Panel A) Solid curve: low-temperature (77 K) isotropic fluorescence emission spectrum from *Rhodospirillum rubrum* 777-(dissoc) and B820. Excitation wavelength 595 nm. Spectral bandwidth of both excitation and detection monochromators was 12 nm. The isotropic signal was calculated from the polarized signals. Dotted curve: polarization of the emission. (Panel B) Solid curve: low-temperature (77 K) isotropic fluorescence emission spectra of 777-(dissoc) and B825 from *Rhodobacter sphaeroides*. Excitation at 595 nm. Spectral bandwidth of excitation and detection monochromators 10 nm. The isotropic signal was calculated from the polarized signals. The polarization is also shown by the dotted curve. (Panel C) Solid curve: low-temperature (77 K) anisotropic fluorescence emission spectrum of 777(dissoc) and B816 from *Rb. capsulatus*. Excitation wavelength 595 nm. The isotropic spectrum was calculated from the polarized spectra. Spectral bandwidth of excitation and detection monochromators 10 nm. The polarization is shown by the dotted curve.

smaller positive peak is present at 790 nm, which is not clearly corresponding to a maximum in the absorption spectrum (see below). If the B820 spectrum is due to exciton interactions among a pair of Bchl *a* molecules, then the two exciton transitions must occur close to 825 nm and slightly red-shifted from 790 nm.

The polarized emission spectrum of a sample containing B820 and some 777(dissoc) from *Rs. rubrum* upon excitation in the Q_x region (around 590 nm) is shown in Figure 3A. Two emission peaks can be observed simultaneously, which do not reflect the total amounts of 777(dissoc) and B820, since 777(dissoc) apparently has a higher fluorescence yield compared to B820. Furthermore, the relative extinction coefficients at 595 nm are also different. Both bands have a strong negative polarization as shown in Figure 3A. The emission from the 777(dissoc) form upon excitation in the Q_x region is less polarized ($p = -0.2$) compared to the emission from Bchl *a* in detergent micelles [$p = -0.24$ (Vermeglio et al., 1978)]. The emission spectra from B825 from *Rb. sphaeroides* are also shown in Figure 3B. The emission spectra were measured upon excitation in the Q_x region. These spectra are very similar to those of *Rs. rubrum* shown in Figure 3A, again displaying a negative polarization.

Upon OG titration of carotenoid-extracted LH1 antenna complexes of *Rb. capsulatus*, a "B820-like" spectral form is obtained that is similar to B820 from *Rs. rubrum* and B825 from *Rb. sphaeroides*. The main absorption peak for this

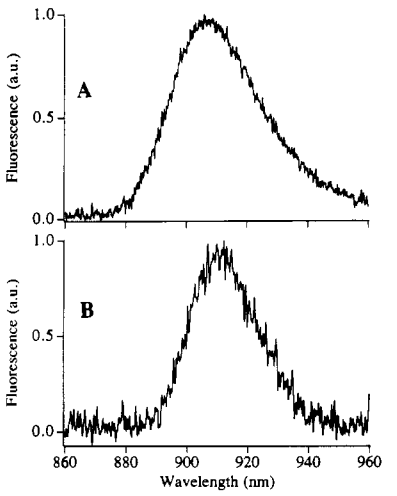

FIGURE 4: (Panel A) Low-temperature (77 K) isotropic fluorescence emission spectrum from *Rhodospirillum rubrum* B873. Excitation wavelength 595 nm. Spectral bandwidth of excitation and detection monochromators was 12 nm. (Panel B) Low-temperature (77 K) fluorescence emission spectrum of B873 from *Rhodobacter sphaeroides*. Excitation at 595 nm. Spectral bandwidth of excitation and detection monochromators 10 nm.

