Reconstitution of Core Light-Harvesting Complexes of Photosynthetic Bacteria Using Chemically Synthesized Polypeptides. 1. Minimal Requirements for Subunit Formation†

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ABSTRACT: Described are the chemical synthesis, isolation, and characterization of each of three polypeptides whose amino acid sequences reproduce portions of the amino acid sequence of the β-polypeptides of the core light-harvesting complex (LH1) of *Rhodobacter sphaeroides* or *Rhodospirillum rubrum*. The native β-polypeptides of LH1 of these organisms contain 48 and 54 amino acids, respectively. The smallest synthetic polypeptide had an amino acid sequence identical to that of the last 16 amino acids of the β-polypeptide of *Rb. sphaeroides* (sphβ16) but failed to form either a subunit- or LH1-type complex under reconstitution conditions. Also, this polypeptide, lengthened on the N terminus by adding the sequence Lys-Ile-Ser-Lys to enhance solubility, failed to form a subunit- or LH1-type complex. In contrast, polypeptides containing either the 31 amino acids at the C terminus of the β-polypeptide of *Rb. sphaeroides* (sphβ31) or the equivalent 31 amino acids of the β-polypeptide of *Rs. rubrum* (rrβ31) were fully competent in forming a subunit-type complex and exhibited association constants for complex formation comparable to or exceeding those of the native β-polypeptides. The absorption and CD spectra of these subunit-type complexes were nearly identical to those of subunit complexes formed with native β-polypeptides. It may be concluded that all structural features required to make the subunit complex are present in the well-defined, chemically synthesized polypeptides. Neither polypeptide appeared to interact with the native α-polypeptides to form a LH1-type complex. However, sphβ31 formed a LH1-type complex absorbing at 849 nm without an α-polypeptide. Although chemical syntheses of polypeptides of this size are common, the purification of membrane-spanning segments is much more challenging because the polypeptides lack solubility in water. The chemical syntheses reported here represent the first such syntheses of membrane-spanning polypeptides which display native activity upon reconstitution.

To fully understand the principles by which supramolecular membrane complexes are constructed, it is very helpful, and perhaps essential, to be able to reconstruct these complexes from their fundamental components. Although much has been learned about bioenergetic membrane systems by a combination of biochemical, electrochemical, molecular genetic, spectroscopic, and, more recently, crystallographic analyses, little progress has been made in reconstitution of major systems from their individual polypeptides and cofactors. The supramolecular system that we have been studying is the photoreceptor complex (PRC)[1] of photosynthetic bacteria consisting of a reaction center (RC) and a core light-harvesting complex (LH1). Our laboratory has successfully reconstituted LH1 from its individual components (*1*−*3*) and has also reassociated this complex with the RC (*4*).

LH1 of photosynthetic bacteria contain two small polypeptides, α and β, that occur in a 1:1 stoichiometry, together with bacteriochlorophyll (Bchl) with an approximate overall stoichiometry of $\alpha_{12}\beta_{12}$·24Bchl per RC based on experiments with intact membranes containing these complexes (*5*−*7*). A somewhat greater stoichiometry has been proposed (16

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[1] Abbreviations: Bchl, bacteriochlorophyll *a*; LH1, core light-harvesting complex (also called B873, named after the far-red absorption maximum); subunit complex (also referred to as B820), subunit form of LH1 either isolated from membranes containing LH1 or prepared by reconstitution using native α- and β-polypeptides and Bchl; subunit-type complex, reconstituted complex exhibiting absorption and CD spectra highly similar to those of the native subunit complex but containing an analogue polypeptide or only a native β-polypeptide or β-polypeptide analogue and Bchl; LH1-type complex, reconstituted complex containing Bchl and an analogue α- or β-polypeptide with a native β- or α-polypeptide, respectively, and displaying absorption and CD spectra highly similar to those of native LH1 [reconstituted systems in which a subunit-type complex or LH1-type complex was formed with only a β-polypeptide or a β-polypeptide analogue are sometimes referred to as a $\beta\beta$ subunit-type complex or a $\beta\beta$ LH1-type complex to distinguish them from the heterologous $\alpha\beta$ complexes]; LH2, accessory light-harvesting complex (also referred to as B800−850); Car, carotenoid; OG, *n*-octyl β-D-glucopyranoside; HFA, hexafluoroacetone trihydrate; near-IR, near-infrared; CD, circular dichroism. The following nomenclature was used to refer to the chemically synthesized polypeptides. sphβ16 and sphβ31 represent the chemically synthesized polypeptides that have amino acid sequences identical to the last 16 or 31 amino acids of the native β-polypeptide of *Rb. sphaeroides*, respectively; rrβ31 represents the chemically synthesized polypeptide that has an amino acid sequence identical to that of the native β-polypeptide of *Rs. rubrum* from residues −20 to +10 (see Figure 1 for numbering of the amino acids in the sequence).

