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Polypeptides and Bacteriochlorophyll Organization in the Light-Harvesting Complex B850 of *Rhodobacter sphaeroides* R-26.1†

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ABSTRACT: The light-harvesting complex (LHC) B850 from *Rhodobacter sphaeroides* was dissociated into several fragments by treatment with sodium dodecyl sulfate. The molecular weight of each fragment was determined by using transverse polyacrylamide gel electrophoresis under nondenaturing conditions and gel filtration techniques. Four B850 LHCs were observed, having molecular weights of 60 000, 72 000–75 000, 105 000, and 125 000–145 000, and two small bacteriochlorophyll (Bchl)–polypeptide complexes having molecular weights of 6000–8000 and 12 000–14 000. Each of the B850 complexes contains ca. one Bchl *a* for each 6.5-kDa protein. The optical absorption and circular dichroism of the B850 LHCs recorded directly from the gels are similar to those measured previously for a 22–24-kDa B850 LHC by Sauer and Austin [(1978) *Biochemistry 17*, 2011–2019]. These data, combined with studies of other groups, indicate that the smallest LHC in LH1 and LH2 is a Bchl–polypeptide tetramer. Each tetramer contains two Bchl dimers that probably have the structure of P-860, the primary electron donor in *Rhodobacter sphaeroides*, and two α–β-polypeptide pairs. Interactions among the paired Bchls shift their individual Q_y transitions from 780–800 to 850–860 nm, and interactions among two such pairs induce the circular dichroism signal of the LHCs. Three Bchl–polypeptide tetramers probably form a dodecamer having C_3 symmetry, and six such dodecamers organize into a large hexagon that can accommodate one or two reaction center complexes.

Biological photosynthesis converts electromagnetic radiation into useful chemical energy by the joint action of two membrane-bound complexes termed "antennas" and "reaction centers" (RCs) (Clayton, 1980). The antennas consist of light-harvesting complexes (LHCs) made of polypeptide networks that bind small clusters of chromophores, mostly chlorophylls in oxygenic photosynthetic organisms and bacteriochlorophylls (Bchls) in nonoxygenic bacteria. Purple non-sulfur bacteria contain three types of LHCs: LH1 or B875, whose pigment centers have maximum optical absorption at 870–890 nm; LH2 or B800–850, whose pigment centers absorb at 800 and 850– nm, and LH3 or B800–820, whose pigment centers absorb at 800 and 820 nm [for leading reviews, see Cogdell and Thornber (1980), Zuber (1985), and Hunter et al. (1989)]. In recent studies, van Grondelle et al. (1988) suggested a fourth kind of pigment center with optical absorption at 895 nm.

The LHCs are packed around the RCs in order of increasing wavelength, and as energy migrates from short-wavelength-absorbing complexes to long-wavelength-absorbing ones, a unidirectional energy flow is established. Captive photons are finally trapped by the long-wavelength-absorbing primary donors in the RCs, where they promote charge separation across the photosynthetic membrane.

Each of the above-mentioned pigment centers is formed by Bchl *a* molecules, which have their lowest energy absorption at 780 nm when isolated as monomers in vitro. Two basically different concepts have been developed to explain the large

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bathochromic shift of the in vivo Bchls [for a leading review, see Pearlstein (1987)]. The first relates the shift to strong interactions of the Bchls with the surrounding protein (Rafferty et al., 1979; Kramer et al., 1984; Thornber, 1986; Brunisholz & Zuber, 1988); the second relates the shift to strong interactions among Bchl molecules organized in dimers that have similar geometry to the primary electron donors of the purple bacteria (Scherz et al., 1985; Scherz & Parson, 1986; Miller et al., 1987; Ghosh et al., 1988; Scherz & Rosenbach-Belkin, 1989).

The different concepts for the origin of the Q_y shift are directly related to the supramolecular organization of Bchls and the surrounding protein networks in the photosynthetic antennas. Following the first concept and recent electrophoretic studies of the LHC B875 from *Rhodobacter* (*Rb.*) *sphaeroides*, Hunter et al. (1988) have suggested that the minimum unit of the LHCs is a α,β-polypeptide trimer. In LH1, each $(\alpha,\beta)_3$ unit is assumed to hold six Bchls that are separated by about 10–15 Å from each other and have their maximum optical absorption at about 870 nm. In LH2, the polypeptide trimers are proposed to hold nine Bchls; six are separated from each other by about 15 Å and have their maximum absorption at 850 nm, and three are separated from the former by a similar distance and have their maximum absorption at 800 nm. Zuber (1985) and Zuber et al. (1987) have also argued that the individual Bchls have maximum light absorption at 850 nm and are separated from each other by 10–15 Å. However, they suggested that the elementary LHC unit is made of six α,β pairs in a cyclic arrangement where each pair hold two Bchls. In each of these models, the individual Bchls are positions in C_{3n} symmetry to account for the CD and LD signals (Breton et al., 1981; Pearlstein, 1987). Since each Bchl is ligated to a polypeptide (either α or β), these polypeptides should be also organized in a C_{3n} symmetry.

The second concept attributes the absorption at 850 nm to Bchl *a* dimers with an approximate C_2 symmetry (Scherz & Parson, 1986; Scherz & Rosenbach-Belkin, 1989) and assumes that pairs of Bchl dimers form a loosely coupled tetramer. According to this hypothesis, the minimum unit of B850 is a polypeptide–Bchl tetramer and the PP–pigment organization in the complete LHCs has C_{4n} symmetry with $n \geq 3$ (Pearlstein, 1987).