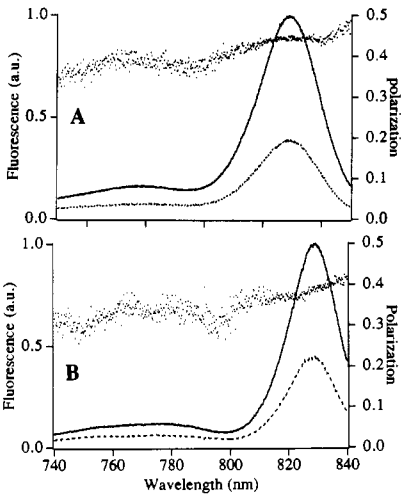

FIGURE 5: (Panel A) Low-temperature (77 K) polarized fluorescence excitation spectra of the Q_y band from *Rhodospirillum rubrum* B820. Detection at 850 nm; spectral bandwidth 10 nm. (—) Detection polarization parallel to excitation polarization; (---) detection polarization perpendicular to excitation polarization. The polarization calculated from these spectra is shown by the dotted curve (Panel B) Low-temperature (77 K) fluorescence excitation spectrum of the Q_y band B825 from *Rhodobacter sphaeroides*. Detection at 840 nm. Detection polarization parallel (—) and perpendicular (---) to excitation polarization. Spectral bandwidth of excitation and detection monochromators 10 nm. The polarization is also shown by the dots.

species is at 816 nm at room temperature (Heller & Loach, 1990). The B816 absorption properties are similar to B820 and B825. Figure 3C shows the emission spectrum of B816 upon excitation in the Q_x region. Although the polarization of the emission of the B816 particle is not as negative as for *Rs. rubrum* or *Rb. sphaeroides*, also in this case the polarization of the emission from B816 is more negatively polarized than the emission from 777(dissoc), indicating similar spectral properties of this subunit form.

Figure 4A shows the 77 K emission spectrum of B873-(reassoc) from *Rs. rubrum* and Figure 4B that from *Rb. sphaeroides*. We note that the emission maximum is some 35 nm to the red compared to the absorbance maximum. This is a much larger red shift as has been previously reported (Chang et al., 1990a) and is comparable to observed emission

maxima for intact LH1 complexes.

Figure 5A shows polarized excitation spectra for the B820 from *Rs. rubrum*, and Figure 5B shows the same spectrum for *Rb. sphaeroides*. We note that the excitation spectra at 77 K are almost "pure". Although in the 77 K absorption spectra of various preparations contributions from the other spectral forms can usually be observed, the 777(dissoc) absorption band does not appear in the excitation spectrum of B820 and also little or no contribution from B820 is observed in the B873(reassoc) emission at 77 K in both species (see below). This indicates that 777(dissoc), B820, and B873-(reassoc) are not electronically coupled, and therefore energy transfer between the different forms does not occur. The most likely explanation for the absence of coupling is that each form represents a truly distinct particle (Miller et al., 1987). The fact that the maximum of the excitation spectrum is slightly red-shifted compared to the maximal absorbance of the B820 spectrum in Figure 1 must be ascribed to the presence of some 777(dissoc) causing a slight blue shift of the absorbance maximum of the sample.

For *Rs. rubrum* and *Rb. sphaeroides*, the polarization is high upon excitation over the B820 absorption band, but a small significant drop can be observed (to 0.37 for *Rs. rubrum* and to 0.32 for *Rb. sphaeroides*) upon excitation around 795 nm. This drop neither coincides with the first vibration band of the B820 band situated at 780 nm in the excitation spectrum of this particle nor can it be associated with some remaining 777(dissoc). Polarized fluorescence excitation spectra of Bchl *a* in 5% OG did not show this drop in the polarization (data not shown). The polarization is constant over the whole excitation band apart from a small increase toward the red wing, which could be due to light scattering. We ascribe the dip in the polarization found upon excitation of the B820 subunit around 795 nm to the same transition that gives rise to the positive CD signal at 790 nm in the low-temperature CD spectrum of this B820 form, i.e., the high exciton component of an interacting pair of Bchl *a* molecules.