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α-polypeptides, 16 β-polypeptides, and 32 Bchl per RC) on the basis of cryoelectron microscopic results with isolated LH1 complexes of *Rhodospirillum rubrum* (*8*). Carotenoid (Car) is also present in LH1 complexes and varies between a 2:1 ratio of Bchl to Car for LH1 in *Rs. rubrum* (*9*, *10*) and a 1:1 ratio for Bchl to Car in LH1 of *Rhodobacter sphaeroides* (*10*−*12*). A stable subunit complex has been isolated from LH1 of many photosynthetic bacteria (*13*−*19*) and most likely has the composition $\alpha_1\beta_1$·2Bchl (*6*).

Although the structure of LH1 has not yet been resolved by crystallography, during the last two years, two crystal structures of an accessory light-harvesting complex (LH2) have been reported (*20*, *21*). LH2 complexes are similar to LH1 complexes in that they contain small α- and β-polypeptides in a 1:1 ratio, but they have a larger Bchl content ($\alpha_1\beta_1$·3Bchl·2Car). The amino acid sequences of LH2 polypeptides generally have a low percent identity (typically about 30%) relative to those of LH1 polypeptides, but share many general features (*22*). Whereas LH1 complexes exhibit a single long-wavelength absorption band (between 865 and 885 nm for Bchl *a*-containing organisms), LH2 complexes have two absorption bands in the near-infrared, one at 800 nm and another near 850 nm (*10*).

In the LH2 structure of *Rhodopseudomonas acidophila*, there are two concentric cylinders of helical protein subunits which enclose the pigment molecules (*20*). At one end of the cylindrical structure, nine Bchl molecules are positioned between the outer helices constituted by the β-polypeptides with the bacteriochlorin rings oriented perpendicular to the transmembrane helix axis. At the other end of the cylindrical structure, 18 additional Bchl molecules are sandwiched between the two rings of helices and form a continuous overlapping ring. The Bchl molecules in this latter ring are most likely responsible for the 850 nm absorption band of LH2 and exist in a structural environment which is believed to be substantially similar to that of the Bchl in LH1. Many of these structural features were also observed in the crystal structure of *Rhodospirillum molischianum* LH2 except that the oligomeric ring contained eight repeating heterodimer units instead of nine (*21*). An overlay of the polypeptide backbones determined for the two LH2 structures shows extremely similar overall conformations.

The ability to reconstitute LH1 from its fundamental components allows the pursuit of detailed structural−functional information. Thus, the relative contributions of various binding elements can be evaluated by utilization of reconstitution with structural analogues of Bchl (*23*, *24*), Car (*3*), and the polypeptides (*2*, *25*, *26*). In this paper, we report the successful chemical syntheses of three polypeptides that were designed to be identical to a portion of the β-polypeptides of either *Rb. sphaeroides* or *Rs. rubrum* LH1. As a result of these studies, it is now possible to evaluate the role of structural features as small as a single atom, as well as to engineer interesting new complexes.

METHODS AND MATERIALS

Chemical Syntheses of Polypeptides. Peptides were synthesized in an atmosphere of ultrapure nitrogen on a Milligen 9600 Peptide Synthesizer using 9-fluorenylmethoxycarbonyl (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Fmoc-BOP) chemistry. Fmoc-L-amino acids were obtained from Perseptive Biosystems, with the exception of Fmoc-Trp(Boc) which was purchased from Novabiochem. Threonine, serine, and tyrosine used *tert*-butyl (tBu) protecting groups, while aspartic acid and glutamic acid used OtBu protecting groups. Arginine was protected with 2,2,5,7,8-pentamethyl chroman-6-sulfonyl (Pmc), and glutamine and histidine were protected with triphenylmethyl (Trt) groups. Fmoc-Phe−polyethylene glycol−polystyrene (Fmoc-Phe−PEG−PS) and Fmoc-Val−PEG−PS from Perseptive Biosystems were used as resins. During the syntheses, the Fmoc protecting groups were removed using a solution of 30% piperidine, 35% dimethylformamide (DMF), and 35% toluene. Amino acid couplings were carried out in solution with a 6.67-fold excess of each of free amino acids, BOP, and 1-hydroxybenzotriazole (HOBT) over the molarity of the nascent polypeptide. The amino acids were activated before coupling with a solution of 2.2% *N*-methylmorpholine in DMF. After synthesis, the resin was washed with methanol and lyophilized to dryness. The peptide was then cleaved from the resin with Reagent R, composed of 90% trifluoroacetic acid (TFA), 5% thioanisole, 3% ethanedithiol, and 2% anisole, to yield the free acid (*27*). The resin was stirred in Reagent R under argon for 4 h, and the mixture was then filtered through glass wool into cold ether. The beads were washed on the glass wool with neat TFA, and the ether solution was stored in the refrigerator for 1 h. The precipitated peptide was filtered out on a sintered glass funnel, washed with cold ether, and dried overnight in a vacuum. Synthesized peptides were purified by reverse phase HPLC on a C-18, C-8, or C-4 column and samples submitted for mass spectral analyses and then tested for their reconstitution activity. The HPLC procedure has been described (*25*) and uses a gradient with 0.1% (v/v) TFA as the aqueous solvent and 0.1% (v/v) TFA in 2:1 (v/v) acetonitrile/2-propanol as the organic solvent. Electrospray ionization mass spectral analyses and determination of amino acid compositions were conducted by the Harvard Microchemistry Facility (Cambridge, MA).