Evidently, the symmetries assumed for the Bchl molecules in the three models coincide for polypeptide–Bchl dodecamers, and the effects of the different symmetries on the spectra of the LHCs cannot be distinguished for larger polypeptide–Bchl units. Therefore, to provide further evidence for any of the discussed models, the spectral properties of smaller LHCs have to be investigated. Such experiments were initiated over a decade ago by Sauer and Austin (1978). These authors reported on a 22-kDa Bchl–protein that had the spectral properties of B850. They suggested that the 22-kDa unit consists of two Bchls and two polypeptides. However, during the last decade, the amino acid sequences of α and β, the two polypeptides that comprise the protein of B850, were determined (Theiler et al., 1984a,b), and the molecular weights were calculated to be 5600 and 5450 for α and β, respectively. With these new numbers, the 22-kDa unit of Sauer and Austin could be a Bchl–polypeptide tetramer, in agreement with the model of Scherz and Parson (1986). The optical absorption and CD of these tetramers are expected to be similar to the spectra of the larger B850 complexes if they are well separated and maintain C_3 symmetry [see Pearlstein (1987)]. Hence, to illuminate the Bchl–polypeptide organization in B850, we set out to isolate B850 fragments of different sizes and compare their optical absorption and CD signals. A preliminary report has been given by Rosenbach-Belkin et al. (1988).

Materials and Methods

Extraction and Purification of B850. Rb. sphaeroides (strain R26.1) was grown anaerobically in Ormerod medium (1961) (Braun, 1988). Wet cells were collected at the stationary growth phase by centrifugation, washed, and lyophilized for storage. Triton X-100–LHC B850 was prepared according to the method of Sauer and Austin (1978) with the following modifications. Isolated chromatophores were treated with 2.5% Triton X-100 (TX-100) as described by Sauer and Austin (1978) layered over a precooled 0.4/0.9 M discontinuous sucrose gradient and centrifuged at 290000*g* for 3 h at 4 °C. The green band at the interface was collected and dialyzed overnight against 10 mM Tris buffer (pH 7.5). TX-100 treatment and the subsequent centrifugation were repeated after concentrating the dialyzed solution to A_{853} = 30–50 cm^{-1} by pressure filtration (Amicon Diaflo membrane PM 10, molecular weight cutoff 10000). TX-100 was replaced by sodium dodecyl sulfate (SDS) by repeated pressure filtration against 10 mM Tris buffer (pH 7.5) containing 0.1 or 1% SDS. The resulting SDS–B850 solution was layered over a 0.4/0.9 M sucrose gradient and centrifuged as described. The green precipitant and the green band at the interface were collected and used for further analyses.

Molecular Weight Determination by Gel Filtration Chromatography. A water-jacketed 1.6 × 70 cm column (Pharmacia K 16/70) was packed with Sephadex G-50 (fine) or Sephacryl 300 (preswollen) beads. The column was equipped with two flow adaptors to allow upward elution. Gels were run in 10 mM Tris buffer (pH 7.5) containing 0.5, 1, or 1.5% SDS (w/v) for chromatography at 14 °C. A calibration curve was prepared with the following proteins in the SDS buffer system: bovine serum albumin (Pharmacia) (67 kDa), ovalbumin (Sigma) (43 kDa), carbonic anhydrase (Sigma) (29 kDa), chymotrypsinogen (Pharmacia) (25 kDa), ribonuclease A (Pharmacia) (13 kDa), and cytochrome *c* (Sigma) (12.4 kDa).

Polyacrylamide Gel Electrophoresis (PAGE). Samples for PAGE were washed with Tris buffer (0.01 M, pH 7.5) containing 1% (w/v) SDS by pressure filtrations and concentrated to A = 10–15 cm^{-1} at 850 nm. Sample buffer that contained 15% (w/v) sucrose, 62.5 mM Tris (pH 6.8), and 0.1 or 1% SDS (w/v) was added to the samples to a final concentration of 30–50% (w/v) when loaded onto gels, and 0.5% pyromin was used as a dye front.

(*A*) *Nondenaturing SDS–PAGE* was carried out according to Laemmli (1975) with the following modifications. SDS was omitted from the resolving gels (1 × 165 × 100 mm), while stacking gels (1 mm × 165 mm × 20 mm), upper and lower reservoir buffer, contained 0.1% or 1% (w/v) SDS. The stacking gel contained 5% polyacrylamide whereas the resolving gel contained a linear gradient of polyacrylamide (8–15%) stabilized by a 5.5–17.5% sucrose gradient [modified after Delepelaire and Chua (1981)]. Electrophoresis was performed at 10 °C in the dark for 6 h. The current used was 10 mA before the dye front entered the resolving gel, and 10–20 mA while it migrated in the resolving gel. Prestained commercial protein molecular weight standards (Bethseda Research Laboratories) were used in all experiments. The standards included cytochrome *c* (12.3 kDa), β-lactoglobulin (18.4 kDa), α-chymotrypsinogen (25.7 kDa), ovalbumin (43 kDa), bovine serum albumin (68 kDa), phosphorylase *b* (97.4 kDa), and myosin H-chain (200 kDa). Under the described conditions, the LHCs retain their long-wavelength absorption

and CD spectra. For their measurements, the positions of the LHCs in unstained lanes of the gel were determined by comparison with the bands in duplicate lanes that were stained with 0.2% Coomassie Brilliant Blue in 45% methanol.

(*B*) *Transverse gradient SDS–PAGE* was modified after Smith and Ellis (1979). The conditions were identical with those described above for nondenaturing SDS–PAGE, with the following modifications. The resolving gel (1 mm × 170 mm × 180 mm) contained an 8–15% polyacrylamide gradient stabilized with a 6.5–17.5% sucrose gradient. Once polymerized, the gel was rotated by 90°, and a 5% (w/v) polyacrylamide slot former was cast along one edge of the gradient gel. Samples and marker mixture were loaded onto the gel in alternating slots.

