Enzymatic and Chemical Cleavage of the Core Light-Harvesting Polypeptides of Photosynthetic Bacteria: Determination of the Minimal Polypeptide Size and Structure Required for Subunit and Light-Harvesting Complex Formation†

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ABSTRACT: To ascertain the minimal structural requirements for formation of the subunit and core light-harvesting complex (LH1), the α- and β-polypeptides of the LH1 from three purple photosynthetic bacteria were enzymatically or chemically truncated or modified. These polypeptides were then used in reconstitution experiments with bacteriochlorophyll *a* (BChl*a*), and the formation of subunit and LH1 complexes was evaluated using absorbance and circular dichroism spectroscopies. Truncation or modification outside of the conserved core sequence region of the polypeptides had no effect on subunit or LH1 formation. However, the extent of formation and stability of the subunit and LH1 decreased as the polypeptide was shortened inside the core region within the N-terminal domain. This behavior was suggested to be due to the loss of potential ion-pairing and/or hydrogen-bonding interactions between the polypeptides. While the spectroscopic properties of the subunit complexes generated using truncated polypeptides were analogous to those obtained using native polypeptides, in some cases the resulting LH1 complex absorption was blue-shifted relative to the control. Thus, truncation within the N-terminal domain may have long-range effects on the immediate BChl*a* binding environment, since the putative BChl*a* binding site resides near the C-terminal end of the polypeptides. It was also demonstrated that the His located within the membrane-spanning domain on the N-terminal end of the β-polypeptide is not participating in ligation of the BChl*a* in the reconstituted subunit and therefore probably not in LH1.

The light-harvesting complexes (LH)[1] found in photosynthetic organisms collect and transfer excited-state energy with high efficiency to the reaction center (RC) (Duysens, 1951; Vredenberg & Duysens, 1963; van Grondelle, 1985). In purple non-sulfur photosynthetic bacteria, the core LH (LH1) is located in close proximity to, and is present in, a fixed stoichiometric ratio with the RC (Aagaard & Sistrom, 1972; Monger & Parson, 1977; Dawkins et al., 1988). LH1 comprises two small polypeptides α and β, which are present in a 1:1 ratio, along with two bacteriochlorophyll (BChl) and one or two carotenoid molecules per αβ pair (Sauer & Austin, 1978; Broglie et al., 1980; Cogdell et al., 1982; Picorel et al., 1983). A property of continuing interest is the substantial red shift of the BChl Q_y absorption band in LH1. Indeed, the BChl in LH1 absorbs between 870 and 1015 nm, whereas monomeric BChl*a* and BChl*b* in acetone absorb at 777 and 790 nm, respectively (Weigl, 1953; Oelze, 1985). Hitherto, information on the structure and function of LH1 has been obtained using biochemical and spectroscopic methods, since the structure of LH1 from purple bacteria is not known to atomic resolution. However, a number of groups have prepared diffracting crystals and are presently working to solve the X-ray crystal structure [see Cogdell and Hawthornthwaite (1993) for a recent review].

LH1 α- and β-polypeptides each have an average molecular weight of 6000 and contain approximately 65% α-helical character based on far-UV CD data (Cogdell & Scheer, 1985; Breton & Nabedryk, 1987; Ghosh et al., 1988) and IR spectroscopy (Theiler & Zuber, 1984). Primary sequence analysis of LH1 polypeptides from several purple bacteria [see Zuber and Brunisholz (1991) for a review and Figure 1 for specific references], along with hydropathy plots, has enabled three distinct domains to be identified: the hydrophobic membrane-spanning domain (primarily α-helical), which is flanked by the highly charged hydrophilic N-terminal domain, and the moderately charged C-terminal domain (Brunisholz et al., 1984a, 1985). Partial proteolysis experiments have demonstrated that the α- and β-polypeptides each span the photosynthetic membrane once, with the N-terminus facing the cytoplasmic side and the C-terminus facing the periplasmic side (Brunisholz et al., 1984b, 1986;

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[1] Abbreviations: BChl, bacteriochlorophyll; BChl*a*, bacteriochlorophyll *a*; BChla_{gg}, bacteriochlorophyll *a* containing geranylgeraniol as the esterifying alcohol; BChl*b*, bacteriochlorophyll *b*; B820, subunit form of the core light-harvesting complex; CB, carboxypeptidase B; CD, circular dichroism; CY, carboxypeptidase Y; HFA, hexafluoroacetone trihydrate; HPLC, high-performance liquid chromatography; HSQC, heteronuclear single quantum coherence; IR, infrared; LH, light-harvesting complex; LH1, core light-harvesting complex; LH2, peripheral light-harvesting complex; MWCO, molecular weight cutoff; NMR, nuclear magnetic resonance spectroscopy; OG, *n*-octyl β-D-glucopyranoside; (PRQ...C-terminus) represents the truncated polypeptide where the first three amino acids are PRQ and the polypeptide extends to the C-terminus; Q_y, $\pi \rightarrow \pi^*$ transition of BChl in the near-IR region of the absorption spectrum; RC, reaction center; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; UV, ultraviolet region of the absorption spectrum.

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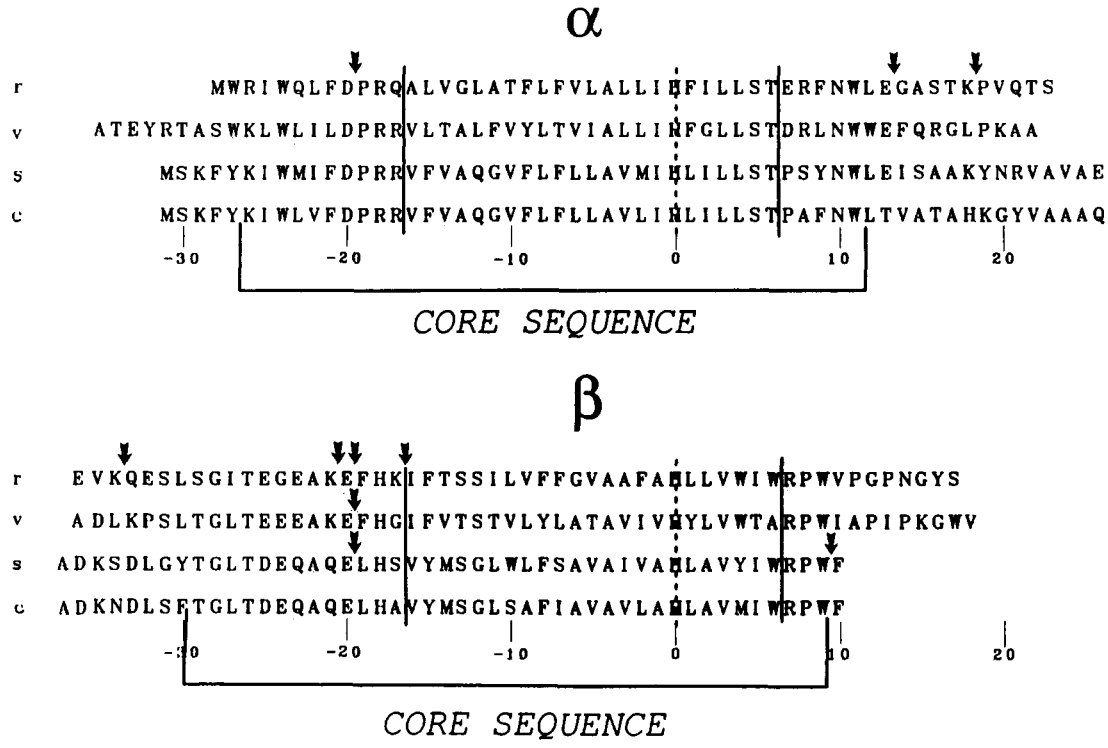

FIGURE 1: Amino acid sequences of the α- and β-polypeptides of (r) *R. rubrum* (Brunisholz et al., 1981, 1984a; Gogel et al., 1983; Bérard et al., 1986), (v) *Rps. viridis* (Brunisholz et al., 1985; Wiessner et al., 1990), (s) *Rb. sphaeroides* (Theiler et al., 1985; Kiley et al., 1987), and (c) *Rb. capsulatus* (Tadros et al., 1984, 1985; Youvan et al., 1984). For ease of comparison, the common His residue nearest the C-terminus was used for alignment and defined as the zero position. Arrows indicate the positions of chemical and protease cleavage sites.

Tadros et al., 1986a,b, 1987, 1988; Takemoto et al., 1987). All LH1 polypeptides contain a conserved His residue (assigned to position 0 in Figure 1), which is proposed to bind one BChl molecule noncovalently via coordination to the Mg atom (Brunisholz et al., 1984a; Robert & Lutz, 1985; Bylina et al., 1988).

One approach to obtaining structural information regarding LH1 has been the isolation and characterization of subunit complexes from the LH1 of a number of purple bacteria (Miller et al., 1987; Chang et al., 1990a; Heller & Loach, 1990; Kerfeld et al., 1992, 1994; Meckenstock et al., 1992; Visschers et al., 1992; Jirsakova et al., 1993). Spectroscopic and biochemical studies of the subunit, which has a Q_y absorbance near 820 nm and has thus been named B820, have indicated that the basic unit of B820 is a protein-bound dimer of BChl molecules that are excitonically coupled (Chang et al., 1990a; van Mourik et al., 1991), with two BChl's per αβ pair (Miller et al., 1987; Loach et al., 1990). The subunit is presumed to comprise either a single αβ pair (van Mourik et al., 1991; Loach & Parkes-Loach, 1995) or an $(\alpha\beta)_2$ unit (Ghosh et al., 1990; Sturgis & Robert 1994). Nevertheless, the subunit is believed to be the "basic building block" of LH1, because spectrally the B820 subunits appear to be quite similar, regardless of different bacterial sources [Visschers et al., 1991; for a review, see Loach and Parkes-Loach (1995)]. Further, the subunit can be reversibly reassociated to the LH1 complex or dissociated to free (unbound) BChl and protein.

The subunit and LH1 can also be reconstituted in vitro from separately isolated α- and β-polypeptides and BChl (Parkes-Loach et al., 1988, 1994; Loach et al., 1994). The reconstitution assay, together with the knowledge of the amino acid sequences of the LH1 α- and β-polypeptides, has enabled structure–function relationships in LH1 to be probed in increasing detail. For example, reconstitution experiments employing BChl analogues have elucidated the key structural binding requirements of BChl for the formation of subunit and LH1 complexes (Parkes-Loach et al., 1990). Moreover, reconstitutions between heterologous α- and β-polypeptide pairs have indicated that the core sequence regions (see Figure 1) of the polypeptides contain all of the necessary components for reconstitution of the subunit and LH1 (Loach et al., 1994). Finally, recent work involving the addition of carotenoid during reconstitution of LH1 has illustrated that the in vivo spectral properties of wild-type LH1 can be reproduced in reconstitution (Davis et al., 1995).

Recent mutagenesis experiments on LH1 polypeptides have illustrated the importance of certain amino acids or specific regions of the polypeptides for LH1 assembly and formation in vivo (Bylina et al., 1988; Dörge et al., 1990; Stiehle et al., 1990; Babst et al., 1991; Richter et al., 1991, 1992; Olivera et al., 1994). Also, mutations of selected highly conserved residues and the attending spectral changes of the LH1 and/or the peripheral LH (LH2) have been used to provide insight into those amino acids that may be proximal to and interacting with the BChl (Babst et al., 1991; Fowler et al., 1992, 1993; Crielaard et al., 1994; Olivera et al., 1994; Olsen et al., 1994; Visschers et al., 1994). The in vitro reconstitution assay complements the mutagenesis studies and offers distinct advantages for structure–function studies, since it relies solely upon the interactions between the α- and β-polypeptides and BChl within a detergent milieu. Thus, the polypeptide components involved in formation and stabilization of the LH1 complex may be examined in vitro without taking into account additional factors that may be operative in vivo [see Kaplan and Donohue (1993) for a recent review].