Reconstitution Assay. The reconstitution procedures used here were described previously (*2*). Although wild-type LH1 complexes contain carotenoid, and we have successfully reconstituted this component (*3*), the presence of carotenoid greatly shifts the subunit complex equilibrium toward LH1 and thus makes it impossible to measure K_{Assoc} for subunit formation. Since the presence of carotenoid causes only a small red shift in the λ_{Max} of the Q_Y band (*3*, *13*), it is assumed that the native Bchl environment is not greatly perturbed by the absence of carotenoid. Also, many bacteria containing mutations that eliminate the carotenoid component have been prepared, and the LH1 component does not seem greatly affected (*10*). In the reconstitution assay used, the native α- and β-polypeptides in 0.75% OG associate with Bchl to form a subunit complex with a red-shifted λ_{Max} at about 820 nm (relative to free Bchl in detergent with a λ_{Max} at 777 nm). An LH1 complex is formed when the OG concentration is decreased by dilution or when the cmc is raised by cooling the sample to 4 °C. The native α-polypeptide alone does not form red-shifted species with Bchl, whereas the native β-polypeptide alone forms a subunit-type complex with Bchl but cannot form a LH1-type complex unless the native α-polypeptide is present. In general, reconstitutions were carried out by dissolving in 5−10 μL of HFA 0.03−

0.07 mg of each polypeptide. The polypeptides had previously been HPLC-purified and partitioned from HFA and dried under vacuum. One-half of a milliliter of 4.5% (w/v) *n*-octyl β-D-glucopyranoside (OG) in 50 mM potassium phosphate buffer (pH 7.5) was then added to the HFA solution followed by 2.0 mL of 50 mM potassium phosphate buffer to bring the OG concentration to 0.90%. Bchl was added to the sample by injecting 5−20 μL of an acetone solution, and an absorption spectrum was taken. The sample was diluted to 0.75% OG, an absorption spectrum taken, the solution diluted again to 0.66% OG to optimize subunit formation, and an absorption spectrum taken. For LH1 formation, the sample was placed on ice for 1 h; an absorption spectrum was taken, and the sample was finally kept at 4−8 °C overnight, after which a final absorption spectrum was taken. Prior to reconstitution, protein concentrations were determined by dissolving the protein in the HFA or HPLC mobile phase, recording the UV absorption spectrum, and calculating the concentration assuming an extinction coefficient at 287 nm of 3400 M^{-1} cm^{-1} per Trp residue (*13*).

An alternative method of reconstitution was employed when phospholipids were included. The protein to be tested was dissolved in HFA, diluted with chloroform/methanol (1:1), and added to phosphatidylcholine previously dried out of hexane onto the glass surface of a round-bottom flask. This solution was then frozen and dried under vacuum. The phospholipid and protein were removed from the glass surface with 0.05 M potassium phosphate buffer (pH 7.5) and sonicated to produce proteoliposomes. Bchl was added and a spectrum taken. OG (20%) was then added to bring the sample OG concentration to 0.90% and a spectrum taken. The sample was diluted to 0.75 and 0.66% OG, taking a spectrum at each concentration. Finally, the sample at 0.66% OG was chilled overnight and another spectrum taken. Control experiments using native polypeptides with this procedure produced normal formation of the subunit complex.

Absorption spectra were recorded with a Shimadzu UV-160 spectrophotometer. To reduce the effects of scattered light, opal glass was placed between the sample and the detector. CD spectra were recorded using a Jasco J500C spectropolarimeter interfaced with a microcomputer.

Determination of Association Constants. Association constants were calculated for the equilibria between the fully dissociated components (Bchl and α- and β-polypeptides) and the subunit complex. On the basis of determinations of stoichiometry (*28*), gel filtration experiments, and various spectroscopic analyses (*6*, *29*, *30*), it is assumed that the native subunit complex has the composition $\alpha_1\beta_1$·2Bchl and that the subunit-type complex formed with only Bchl and the β-polypeptide has the composition β_2·2Bchl. Thus,

$$K_{\mathrm{Assoc}} = \frac{[\text{subunit complex}]}{[\alpha][\beta][\mathrm{Bchl}]^2}$$

and for formation of a similar complex with Bchl and only the β-polypeptide

$$K_{\mathrm{Assoc}} = \frac{[\text{subunit-type complex}]}{[\beta]^2[\mathrm{Bchl}]^2}$$

The concentrations of subunit complex and uncomplexed Bchl were determined by deconvolution of the absorption spectrum in the Q_Y region (*28*). The data were fit to two components using a molar absorptivity for Bchl in OG of 55 mM^{-1} cm^{-1} at 777 nm and a molar absorptivity for the subunit complex of 172 mM^{-1} cm^{-1} at 824 nm (*Rb. sphaeroides*) or 820 nm (*Rs. rubrum*) based on 2 Bchl per complex (*31*).