Molecular Weight Determination Using Ferguson Plots. The prestained standard proteins were separated by transverse PAGE, and their mobilities (R_f) were measured relative to the dye front (Hames & Rickwood, 1985). A Ferguson plot [100 × log $R_f = Y_0 - K_r T$ where Y_0, K_r, and T are the mobility of the free protein, the retardation coefficient, and the polyacrylamide percentage (w/v), respectively] was generated for each standard protein. The different curves were linear and intersected at 1.5–2% polyacrylamide. A plot of the K_r values for the different protein curves against the corresponding molecular weights provided a linear calibration curve.

Light Absorption and CD Measurements. Light absorption was measured by computerized Milton-Roy Spectronics 1001 and 1201 spectrophotometers, to which we attached a linear transport device. The light absorption of electrophoretic complexes was recorded directly from the gels by using the transport device in intervals of 1–2 mm.

CD spectra were measured with a home-built spectrograph (Scherz & Rosenbach-Belkin, 1989). Pigmented regions identified in the gel by their light absorption were excised and used for these measurements. The ellipticity of 1 mg/mL *d*-camphorsulfonic acid in water ($\theta_{292nm} = 0.308°$) was used for calibration. Convolution of CD spectra was carried out by using the CURFIT program (written in Fortran) and the CDMASTER program (written in Turbo Pascal, version 4) of the Weizmann Institute.

Measurements of Bchl-to-Protein Ratio. The LM subunit of the RC, separated on native gels from different fragments of B850, was eluted from the gel bands into Tris-buffered solutions that contained 0.05% SDS. The Bchl and protein concentrations were calculated from the absorption at 855 nm by using an extinction coefficient of 1.3×10^5 M^{-1} cm^{-1} [after Straely et al. (1973)]. Twelve protein standards were prepared from bovine serum albumins as recommended by Bio-Rad Laboratories for microassay calibration with the Bradford reagent. Known concentrations of the LM subunit extract were mixed with the Bradford reagent, and their optical absorption spectra were recorded. The protein amount in each sample calculated from the absorption at 595 nm (using albumin as a standard) was 2.09 times the concentration calculated from the 855-nm absorption (an average of four measurements). This factor was used to correct the protein amounts calculated for the B850 fragments in a similar manner.

RESULTS

Near-IR Optical Absorption and CD of Chromatophores and the TX-100–B850 LHC. Optical absorption and CD spectra of isolated chromatophores from R-26.1 were similar to those reported by Sauer and Austin (1978). Treatment of the chromatophores with TX-100 and subsequent purification

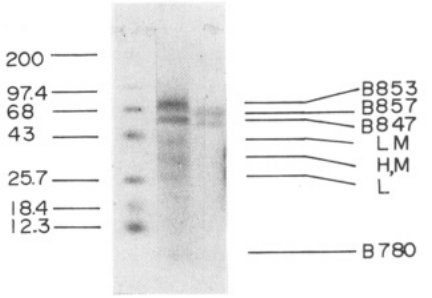

FIGURE 1: Polyacrylamide gel of the nondenatured SDS–B850 LHCs after being stained with Coomassie brilliant blue. (Right lane) SDS–B850 that was collected from the interface of sucrose gradient (0.4/0.9 M) ultracentrifugation after treatment with 1% sodium dodecyl sulfate (SDS) in Tris buffer solution (pH 7.5) for about 2 h. (Middle lane) SDS–B850 that was collected from the interface of the 0.4/0.9 M sucrose gradient after treatment with 0.1% SDS. Running buffer, upper and lower reservoir contained 0.1% SDS. The left lane shows the molecular size markers ($\times 10^{-3}$). The assignment of the LHCs and LM bands is based on the optical absorption of lanes that were not stained with Coomassie blue. The assignment of the M and H subunits (the heavy and medium subunits of the RCs, respectively) is based on the apparent sizes of the stained proteins. LM stands for the L and M polypeptide complex of the RC and is identified by the triple near-IR absorption pattern of the RC. For the LHC bands, the numbers indicate the long-wavelength absorption maximum. Note that the 1% SDS treated samples contain only the B857 and B847 LHCs.

resulted in several LHC preparations with slightly different spectra. The LHC collected from the interface of the first sucrose gradient had maximum optical absorption at 855 nm, and the complex that was collected from slightly above that interface had its absorption maximum at 850 nm. Precipitation of the two complexes by dialysis against 10 mM Tris buffer solution (pH 7.5) and resuspension in the Tris buffer solution resulted in LHCs that had maximum absorption at 858–860 nm.

Preparation of SDS–B850 LHCs. The LHC B850 was dissociated into smaller Bchl–proteins by adding 10 mM Tris buffer solution (pH 7.5) that contained 0.1 or 1% SDS or 1% LDS after the TX-100 was removed. These SDS–B850 fragments were purified by ultracentrifugation on 0.4/0.9 M sucrose gradient as described and examined by PAGE under nondenaturing conditions (Figure 1).

The 1% SDS-treated LHCs, that were collected from the sucrose gradient interface, were separated into two pigmented proteins having apparent sizes of 65 and 50 kDa and maximum optical absorption at 857 and 847 nm, respectively (Figure 1, right lane). The 0.1% SDS treated LHCs that were collected from the sucrose gradient interface separated into six pigmented proteins with apparent sizes of 78, 65, 50, 40, 10, and 6–8 kDa in addition to two nonpigmented proteins (~30 and 28 kDa). The respective wavelengths for maximum absorption of the pigmented proteins were 853, 857, 847, 800, 780, and again 780 nm. Identification of the 40-kDa pigment protein as the LM subunit of a RC was based on its optical absorption in the VIS–NIR which was identical with the absorption of the intact RCs of R-26.