Our goal in this study is to determine the minimal polypeptide size and structure necessary for binding BChl*a* and forming a subunit and LH1 in vitro. Our approach involves enzymatically or chemically truncating or modifying LH1 polypeptides within the core sequence region and using these altered polypeptides to reconstitute the subunit and LH1 with BChl*a*.

MATERIALS AND METHODS

Materials. *n*-Octyl β-D-glucopyranoside (OG) was obtained from Sigma Chemical Company. Hexafluoroacetone trihydrate 98% (HFA) and trifluoroacetic acid (TFA) (protein sequencing grade) were purchased from Aldrich. [1,1′-$^{13}C_2$]-Acetic anhydride (minimum 99% labeled) and [^{15}N]-L-phenylalanine (minimum 99% labeled) were purchased from Isotec, Inc. [^{15}N]Ammonium sulfate (minimum 98% labeled) was a gift from Dr. David LeMaster of Northwestern University. High-performance liquid chromatography (HPLC) solvents were HPLC grade and were obtained from Burdick & Jackson.

Bacteriochlorophyll *a* containing geranylgeraniol as the esterifying alcohol (BChla_{gg}) was isolated and purified from the carotenoidless mutant G-9 of *Rhodospirillum rubrum* following procedures based on Berger et al. (1987) and Michalski et al. (1988). Since BChla_{gg} is air and light sensitive, all manipulations were performed under an argon atmosphere in dim red fluorescent light, and all solvents were degassed and kept on ice throughout the procedure. Pelleted whole cells (1 g) were introduced into a homogenizing tube and resuspended in 4 mL of methanol. Following 10 min of centrifugation in a table-top centrifuge, the supernatant was removed and maintained on ice. The resulting pellet was extracted with 3 × 4 mL of 80% (v/v) methanol, and all of the supernatants were combined. Deionized water was added to the pigment-containing supernatant to decrease the methanol concentration to 70% (v/v). The supernatant was then loaded onto a Sep-Pak Plus C-18 cartridge (Waters Chromatography) that had been prewashed with 70% methanol. The BChla_{gg} was washed with 2 × 10 mL of 70% methanol and eluted with 10 mL of 100% ethanol. The ethanol was removed in vacuo leaving a dark green residue. Quantification of the BChla_{gg} in ether was accomplished using ϵ_{770} = 91 000 M^{-1} cm^{-1} (Smith & Benitez, 1955). The quality of the BChla_{gg} was assessed via analytical HPLC on a Beckman Ultrasphere ODS column (250 × 4.6 mm, 5 μm, 80 Å), with a mobile phase consisting of 72:20:8 acetone/ethanol/water (v/v) and a flow rate of 0.8 mL/min. The pigment was purified further by preparative HPLC on a Zorbax ODS column (MAC-MOD Analytical) (250 × 9.4 mm, 5 μm, 70 Å), using the mobile phase described previously and a flow rate of 2 mL/min. Detection was monitored at 380 nm during both HPLC procedures. Bacteriochlorophyll *a* containing phytol as the esterifying alcohol (BChla_p), obtained from Sigma Chemical Company and used without further purification, was used only in those reconstitutions involving the acetylated α-polypeptide of *R. rubrum*.

R. rubrum (strain S1) wild-type cultures were propagated, and the α- and β-polypeptides of the LH1 complex were isolated by procedures described previously (Tonn et al., 1977; Brunisholz et al., 1981, 1984a; Gogel et al., 1983; Miller et al., 1987). Cultures of the PUC705-BA mutant (lacking the LH2 complex) of *Rhodobacter sphaeroides* (Lee et al., 1989) were grown, and the α- and β-polypeptides of the LH1 complex were isolated by previously reported procedures (Chang et al., 1990b; Davis et al., 1995). *Rhodopseudomonas viridis* wild-type ATCC 19567 whole cells were grown, and the α- and β-polypeptides were isolated according to Brunisholz et al. (1985) and Parkes-Loach et al. (1994).

Table 1: HPLC Gradients[a]

%B solvent[b]	time (min) G1	G2	G3	G4
50	0	0	0	0
70	10	30	40	20
90	30	45	55	45
100[c]	32	50	60	50

[a] Gradients are named G1, G2, G3, and G4. %B solvent indicates the percentage of solvent B (0.1% TFA in 2:1 acetonitrile/2-propanol). Example of how to read table: in gradient G2, at time = 0 min, %B = 50 and increases linearly to %B = 70 in 30 min. [b] Each %B solvent step is linear. [c] Upon reaching %B = 100, each gradient includes a 12 min reequilibration step back to time = 0 min conditions.

Prior to use, the α- and β-polypeptides were purified using reverse-phase HPLC according to methods outlined in Tarr and Crabb (1983) and Parkes-Loach et al. (1988), with modifications. The lyophilized polypeptides were dissolved in HFA prior to injection onto either a Perkin-Elmer Pecosphere HS-5 HCODS C-18 cartridge column (250 × 4.6 mm, 5 μm, 300 Å, flow rate = 0.7 mL/min) or a Waters RCM 8 × 10 Module fitted with a Radial-Pak Delta-Pak C-18 cartridge (100 × 8 mm, 15 μm, 300 Å, flow rate = 2.8 mL/min). The HPLC solvent system consisted of (A) 0.1% (v/v) TFA as the aqueous solvent and (B) 0.1% (v/v) TFA in 2:1 (v/v) acetonitrile/2-propanol as the organic solvent. Gradient G4 in Table 1 was used during purification. The wavelength of detection was either 220 or 280 nm. Following purification, the polypeptides were lyophilized and stored at −20 °C.

Proteolysis of the LH1 α- and β-Polypeptides. Two enzymes were utilized to specifically cleave the α- and β-polypeptides. Endoproteinase Glu-C protease V8 from *Staphylococcus aureas V8* was purchased from Boehringer Mannheim. Endoproteinase Lys-C, sequencing grade, from *Lysobacter enzymogenes* was obtained from Sigma Chemical Company and Calbiochem. In a typical experiment, enzymatic digestion of the α- or β-polypeptide (approximately 50 μM) was carried out in the incubation buffer specified in the following, which contained 2% (w/v) OG, using an enzyme:polypeptide ratio ranging from 1:100 to 1:25 (w/w) for 24 h in a shaking water bath. In those experiments using endoproteinase Glu-C, the incubation buffer was 50 mM ammonium bicarbonate (pH 7.8) and the incubation temperature was 25 °C. With endoproteinase Lys-C, the incubation was performed in 50 mM Tris·HCl (pH 8.5) at 37 °C. The extent of cleavage was monitored by reverse-phase HPLC using the methods described earlier and the gradients shown in Table 1. After 24 h, the incubation solution was diluted with deionized water to 3.5 mL, bringing the concentration of OG below the critical micellar concentration. The solution was then dialyzed (Spectra/Por 3 Membranes 3500 MWCO from Spectrum Medical) for 4 h against deionized water, with water changes every 30 min, to remove a significant amount of the OG. The resulting

truncated polypeptides were lyophilized and stored at −20 °C.

Carboxypeptidase Y (CY) from baker's yeast (Sigma) was used to cleave the C-terminal Phe residue from the β-polypeptide of *Rb. sphaeroides* (see Figure 1 for sequences of the polypeptides used in this study). The method followed was based upon that of Klemm (1984). Enzymatic digestion of the β-polypeptide (118 μM) was carried out in 0.1 M pyridine acetate (pH 5.6) containing 1% (w/v) sodium dodecyl sulfate (SDS). A CY:polypeptide ratio of 1:100 (w/w) was employed with a 3 h incubation at 25 °C in a shaking water bath. The cleavage reaction was monitored via HPLC, using the methods described earlier and gradient G2 from Table 1. The reaction was quenched by the addition of glacial acetic acid followed by lyophilization.

Cleavage of the Asp−Pro Bond in the α-Polypeptide of R. rubrum. Mild acid hydrolysis was used to selectively cleave the Asp−Pro bond in the α-polypeptide of *R. rubrum*. Two methods were employed. In the first, a 0.1 mM solution of the α-polypeptide in 50% (v/v) acetic acid and 10% (v/v) HFA was incubated for 2 h at 96 °C. The second method involved incubating a 0.1 mM solution of the polypeptide in 75% (v/v) formic acid for 48 h at 40 °C (Jauregui-Adell & Marti, 1975; Landon, 1977). The cleavage reaction was monitored via reverse-phase HPLC using gradient G1 from Table 1. The reaction mixture was lyophilized following cleavage.

Isolation, Purification, and Identification of Truncated Polypeptides. The truncated polypeptides were isolated and purified from the digest mixture using reverse-phase HPLC with the gradient programs shown in Table 1. Polypeptides were identified and their purity was assessed by N-terminal amino acid sequencing (typically 5−15 residues), amino acid composition, electrospray ionization mass spectrometry, and C-terminal sequencing with carboxypeptidase B (CB) (porcine pancreas, Boehringer Mannheim). The N-terminal sequencing and amino acid analyses were performed by Dr. Joseph Leykam at the Michigan State University Macromolecular Structure Facility (East Lansing, MI) or by Dr. Ka-Leung Ngai at the Northwestern University Biotech Facility. Amino acid analyses and mass spectrometry were done by Dr. William Lane at the Harvard Microchemistry Facility (Cambridge, MA). The CB assay was performed according to the procedures described in Ambler (1967) and Brunisholz et al. (1985).

Chemical Modification of the α-Polypeptide of R. rubrum. The Lys residue located in the C-terminal domain of the α-polypeptide at position +18 of *R. rubrum* was acetylated following the procedure of Riordan and Vallee (1967). The procedure involved dissolution of the polypeptide in a small volume of 2% OG, followed by the addition of an equal volume of half-saturated sodium acetate (0.5 g of sodium acetate in 0.8 mL of deionized water). The final polypeptide concentration was typically 410 μM. While the sample was stirred in an ice−water bath, aliquots of acetic anhydride were added every 9 min over a 1 h period, resulting in a total addition of approximately 9 mmol of acetic anhydride/μmol of polypeptide. The sample was allowed to stir on ice for an additional hour and was then dialyzed overnight against cold (4 °C) deionized water prior to lyophilization. The progress of the modification reaction was monitored by reverse-phase HPLC utilizing gradient G1 from Table 1.

R. rubrum wild-type cells were grown on RCV minimal medium (Marrs et al., 1972) with [^{15}N]ammonium sulfate to uniformly label the polypeptides with ^{15}N. The α-polypeptide was isolated and purified as described earlier for unlabeled *R. rubrum* α-polypeptide. The acetylation of ^{15}N-labeled *R. rubrum* α-polypeptide, modified with [1,1′-$^{13}C_2$]-acetic anhydride, was confirmed by high-resolution NMR. ^{1}H−^{15}N shift correlation NMR spectra were acquired on a Bruker 600 MHz spectrometer at Northwestern University's Spectrometer Facility. The protein was dissolved in 350 μL of HFA containing 20% (v/v) deuterated acetone for signal locking. The resulting protein concentration was 1.7 mM. The pulse sequence used in these heteronuclear single quantum coherence (HSQC) experiments was that of Bax et al. (1990). HSQC experiments with and without ^{13}C decoupling were performed. Each experiment required approximately 9 h of collection time and was run at 40 °C to minimize line broadening. The sequence included 100 preparative scans to achieve thermal equilibrium in the sample and a presaturation pulse (65 dB) of 3.0 s to suppress the solvent peaks. The pulse frequencies were 600.142 667 6 MHz for ^{1}H, 60.818 620 0 MHz for ^{15}N, and 150.931 562 5 MHz for ^{13}C. The spectral width in the proton dimension was 12.074 ppm (7245.38 Hz), in which 4096 data points were acquired. The nitrogen dimension collected 600 t_1 points with a spectral window of 40.0 ppm (2432.74 Hz).