Because the presence of low percentages of impurities in the Bchl and polypeptides can have a major effect on determinations of K_{Assoc}, and because Bchl is especially difficult to maintain at >98% purity, control reconstitution assays (native polypeptides and Bchl) were always conducted in parallel with reconstitutions utilizing synthetic polypeptides. In these control assays, all components were identical except for the polypeptide being tested. Furthermore, if any component in the control experiments showed evidence of being less than 95% pure, new materials were isolated.

RESULTS

As a result of reconstitution studies involving protease-truncated polypeptides, core regions of the α- and β-polypeptides have been identified that contain all the structural elements necessary for forming a subunit-type complex with a value for K_{Assoc} equivalent to that of systems containing native polypeptides (*6*, *25*). On the basis of these results, 31-amino acid portions of the β-polypeptides of *Rb. sphaeroides* and *Rs. rubrum* were selected for our first syntheses, as they would be expected to form stable subunit complexes. The amino acid sequences of the polypeptides synthesized are shown in Figure 1 along with the sequences of the native β-polypeptides. Because these membrane-spanning polypeptides are insoluble in water, chemical synthesis and isolation of the pure polypeptides were anticipated to be more difficult than in the case of water-soluble polypeptides. This has certainly been the case, but the methodology developed (*1*, *2*) for isolation of native α- and β-polypeptides has proven to be very useful in the isolation of the synthetic polypeptides.

Chemical Synthesis of sphβ16. A relatively short polypeptide of 16 amino acids was first synthesized with the idea that we might subsequently couple two such polyeptides together to achieve a larger polypeptide. The synthesized 16mer reproduced the last 16 amino acids of the β-polypeptide of *Rb. sphaeroides* (Figure 1). HPLC analysis of the product of this synthesis indicated that the desired polypeptide was approximately 75% of the crude synthesized material, and it was easily purified by HPLC using a Vydac C-8 column with a G3 solvent gradient (*25*). sphβ16 would not readily dissolve in OG solution under the conditions of our standard reconstitution assay, and an alternative method of assay using phospholipids (*4*) was also tried to test its activity. No activity was observed, with or without a native α-polypeptide, either in our standard assay system or in the phospholipid assay system.

After experiencing the severe solubility problems with sphβ16, we decided to extend this synthesis by adding four more amino acids to the N terminus of about half of the material which had not been removed from the resin. The N-terminal sequence added was Lys-Ile-Ser-Lys, making the polypeptide 20 amino acids long. The two Lys were selected to improve solubility, and their locations were chosen (on the basis of the crystal structure of LH2) so that the side

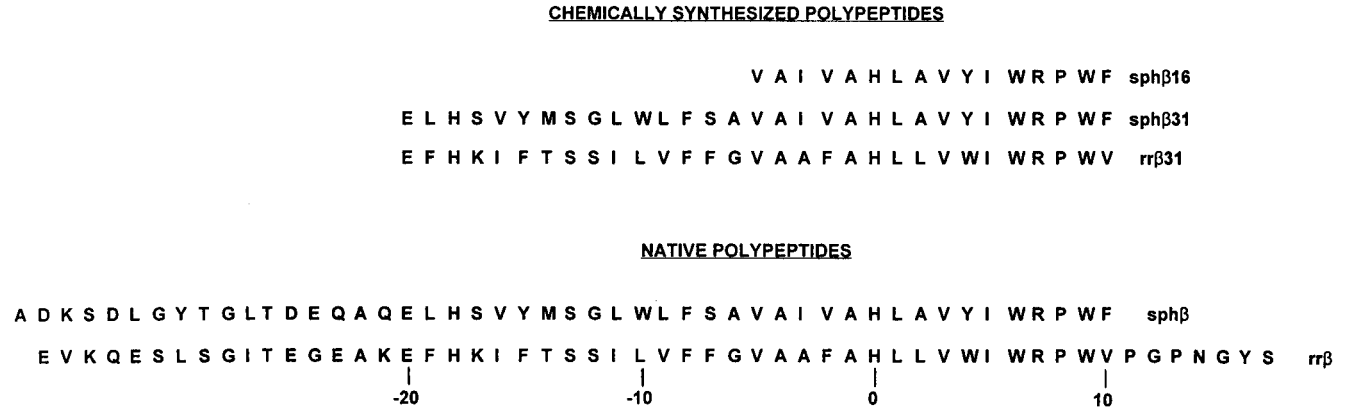

FIGURE 1: Amino acid sequences of chemically synthesized and native β-polypeptides of LH1 of *Rs. rubrum* (rrβ) (*32*, *33*) and *Rb. sphaeroides* (sphβ) (*34*, *35*). The His to which Bchl is coordinated was assigned position 0 and each polypeptide aligned accordingly. The abbreviation used for each polypeptide in the text is indicated on the right side.