Molecular Weight Determination of SDS–B850 LHCs by Transverse PAGE. The sizes of the SDS–B850 LHCs were more accurately determined by transverse PAGE under nondenaturing conditions. Gels that contained small amounts of the LM subunits (for calibration) were stained with Coomassie brilliant blue (Figure 2) and used to generate Ferguson plots for pigmented proteins that have the spectra of B850 and RC. The apparent molecular weight of the LM complex determined by this method was about 60 000, and the molecular weights calculated for the different SDS–LHC frag-

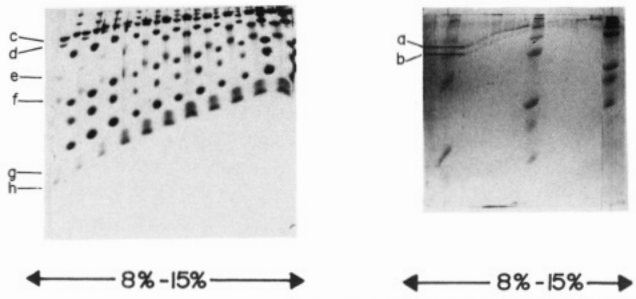

FIGURE 2: Transverse polyacrylamide gel electrophoresis (PAGE) of SDS–B850 fragments under nondenaturing conditions and after being stained with Coomassie brilliant blue. (Right panel) SDS–B850 fragments that were precipitated during 0.4/0.9 M sucrose gradient ultracentrifugation (0.1% SDS in running buffer): (a) 125-kDa unit; (b) 105-kDa unit; lanes 1, 7, and 12 contain marker proteins only. (Left panel) SDS–B850 fragments that were collected from the interface of 0.4/0.9 M sucrose gradient ultracentrifugation and contained traces of LM complex for calibration (0.1% SDS in the running buffer). Even lanes contain molecular size markers; odd lanes are LHC and RC fragments: (c) 72–75-kDa B850; (d) 60-kDa B850; (e) 60-kDa LM subunit of the RC; (f) H and L subunits of the RC; (g) 11-kDa B780 unit; (h) 6–8-kDa B780 unit and dye front. Even lanes contain marker proteins only.

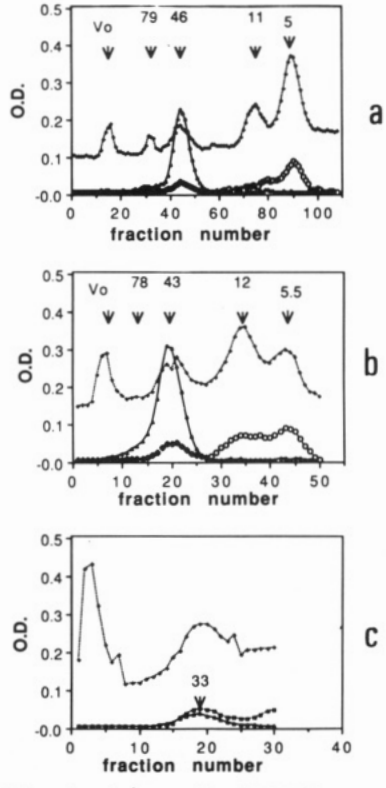

FIGURE 3: Gel filtration elution profile of B850 fragments. (a) Elution profile of 1% SDS treated B850; (b) elution profile of 0.5% SDS-treated B850; (c) elution profile of the LM subunit in 0.5% SDS; V_0, void volume. Numbers above the absorption maxima give the pigment–protein sizes ($\times 10^{-3}$) using the denatured globular proteins for calibration. (+) 280-nm absorption; (△) 850–860-nm absorption; (●) 780-nm absorption; (○ or □) 760-nm absorption.

ments were 6000, 13 000, 60 000, 72 000–75 000, 105 000, and 125 000.

Molecular Weight Determination of SDS–B850 LHCs by Gel Filtration. The SDS-treated LHCs were separated into four pigmented proteins by gel filtration (Figure 3a,b). By use of the globular proteins for calibration, the apparent sizes of these pigmented proteins were found to be 78–79 (minor amount), 43–46 (major amount), 11–12 (intermediate amount), and 5–5.5 kDa (major amount). Under similar conditions, the LM subunit appeared as a 33-kDa protein

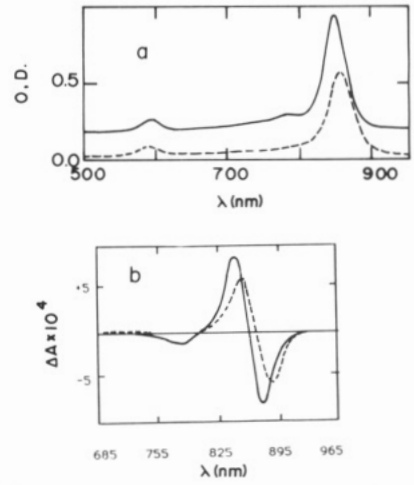

FIGURE 4: (a) Absorption and (b) circular dichroism of SDS–B850 LHCs. Spectra were recorded directly from polyacrylamide gels. Solid lines, B847 (60 kDa); dashed lines, B857 (72–75 kDa). ΔA is the difference between the absorption of left and right circularly polarized light.

(Figure 3c) instead of the calculated 60 kDa using transverse PAGE, or amino acid composition. Therefore, the 78–79- and 43–46-kDa units were multiplied by 60/33 to give the corrected size of the B850–LHCs after SDS treatment. The resulting (~140, ~76 kDa) correspond well to the sizes determined by transverse PAGE. Note that the optical absorption of both complexes peaked at ~850 nm and could not be further resolved.