Reconstitution Assay. The procedure used here was essentially that of Loach et al. (1994), with modifications. The protein was quantified in HFA using the reported molar absorptivity value at 290 nm of $\epsilon_{290} = 3400\ M^{-1}\ cm^{-1}$/Trp contained in the polypeptide (Miller et al., 1987). Following quantification, the polypeptides were aliquoted and the HFA was removed on a vacuum line. For the reconstitution assay, the polypeptide(s) was(were) dissolved in 0.5 mL of 50 mM potassium phosphate buffer (pH 7.5) containing 4.5% (w/v) OG. In some cases, the truncated polypeptides displayed limited solubility in 4.5% OG. In order to solubilize these polypeptides, 5−10 μL of HFA was added to the polypeptide prior to OG. The concentration of OG was then reduced to 0.9% (w/v) via the addition of 50 mM potassium phosphate buffer (pH 7.5), resulting in a total protein concentration of approximately 4 μM, unless indicated otherwise. Aliquots of a 0.50 mM BChl*a* solution (typically 10−20 μL) in acetone were then added to the polypeptide mixture, resulting in a pigment absorbance between 0.1 and 0.2 at 777 nm (BChl*a* concentration between 20 and 40 μM at 0.9% OG). The concentration of OG was further reduced to approximately 0.75% (w/v), and finally to approximately 0.66% (w/v) or lower to optimize formation of the subunit complex, which has a BChl*a* Q_y absorbance at approximately 820 nm. After the subunit was chilled at 4 °C for 1 h, an absorption spectrum was recorded. The sample was then incubated at 4 °C overnight and an absorption spectrum was taken again. LH1 formation was indicated by a bathochromic shift of the BChl*a* Q_y absorbance to approximately 870 nm.

Instrumentation. Absorption spectra were recorded with a Shimadzu UV-160 spectrophotometer interfaced to a Goldstar microcomputer. Data were stored using the PC-160 Spectroscopy Interface Software Package version 3.2, supplied by Shimadzu Scientific Instruments, Inc. During the reconstitution assay, opal glass was placed in the sample and reference beams of the spectrophotometer in order to reduce the effects of light scattering. Circular dichroism

(CD) spectra were recorded on a Jasco J-500C spectropolarimeter interfaced with an IF500 Interface Unit to a Leading Edge microcomputer. Spectra were recorded using either a 2 or 1 cm path length cell at 25 °C or at approximately 4 °C. Two scans were averaged for each spectrum. An absorption spectrum of each sample was recorded immediately before and after each CD spectrum to ensure that no significant degradation of the sample had occurred during the measurement. HPLC was performed either on a Waters system consisting of two 501 pumps, a system interface module, a 486 tunable absorbance detector, a U6K injector, and Waters Baseline chromatography software or on a Perkin-Elmer Series 4 liquid chromatograph with an LC-85B spectrophotometric detector and an LCI-100 laboratory computing integrator.

RESULTS

The extent of formation of the reconstituted subunit and LH1 was assessed qualitatively by comparing the relative amounts of unbound BChl*a* (absorbance at 777 nm) and subunit or LH1 (absorbances at 820 or approximately 870 nm, respectively) present in the sample. For the subunit complex, this comparison was done first at 0.75% OG and then again once the subunit spectrum was optimized (such that the 777 nm band was as small as possible relative to the 820 nm band), usually at or below 0.66% OG. For the LH1 complex, the comparison was done after storage at 4 °C overnight. Absorption and CD spectra of a subunit and LH1 generated in a reconstitution using native α- and β-polypeptides from *R. rubrum* are illustrated in Figure 2. Although the λ_{max} of the BChl*a* Q_y absorption band of LH1, generated by reconstitution utilizing the native α- and β-polypeptides from *R. rubrum*, exhibited only small variations (typically 869 ± 5 nm), the positions of the peak and trough in the CD spectrum of LH1 ranged from 845 to 860 nm for the trough and from 876 to 897 nm for the peak. The results presented in this paper are tabulated in Table 2.

Acetylation of the α-Polypeptide of R. rubrum. The α-polypeptide of *R. rubrum* contains one Lys residue located in the C-terminal domain at position +18, as shown in Figure 1. Acetylation was used to neutralize the positive charge of the ε-amino group of this Lys residue. Since the N-terminus of the α-polypeptide of *R. rubrum* is blocked with a formyl group (Cuendet & Zuber, 1981), the ε-amino group of the Lys residue is the sole potential acetylation site. In accordance with this, the HPLC of the acetylated polypeptide showed a single product with no native α-polypeptide remaining (see Table 2, experiment 1, for peak position). Modification of the Lys ε-amino group was confirmed using high-resolution NMR. In the HSQC experiment, protons directly bonded to nitrogen, such as in amide bonds, are observed. Without ^{13}C decoupling, the spin states of the carbon and proton nuclei can mix, and the proton signal will split into a doublet. However, when the experiment is performed with ^{13}C decoupling, the carbon spin states cannot interact with those of the protons, and a single proton resonance signal is observed. This phenomenon was observed in the two HSQC experiments of the *R. rubrum* ^{13}C,^{15}N-labeled, acetylated α-polypeptide. The HSQC spectrum without ^{13}C decoupling showed a doublet with chemical shifts as follows: δ_H = 6.726 ppm and δ_N = 125.388 ppm for one signal, and δ_H = 6.720 ppm and δ_N = 125.16 ppm for the other. The proton chemical shift of 6.7 ppm was

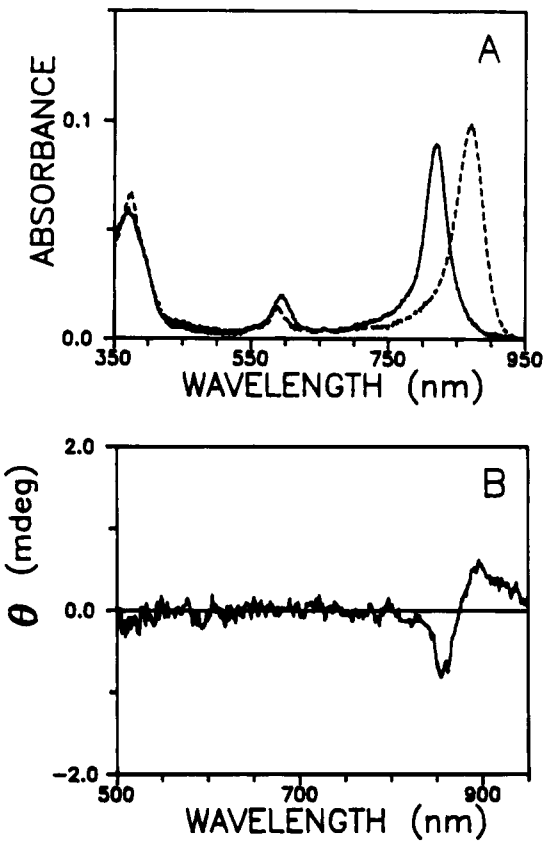

FIGURE 2: Reconstitution using the native α- and β-polypeptides from *R. rubrum* and BChla_{gg}. (A) Absorption spectra under optimized subunit conditions of 0.66% OG, λ_{max} = 821 nm (solid line) (note that the spectrum at 0.75% OG is not shown because it is identical to the spectrum at 0.66% 0G), and at 0.66% OG stored at 4 °C overnight, λ_{max} = 870 nm (dashed line). Concentrations: α = β = 1.5 μM, BChl*a* = 1.3 μM at 0.66% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the LH1 complex and multiplied by a dilution factor for ease of comparison. Spectra were recorded in 1 cm cuvettes. (B) CD spectrum of the LH1 complex at 0.66% OG after storage at 4 °C overnight (trough at 849 nm, peak at 887 nm). Concentrations: α = 1.6 μM, β = 1.5 μM, BChl*a* = 2.3 μM at 0.66% OG. The CD spectrum was scaled to correspond to an absorbance of 0.1 cm^{-1} at 870 nm. CD parameters were as follows: time constant = 4 s, slit width = 160 μm, sensitivity = 2 mdeg/cm, step resolution = 1 nm; spectrum was recorded in a 1 cm cuvette at approximately 0 °C.

appropriate for a side chain amide (Wüthrich, 1986). The observed ^{15}N chemical shift of 125 ppm was about 5 ppm downfield from the chemical shift of *N*-butylacetamide (Westerman & Roberts, 1978), a comparable test compound for N_ϵ-acetyl-Lys. The increased chemical shift in the polypeptide probably is not notable because of the sensitivity of ^{15}N chemical shifts to solvent, temperature, and chemical environment (Srinivasan & Lichter, 1977; Witanowski et al., 1977). The spin−spin coupling constants $^1J_{CN}$ = 13.8 Hz and $^2J_{CH}$ = 3.5 Hz were within the expected ranges for amides (Bystrov, 1976). In the ^{13}C-decoupled spectrum, the doublet collapsed into a singlet, which was positioned approximately in the center of the former doublet with chemical shifts of δ_H = 6.722 ppm and δ_N = 125.275 ppm. No other changes in the spectrum were observed upon removing the ^{13}C spin interactions. Thus, the Lys amino group was modified to an amide. As shown in Table 2, experiment 1, when the acetylated α-polypeptide from *R. rubrum* was combined in a reconstitution with the native β-polypeptide from *R. rubrum* and BChla_p, a subunit