Figure 5B shows the polarized excitation spectrum of the B825 form of *Rb. sphaeroides*. Like the excitation spectrum of the *Rs. rubrum* B820 form, the excitation spectra of *Rb. sphaeroides* B825 have no detectable 777(dissoc) contribution. The polarization of the B825 emission upon excitation in the Q_y band is comparable ($p = 0.38$) with the value found for the B820 form of *Rs. rubrum*. Again, the polarization shows a clear dip on the blue side of the NIR excitation band at 795 nm; also for the B825 of *Rb. sphaeroides*, we ascribe this dip to the presence of a weak high-energy exciton component, similar to B820 from *Rs. rubrum*.

A marked change is observed in the fluorescence polarization upon aggregation of the B820 form into B873(reassoc) by removal of the OG. The polarization decreases to about 0.12 for the B873(reassoc) form and in the case of *Rs. rubrum* is nearly constant, upon changing the excitation wavelength over the B873 absorption band (Figure 6A). The observed p value is in good agreement with earlier observations (Bolt et al., 1981; Kramer et al., 1984b) and indicates that the B873(reassoc) consists of a number of interacting Bchl *a* molecules with a nonparallel configuration. The B873 form emits maximally at 912 nm, and only a limited increase of the polarization is observed upon excitation in the red wing of the absorption band. The small increase in the polarization toward the red side of the excitation band (apart from a light-scattering contribution) in LH1 of *Rs. rubrum* is in contrast to what is observed in the *Rb. sphaeroides* B873(reassoc) (see below). This is probably due to a relatively small energy

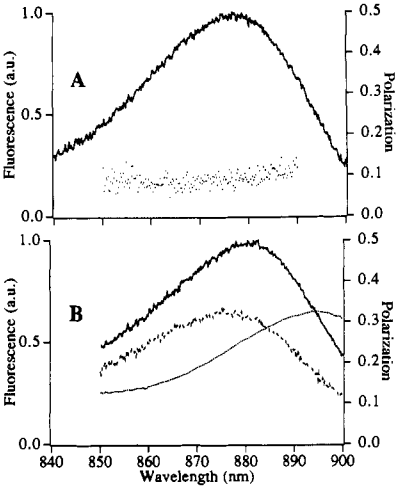

FIGURE 6: (Panel A) Low-temperature (77 K) polarized fluorescence excitation spectra of the Q_y band from *Rhodospirillum rubrum* B873 (reassociated from B820). Detection at 920 nm. (Solid line) Spectral bandwidth of excitation and detection monochromators 10 nm. The polarization is shown by the dots. (Panel B) Low-temperature (77 K) fluorescence excitation spectrum of B873 from *Rhodobacter sphaeroides*. Detection at 920 nm. Spectral bandwidth of excitation and detection monochromator 10 nm. Detection polarization parallel (—) and perpendicular (---) to excitation polarization. Spectral bandwidth of excitation and detection monochromators 10 nm. The polarization (dotted curve) is also shown by the unmarked curve (the signal was smoothed by a polynomial smoothing algorithm to reduce noise).

separation between B896 absorption and B873 absorption at 77 K in *Rs. rubrum*, in agreement with 77 K picosecond absorption (Visscher et al., 1989) and singlet–singlet annihilation measurements (Deinum et al., 1989).

The polarization of the B873(reassoc) upon excitation in the Q_y band from *Rb. sphaeroides* (Figure 6B) again is low ($p = 0.11$) upon excitation in the Q_y band, probably indicating the formation of a larger complex in which several Bchl *a* molecules interact. Contrary to the results for *Rs. rubrum*, a distinct increase in the polarization is observed in the red wing of the Q_y band of B873(reassoc) from *Rb. sphaeroides*. This is indicative of the heterogeneous nature of this absorption band, and the increase in polarization can be ascribed to the presence of the long-wavelength B896 species. The stronger manifestation of B896 in *Rb. sphaeroides* is in good agreement with 77 K picosecond absorption measurements on detergent-solubilized LH1 complexes and chromatophores from *Rb. sphaeroides* that indicate a larger energy separation between B896 and the bulk LH1 absorption in *Rb. sphaeroides* than in *Rs. rubrum* (Visscher et al., 1989).