The apparent size of the smaller pigment proteins corresponds quite well to the calculated size of the α or β subunits (~5.5 kDa) and their dimers (ca. 11 kDa) with no further correction. One way to account for the need to correct the higher MW is that the monomeric or dimeric subunits are fully exposed to hydrophobic interactions with the SDS molecules. Therefore, their binding capacity is similar to that of the globular protein after denaturation (~1.3 mg of SDS to each milligram of protein). The aggregation of the α and β subunits to larger LHCs as well as the folding of the five helices in the L and M polypeptides saturates about half of their hydrophobic sites and reduces the number of SDS molecules that are needed to saturate the hydrophobic sites of each milligram of protein. Therefore, their apparent size appears lower on the globular protein calibration curve.

There is a significant decrease in the number of pigments per proteins followed by pheophitinization when the 5.5- and 11-kDa fractions are compared with the 76- and 140-kDa LHCs. The pigments that are held by the 5.5- and 11–12-kDa proteins have the spectral features of Bphe *a* and oxidized Bchl *a* probably due to a degradation of the chromophores. The elution profile of the SDS–B850 LHC complexes may point to the presence of a minor SDS–B850 LHC with a molecular weight of ca. 25 000–30 000 (shoulder to the right of the ca. 76-kDa protein). This third component is probably equivalent to the 22-kDa unit reported by Sauer and Austin (1978), but it contributes only 5–10% of the total SDS–B850 LHCs. We could not isolate this complex on the native gels.

Optical Absorption and CD Spectra of SDS–B850 LHCs in the Near-IR. The absorption and CD spectra of the smallest Bchl–protein unit on the nondenaturing gel (with apparent size of 6–8 kDa) are identical with the spectra of Bchl *a* monomers in vitro in protic solvents (Rosenbach-Belkin, 1989). It was, therefore, termed B780. The 60-kDa fragment (termed B847) has maximum absorption at 847 nm (Figure 4a, solid line) and a satellite absorption at 780 nm. It shows relatively strong

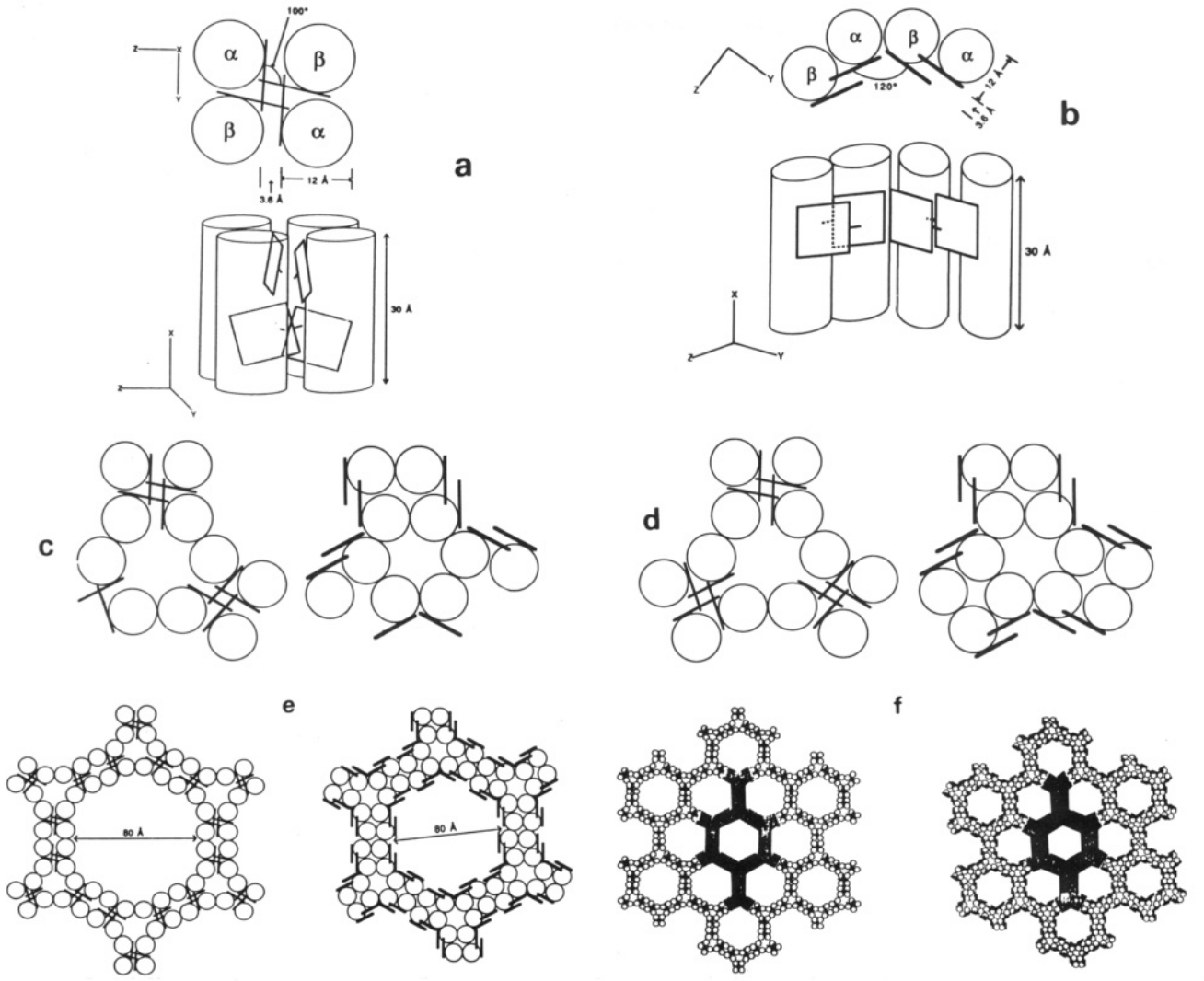

FIGURE 5: Bchl and polypeptide organization in B850 and the complete LHC–RC unit. (a) Four Bchls (squares) attached to four polypeptides in a heterodimer configuration. (b) Four Bchls attached to four polypeptides in a homodimer configuration. (c) Proposed structures of B847 made of homo (left) or hetero (right) polypeptide dimers. (d) Proposed structure for B857. To obtain B847, two PPs and the attached Bchls should be removed. (e) Complete B850 LHC for homodimers (left) and heterodimers (right). (f) Supramolecular organization of several B850 LHCs, B870 LHCs (shaded area), and RCs.