Table 2: Biochemical and Spectral Data from Chemical and Enzymatic Modification of LH1 Polypeptides

experiment no.	polypeptide (Figure 1)	treatment	gradient (Table 1)	retention time (min)	optimized subunit (nm)	LH1-type complex (nm)	LH1 CD trough (nm)	LH1 CD peak (nm)
1	rub α K 18 acetylation + rub β	see Materials and Methods	G1	27.2 (25.8)[a]	818 (818)	865 (864)	ND[b]	ND
2	rub α (−28 to +18) + rub β	endolys C	G1	27.2 (27.0)	818 (819)	871 (871)	849 (850)	887 (885)
3	rub α (−28 to +13) + rub β	endoglu C	G1	35.8 (32.7)	818 (819)	869 (872)	846 (850)	882 (885)
4	rub α PRQ (−19 to +23) + rub β	mild acid	G1	21.6 (27.9)	818 (817)	862 (870)	840 (845)	885 (876)
5	rub α PRQ alone	mild acid	G1	21.6 (27.9)	NA[c]	871 (850)	850 (823)	885 (855)
6	rub β QES (−33 to +17) + rub α	endolys C	G3	42.6 (36.9)	820 (820)	869 (871)	843 (846)	876 (883)
7	rub β EFH (−20 to +17) + rub α	endolys C	G3	35.7 (36.9)	818 (820)	870 (871)	834 (846)	873 (883)
8	rub β EFH alone	endolys C	G3	35.7 (36.9)	819 (819)	NA	NA	NA
9	rub β FHK (−19 to +17) + rub α	endoglu C	G1	17.3 (18.5)	816 (820)	862 (872)	839 (845)	874 (880)
10	rub β FHK alone	endoglu C	G1	17.3 (18.5)	817 (819)	NA	NA	NA
11	rub β IFT (−16 to +17) + rub α	endolys C	G3	41.0 (36.9)	816 (820)	NA	NA	NA
12	rub β IFT alone	endolys C	G3	41.0 (36.9)	817 (819)	NA	NA	NA
13	rub α PRQ + rub β FHK	see above	see above	see above	816 (820)	864 (872)	ND	ND
14	sph β LHS (−19 to +10) + rub α	endoglu C	G3	35.1 (37.2)	823 (825)	847 (874)	830 (855)	851 (896)
15	sph β LHS alone	endoglu C	G3	35.1 (37.2)	823 (823)	847 (NA)	829 (NA)	851 (NA)
16	sph β (−37 to +9) + rub α	CY	G2	35.8 (38.7)	822 (827)	871 (874)	846 (859)	872 (898)
17	sph β alone (−37 to +9)	CY	G2	35.8 (38.7)	821 (823)	NA	NA	NA
18	vir β FHG (−19 to +18) + rub α	endoglu C	G2	28.7 (30.0)	810 (815)	865 (877)	882 (883)	849 (845)
19	vir β FHG alone	endoglu C	G2	28.7 (30.0)	NA	872 (874)	882 (883)	851 (854)

[a] Values in parentheses represent those obtained for the control. [b] ND, not determined. [c] NA, not applicable.

complex was formed that, upon chilling overnight at 4 °C, yielded an LH1 complex. The results obtained with the modified polypeptide were equivalent to those obtained in the control reconstitution using native α- and β-polypeptides from *R. rubrum* and BChla_p (Table 2, experiment 1, values in parentheses).

Biochemical Characterization of the Truncated Polypeptides. In the proteolysis experiments described in this paper, verification that the polypeptides were not altered, as a result of the incubation procedure alone, was provided by subjecting the native polypeptides to incubation and work-up conditions identical to those employed during the enzyme treatment, but in the absence of the enzyme. Reconstitution utilizing the incubated native polypeptides resulted in the formation of subunit and LH1 complexes that were spectrally indistinguishable from those obtained using native polypeptides that were not subjected to incubation. The values displayed in parentheses in Table 2 are those generated in controls where the native α- or β-polypeptide was subjected to incubation conditions without the enzyme, where applicable. Further, in all cases, the incubated polypeptide coeluted with a native sample of the polypeptide on HPLC.

The identities of the polypeptides truncated in the N-terminal domain were verified by N-terminal sequencing of the first 5−15 residues. Contamination by another polypeptide was observed only in the truncated β-polypeptide from *Rb. sphaeroides* (LHS...C-terminal) (residues −19 through +10), where N-terminal sequencing indicated the presence of less than 10% of the native uncleaved β-polypeptide. In all other cases, there was no evidence of any contaminating sequences, as indicated by the results from N-terminal sequencing, amino acid analysis, and HPLC. The identities and purities of those polypeptides truncated in the C-terminal domain were verified by mass spectral analysis, amino acid composition, C-terminal analysis by CB, and HPLC. A representative chromatogram of one of the purified, truncated polypeptides is shown in Figure 3A.

C-Terminal Cleavage of the α-Polypeptide of R. rubrum. Treatment of the α-polypeptide of *R. rubrum* with endoproteinase Lys-C resulted in cleavage on the C-terminal side of the Lys residue in position +18 and quantitative removal of the PVQTS peptide fragment from the C-terminus (refer to Figure 1 for sequence information). C-Terminal amino acid sequencing of the truncated α-polypeptide with CB verified Lys as the C-terminal amino acid and Thr as the penultimate residue.

When the truncated α-polypeptide (residues −28 through +18) was reconstituted with the native β-polypeptide from *R. rubrum* and BChla_{gg}, a subunit and an LH1 complex were formed that were spectrally indistinguishable from those

generated in the control reconstitution utilizing α- and β-polypeptides from *R. rubrum*. The results are shown in Table 2, experiment 2.

Incubation of the α-polypeptide of *R. rubrum* with endoproteinase Glu-C yielded quantitative cleavage on the C-terminal side of the Glu residue located in position +13 and resulted in the polypeptide (residues −28 through +13), as confirmed by molecular weight determination by electrospray ionization MS (predicted mass = 5146 Da; actual mass = 5150 Da) and amino acid analysis. When the truncated α-polypeptide (residues −28 through +13) was reconstituted with the native β-polypeptide from *R. rubrum* and BChla_{gg}, a subunit and an LH1 complex were formed that were equivalent to those generated in the control using native α- and β-polypeptides. The results are reported in Table 2, experiment 3.

Attempts to cleave at the Glu residue in position +7 by the usual conditions (see Materials and Methods section), by changing the α-polypeptide:endoproteinase Glu-C ratio from 50:1 to 25:1 (w/w), or finally by treatment of the α-polypeptide with endoproteinase Glu-C in 0.5% (w/v) SDS instead of 2.0% (w/v) OG proved unsuccessful. In another experiment, the α-polypeptide was first treated with endoproteinase Glu-C in 2.0% (w/v) OG to yield the truncated polypeptide having Glu +13 as the C-terminal residue. Upon isolation and purification of this polypeptide, a second treatment with endoproteinase Glu-C in 0.5% (w/v) SDS was conducted. Again, no further cleavage was observed.

N-Terminal Cleavage of the α-Polypeptide of R. rubrum. All α-polypeptides of LH1 for which amino acid sequence information is available contain an Asp−Pro bond within the N-terminal domain (Zuber & Brunisholz, 1991). The Asp−Pro linkage is especially labile to mild acid hydrolysis and can therefore be selectively cleaved (Jauregui-Adell & Marti, 1975; Landon, 1977). Mild acid hydrolysis of the α-polypeptide from *R. rubrum* resulted in the formation of the polypeptide (PRQ...C-terminus) (residues −19 through +23). A chromatogram of the purified polypeptide (PRQ...C-terminus) is shown in Figure 3A and is representative of the chromatograms routinely obtained for the purified, truncated polypeptides prepared in this study.

Reconstitution of the truncated α-polypeptide (PRQ...C-terminus) with the native β-polypeptide from *R. rubrum* resulted in the formation of a subunit and an LH1 complex, as shown in Figure 3B and Table 2, experiment 4. When compared to the control using native polypeptides from *R. rubrum*, formation of both the subunit and LH1 complex was not complete. In addition to the absorption bands attributable to the subunit and LH1 complexes, a significant absorbance at 780 nm remained, indicative of unbound BChl*a*. Furthermore, the LH1 complex was not stable since, upon incubation at 4 °C overnight, the absorbance of the 862 nm band decreased significantly relative to that of the 780 nm band. Despite the observed lack of stability of the LH1 complex, when the CD spectrum of the LH1 complex prepared with the truncated α-polypeptide was compared with the CD of the LH1 complex generated in a native reconstitution, the peak and trough positions, shape, and rotational strength were quite similar (see Table 2 for peak and trough positions). The only notable difference was that the CD spectrum of the LH1 complex prepared using the (PRQ...C-terminus) α-polypeptide was somewhat broader

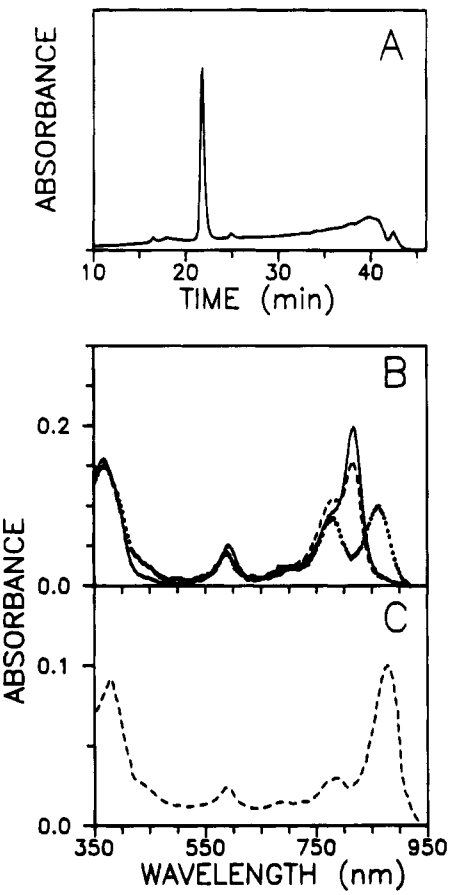

FIGURE 3: (A) HPLC chromatogram of purified (PRQ...C-terminus) α-polypeptide from the α-polypeptide of *R. rubrum*. Retention time was 21.6 min on gradient G1 from Table 1 (see Materials and Methods for further details). (B) Absorption spectra of a reconstitution using (PRQ...C-terminus) and the native β-polypeptide from *R. rubrum* with BChla_{gg} at 0.75% OG, shoulder at 780 nm, λ_{max} = 818 nm (dashed line); under optimized subunit conditions of 0.66% OG, λ_{max} = 818 nm (solid line); and at 0.59% OG after storage at 4 °C overnight, λ_{max} = 782 and 862 nm (dotted line). Concentrations: (PRQ...C-terminus) = β = 2.0 μM, BChl*a* = 2.9 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 862 nm species and multiplied by a dilution factor. (C) Absorption spectra of a reconstitution using the truncated (PRQ...C-terminus) α-polypeptide and BChla_{gg} at 0.59% OG after storage at 4 °C overnight, λ_{max} = 871 nm (dashed line). Concentrations: (PRQ...C-terminus) = 1.5 μM, BChl*a* = 1.3 μM at 0.59% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 871 nm species.

than that of the control and appeared to contain a small contribution from the subunit complex.

Most unexpectedly, the truncated (PRQ...C-terminus) α-polypeptide alone (in the absence of the β-polypeptide) was able to form an LH1-like complex upon dilution to 0.59% OG (λ_{max} = 867 nm), which was further red-shifted following storage at 4 °C overnight (shown in Figure 3C and Table 2, experiment 5). The LH1-like complex contained a small amount of unbound BChl*a* since an absorbance band at 783 nm was present in the absorption spectrum. However, there was no evidence of subunit formation. In comparing Figure 3B with 3C, the LH1-like complex prepared in Figure 3C was formed more completely, was more stable, and was further red-shifted than the LH1 complex that was formed when the truncated (PRQ...C-terminus) α-polypeptide was combined with the native β-polypeptide from *R. rubrum* in a reconstitution. The CD spectra of the LH1 complexes prepared using the truncated

α-polypeptide with and without the native β-polypeptide were similar to each other and to that of the control using native α- and β-polypeptides in size, shape, and peak position (data not shown); thus, the environment of the BChl*a* appears to be the same regardless of whether the β-polypeptide is present or not. As a control, the native α-polypeptide from *R. rubrum*, when subjected to the same reconstitution conditions as the truncated α-polypeptide (in the absence of the β-polypeptide), formed low concentrations of a red-shifted complex with BChl*a* having a near-IR absorption at 850 nm, following storage at 4 °C overnight (spectra not shown, but peak positions are given in parentheses in Table 2, experiment 5). Previous work, however, has demonstrated that a common aggregated form of BChl*a* absorbs at 850 nm (Gottstein & Scheer, 1983; Parkes-Loach et al., 1988; Scherz & Rosenbach-Belkin, 1989).