We also measured the fluorescence polarization of the B820(recon) and B873(recon) forms that were reconstituted from purified α- and β-polypeptides from *Rs. rubrum* and Bchl *a* [for details, see Parkes-Loach et al. (1988) and Chang et al. (1990a)]. The 77 K absorption spectra of these reconstituted forms were nearly identical with those of B820 and B873(reassoc) (data not shown). The polarization value for the B820(recon) form was the same as for B820. The slightly higher polarization values that were found upon Q_x and Q_y excitation for the B873(recon) (see Table I) indicate that the B873(recon) probably is less well structured than the B873-(reassoc). Possibly the correct association of the B820(recon) into B873(recon) requires specific stoichiometries of all the components or may require additional components or membrane structures.

Finally, we have measured the excitation spectra and polarization of a B820 subunit form that can be reconstituted

Table I: Polarization Values for the Different Subunits from the Core Complex of Three Purple Bacteria

	Q_x excitation			Q_y excitation		
	λ_{exc} (nm)	λ_{em} (nm)	p (±0.02)	λ_{exc} (nm)	λ_{em} (nm)	p (±0.02)
B873 *Rs. rubrum* wt	595	920	−0.215	884	920	0.11
B873 *Rb. sphaeroides*	595	920	−0.215	888	920	0.11
B873 (recon from *Rs. rubrum* α and β)	588	900	−0.09	857	920	0.19
B820 *Rs. rubrum* wt	595	825	−0.195	831	850	0.40
B825 *Rb. sphaeroides*	595	830	−0.17	830	840	0.38
B816 *Rb. capsulatus*	595	820	−0.10	ND	ND	ND
B820 (recon α and β)	600	840	−0.20	831	840	0.36 ± 0.05
B820(recon β only)	ND[a]	ND	ND	820	840	0.42
777(dissoc) *Rs. rubrum* wt	596	785	−0.17	770	800	0.45
Bchl *a* in 5% OG	590	800	−0.24	792	800	0.49

[a] Not determined.

from purified β-polypeptides and Bchl *a* (data not shown) (Parkes-Loach et al., 1988). The emission spectrum obtained from this subunit is quite similar to the spectrum shown for B820. The polarization of the Q_y band of this particle also displays the small depolarization that can be observed in the B820 form in Figure 4a. The overall polarization of this particle is comparable to the B820 form (see Table I).

Discussion

In this paper, the spectral properties at low temperature are described for the three forms B873(reassoc), B820, and 777-(dissoc) that can be obtained from the core antenna from the photosynthetic purple bacteria *Rs. rubrum*, *Rb. sphaeroides*, and *Rb. capsulatus*. Our results give support for a strong spectral similarity between the forms obtained from each species. Therefore, we restrict the discussion below to the properties of the spectral forms of *Rs. rubrum*.

777(dissoc). The 777(dissoc) has previously been described as identical with Bchl *a* in detergent micelles (Parkes-Loach et al., 1988; Chang et al., 1990a). It is not clear whether the bacteriochlorophyll *a* molecules are completely dissociated from the protein. The polarization upon Q_x excitation of Bchl *a* is slightly lower than the polarization of the 777(dissoc) emission (see Table I). One possible explanation for this might be a putative weak interaction between chlorophylls in the 777(dissoc) state. For *Rb. sphaeroides*, we noted that a fourth spectral form is sometimes obtained with maximal absorbance at approximately 850 nm. Although we have not been able to fully resolve the spectral properties of this species, it may be a detergent-solubilized Bchl *a* dimer or aggregate that has a red-shifted Q_y absorption (Scherz & Rosenbach-Belkin, 1989; Fisher et al., 1990).