and slightly nonconservative CD signals at 840 (+) and 871 (−) nm (Figure 4b, solid line). By use of the CURFIT and CDMASTER programs, the CD signal was deconvoluted into three Gaussians that peak at 872 nm (negative), 854 nm (positive), and 780 nm (negative). The absorption band at 780 nm (Figure 4) is typical of Bchl monomers in vitro. The extinction coefficient of such monomers is about half the extinction coefficient of a dimerized Bchl (Scherz & Rosenbach-Belkin, 1989). Taking this into account, the 780-nm-absorbing Bchls make up about one-fourth of the B847 Bchls. The CD signal of B847 at 780 nm (Figure 4b, solid line), which is about 10 times the signal of Bchl monomers in vitro and opposite in sign, suggests that the "monomeric" Bchls are loosely coupled to the 847-nm-absorbing pigments and thereby gain their optical activity [for a discussion of this "intensity borrowing", see Scherz and Parson (1986)].

The 72–75-kDa unit (termed B857) has its maximum optical absorption at 857 nm (Figure 4a, dashed line) and CD signals at 847 (+) and 883 (−) nm (Figure 4b, dashed line). Maximum absorption at 855 nm and almost conservative CD signals at 845 (+) and 881 (−) nm characterize the 105- and 125-kDa fragments of the B850 LHC (data not shown).

The near-IR spectra (optical absorption and CD) for the 76- and 140-kDa fragments of B850 separated by gel filtration resemble the near-IR spectra of the large (>120 kDa) SDS–B850 separated by native gels.

Bchl-to-Protein Ratio in B850 Fragments after SDS Treatment. SDS-treated fragments of the LHC B850 were extracted from the corresponding gel slabs. The chromophore concentration in each sample was determined from the maximum absorption in the near-IR (847, 857, or 853 nm), using an extinction coefficient of 1.13×10^5 M^{-1} cm^{-1}. This value equals the extinction coefficient of B850 in B800–850 of *Rb. sphaeroides* strain 2.4.1 (1.8×10^5 M^{-1} cm^{-1}, Clayton & Clayton, 1981) divided by the corresponding bandwidth (360 cm^{-1}) and multiplied by the bandwidth of B850 in *Rb. sphaeroides* strain R-26.1 (561 cm^{-1}) found in the present study.

Known volumes of the B847 and B857 extracts were mixed with the Bradford reagent, and the apparent protein concentration was calculated by using the bovine serum albumin standards. The result was divided by 2.09. For both complexes, we found one molecule of Bchl *a* per each 6.5-kDa protein.

DISCUSSION

Polypeptide and Bchl Contents of SDS–B850 Fragments. Theiler et al. (1984a,b) have shown that B850 from R-26.1

and B800–850 from wild-type *Rb. sphaeroides* consist of similar α- and β-polypeptides with molecular weights of 5600 and 5450, respectively. We therefore presume that B780 is a Bchl-α or Bchl-β subunit and that the 11–12-kDa fraction is a polypeptide dimer. Note that the average difference between the molecular weights of the SDS–B850 fragments is $n \times 12000$ where n is an integer.

The gel filtration elution profile of Sauer and Austin (1978) suggest that the smallest SDS–B850 fragment that absorbs at 850 nm has a molecular weight of ca. 22000–24000 and should therefore contain about four polypeptides and four Bchl molecules. In our hands, the concentration of this component relative to other SDS–B850 fragments is very small. However, Sauer and Austin (1978) were able to isolate an SDS–B850 LHC of similar molecular weight in very high yield from their *Rb. sphaeroides* mutants. Although they referred to these mutants as R-26, their spectra fit the criteria of strain R-26.1 (Theiler et al., 1984a).

Following similar argumentation, B847 (60 kDa) is probably a Bchl–polypeptide decamer, and B857 (72–75 kDa) is probably a dodecamer. The larger Bchl–polypeptide complexes are probably aggregates of these B850 fragments. Note that the smallest B800–850 LHC studied by Hunter et al. (1988) had an apparent molecular weight of 145000. The molecular weight after subtracting the contribution of the 800-nm-absorbing Bchls and the carotenoids from this complex is approximately 125000, similar to the largest B850 fragment that we have separated by gel filtration (Figure 3a,b) and transverse PAGE techniques.

The optical absorption and CD signals of the different SDS–B850 LHCs in the near-IR region are practically identical with the spectra described by Sauer and Austin (1978) for the 22–30-kDa SDS–B850 LHC, which we take to contain four Bchls. This implies that the spectral properties of B850 are governed primarily by interactions within Bchl tetramers, as suggested by Scherz and Parson (1986). These tetramers need to form cyclic arrays of 30–50 molecules each to account for the fluorescence annihilation observed by van Grondelle et al. (1988). Yet, they need to be well separated from each other to conserve their absorption and to maintain a C_3 symmetry to conserve the double-banded CD (Pearlstein, 1987). In the following discussion, we shall consider a model that has these required properties.