N-Terminal Cleavage of the β-Polypeptide of R. rubrum. The β-polypeptide of *R. rubrum* contains three Lys residues, one of which is located very close to the N-terminus at position −34 and two of which reside within the core region at positions −21 and −17. Endoproteinase Lys-C was used to cleave on the C-terminal side of each Lys residue. Reconstitution using the β-polypeptide generated from cleavage at the Lys in position −34 (QES...C-terminus) and the native α-polypeptide from *R. rubrum* resulted in the formation of a subunit and an LH1 complex that were spectrally indistinguishable from the control, as shown in Table 2, experiment 6.

As shown in Figure 4A and Table 2, experiment 7, use of the (EFH...C-terminus) polypeptide (residues −20 through +17) in a reconstitution with the native α-polypeptide from *R. rubrum* gave a subunit that, upon chilling at 4 °C for 1.5 h, yielded an LH1 complex. The intensity of the LH1 absorption band decreased relative to the 780 nm band following storage at 4 °C overnight. Both the subunit and LH1 spectra contained a significant absorption band at 777 nm, indicative of the presence of unbound BChl*a*. The CD spectrum of the subunit complex was identical to that obtained in the control reconstitution, while the CD spectrum of the LH1 complex was shifted hyposchromically with respect to the control (see Table 2).

In a reconstitution using the β-polypeptide (EFH...C-terminus) alone (in the absence of the α-polypeptide), a subunit was formed that exhibited a shoulder at 783 nm. The data are shown in Table 2, experiment 8. Upon chilling overnight, the subunit degraded, as evidenced by the disappearance of the subunit peak and concomitant increase in intensity of the 780 nm absorption peak (data not shown). The lack of LH1 formation is in accord with results obtained in the control reconstitution using the native β-polypeptide from *R. rubrum* in the absence of the α-polypeptide (Parkes-Loach et al., 1988; Loach et al., 1994). By comparing the subunit complexes prepared using the (EFH...C-terminus) polypeptide with and without the α-polypeptide at 0.75% (w/v) OG, the subunit formed in the absence of the α-polypeptide was formed more completely than that formed with the α-polypeptide. However, upon optimization, both subunit complexes appeared to be formed to the same extent and contained comparable amounts of unbound BChl*a*.

In order to assess the importance of the Glu residue in position −20 of the β-polypeptide of *R. rubrum*, the truncated β-polypeptide (FHK...C-terminus) (residues −19 through +17) was prepared using endoproteinase Glu-C. In Figure 4 and Table 2, experiment 9, reconstitution results using the truncated (FHK...C-terminus) β-polypeptide are compared with those obtained using the truncated (EFH...C-terminus) β-polypeptide. As was observed with the (EFH...C-terminus) polypeptide, the (FHK...C-terminus) polypeptide was able to form a subunit complex when reconstituted with the native α-polypeptide from *R. rubrum*, albeit somewhat incompletely, as shown in Figure 4B. The spectrum recorded at 0.75% (w/v) OG, as well as the optimized spectrum, showed that the subunit prepared with the (FHK...C-terminus) polypeptide was not formed as completely as that formed using the (EFH...C-terminus) polypeptide. Formation of the

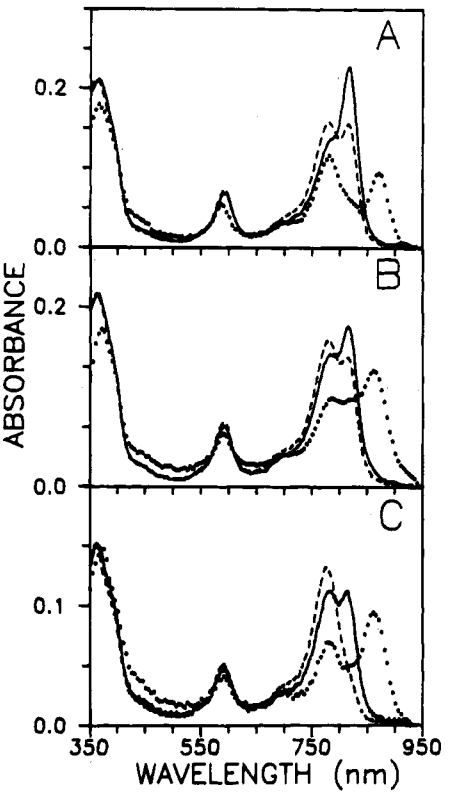

FIGURE 4: Absorption spectra. (A) Reconstitution of the native α-polypeptide from *R. rubrum* with the (EFH...C-terminus) β-polypeptide from *R. rubrum* and BChla_{gg} at 0.75% OG, λ_{max} = 780 and 816 nm (dashed line); under optimized subunit conditions of 0.63% OG, λ_{max} = 818 nm (solid line); and at 0.59% OG after storage at 4 °C overnight, λ_{max} = 782 and 870 nm (dotted line). Concentrations: (EFH...C-terminus) = 3.1 μM, α-polypeptide = 1.7 μM, BChla_{gg} = 2.7 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 870 nm species and multiplied by a dilution factor for ease of comparison. (B) Reconstitution of the native α-polypeptide from *R. rubrum* with the (FHK...C-terminus) β-polypeptide from *R. rubrum* and BChla_{gg} at 0.75% OG, λ_{max} = 781 and 815 nm (dashed line); under optimized subunit conditions of 0.66% OG, λ_{max} = 784 and 816 nm (solid line); and at 0.59% OG after storage at 4 °C overnight, λ_{max} = 788 and 862 nm (dotted line). Concentrations: (FHK...C-terminus) = 2.4 μM, α-polypeptide = 1.7 μM, BChla_{gg} = 2.8 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 862 nm species and multiplied by a dilution factor for ease of comparison. (C) Reconstitution of the (PRQ...C-terminus) α-polypeptide with the (FHK...C-terminus) β-polypeptide (both from *R. rubrum*) and BChla_{gg} at 0.75% OG, λ_{max} = 779 nm (dashed line); under optimized subunit conditions of 0.59% OG, λ_{max} = 786 and 816 nm (solid line); and at 0.56% OG after storage at 4 °C overnight, λ_{max} = 784 and 864 nm (dotted line). Concentrations: (PRQ...C-terminus) = 2.6 μM, (FHK...C-terminus) = 3.2 μM, BChla_{gg} = 1.5 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 864 nm species and multiplied by a dilution factor.

LH1 complex was incomplete in each case, since there was a prominent band at 780 nm present in each absorption spectrum. The subunit complex yielded a CD spectrum identical to that obtained in a control reconstitution with native polypeptides. The CD spectrum of the LH1 complex prepared with the (FHK...C-terminus) polypeptide was similar to that generated using (EFH...C-terminus) in terms of peak and trough positions (see Table 2) and shape.

When the (FHK...C-terminus) β-polypeptide was tested in a reconstitution with BChla_{gg} (in the absence of the α-polypeptide), a subunit complex was formed that exhibited a shoulder at 780 nm (Table 2, experiment 10). This subunit was not formed as completely as the subunit prepared using the (EFH...C-terminus) polypeptide alone, on the basis of comparison of the absorption spectra at 0.75% OG (data not shown). However, upon optimization, both subunit complexes were formed to the same extent and contained comparable amounts of unbound BChl*a*. Again, storage at 4 °C did not yield an LH1 complex (data not shown). Finally, the subunit complex prepared using the (FHK...C-terminus) polypeptide in the absence of the α-polypeptide at 0.75% OG and under optimized conditions was formed more completely and contained less unbound BChl*a* than the subunit generated in the presence of the α-polypeptide.

Initial attempts to prepare the truncated (IFT...C-terminus) β-polypeptide from *R. rubrum* (residues −16 through +17), resulting from cleavage at the Lys residue in position −17 with endoproteinase Lys-C, yielded a 1:1 mixture of products consisting of the (QES...C-terminus) polypeptide (residues −33 through +17) and the desired (IFT...C-terminus) polypeptide. The (IFT...C-terminus) polypeptide could be isolated from the mixture via reverse-phase HPLC using gradient G3 from Table 1. However, in order to eliminate the possibility of contamination with the (QES...C-terminus) polypeptide, the β-polypeptide of *R. rubrum* was first treated with endoproteinase Glu-C to isolate the (FHK...C-terminus) polypeptide (residues −19 through +17). This polypeptide was then treated with endoproteinase Lys-C, which resulted in the formation of the (IFT...C-terminus) polypeptide.

Combination of the (IFT...C-terminus) polypeptide with the native α-polypeptide from *R. rubrum* resulted in the generation of a subunit complex, as illustrated in Figure 5A and Table 2, experiment 11. However, the formation of the subunit was not quantitative, based on the presence of unbound BChl*a*. Despite incomplete formation, the CD spectrum of the subunit was comparable to the control (data not shown). Upon chilling of the sample at 4 °C overnight, the subunit band shifted virtually completely to 780 nm, as shown in Figure 5A, indicating degradation of the subunit complex without evidence of LH1 formation.

When the (IFT...C-terminus) β-polypeptide was reconstituted with BChla_{gg}, in the absence of the α-polypeptide, a subunit was formed as shown in Figure 5B and Table 2, experiment 12. Storage of this subunit complex at 4 °C overnight resulted in the disappearance of the subunit absorbance, leaving only the band at 784 nm (data not shown). Comparison of Figure 5A with 5B illustrates that the subunit was formed more completely in the absence of the α-polypeptide than in its presence, as indicated by the relative sizes of the 777 and 816-817 nm bands in the absorption spectra.

Reconstitution Combining Truncated α- and β-Polypeptides from R. rubrum. A reconstitution using the truncated

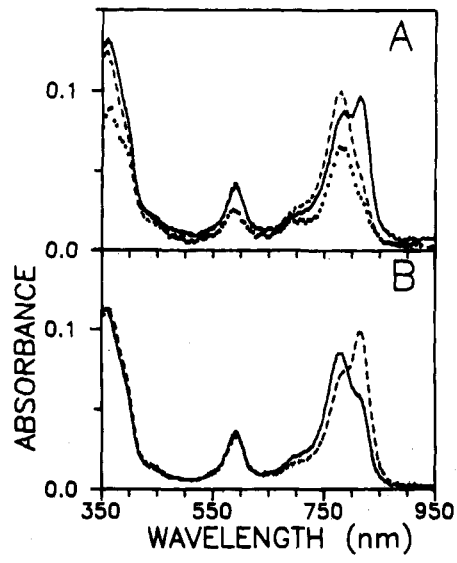

FIGURE 5: Absorption spectra. (A) Reconstitution of the native α-polypeptide from *R. rubrum* with the (IFT...C-terminus) β-polypeptide from *R. rubrum* and BChla_{gg} at 0.75% OG, λ_{max} = 779 nm (dashed line); under optimized subunit conditions of 0.61% OG, λ_{max} = 784 and 816 nm (solid line); and at 0.59% OG after storage at 4° C overnight, λ_{max} = 780 nm (dotted line). Concentrations: (IFT...C-terminus) = 2.7 μM, α-polypeptide = 1.7 μM, BChla_{gg} = 1.9 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 816 nm species and multiplied by a dilution factor. (B) Reconstitution of (IFT...C-terminus) β-polypeptide with BChla_{gg} at 0.75% OG, λ_{max} = 779 nm (solid line); and under optimized subunit conditions of 0.61% OG, λ_{max} = 817 nm (dashed line). Concentrations: (IFT...C-terminus) = 6.2 μM, BChla_{gg} = 1.9 μM at 0.75% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 817 nm species and multiplied by a dilution factor.