B820. The LH1 antenna complex from the photosynthetic bacteria is dissociated upon titration of the carotenoid-extracted complexes with the detergent OG, and a subunit form absorbing maximally at 820 nm is obtained. This subunit has spectroscopic properties that are remarkably different from the original LH1 antenna complex. To account for the optical and biochemical properties of this B820 subunit, three different models have been suggested (Loach et al., 1985; Chang et al., 1990a,b). The first model, in which one Bchl *a* ligates to the protein in such a way that it absorbs at 820 nm and that another Bchl *a* absorbs at 777 nm without any interaction with the protein, was already regarded highly unlikely. This model does not explain most of the biochemical data, showing that both Bchl *a* molecules are bound to the protein, and also fails to account for the observed increase in the intensity of the CD spectrum.

The second model assumed two protein-bound, weakly coupled Bchl *a* molecules, with different absorbance maxima. The difference in absorbance maxima would be due to differences in the environment of the two Bchl *a* molecules causing one of the Bchl *a* molecules to absorb maximally at 820 nm. The other Bchl *a* molecule would have an absorbance maximum at 777 nm, or perhaps, due to environmental effects, slightly red-shifted from this wavelength, coinciding with the NIR maximum of the CD spectrum of the B820 subunit (see Figure 2, 795 nm at 77 K). This model would thus comprise two Bchl *a* molecules in different environments both red-shifted, but to a different extent from the initial absorbance maximum (777 nm). The changes in the absorbance properties would be caused by interaction of the Bchl *a* molecules with the α- and β-polypeptides solely. Theoretical calculations (Eccles & Honig, 1983; Gudowska-Nowak et al., 1990) and empirical work (Wright & Boxer, 1981) have indeed shown that the protein interaction with bacteriochlorophyll derivatives may cause red shifts of the absorbance maxima of the chromophores and can induce CD. The extreme nonconservativeness of the CD spectrum shown in Figure 2 could be attributed to this effect. The increase of the CD intensity of the B820 as compared to the 777(dissoc) could also be ascribed to distortion of the chromophore by chromophore–protein interaction. However, the contribution of the "putative" 777 monomeric Bchl *a* to the B820 absorption spectrum must be very low. Since there is a reasonable spectral overlap between the emission of the 795-nm Bchl *a* and the absorption of the 820-nm Bchl *a* and as both Bchl *a* molecules are bound to an αβ-polypeptide pair (i.e., the distance between them is probably less than 2 nm), a significant contribution of the 795-nm Bchl *a* molecule should be expected in the excitation spectrum of the B820 subunit. This is not in line with our observations, and therefore we regard this hypothesis unlikely. Furthermore, we have found that the 777-nm contribution to the B820 absorption spectrum is far from constant in different preparations and is sometimes virtually absent in preparations with a high concentration of B820 (van Mourik et al., unpublished results). We therefore suggest that the 777-nm absorbance observed in most of the samples is simply caused by a contamination of the B820 preparation with detergent-solubilized Bchl *a* absorbing at 777 nm.

The third model assumed two excitonically coupled Bchl *a* molecules, one bound to the α-polypeptide and one to the β-polypeptide. This model has not been discussed in great detail previously, but it describes the results presented in this paper rather well. Starting from the 77 K CD spectrum shown in Figure 2, it follows that the high- and low-exciton components of this Bchl *a* dimer are situated at approximately 790 and 825 nm, respectively, at 77 K. The dip in the fluorescence polarization of the Q_y excitation of the B820 subunit is in good agreement with the position of the high-energy component at 795 nm in the 77 K CD spectrum. The position of the low-energy exciton component in the CD spectrum coincides with

the maximum in the 77 K absorbance spectrum shown in Figure 1. These results put the monomeric transition dipole at 807 nm and yield a dipole–dipole interaction of $2V_{dimer}$ = 457 cm^{-1}. The shift of the monomeric absorbance to 807 nm could arise from interactions with neighboring protein residues as discussed previously.