Table 1: Association Constants of Reconstituted Subunit Complexes

system	polypeptide (μM at 0.75% OG)	K_{Assoc} ($M^3 \times 10^{-16}$) 0.90% OG	0.75% OG
Rs. rubrum	native β (2.0) + native α (1.7)	126	≥300
	native β (3.7)	2	24
enzyme-truncated *Rs. rubrum*	truncated $\beta_{\text{from EFHK to the C terminus}}$ (3.0) + native α (1.6)	1.1	9
	truncated $\beta_{\text{from EFHK to the C terminus}}$ (4.4)	0.6	3
chemical synthesis *Rs. rubrum.*	rrβ31 (2.6) + native α (2.2)	8	85
	rrβ31 (4.9)	12	120
Rb. sphaeroides	native β (1.8) + native α (1.2)	38	≥300
	native β (6.1)	23	200
enzyme-truncated *Rb. sphaeroides*	truncated $\beta_{\text{from LHSV to the C terminus}}$ (2.3) + native α (1.7)	16	139
	truncated $\beta_{\text{from LHSV to the C terminus}}$ (6.0)	72	≥300
chemical synthesis *Rb. sphaeroides*	sphβ31 (3.3) + native α (3.0)	10	78
	sphβ31 (5.5)	40	≥300

chains should not interfere with formation of a subunit-type complex. This polypeptide was purified by reverse phase HPLC using a C-18 column. It was well-behaved and readily dissolved in our standard assay media. Once again, however, neither subunit- nor LH1-type complexes could be formed.

Chemical Synthesis of sphβ31. From our experience with the synthesis of sphβ16, it seemed feasible to directly synthesize polypeptides 30−35 amino acids in length. We chose to synthesize the sequence equivalent to the last 31 amino acids of the β-polypeptide of *Rb. sphaeroides* (from ELHS to the C terminus) which we could compare effectively to data for the truncated polypeptide containing 30 amino acids (from LHSV to the C terminus) (*25*). One of the reasons for choosing this polypeptide for synthesis is the very large K_{Assoc} the truncated polypeptide exhibits (as a β-only system) for formation of a subunit-type complex (Table 1). The products of the chemical synthesis were separated by HPLC using a Perkin-Elmer Pecosphere HS-5 HCODS C-18 column with a G3 solvent gradient (*25*), and the material of the major peak was collected. Upon rerunning this material on HPLC, we obtained a single, well-defined peak which gave a value of 3664.1 Da on mass spectral analysis, in excellent agreement with the expected mass (3663.5 Da). When assayed for its ability to form a subunit-type complex, as well as a LH1-type complex, its activity and spectral properties matched those of the protease-truncated native polyeptide (from LHSV to the C terminus) (compare panels A and B of Figure 2). A subunit-type complex readily formed with a λ_{Max} of 820 nm and with a K_{Assoc} very similar to that obtained using the sequence from LHSV to the C terminus (Table 1). Its CD spectrum (Figure 2C) is very similar to those of native subunit complexes (*14*). Upon the solution being subjected to LH1-forming conditions, a LH1-type complex was produced even in the absence of an α-polypeptide (Figure 2B) and displayed a λ_{Max} of 849 nm. The CD spectrum of this LH1-type complex (Figure 2C) is different from that of the subunit, and also somewhat different from that of native LH1 (*14*). Reconstitutions with sphβ31, Bchl, and the native *Rb. sphaeroides* α-polypeptide gave results very similar to those obtained with only sphβ31 and Bchl. The λ_{Max} of the LH1-type complex was 849 nm, greatly blue-shifted from that of native LH1 (λ_{Max} = 874 nm).

Chemical Synthesis of rrβ31. The native β-polypeptide of *Rs. rubrum* exhibits properties similar to those of the native β-polypeptide of *Rb. sphaeroides*; it forms a subunit-type complex without an α-polypeptide but will not form a LH1-type complex alone (*1*). From our experiments with protease-truncated β-polypeptides of *Rs. rubrum*, the polypeptide from EFHK to the C terminus (which has the native amino acid sequence from residue −20 to +17) was effective both in forming a subunit-type complex and in forming a LH1-type complex in the presence of the native α-polypeptide (*25*). Therefore, it was of interest to chemically synthesize a 31mer that begins at residue −20 and ends at residue +10 (rrβ31), thus allowing a comparison to the same polypeptide region as sphβ31 (see Figure 1). The synthesis, isolation, and manipulation proceeded smoothly. The product was isolated as a major peak on HPLC using a Perkin-Elmer Pecosphere HS-5 HCODS C-18 column with a G3 solvent gradient (*25*). The isolated peak was shown to be chromatographically pure by rerunning it on HPLC. Mass spectral analysis gave a value of 3719.1 Da, in excellent agreement with the expected mass (3718.5 Da). The activity of this polypeptide in reconstitution assays has proven to be very informative. When reconstituted with Bchl without an α-polypeptide, the polypeptide displayed an excellent ability to form a subunit-type complex (Figure 3 and Table 1). Interestingly, the K_{Assoc} for formation of a $\beta\beta$ subunit-type