(PRQ...C-terminus) α-polypeptide (residues −19 through +23) and the truncated (FHK...C-terminus) β-polypeptide (residues −19 through +17), both from *R. rubrum*, was performed to observe the effect of combining two truncated polypeptides that both lack a significant portion of their N-terminal domains on subunit and LH1 formation. As shown in Figure 4C and Table 2, experiment 13, this reconstitution resulted in the formation of a subunit and an LH1 complex. The absorbance of the LH1 complex was slightly blue-shifted relative to a control reconstitution using native α- and β-polypeptides from *R. rubrum*. The extent of formation of the LH1 complex in this case was comparable to what was observed in those reconstitutions utilizing the aforementioned truncated polypeptides in combination with their native (uncleaved) partners (Figures 3B and 4B). By comparing the data at 0.75% OG and under optimized conditions, the reconstitution using the two truncated polypeptides (PRQ...C-terminus) and (FHK...C-terminus) yielded the most poorly formed subunit. The subunit was formed most completely in the reconstitution where (PRQ...C-terminus) was combined with the native β-polypeptide from *R. rubrum*, followed by the reconstitution of the (FHK...C-terminus) polypeptide alone and the reconstitution of the (FHK...C-terminus) polypeptide with the native α-polypeptide from *R. rubrum*.

N-Terminal Cleavage of the β-Polypeptide of Rb. sphaeroides. Endoproteinase Glu-C was used to cleave the β-polypeptide of *Rb. sphaeroides* on the C-terminal side of the Glu residue in position −20, which yielded the (LHS...C-terminus) polypeptide (residues −19 through +10). The

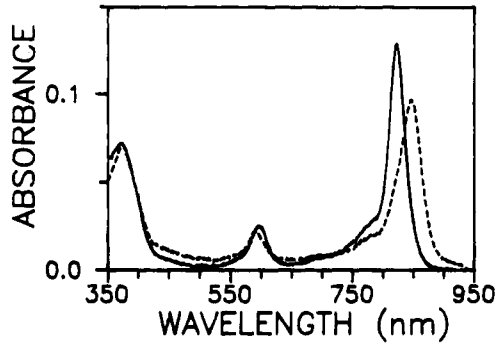

FIGURE 6: Absorption spectra of a reconstitution using the native α-polypeptide of *R. rubrum* and the (LHS...C-terminus) β-polypeptide from *Rb. sphaeroides* under optimized subunit conditions of 0.66% OG, λ_{max} = 823 nm (solid line); and at 0.66% OG after storage at 4 °C overnight, λ_{max} = 847 nm (dashed line). Concentrations: α-polypeptide = 1.5 μM, (LHS...C-terminus) = 2.8 μM, BChla_{gg} = 2.6 μM at 0.66% OG. Spectra were normalized to an absorbance of 0.1 cm^{-1} for the 847 nm species.

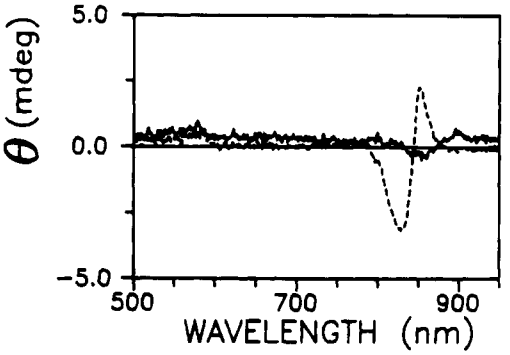

FIGURE 7: CD spectrum (dashed line) of the 847 nm species generated in reconstitution using the native α-polypeptide from *R. rubrum* and the (LHS...C-terminus) β-polypeptide at 0.66% OG after storage at 4 °C overnight (trough at 830 nm, peak at 851 nm). Concentrations: α = 1.5 μM, (LHS...C-terminus) = 2.8 μM, BChla_{gg} = 2.6 μM at 0.66% OG. The CD spectrum was scaled to correspond to an absorbance of 0.1 cm^{-1} at 847 nm. CD spectrum (solid line) of a reconstitution using the native α-polypeptide from *R. rubrum* and the native β-polypeptide from *Rb. sphaeroides* at 0.66% OG after storage at 4 °C overnight (trough at 855 nm, peak at 896 nm). Concentrations: α = 1.5 μM, β = 1.9 μM, BChla_{gg} = 2.0 μM at 0.66% OG. The CD spectrum was scaled to correspond to an absorbance of 0.1 cm^{-1} at 875 nm. The CD parameters were those used for Figure 2B.

majority of the reconstitution experiments were performed with the α-polypeptide from *R. rubrum* instead of the α-polypeptide from *Rb. sphaeroides*. The α-polypeptide from *R. rubrum* was selected because it was observed to interact strongly with all of the LH1 β-polypeptides tested in hybrid experiments (Loach et al., 1994). Also, more direct comparisons could be made between the truncated β-polypeptide from *R. sphaeroides* and the truncated β-polypeptides from *R. rubrum* when reconstituting with the same α-polypeptide.

Previous work has shown that reconstitution of the native β-polypeptide from *Rb. sphaeroides* with the α-polypeptide from *R. rubrum* and BChl*a* results in the formation of subunit and LH1 complexes with near-IR absorbances at 824 and 875 nm, respectively, with no evidence of a 780 nm band (Loach et al., 1994). When the (LHS...C-terminus) polypeptide was combined with the α-polypeptide of *R. rubrum* and BChla_{gg} in a reconstitution, a subunit was formed, as illustrated in Figure 6 and Table 2, experiment 14. Upon the subunit chilling overnight at 4 °C, the absorption band shifted to 847 nm almost completely, as shown in Figure 6. Comparable results were obtained when the α-polypeptide from *Rb. sphaeroides* was used in place of the α-polypeptide from *R. rubrum* (data not shown). The CD spectrum of the subunit was identical to that obtained in the control reconstitution using the native β-polypeptide from *Rb. sphaeroides* and the native α-polypeptide from *R. rubrum* (Chang et al., 1990b). The 847 nm species had a CD spectrum that was quite different from that observed for the LH1 complex formed in the control reconstitution, as shown in Figure 7.

When the truncated (LHS...C-terminus) β-polypeptide was reconstituted with BChla_{gg} (in the absence of α-polypeptide), an archetypal subunit was formed (Table 2, experiment 15). The subunit generated with the (LHS...C-terminus) polypeptide in the absence of the α-polypeptide was formed more completely at 0.75% OG than in the reconstitution where the α-polypeptide was present (data not shown). However, in each case, under optimized conditions, the size of the 777 nm band was insignificant. Upon chilling of the subunit, an LH1-like complex was formed that yielded a CD spectrum identical to that observed for the LH1-like complex formed when the (LHS...C-terminus) polypeptide was combined in a reconstitution with the α-polypeptide from *R. rubrum* (see Figure 7). The native β-polypeptide from *Rb. sphaeroides* is unable to form an LH1 complex in reconstitution without the α-polypeptide (Loach et al., 1994). Also, this result is unlike what was observed for the cleavage products of the β-polypeptide from *R. rubrum*, where the reconstitutions using the native or the truncated β-polypeptides in the absence of the α-polypeptide yielded only the subunit and no LH1 complex.

C-Terminal Cleavage of the β-Polypeptide of Rb. sphaeroides. CY was used to cleave the C-terminal Phe residue from the β-polypeptide of *Rb. sphaeroides*. Electrospray ionization MS (predicted mass = 5310 Da; actual mass = 5327 Da) and amino acid analysis verified the cleavage site. The observed difference in predicted versus actual mass may be due to a bound water molecule. The yield of this reaction was rather small (34%) and further cleavage of the penultimate residue (Trp) from the β-polypeptide was attempted using CY, but the yields were exceedingly poor based upon HPLC analysis.

Reconstitution of the truncated β-polypeptide lacking the C-terminal Phe (residues −37 through +9) with the α-polypeptide from *R. rubrum* yielded a subunit that, upon chilling at 4 °C overnight, resulted in the formation of an LH1 complex, as shown in Table 2, experiment 16. The CD spectrum of the subunit prepared using the truncated polypeptide was analogous to that of the control (data not shown). However, the CD spectrum of the LH1 complex formed using the truncated polypeptide was hypsochromically shifted relative to that of the control, as shown in Table 2 (see Figure 7 for the representative spectrum of the control).

In a reconstitution combining the truncated β-polypeptide lacking the C-terminal Phe with BChla_{gg} (in the absence of the α-polypeptide), a subunit was formed and there was no change in the spectrum upon chilling at 4 °C overnight (Table 2, experiment 17). These results were comparable to those obtained in a control reconstitution using the native β-polypeptide from *Rb. sphaeroides*.

N-Terminal Cleavage of the β-Polypeptide of Rps. viridis. *Rps. viridis* contains BChl*b* as its light-absorbing pigment

instead of BChl*a*. Recent work in our laboratory has demonstrated that, in vitro, the α- and β-polypeptides of *Rps. viridis* are capable of binding BChl*a* in a way that is similar to that observed with the polypeptides of *R. rubrum*, *Rb. sphaeroides*, and *Rb. capsulatus* (Parkes-Loach et al., 1994). In contrast to what has been observed during reconstitution using the β-polypeptides from the three aforementioned bacteria (in the absence of an α-polypeptide), where LH1 formation did not occur, reconstitution utilizing the native β-polypeptide alone from *Rps. viridis* with BChl*a* resulted in the formation of an LH1-like complex with a near-IR absorption at 872 nm. There was only a small amount of subunit formed (Parkes-Loach et al., 1994). Recall that formation of an LH1 complex in the absence of an α-polypeptide had not hitherto been observed in reconstitutions with native polypeptides. In order to ascertain whether the N-terminal region of the β-polypeptide from *Rps. viridis* was important for LH1 formation and/or was a hindrance to subunit generation (and thus responsible for the unique behavior of the *Rps. viridis* β-polypeptide), the β-polypeptide was cleaved on the C-terminal side of the Glu residue in position −20 with endoproteinase Glu-C, which yielded the (FHG...C-terminus) polypeptide (residues −19 through +18).

Reconstitution using the truncated (FHG...C-terminus) β-polypeptide and BChla_{gg} (without the α-polypeptide) resulted in the formation of an LH1 complex comparable to the control, as shown in Table 2, experiment 19. The CD spectrum of the LH1 complex formed from the (FHG...C-terminus) polypeptide was similar in shape and in peak and trough position to those of the control [see Parkes-Loach et al. (1994) for the CD spectrum of the native β-polypeptide from *Rps. viridis* reconstituted with BChl*a*]. Apparently, the moieties and/or structural motifs responsible for the unusual behavior of the β-polypeptide of *Rps. viridis* in reconstitution with BChl*a* do not reside on the portion of the N-terminus that was removed from the polypeptide.