We can further estimate the oscillator strength of the high-energy component from the amplitude of the dip in the fluorescence polarization at 795 nm. It is not possible to estimate the magnitude of the oscillator strength from the 77 K absorbance spectrum in view of the presence of 777(dissoc) in the preparations. However, since the 777(dissoc) is not detected in the fluorescence excitation spectrum of B820, it is possible to estimate the contribution of this high-energy component. The decrease observed in the polarization could arise from a transition that has approximately 3% of the total oscillator strength, since the high-energy exciton component is perpendicular to the low-energy component, assuming that the excitation transfer between the two participating dipoles is of the "Förster-type" (i.e., $p = -0.33$ for perpendicular dipoles). This large intensity ratio with virtually all of the oscillator strength in the redmost transition corresponds with a close to parallel or antiparallel dimer. To estimate an angle between the original transition dipoles, we considered only the original Q_y transition dipoles without any exciton interaction. Upon excitation of one of the pigment molecules, the excited state might be transferred to the other pigment molecule, and either of these emits the fluorescence. This causes a depolarization that depends on the angle between the Q_y dipoles. An angle between the initial dipoles of 33° would yield a polarization of 0.40. Although this model is not fully correct, it probably puts an upper limit of 33° to the angle between the dipoles.

To evaluate possible configurations of Bchl *a* molecules that have spectral properties similar to B820, i.e., 15% hyperchromicity of the B820 Q_y region over the B777, a CD spectrum in the Q_y region with $[\theta]_{820} = -100$ mM^{-1} and $[\theta]_{795}$ = 50 mM^{-1}, a ratio between low- and high-exciton components of approximately 30, and an angle between the contributing dipoles of 25°, we have used exciton calculations and dipoles for Bchl *a* as described by Scherz and Parson (1984a). In these calculations, we included contributions from nondegenerate states (Q_x, Q_y, B_x, B_y). We could reproduce the results obtained by Scherz and Parson (1986) for closer coupled dimers using this formalism. In Figure 7, we show a possible geometry that yields optical properties similar to those of the B820 subunit. In this model, the two Bchl *a* molecules are placed at a distance of 11 Å with an angle between the Q_y transition dipoles of 24°. Further details are given in the legend to Figure 7.

From absorbance and CD spectra of different antenna complexes, Scherz and Parson (1986) suggested that a strongly interacting Bchl *a* dimer could give rise to the observed bathochromic shift of approximately 70 nm from the initial absorbance maximum. In order to generate a proper CD spectrum, they concluded also that weaker interactions between these strongly interacting dimers contribute to the final optical properties of these complexes. Under favorable conditions, Bchl *a* indeed self-aggregates into dimers having optical properties similar to those in the antenna complexes and reaction centers of purple bacteria (Scherz & Rosenbach-Belkin, 1989). The isolation of a subunit form of the core antenna complex with an absorbance maximum at significant lower wavelength, having CD and highly polarized fluorescence, is not predicted by this model, since these dimers are already absorbing at the native, completely red-shifted wavelength.