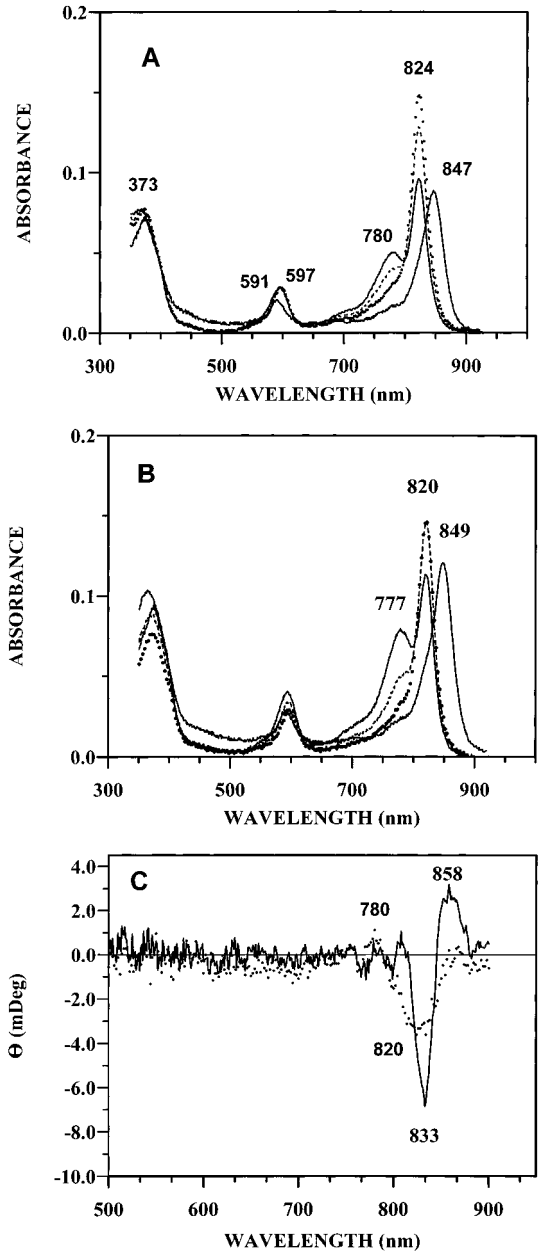

FIGURE 2: (A) Absorption spectra of a reconstitution experiment using the 30-amino acid, protease-truncated β-polypeptide of *Rb. sphaeroides* (from LHSV to the C terminus) (*25*). Spectra were recorded at 0.90% OG (solid curve with λ_{Max} at 780 and 824 nm), 0.75% OG (dashed curve), 0.66% OG (dotted curve), and 0.66% OG stored at 4 °C overnight (solid curve with a λ_{Max} of 847 nm). Concentrations were as follows: α-polypeptide, 1.5 μM; sequence from LHSV to the C terminus, 2.8 μM; and Bchl, 1.9 μM at 0.66% OG. Spectra were recorded in 1 cm cuvettes and multiplied by an appropriate dilution factor to normalize the spectra to a constant Bchl concentration. (B) Absorption spectra of a reconstitution experiment using sphβ31 and Bchl. Spectra were recorded at 0.90% OG (solid curve with absorption peaks at 777 and 820 nm), 0.75% OG (dashed curve), 0.66% OG (dotted curve), and 0.66% OG stored at 4 °C overnight (solid curve with a λ_{Max} of 849 nm). Concentrations were as follows: sphβ31, 4.8 μM; and Bchl, 1.9 μM at 0.66% OG. Spectra were recorded in 1 cm cuvettes and multiplied by an appropriate dilution factor to normalize the spectra to a constant Bchl concentration. (C) Corresponding CD spectra of the subunit-type complex (dotted curve) at 0.66% OG at room temperature formed using sphβ31 and Bchl and the LH1-type complex (solid curve) formed after storage of the sample at 4 °C for 2 days. Concentrations under subunit-forming conditions were as follows: sphβ31, 5.5 μM; and Bchl, 1.3 μM. Spectra were recorded in 1 cm cuvettes.