In an effort to further compare the performance of the truncated β-polypeptide from *Rps. viridis* with the behavior observed using the truncated β-polypeptides from *R. rubrum* and *Rb. sphaeroides* in reconstitution, the truncated (FHG...C-terminus) β-polypeptide of *Rps. viridis* was combined with the α-polypeptide from *R. rubrum* and BChla_{gg} in a reconstitution. Subunit formation was impeded somewhat relative to the control, as illustrated in Figure 8 and Table 2, experiment 18. In the control reconstitution, where the native β-polypeptide from *Rps. viridis* was combined with the α-polypeptide from *R. rubrum*, a subunit was formed that contained only a slight shoulder at 780 nm [see Loach et al. (1994) for the control spectrum]. Cleavage of the β-polypeptide also had a deleterious effect on LH1 complex formation, since there was a significant band at 782 nm present in the absorption spectrum, as shown in Figure 8. Relative to the absorption spectrum of the LH1 complex formed in the control reconstitution (Loach et al., 1994), the absorption spectrum of the LH1 prepared using the truncated polypeptide was shifted hyposchromically, as shown in Table 2. Despite the differences described earlier, the CD spectrum of the LH1 complex resulting from reconstitution using the truncated β-polypeptide and the α-polypeptide from *R. rubrum* (data not shown) was quite comparable to the control CD spectrum in shape and in peak and trough position and was analogous to the CD spectra of the LH1 complexes generated with the native α- and β-polypeptides from *Rps. viridis* and BChl*a* and with the β-polypeptide from *Rps. viridis* alone with BChl*a* (Parkes-Loach et al., 1994).

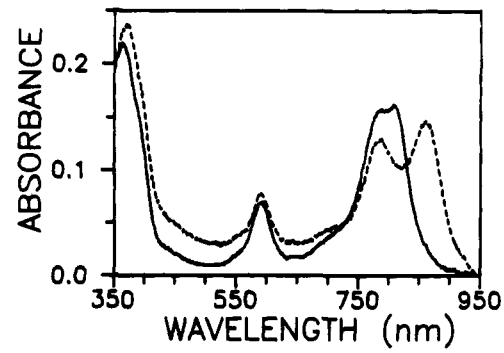

FIGURE 8: Absorption spectra of a reconstitution using the native α-polypeptide from *R. rubrum* and the (FHG...C-terminus) β-polypeptide from *Rps. viridis* at optimized subunit conditions of 0.61% OG, λ_{max} = 783 and 810 nm (solid line); and at 0.58% OG after storage at 4 °C overnight, λ_{max} = 782 and 865 nm (dashed line). Concentrations: α = 1.6 μM, (FHG...C-terminus) = 2.0 μM, BChla_{gg} = 2.4 μM at 0.61% OG. The spectra were normalized to an absorbance of 0.1 cm^{-1} for the 865 nm species and multiplied by a dilution factor.

DISCUSSION

The subunit complex B820, which can be formed by titration in vivo of LH1 with detergent, contains the LH1 α- and β-polypeptides in a 1:1 ratio (Miller et al., 1987; Chang et al., 1990b; Heller & Loach, 1990; Meckenstock et al., 1992). In contrast, in vitro reconstitution of the subunit requires only the presence of the β-polypeptide from *R. rubrum*, *Rb. sphaeroides*, *Rb. capsulatus*, or *Rps. viridis*, although with *Rps. viridis* the subunit is not formed quantitatively (Parkes-Loach et al., 1988, 1994; Loach et al., 1994). The α-polypeptides from these bacteria are unable to form a subunit complex in reconstitution in the absence of a β-polypeptide (Parkes-Loach et al., 1988; Loach et al., 1994). Spectroscopically, the subunit prepared from the β-polypeptide is indistinguishable from that formed when both the α- and β-polypeptides are present (Parkes-Loach et al., 1988; Visschers et al., 1991; Loach et al., 1994). Thus, it is useful to closely examine both the extent to which truncated β-polypeptides are able to form a subunit complex and their reactivity with native α-polypeptides.

Polypeptide Requirements for Subunit Formation. Reconstitutions employing hybrid α- and β-polypeptide pairs from four purple non-sulfur bacteria have suggested the importance of amino acid residues located within the highly conserved core region of the polypeptides (see Figure 1) for subunit formation and, concomitantly, the relative insignificance of those areas on the polypeptide that lie outside of this region (Loach et al., 1994). In the present study, those reconstitutions utilizing either the acetylated α-polypeptide from *R. rubrum* or the truncated polypeptides where the cleavage site resides outside of the core region result in subunit complexes that are indistinguishable from controls where native polypeptides are utilized. However, while the formation of a subunit complex is observed in every reconstitution involving a truncated β-polypeptide, the extent of formation varies depending upon the location of the cleavage within the core sequence region and the identity of the polypeptide itself.

On the basis of those experiments where subunit formation is substantially reduced (as indicated by the presence of a sizeable band at approximately 780 nm in the subunit absorption spectrum under the preselected conditions described in the Results section), the N-terminal regions of the β-polypeptides from *R. rubrum* (positions −29 through −17) and *Rps. viridis* (positions −29 through −20) appear to be important for stabilizing subunit formation. For example, in the *R. rubrum* system, comparison of the absorption spectra of the subunit complexes prepared at 0.75% OG (see Figures 2A, 4A,B, and 5A,B) shows that the extent of formation of the subunit complexes prepared using truncated β-polypeptides decreases as the polypeptide is shortened, both in the presence and absence of the native α-polypeptide from *R. rubrum*. Furthermore, reconstitutions using the truncated β-polypeptide in the absence of the native α-polypeptide result in a more completely formed subunit complex than when the α-polypeptide is present. This behavior is in contrast to that observed for reconstitutions using native, uncleaved polypeptides from *R. rubrum*, where the subunit prepared from the α- and β-polypeptides is formed more completely than the subunit generated using only the β-polypeptide (Parkes-Loach et al., 1988). The apparent stabilization of the subunit complex in the native system by the α-polypeptide may be attributed to ion-pairing and hydrogen-bonding interactions between the α- and β-polypeptides resulting from the presence of complementary N-terminal domains on each polypeptide.

Differences in inter-polypeptide ion pairing, hydrogen-bonding capability, and the extent of association between the side chains of the amino acids [resulting in knobs-into-holes packing of the α-helices (Dunker & Jones, 1978)] have been invoked to explain the disparities in relative stability observed in reconstituted subunit (and LH1) complexes (Loach et al., 1994). Thus, the association between the α- and β-polypeptides is more favorable than that between the β-polypeptides. However, when the N-terminal domain of the β-polypeptide is shortened, 3−6 charged residues are removed and the interaction between the cleaved β-polypeptide and the uncleaved α-polypeptide apparently is disrupted, perhaps because of the removal of one or more potential ion-pairing and/or hydrogen-bonding residues from the β-polypeptide. Moreover, if the molecularity of the subunit is $(\alpha\beta)_2$ (Ghosh et al., 1990; Sturgis & Robert, 1994), an additional unfavorable interaction might consist of charge repulsion between the highly charged N-terminal domains of the two uncleaved α-polypeptides. Finally, because the combination of two truncated polypeptides in a reconstitution does not yield a subunit that is as completely formed as that between native polypeptides (subunits formed either from αβ pairs or from the β-polypeptide only), the interaction between the two N-terminal domains on the polypeptides making up the subunit appears to have a significant effect upon relative subunit stability. These same arguments are applicable to the results obtained using the *Rps. viridis* β-polypeptide in reconstitution with the α-polypeptide from *R. rubrum* or *Rps. viridis*, where again the α-polypeptide stabilizes the formation of the subunit (Parkes-Loach et al., 1994; Loach et al., 1994). However, the subunit formed with the truncated (FHG...C-terminus) β-polypeptide from *Rps. viridis* and the native α-polypeptide from *R. rubrum* is quite incomplete, as shown in Figure 8.

As is illustrated by comparison of Figures 4A,B with 5A,B, the subunit complex generated with the (IFT...C-terminus) β-polypeptide from *R. rubrum* is not formed as completely as the subunits prepared with either the (EFH...C-terminus) or the (FHK...C-terminus) polypeptides, regardless of whether the α-polypeptide is present or not. By comparing the sequences of the four native β-polypeptides shown in Figure 1, neither the Phe nor the Lys residue, at positions −19 and −17, respectively, in the β-polypeptide of *R. rubrum*, is strictly conserved, thus suggesting the importance of the highly conserved His at position −18. The ability of the (IFT...C-terminal) β-polypeptide to form a subunit complex, with or without the α-polypeptide from *R. rubrum*, indicates that the His residing at position −18 is not directly participating (i.e., is not providing a coordinating ligand to the Mg) in the binding of the excitonically coupled BChl*a* molecules of the subunit complex, but it may influence the completeness of formation of the complex due to ion-pairing and/or hydrogen-bonding interactions.

In regard to truncation of the α-polypeptide from *R. rubrum* and the resultant effects on subunit formation, the (PRQ...C-terminus) polypeptide in combination with the native β-polypeptide from *R. rubrum* gives an incompletely formed subunit complex, as shown in Figure 3B. As suggested earlier, a reduction in N-terminal ion pairing and/or hydrogen bonding, brought about by the removal of a portion of the N-terminal domain of the α-polypeptide, may be the cause. As illustrated in Figure 3C, in a reconstitution using the (PRQ...C-terminus) polypeptide alone, without the β-polypeptide, there is no evidence of subunit formation. Thus far, we have not observed the formation of a subunit complex in the absence of a native or truncated β-polypeptide.

The observed CD spectra of the subunits generated with truncated polypeptides closely resemble the spectrum reported for *R. rubrum* (Parkes-Loach et al., 1988). Thus, it appears that, while truncation within the portion of the N-terminal domain inside the core region of the polypeptides affects the extent of formation of the subunit in some cases, the BChl*a* environment remains essentially unperturbed.

Polypeptide Requirements for in Vitro LH1 Formation. It has been demonstrated that reconstitution of an LH1 complex requires the presence of both the native α- and β-polypeptides from *R. rubrum*, *Rb. sphaeroides*, or *Rb. capsulatus* (Parkes-Loach et al., 1988; Loach et al., 1994). Ion pair associations between selected amino acids within the core regions of the oppositely charged N-terminal domains of the α- and β-polypeptides have been suggested to contribute to the association between the polypeptides, leading to stabilization of LH1 complex formation in vitro (Loach et al., 1994).

The extent of formation and relative stability of the reconstituted LH1 complex can be estimated by comparing the relative amounts of unbound BChl*a* and LH1 by their absorbances at 780 and 870 nm, respectively, after storage at 4 °C. Using these criteria, truncation of 10 amino acids or chemical modification of the Lys residue, both within the C-terminal domain of the α-polypeptide from *R. rubrum*, and removal of the C-terminal Phe from the β-polypeptide of *Rb. sphaeroides* have no effect on the formation and stability of the reconstituted LH1 complex. These results are in accord with the locations of the cleavages and the

chemically modified Lys residue outside of the core region of the polypeptides, within areas of limited homology.

Those cleavages within the core region of the N-terminal domains of the polypeptides from *R. rubrum* and *Rps. viridis*, which lead to incomplete formation of the subunit complex, also have an impact on LH1 formation and stability. In the *R. rubrum* system, the extent of formation and the stability of the LH1 complex generated using a truncated β-polypeptide and the native α-polypeptide depend upon the position of the cleavage within the N-terminal domain. Indeed, cleavage in the N-terminal domain within the core region, giving the shortened polypeptides (EFH...C-terminus) and (FHK...C-terminus), results in a marked reduction in the formation and stability of the reconstituted LH1 complexes when compared with the control, as shown in Figures 2 and 4. Moreover, as illustrated in Figure 5A, removal of the strictly conserved His residue at position −18 from the β-polypeptide of *R. rubrum*, yielding the (IFT...C-terminus), polypeptide results in the inability to reconstitute LH1. Combination of two truncated polypeptides in a reconstitution, namely, the (PRQ...C-terminus) polypeptide from the α-polypeptide of *R. rubrum* and the (FHK...C-terminus) polypeptide from the β-polypeptide of *R. rubrum*, results in the formation of an LH1 complex that is quite comparable to those LH1 complexes that are formed between the truncated polypeptides and their native partner polypeptides, yet is inferior to the LH1 formed between native α- and β-polypeptides. Thus, the absence of a portion of the N-terminal domain within the core sequence region of the α- and/or β-polypeptides may remove stabilizing interactions between heterologous polypeptides involving ion-pairing and/or hydrogen-bonding residues, thus resulting in LH1 instability. Further, in LH1 at least two α- and β-polypeptide pairs must be closely associated (van Mourik et al., 1992). Therefore, in those reconstitutions involving the combination of truncated polypeptides with native, uncleaved polypeptides, charge repulsion between the proximal N-terminal regions of the uncleaved polypeptides may also act to further destabilize the complex.