Pigment Organization in Bchl–Polypeptide Tetramers. The double-Cotton effect at 860 nm observed by Sauer and Austin in their SDS–B850 LHC indicates that the complex contains two pigment centers that have maximum optical absorption at 860 nm. Evidently, each pigment center consists of two Bchls. There are several indications that the maximum absorption of the individual Bchls is at 780 nm. First, this is the absorption wavelength for the B780 unit, which probably contains one polypeptide and one Bchl. Second, approximately 2 of the 10 Bchls in B847 absorb at 780 nm although they are probably ligated to the protein. By addition of two more Bchls, the maximum absorption of these Bchls is shifted to 857 nm (in B857), possibly due to their dimerization with the added Bchls. Third, there is no indication that the protein environment of the Bchl molecules in the LHCs can induce a large bathochromic shift of their Q_y transition (Scherz & Parson, 1986). On the other hand, in vitro dimers of Bchl *a* can mimic the Q_y shift and the accompanying CD signal of the Bchl dimers in vivo (Scherz & Rosenbach-Belkin, 1989). Hence, the bathochromic shift of the long-wavelength transition in each pigment center of the 22–30-kDa SDS–B850 is most likely due to the dimerization of the Bchls (Scherz et al., 1985; Scherz & Parson, 1986; Miller et al., 1988; Ghosh et al., 1988; Scherz & Rosenbach-Belkin, 1989). The Bchl dimers probably have a geometry similar to that of the primary donor P-860 (Scherz & Parson, 1986; Scherz & Rosenbach-Belkin, 1989), and therefore have a single positive CD band at about 855 nm.

Table I: Calculated[a] Spectroscopic Properties of Bchl Tetramers and Experimental Properties of the 72–75-kDa B850 Light-Harvesting Complex

	λ_{max} (nm)	dipole strength (D^2)	rotational strength ($D\text{-}\mu_B$)
configuration A[b]	872	61	−1.4
	854	179	+1.4
configuration B[c]	864	66	−30.0
	857	133	+30.0
experimental	872	≤40	−1.02
	854	≥160	+1.18

[a] Only the lowest energy Q_y excitonic transitions of two Bchl molecules have been considered. The four Bchls are assumed to construct two tight pairs, each with the geometry of P-860 (Scherz & Parson, 1986; Scherz & Rosenbach-Belkin, 1989). For the calculation details, see Scherz and Parson (1986). [b] Figure 5a. [c] Figure 5b.

Interactions between the Bchl dimers in each 22–30-kDa SDS–B850 unit provide a mechanism for the strong CD induction (Scherz & Parson, 1986). Two possible configurations of the proposed tetramers are shown in Figure 5a. The upper tetramer is made of two homodimers (α–β), and the lower one is made of two heterodimers. In either geometry, self-assembly of the Bchls, which is driven by a free energy change of −5 kcal/mol (Scherz et al., 1989, 1990), would promote the association of the attached polypeptides. Assuming that the Bchl dimers have the geometry of P-860, their macrocycles should be tilted relative to each other by 15–20° (Deisenhofer et al., 1985; Feher et al., 1989). Hence, the polypeptides should be slightly nonparallel. Note that in the heterodimers all the Bchls bind to the cytoplasmic side of the polypeptides, where in the homodimer arrangement one dimer is bound to the periplasmic side of the β-polypeptides and the other Bchl dimer binds to the cytoplasmic side of the α-polypeptides. In each case, exciton coupling among the 860-nm transition dipoles introduces strong CD signals (Table I), but the rotational strength is 10 times larger for the homodimers. Since the splitting between the resulted transitions to the excitonic states is smaller from the splitting in the heterodimers (Table I), the observed CD signal can have the same magnitude.

The organization of the Bchl dimers in each tetramer can account for the ultrafast fluorescence depolarization in B800–850 (Kramer et al., 1984; van Grondelle et al., 1988) because the transition dipoles of the lowest energy transitions in adjacent dimers are roughly perpendicular and their centers are separated by about 15–17 Å.

Bchl and PP Organization in B847 and B857. Our data indicate that B847 probably contains 5 α–β pairs and 10 Bchl molecules. These Bchls and polypeptides should include two tetramers that have maximum absorption at 850–860 nm and strong CD signals whereas two Bchls should stay as single units to account for the absorption at 780 nm and the CD signal at that wavelength (Figure 5c).

B857 (72–75 kDa) probably consists of 12 polypeptides and 12 Bchls organized into 3 tetramers. In that case, we expect no absorption or CD signal at 780 nm (Figure 4a,b). A structure that positions the hetero- and homotetramers in C_3 symmetry as required for maintaining their double-Cotton effect in the near-IR region (Pearlstein, 1987) is shown in Figure 5d. The fragmentation of the prolonged SDS-treated

LHCs into α or β monomers, that contained less than a single Bchl to each polypeptide unit and a much larger B850 complex, indicates the Bchl association is not important for the polypeptide dimerization but essential to the formation of large pigment–protein complexes. As the homotetramers are packed, their Bchls could be pushed slightly closer to each other in the XY plane and thereby have their wavelength for maximum absorption further shifted to the IR. A similar phenomenon but due to thermal expansion was proposed to account for the bathochromic shift of the RCs lowest energy transition (Parson & Warshel, 1987; Won & Friesner, 1988).