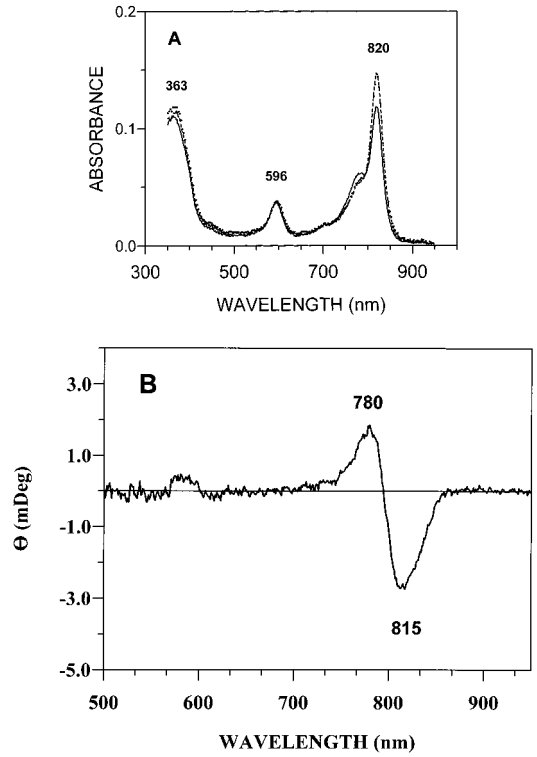

FIGURE 3: Reconstitution using rrβ31 and Bchl. (A) Absorption spectra at 0.90% (solid curve), 0.75% (dashed curve), and 0.68% OG (dotted curve). Concentrations were as follows: rrβ31, 4.9 μM; and Bchl, 1.7 μM at 0.75% OG. Spectra were recorded in 1 cm cuvettes and multiplied by an appropriate dilution factor to normalize the spectra to a constant Bchl concentration. (B) CD spectrum of the subunit-type complex at 0.66% OG. Concentrations were as follows: rrβ31, 10.1 μM; and Bchl, 2.2 μM. Four spectra were recorded and averaged. Spectra were recorded in a 2 cm cuvette at room temperature.

complex was severalfold greater than that of the native β-polypeptide of *Rs. rubrum* and was similar to that exhibited by the native β-polypeptide of *Rb. sphaeroides* (Table 1). The CD spectrum (Figure 3B) of the subunit-type complex is again very similar to those of native subunit complexes (*14*). Thus, the N-terminal region and the seven additional amino acids at the C terminus are, as expected, not required for forming the subunit complex. However, in contrast to the results with the sphβ31, no LH1-type complex could be formed either in the presence of a native *Rs. rubrum* α-polypeptide or without it. By comparison to the results obtained with the protease-truncated sequence from EFHK to the C terminus in which a LH1-type complex was formed (*25*), the lack of LH1 formation in the system containing rrβ31 and the native α-polypeptide presumably means that some portion of the seven C-terminal amino acids of the native β-polypeptide interacts with the C terminus of the α-polypeptide and/or Bchl to help stabilize LH1 formation.

DISCUSSION

In this paper, we have reported the successful chemical synthesis and isolation of three polypeptides, each of which reproduces portions of the amino acid sequence of the β-polypeptides of LH1 of *Rb. sphaeroides* or *Rs. rubrum*. The fact that sphβ31 and rrβ31 have properties in the reconstitution assay that are very similar to those of the protease-truncated native polypeptides allows several important conclusions. First, since chemical synthesis provides

a polypeptide whose primary structure is completely defined, it can be safely concluded that the native β-polypeptide does not contain unique structural features such as a *cis*-proline linkage, amidation of carboxyl groups, phosphorylation of a side chain, or other post-translational modifications that are required for reconstitution to form a subunit-type complex.

Second, a requirement for small quantities (e.g., <10% compared to the amount of β-polypeptide) of a hypothetical component in addition to the β-polypeptide and Bchl can be excluded. This is an important point because of the unknown role for the *puf x* gene product in the in vivo LH1 complex (*36*, *37*) and the possibility that small quantities of a small molecule (e.g., phospholipid) could have been a structural requirement. This is not to say that some such components may not be involved with the LH1 complex in vivo, but that no extra component is necessary with the β-polypeptides for forming $\beta\beta$ subunit-type complexes.

A third point is that the chemical syntheses of these polypeptides represents the first chemical syntheses of membrane-spanning polypeptides which have activity identical to that of the native system. Although chemical syntheses of polypeptides of this size are common, the purification of chemically synthesized, membrane-spanning segments is much more challenging because the polypeptides lack solubility in water. The methodology we have previously developed for isolation and assay of such polypeptides (*1*, *13*, *25*, *38*) has been immensely important to our success in this regard. The ability to chemically synthesize active polypeptides allows one to prepare peptide analogues that cannot be produced by site-directed mutagenesis. In addition, analogues in which a single atom is changed can be synthesized.

A fourth important result of the successful chemical syntheses of selected polypeptides is the ability to conduct characterization experiments that require large quantities of protein. Typically, isolation of pure native polypeptides provides quantities of protein of only 1−2 mg from several weeks of work, and yields of chemically or enzymatically modified polypeptides rarely exceed a few tenths of a milligram. On the other hand, chemical synthesis can routinely provide more than 100 mg of product, making extensive characterization of interesting systems possible.