The lack of LH1 formation with the (IFT...C-terminus) β-polypeptide can perhaps be attributed to the combination of the removal of the majority of the N-terminal domain and the loss of the His residue at position −18, since the truncated β-polypeptide of *R. rubrum* containing just three more amino acid residues (FHK) is able to form an LH1 complex, as are the β-polypeptides from *Rb. sphaeroides* and *Rps. viridis*, which are cleaved in comparable positions [the (LHS...C-terminus) polypeptide in the former case and (FHG...C-terminus) in the latter in Figures 6 and 8]. As discussed earlier as a possible reason for the poor formation of the subunit prepared with the (IFT...C-terminus) polypeptide, the loss of a potential ion-pairing and/or hydrogen-bonding residue in this region of the polypeptide, by removal of the His residue at position −18, presumably is even more detrimental to the formation of the LH1 complex. Another important consideration involves the possibility of the His at position −18 participating as a ligand in the binding of the excitonically coupled BChl*a* molecules in reconstituted LH1. A model proposed for the BChl*a* dimer in the subunit complex indicates that the observed optical properties are best explained by having each BChl*a* molecule bound to the His at position 0 (van Mourik et al., 1990; Visschers et al., 1991). Association constants (K_{assoc}) for the formation of the reconstituted subunit complex using native α- and β-polypeptides from *R. rubrum* indicate a high affinity and presumably a high specificity of binding of the BChl*a* to the polypeptides (K_{assoc} on the order of 10^{17} M^{-3}) (Loach & Parkes-Loach, 1995). If it is assumed that the LH1 complex is an aggregate comprising several subunit complexes, such that the subunit is the basic unit of LH1 (Miller et al., 1987; Parkes-Loach et al., 1988; van Mourik et al., 1992), the probability of multiple BChl binding sites is unlikely and His −18 probably is not a ligand to the excitonically coupled BChl*a* in the reconstituted subunit or LH1.

Truncations That Result in Hypsochromically Shifted LH1 BChla Q_y Absorption Bands. Two of the truncated polypeptides, which form LH1 complexes of limited stability when reconstituted with native, uncleaved partner polypeptides, also exhibit concomitant blue shifts of the BChl*a* Q_y absorption band. These polypeptides are the (PRQ...C-terminus) polypeptide from the α- polypeptide of *R. rubrum* and the (FHG...C-terminus) polypeptide from the β-polypeptide of *Rps. viridis* (see Figures 3 and 8). The truncated β-polypeptide of *Rb. sphaeroides*, (LHS...C-terminus), when reconstituted with the α-polypeptide from *R. rubrum* or *Rb. sphaeroides*, also results in the formation of an LH1 complex that is hypsochromically shifted to 847 nm, as shown in Figure 6 (which is discussed in more detail later). However, this complex appears to be quite stable and forms completely, in contrast to the results discussed earlier for the *R. rubrum* and *Rps. viridis* systems. The observed blue shifts indicate that truncation within the core region in the N-terminal domain not only affects the interaction between the polypeptides but may also have long-range effects on the immediate BChl*a* binding environment, since the putative binding site resides on the end of the complex opposite where cleavage is occurring. A similar effect has been observed when the β-polypeptide of LH2 from *Rb. sphaeroides* is subjected to site-directed mutagenesis. In this case, when the Lys in position −17 is mutated to a Gln, the Q_y absorption peak is blue-shifted 18 nm from 855 to 837 nm (Fowler et al., 1993). Interestingly, the mutated Lys residue is located on the N-terminal side of the membrane-spanning domain of the polypeptide, 17 residues away from the putative BChl*a* binding site. Here, the authors have attributed the observed blue shift to long-range effects on the BChl binding pocket.

LH1-like Complexes Prepared Using a Single Polypeptide. Three of the truncated polypeptides prepared in this study are able to form LH1-like complexes in the absence of another polypeptide (resulting in α- or β-only LH1-like complexes). Hitherto, the only example of an LH1-like complex formed from one polypeptide was that formed by the native β-polypeptide from *Rps. viridis* with BChl*a* (Parkes-Loach et al., 1994). The ability of the β-polypeptide from *Rps. viridis* to form an LH1 complex (in the absence of an α-polypeptide) has been suggested to be due to the presence of three additional amino acid residues proximal to the BChl*a* binding site, which have hydrogen-bonding capability, when compared to the β-polypeptide from *R. rubrum* (Parkes-Loach et al., 1994). Consistent with this view, the removal of a significant portion of the N-terminal domain from the β-polypeptide of *Rps. viridis*, resulting in the (FHG...C-terminus) polypeptide, does not significantly alter the association between the β-polypeptides, since the truncated polypeptide did not lose the ability to form an LH1-like complex in the absence of an α-polypeptide.

Another example of an LH1-like complex being generated exclusively from a β-polypeptide is that prepared with the (LHS...C-terminus) polypeptide from the β-polypeptide of *Rb. sphaeroides*. This truncated polypeptide is able to form an LH1-like complex with a Q_y absorption band at 847 nm, both in the presence (discussed earlier) and absence of an α-polypeptide. Because the CD spectrum of the LH1-like complex (see Figure 7) resembles the CD spectra of long-wavelength-absorbing forms of BChl*a* aggregates in Triton X-100 (Gottstein & Scheer, 1983), in 3:1 (v/v) formamide/water (Scherz & Rosenbach-Belkin, 1989), and in 0.66% OG after chilling at 4 °C overnight (unpublished results), it is possible that the species absorbing at 847 nm is an aggregate of BChl*a* and not a pigment/protein complex. However, because a subunit readily forms with this truncated β-polypeptide during reconstitution, with no evidence of unbound BChl*a* (see Figure 6), and because the CD spectrum indicates that the BChl*a* environment is what is expected in a B820 subunit complex, binding between the BChl*a* pigment and the polypeptide occurs with high affinity and in the correct orientation under subunit-forming conditions. Therefore, any aggregation that may be occurring under LH1-forming conditions probably involves the BChl*a* bound to the polypeptide, rather than free BChl aggregation. Perhaps the exciton-paired BChl*a* pigments are oriented in a face-to-face dimer (with significant π orbital overlap), similar to what is proposed for BChl*a* aggregates (in the absence of protein) (Scherz & Rosenbach-Belkin, 1989) but unlike what is postulated for the pigments in LH1, where the excitonically coupled BChl*a* pigments are proposed to be approximately 11 Å apart without significant π orbital overlap (Visschers et al., 1991). Finally, since the LH1-like complex is formed both with and without the α-polypeptide present, the α-polypeptide may not be participating in complex formation at all. In other words, the BChl*a* may be bound exclusively by the β-polypeptide. If this is indeed the case, then perhaps removal of the N-terminal domain from the β-polypeptide of *Rb. sphaeroides* enables the ββ subunits to associate together (presumably by attenuating charge repulsion), such that oligomerization to an LH1-like complex is favorable.

Lastly, when the truncated (PRQ...C-terminus) α-polypeptide from *R. rubrum* is combined under reconstitution conditions with BChl*a*, in the absence of a β-polypeptide, an LH1-like complex is formed quite completely as illustrated in Figure 3C. Since the absorption and CD spectra of this complex are similar to those obtained with native α- and β-polypeptides, it appears that this complex may represent the first example of an LH1-like complex prepared with an α-polypeptide derivative in the absence of a β-polypeptide. On the basis of comparison of the experimental CD spectrum obtained here (data not shown) with that representing an aggreaged form of BChl*a* in OG (unpublished results) and with published CD spectra of BChl*a* aggregates (Gottstein & Scheer, 1983; Scherz & Rosenbach-Belkin, 1989), it does not appear that the LH1-like complex formed in this case is simply an aggregate of BChl*a*. The ability of the truncated α-polypeptide from *R. rubrum* to generate an LH1-like complex may be attributed to the removal of a large portion of the N-terminal domain, which in turn may alleviate charge repulsion between the polypeptides and allow the associative interactions required for the formation of an LH1-like BChl*a* environment. Presumably, the repulsion between the native α-polypeptides is more significant than that between the β-polypeptides, since a subunit complex exclusively made up of α-polypeptides has not yet been demonstrated. Thus, in order to form an LH1-like complex between BChl*a* and the α-polypeptide, the combined effects of removal of a portion of the N-terminal domain and operation under LH1-forming conditions (below the critical micellar concentration of OG) may be required.

In summary, removal of the portion of the N-terminal domain located within the core region of the α- and β-polypeptides appears to have a deleterious effect on reconstitution of the LH1 complex. Previous mutagenesis experiments have demonstrated the importance of this region of the polypeptides for in vivo LH1 assembly (Richter et al., 1992). Based upon the experiments using the (IFT...C-terminus) β-polypeptide from *R. rubrum*, the Mg atoms of the excitonically paired BChl*a* most probably are coordinated to the imidazole groups of the His at position 0. However, removal of the FHK fragment (positions −19 through −17) from the β-polypeptide of *R. rubrum* has proven to be lethal for LH1 formation, presumably because of the removal of the His residue at position −18, which may participate in an ion pair and/or a hydrogen bond. Finally, those cases where the Q_y absorption band of the BChl*a* in the LH1 complex is blue-shifted indicate that alteration of the polypeptide within the core region of the N-terminal domain can perturb the BChl*a* binding site, which is located on the opposite end of the polypeptide near the C-terminal domain, demonstrating long-range polypeptide effects on pigment binding in vitro.

Conclusions. The results presented here indicate that the minimal structural requirements for the formation of an LH1 or LH1-like complex is a single LH1 membrane-spanning α-helical polypeptide that can associate with itself, giving reasonable knobs-into-holes fit without significant charge repulsion. Also, a His residue located approximately one-third of the way into the apolar region is a prerequisite. The presence of amino acid residues (approximately positions 0 to +9) that have the potential to participate in hydrogen bonding proximal to the BChl*a* binding site is probably significant. The portions of the highly charged and hydrophilic N-terminal domains located within the core regions of the polypeptides (approximately positions −28 through −20 on the α-polypeptides and −30 through −20 on the β-polypeptides) are not required for LH1 formation, but appear to be significant for the resulting stability of the reconstituted LH1.

Recent site-directed mutagenesis of the Trp residue located at position +11 in the LH1 α-polypeptide of *Rb. sphaeroides* has illustrated that specific interactions between BChl*a* and the C-terminal regions of the polypeptides within the core sequence are occurring (Olsen et al., 1994). Thus, this region of the LH1 polypeptides will be the focus of future truncation and mutagenesis studies. Also, the demonstrated ability of the truncated polypeptides prepared here to reconstitute the subunit and LH1 will be used in combination with solid-phase peptide synthesis to examine the functions of specific amino acid residues within the LH1 polypeptides.

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