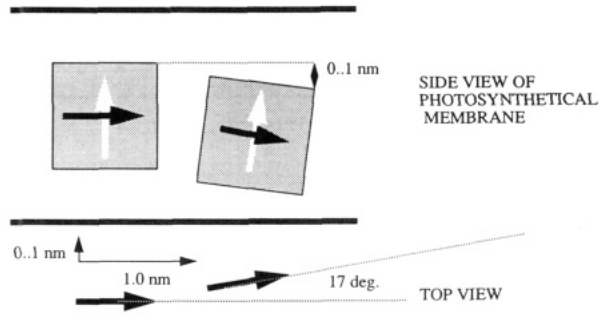

FIGURE 7: Model of a Bchl *a* dimer as present in the B820 subunit of LH1 used for exciton calculations. Q_y transitions are depicted by black arrows, Q_x transitions by white arrows. The dimer is represented by two bacteriochlorophyll *a* molecules (Scherz & Parson, 1984a). For each bacteriochlorophyll *a*, four transitions were considered with the following dipole strengths and maxima: Q_y, 807 nm, 39.7 dB^2; Q_x, 600 nm 3.5 dB^2; B_y, 390 nm, 49 dB^2; B_x, 360 nm, 81 dB^2. To obtain the proper dipole–dipole interaction, one Bchl a molecule is first translated 1.1 nm along the direction of the Q_y transition, resulting in two exciton states at approximately 828 and 788 nm. Second, one of the Bchl *a* molecules is rotated 17° along an axis perpendicular to the membrane plane and rotated 17° along an axis parallel to the membrane plane and perpendicular to the direction of the Q_y transition to obtain a CD signal with reasonable dipole strengths. This yields a final angle of 24° between the Q_y's. Subsequently, this Bchl *a* molecule is translated 0.1 nm perpendicular to the direction of the Q_y transition in the membrane plane and translated 0.1 nm out of the membrane plane to obtain a Q_y CD signal of the proper intensity ratio. Finally, the Q_x transitions are rotated only 2° to introduce a proper CD in these bands. This model thus yields the following maxima, dipole strengths, and rotational strengths for the Q_x and Q_y bands: Q_y(low), 824 nm, 88 dB^2, −0.35 DBM; Q_y(high), 791 nm, 2.9 dB^2, 0.22 DBM; Q_x(high), 602 nm, 0.5 dB^2, −0.08 DBM; Q_x(low), 598 nm, 23 dB^2, 0.05 DBM. The rotational strength of the Q_y(low) band of 0.38 DBM would correspond to a molar ellipticity of approximately 0.1, in good agreement with results from Chang et al. (1990a). The hyperchromicity is about 12%, in reasonable agreement with the experimental results.

It was reported earlier that a subunit very similar to B820 can be formed upon reassociating only the β-polypeptides and Bchl *a*. A further reassociation into B873(recon) upon lowering the OG concentration did not occur in this case as the α-peptide is required (Parkes-Loach et al., 1988). Our results from fluorescence spectroscopy of this particle confirm a strong similarity between the normal B820 complex and this β-only B820. This is in good agreement with the observation that in this complex the ratio between the Bchl *a* pigments and β-polypeptides is approximately 1:1 (Parkes-Loach et al., 1988). In this particle, one of the β-polypeptides probably functions as an α-polypeptide. The lack of α-polypeptides probably pevents further association of the particle to form B873(recon).

B873(reassoc). Reassociation of the B820 form into the B873(reassoc) form enforces a profound lowering of the polarization value to 0.11 (see Figure 6), similar to values reported for detergent-solubilized LH1 complexes (Picorel et al., 1983), for LH1-only mutants of *Rb. sphaeroides* M2192 (Hunter et al., 1988), and for intact chromatophores (Kramer et al., 1984b). This value is indicative of a number of interacting bacteriochlorophylls with a circular degenerate configuration. Thus, upon formation of the B873 form, the B820 aggregates into larger complexes. This is in agreement with studies showing that the bacteriochlorophylls are more exposed in the B820 form than in the B873 form (Miller et al., 1987). In order to rule out the possibility of a difference in aggregation state between the native LH1 complexes and the reassociated B873 particle, we also compared the absorbance and fluorescence of these particles, but were not able to resolve

a systematic difference. We thus propose that upon association of B820 into B873(reassoc) a complex is obtained that is close to identical with the LH1.

It was shown that circular arrangements of 12 dimers could be used to calculate the spectral properties of aggregated forms (van Mourik et al., 1990). It is clear from these calculations that a simple aggregation of dimers can produce an additional red shift of the absorbance to 873 nm without affecting the CD shape and intensity to a large extent. The calculations do not rule out the existence of other aggregate forms but illustrate the possibility of obtaining aggregates from B820 that have spectral properties similar to the properties measured for the B873 species.