Association of six polypeptide–Bhcl dodecamers (Figure 5) could lead to the large and cyclic Bchl ensembles suggested by singlet photon annihilation experiments on B800–850 LHCs (van Grondelle et al., 1988; Hunter et al., 1989). Each ensemble shown in the figure contains 48 Bchls. In the hexagon formed by the homodimers, half of the Bchl molecules form a ring that is parallel to the hexagon perimeter and close to the periplasmic side of the LHC. The other dimers are perpendicular to the hexagon perimeter and form a ring that is close to the cytoplasmic side of the LHC. In the other configuration, all 48 Bchls face the cytoplasmic side of the network, but only half face the inner perimeter of each hexagon.

Since the α and β PPs of B870 are homologous to the α and β PPs of B800–850 (Zuber, 1985; Zuber et al., 1987), we expect a similar structural organization. Indeed, Miller et al. (1988) and Ghosh et al. (1988) have concluded that B870 or *Rhodospirillum rubrum* contains Bchl–polypeptide tetramers.

However, these authors have noted that the Bchl–PP tetramers do not provide the full shift of the Q_y transition (upon reconstitution from single α- and β-Bchl units). This could mean that the geometry of the Bchl dimers depends (probably in a cooperative manner) upon the PP packing in B870.

A supramolecular organization of B850, B870, and the RC complex based on the expansion of the homo- and heterotetramers is illustrated in Figure 5f. Oligomerization of the 48 Bchl hexagons could generate large honeycomb structures that can accommodate the bacterial RCs, which are 80 Å long and 40 Å wide (Feher et al., 1989). These structures are similar to the RC–LHC units described by Stark et al. (1984) and agree with the supramolecular organization that has been proposed by Zuber and collaborators (Zuber et al., 1987). The hexagons of LH1 containing the RCs could be surrounded by LH2 hexagons, as shown in Figure 5c.

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Fluorescence of Tryptophan Dipeptides: Correlations with the Rotamer Model

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ABSTRACT: The multiexponential decay of tryptophan derivatives has previously been explained by the presence of rotamers having different fluorescence lifetimes, but it has been difficult to correlate rotamer structure and physical properties. New time-resolved and static data on dipeptides of the type Trp-X and X-Trp, where X is another aminoacyl residue, are consistent with the rotamer model and allow some correlations. That a dominant rotamer of Trp-X zwitterion has the $-NH_3^+$ group near the indole ring was inferred from absorption and fluorescence spectra, titrimetric determination of pK_a values, photochemical hydrogen–deuterium-exchange experiments, decay-associated spectra, quantum yields, and decay kinetics. Analysis of the lifetime and quantum yield data for Trp dipeptides, especially X-Trp, suggests that static self-quenching is not uncommon. Highly quenched and weak components of the fluorescence do not contribute to the calculated mean lifetime, thus resulting in apparent static quenching. We propose the term *quasi-static self-quenching* (QSSQ) to distinguish this phenomenon from quenching due to ground-state formation of a dark complex. Mechanisms of quenching and the structure of statically quenched rotamers are discussed. The occurrence of QSSQ supports the idea that rotamers interconvert slowly. A major perceived deficiency of the rotamer model, namely, the apparent inability to predict reasonable rotamer populations from fluorescence decay data, may result from the presence of statically quenched species, which do not contribute to the fluorescence.

The idea that different conformations of a given protein coexist in solution has been supported by fluorescence decay measurements. The fluorescence decay of a single species should be monoexponential [e.g., Pringsheim (1949)], so it might be supposed that proteins having a single tryptophan responsible for their fluorescence should each have monoexponential decay. However, most such proteins exhibit multiexponential behavior (DeLauder & Wahl, 1971; Conti & Forster, 1975; Formoso & Forster, 1975; Grinvald & Steinberg, 1976), suggesting the presence of conformers. Tryptophan, its derivatives, and other indole-containing compounds have often been used as models for the study of protein fluorescence. Many of these compounds exhibit nonexponential decay, in analogy with that of single-tryptophan proteins. Rayner and Szabo (1978) first showed that tryptophan zwitterion had biexponential decay kinetics, and others have noted that the decays of tryptophan derivatives were multiexponential (Werner & Forster, 1979; Petrich et al., 1983). Similar nonexponential decay has been noted for tyrosine and related compounds (Gauduchon & Wahl, 1978; Ross et al., 1986; Laws et al., 1986).

These phenomena are most easily explained on the basis of the presence of several conformers, each with different fluorescence lifetimes. In the case of aromatic amino acids, these conformers are postulated to be formed by rotation of carbons about the C_α–C_β and/or C_β–C_γ bonds (Donzel et al., 1974; Szabo & Rayner, 1980; Robbins et al., 1980; Ross et al., 1981). Attempts have been made (Szabo & Rayner, 1980; Petrich et al., 1983; Gudgin-Templeton & Ware, 1984; Beechem & Brand, 1985) to correlate lifetime components with rotamer populations inferred from NMR measurements (Dezube et al., 1981).

Investigation of the rotamer model is clearly desirable for an understanding of protein fluorescence kinetics. Here, we report spectral and lifetime data for tryptophan dipeptides of the form Trp-X and X-Trp, where X is another amino acid, to see if the properties fit the rotamer model. We provide evidence that a single rotamer predominates in Trp-X dipeptides, in which the indole and amino groups are close and influence each other's properties. Quantum yield and lifetime data show that X-Trp zwitterions regularly exhibit static self-quenching. Evidence for such static quenching has been sought unsuccessfully in the past, possibly due to poor precision in lifetime and quantum yield measurements. These findings have interesting implications for the interpretation of protein fluorescence.

Materials and Methods

Peptides were obtained from the following sources: Trp-Val and Trp-β-Ala were from U.S. Biochemical Corp. Trp-Lys, Tyr-Trp, Glu-Trp, and Lys-Trp were from Research Plus, Inc. Gly-Trp-Gly, Trp-Ser, and Phe-Trp were obtained from

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