Minimal Requirements for Subunit Formation. By the use of specific proteases (*25*), limited acid hydrolysis (*25*), heterologous reconstitution (*2*), and site-specific mutagenesis (*26*), a core amino acid sequence was initially defined which contained the necessary structural features for formation of a LH1 subunit-type complex (*6*). These minimal requirements have been further supported by the chemical syntheses of sphβ31 and rrβ31. In both cases, these polypeptides showed excellent ability to form a subunit-type complex with Bchl, exhibiting absorbance and CD properties very similar to those of the native β-polypeptides. Association constants (K_{Assoc}) obtained for reconstitutions with sphβ31 were the same as those of systems utilizing the native *Rb. sphaeroides* β-polypeptide, whereas those obtained for rrβ31 were actually somewhat larger. The enhanced interaction in forming the subunit complex with rrβ31 would seem to be due to the lack of the seven amino acids at the C terminus of the native *Rs. rubrum* β-polypeptide (see ref *39*). On the other hand, sphβ16 and sphβ16 with its four-amino acid extension were unable to form a subunit-type complex under our reconstitution conditions. This is interesting because amino acids whose side chains are now known to be important for subunit stabilization (βHis$_0$, βTrp$_{+9}$, βTyr$_{+4}$, βTrp$_{+6}$, and βArg$_{+7}$; see refs *26* and *39*) are all present in sphβ16. Two amino acids in the N-terminal region (βGlu$_{-20}$ and βHis$_{-18}$) have small effects on subunit stabilization (*25*), but their absence would not be expected to completely eliminate the ability to form the subunit complex. Thus, additional specific interactions or general stabilization due to packing in the additional length of α-helical polypeptides may be important (*40*). It will be interesting to explore portions of the native polypeptide with lengths between those of sphβ16 and sphβ31.

Comparison of β-Polypeptides of Rs. rubrum and Rb. sphaeroides. Comparative biochemistry is a powerful tool for understanding structure−function relationships. Although the amino acid sequences of the β-polypeptides of LH1 of *Rs. rubrum* and *Rb. sphaeroides* are only 35% identical, they exhibit highly similar behavior in forming a $\beta\beta$ subunit-type complex and LH1 (*2*). Amino acid sequence identity of 42% is found between sphβ31 and rrβ31. Even if highly conservative differences in the amino acid sequence are also considered, there is still about a 50% difference in their amino acid sequence. One could conclude from this that, in the regions of variation, there is little specific interaction in the subunit structure.

Although homologous $\beta\beta$ subunit-type complexes form with either sphβ31 or rrβ31 and have comparable values for K_{Assoc} (Table 1), it should be noted that only sphβ31 forms a homologous $\beta\beta$ LH1-type complex. Neither polypeptide appears to interact with the corresponding native α-polypeptide to form a heterologous $\alpha\beta$ LH1-type complex. In the case of rrβ31, the lack of the 16 N-terminal amino acids was expected to weaken LH1 formation, but from the results with the protease-truncated *Rs. rubrum* β-polypeptide (*25*), it was expected that some LH1 would form. The fact that no LH1 could be formed indicated that the seven amino acids at the C-terminal end of the protease-truncated β-polypeptide of *Rs. rubrum*, which were not present in rrβ31, must play a role in stabilizing LH1 formation. In the case of sphβ31, formation of a homologous $\beta\beta$ LH1-type complex with a λ_{Max} of 849 nm and a unique CD spectrum (Figure 2C) seems to occur even in the presence of the native α-polypeptide of *Rb. sphaeroides*; if a heterologous $\alpha\beta$ LH1-type complex had formed, its λ_{Max} would be expected to be near 875 nm with a corresponding native-like CD spectrum (*2*).

One might inquire why the homologous $\beta\beta$ subunit-type complex formed with sphβ31 oligomerizes to form a homologous $\beta\beta$ LH1-type complex whereas that formed from rrβ31 does not. In consideration of what differences between these two polypeptides might account for this rather different behavior, the Lys residue at position −17 in rrβ31 would seem significant (Figure 1). The ϵ-amino group of this residue would be protonated at pH 7 and thus carry a positive charge. However, according to the backbone structure of the β-polypeptide of *Rps. acidophila* LH2, which also has a Lys at this position, the charged ϵ-amino group projects into the aqueous space on the outside of the ring structure, and thus would seemingly not interfere with formation of either subunit- or LH1-type complexes. On the other hand, because in LH1 there is no B800 Bchl, the β-polypeptides must approach each other and the α-polypeptides more closely

than in LH2, and consequently, it is possible that charge repulsion may destabilize the oligomerization of the homologous $\beta\beta$ subunit-type complex. Requirements for formation of native LH1-type complexes are further discussed in the following paper (*39*).

Advantages of Chemical Syntheses. With the demonstration that polypeptides with native activity can be chemically synthesized, it is now possible to systematically address fundamental relationships between structure and function. Although site-directed mutagenesis is quite useful in producing changes in specific amino acid residues, it is limited in several respects: (1) Some key mutations are critical for LH1 formation, and the changed protein cannot be expressed; (2) the amount of LH1 protein which can be isolated from many mutant cells is very small, making extensive biochemical characterization difficult; (3) the smallest unit of structure that can be changed is an entire amino acid; and (4) possible contamination by other tightly bound proteins or factors may make evaluation difficult. On the other hand, chemical synthesis can provide large quantities of pure polypeptides in which it is possible to test the effect of changes as small as a single atom. In the following paper (*39*), chemical syntheses were used to examine the effect of changing specific amino acids in the sequence and to systematically lengthen the N- and C-terminal portions of the polypeptide to initially define structural features necessary to recover native behavior in forming LH1.

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