The B873(recon) complex formed from α, β, and Bchl *a* is quite similar to the B873(reassoc). A comparison of the data presented in Table I shows that the excitation in the Q_y region the polarization is less compared to the B873(recon) complex. This indicates that in the former B873(recon) complex, interactions between the pigments are not as good as in the B873(reassoc). Probably the reassociation requires a more specific stoichiometry of the different constituents, or the protein is affected by the purification of the constituents. Although different CD signals have been observed for reconstituted B820, our fluorescence data indicate that there is no major difference in structure between these complexes and B820. It is however possible that, as CD is more sensitive to minor structural changes, small structural changes are not reflected in the fluorescence polarization.

Conclusions

The data from fluorescence spectroscopy on the subunit form of LH1 presented here clearly show that a specific organization of the pigments in antenna systems from photosynthetic purple bacteria exists. Much data have been collected on the spectroscopic and biochemical nature of the B820 subunit form. This has yielded two models describing the optical properties of the B820 subunit form. Both models comprise two Bchl *a* molecules bound to an $\alpha\beta$-polypeptide pair. The main difference in the two models is the extent to which the two Bchl *a* molecules are interacting. The two most extreme explanations are that the optical properties of the B820 subunit forms are fully determined either by the pigment–protein interaction or by a model in which the pigment–pigment interaction accounts for all the spectroscopic properties of the B820 subunit form.

The spectroscopic data presented here clearly support a model in which two Bchl *a* molecules are excitonically coupled. The model thus derived, comprising two coupled Bchl *a* molecules in a nearly parallel or antiparallel configuration, enables us to account for most of the optical properties of the B820 subunit. More specifically, we have discussed how this model yields the specific high polarization of the Q_y band and the systematic dip in this signal around 795 nm. Second, we are able to show that the CD of such a dimer is qualitatively comparable to the CD of the B820 subunit assuming that contributions from higher excited states are mixed with the lower states. Finally, we can also account for a red shift of the absorbance of the monomer absorbance and the observed hyperchromicity. We place the absorbance of the monomer Bchl *a* molecule, bound to either α- or β-polypeptide, at 807 nm. Obviously, exciton theory does not provide a means to account for this red shift. Therefore, we assume that this initial red shift is caused by pigment–protein interactions. We can not estimate the extent to which the CD spectrum of the B820 contains additional contributions from protein–chromophore interaction.

The results show that the LH1 complexes from the three different phototrophic bacteria *Rs. rubrum*, *Rb. sphaeroides*, and *Rb. capsulatus* all have consistent, well-defined spectroscopic properties which are characteristic for the underlying principles of pigment–protein organization in bacterial photosynthesis. Since there is a large amino acid sequence homology between LH1 complexes of different photosynthetic purple bacteria, it is interesting to note that hitherto B820 subunit forms have been isolated from three species. As confirmed by our studies, subunit forms of *Rs. rubrum*, *Rb. sphaeroides*, and *Rb. capsulatus* also exhibit very similar spectral properties. We propose that the B820 subunit is a simple building block consisting of an interacting bacteriochlorophyll dimer. In membranes, these small building blocks probably aggregate into larger units making up the core antenna whose spectroscopic properties are determined by interactions between these small subunits.

Acknowledgments

We thank Dr. K. J. Visscher and Dr. J. P. Dekker for critically reading the manuscript. We also express our gratitude to Dr. Barry Marrs, Du Pont Corp., for his kind gift of the *Rb. capsulatus* mutant MW442 and to Dr. Samual Kaplan for the generous gift of the *Rb. sphaeroides* mutant puc 705-BA. Thanks are also due to Peggy Bustamante for help in sample preparation.

Registry No. Bchl *a* dimer, 18025-10-0.

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