Interactions Stabilizing the Structure of the Core Light-Harvesting Complex (LH1) of Photosynthetic Bacteria and Its Subunit (B820)†

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ABSTRACT: Reconstitution experiments with a chemically synthesized core light-harvesting (LH1) β-polypeptide analogue having 3-methylhistidine instead of histidine in the position that normally donates the coordinating ligand to bacteriochlorophyll (Bchl) have provided the experimental data needed to assign to B820 one of the two possible $\alpha\beta\cdot$2Bchl pairs that are observed in the crystal structure of LH2 from *Phaeospirillum* (formerly *Rhodospirillum*) *molischianum*, the one with rings III and V of Bchl overlapping. Consistent with the assigned structure, experimental evidence is provided to show that significant stabilizing interactions for both the subunit complex (B820) and LH1 occur between the N-terminal regions of the α- and β-polypeptides. On the basis of the results with the chemically synthesized polypeptides used in this study, along with earlier results with protease-modified polypeptides, mutants, and chemically synthesized polypeptides, the importance of a stretch of 9−13 amino acids at the N-terminal end of the α- and β-polypeptides is underscored. A progressive loss of interaction with the LH1 β-polypeptide was found as the first three N-terminal amino acids of the LH1 α-polypeptide were removed. The absence of the N-terminal formylmethionine (fMet), or conversion of the sulfur in this fMet to the sulfoxide, resulted in a decrease in LH1 formation. In addition to the removal of fMet, removal of the next two amino acids also resulted in a decrease in K_{assoc} for B820 formation and nearly eliminated the ability to form LH1. It is suggested that the first three amino acids (fMetTrpArg) of the LH1 α-polypeptide of *Rhodospirillum rubrum* form a cluster that is most likely involved in close interaction with the side chain of His −18 (see Figure 1 for numbering of amino acids) of the β-polypeptide. The results provide evidence that the folding motif of the α- and β-polypeptides in the N-terminal region observed in crystal structures of LH2 is also present in LH1 and contributes significantly to stabilizing the complex.

The core light-harvesting complex (LH1)[1] of photosynthetic bacteria closely interacts with the reaction center (RC) to provide a highly efficient light-harvesting system. LH1 is thought to encircle (*1*−*6*), or nearly encircle (*7*−*11*), the RC. Many bacteria also contain accessory light-harvesting complexes (e.g., LH2) that function to increase the antenna to RC ratio. Crystal structures of RC (*12*−*14*) and LH2 (*15*, *16*) have provided important details for elucidating structure−function relationships. On the basis of spectroscopic information (*17*, *18*), high-resolution X-ray structures of LH2 complexes (*15*, *16*), and low-resolution electron microscopy studies of LH1 complexes (*1*), the structure of the LH1 complex has been modeled (*19*).

Especially useful for probing structure−function relationships in LH1 has been the ability to reversibly dissociate the complex into subunits (B820) (*20*−*26*) and to further reversibly dissociate B820 into its fundamental components (*27*). The ability to reconstitute B820 of both LH1 (*27*−*30*) and LH2 (*31*) under equilibrium conditions allows the determination of thermodynamic parameters such as specific interaction energies (*28*, *32*−*35*). From these studies, His 0 (see Figure 1 for numbering) and Trp 9 of the β-polypeptide and His 0 and Trp 11 of the α-polypeptide have been shown to play major roles in bacteriochlorophyll (Bchl) binding and subunit stabilization. Additional interactions that seem not to be significant for subunit stability have been found to be important for oligomerization of B820 to form LH1. As an example, His −18 was found to be important for oligomerization of B820 to form LH1 (*35*). Furthermore, removal of an N-terminal segment of either the LH1 α-polypeptide of *Rhodospirillum rubrum* or the LH1 β-polypeptide of *Rs.*

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[1] Abbreviations: Bchl, bacteriochlorophyll *a*; LH1, core light-harvesting complex (also referred to as B875 after the near-infrared absorption maximum); LH2, peripheral light-harvesting complex (also referred to as B800−850 after the near-infrared absorption maxima); B820, subunit form of LH1 or LH2 either isolated from membranes or prepared by reconstitution using native α- and β-polypeptides and Bchl; B820-type complex, in vitro reconstituted complex exhibiting absorption and CD spectra very similar to those of the native subunit complex but containing a non-native α- and β-polypeptide combination or only a native β-polypeptide and Bchl; CD, circular dichroism; OG, *n*-octyl β-D-glucopyranoside; HFA, hexafluoroacetone trihydrate; Me-His, 3-methylhistidine.

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FIGURE 1: Amino acid sequences of the native LH1 α-polypeptide of *Rs. rubrum* (top sequence) and the native LH1 *β*-polypeptide of *Rb. sphaeroides* (top sequence) and derivatives. LH1 α-polypeptide derivatives prepared are the sulfoxide derivative of the native polypeptide (indicated by the arrow pointing at M in the top sequence), the native polypeptide minus the first three amino acids (endoproteinase Arg-C split indicated by △ in the top sequence), and the chemically synthesized 42mer (second sequence under α). A previously reported (*36*) C-terminal truncated polypeptide prepared by endoproteinase Glu-C hydrolysis is shown as the third sequence under α, and the fourth sequence under α is that of a previously reported core fragment prepared by limited acid hydrolysis of the native α-polypeptide (*36*). Chemically synthesized LH1 *β*-polypeptide derivatives prepared are the complete amino acid sequence of the *Rb. sphaeroides* LH1 *β*-polypeptide except for His 0, which was changed to Gly (indicated by the arrow in the second sequence under *β*), and a 31mer with His 0 changed to 3-methylhistidine (indicated by X in the fourth sequence under *β*). A previously reported synthetic polypeptide of 41 amino acids (7 fewer amino acids at the N-terminus relative to the native LH1 *β*-polypeptide) is shown as the third sequence under *β* (*35*).

rubrum and *Rhodobacter sphaeroides* results in peptides that no longer recognize (interact with) the native partner to form LH1 but rather self-associate to form LH1-like complexes (*36*). On the other hand, part of the C-terminal portion of the LH1 α-polypeptide can be removed without effect on expression (*37*) or on reconstitution to form B820 and LH1 (*32*). In this paper, we report the chemical synthesis of a large segment of the native LH1 α-polypeptide and explore the importance of the N-terminal amino acids in reconstitution of B820 and LH1.

On the basis of the two crystal structures of LH2 (*15*, *16*), the structure of the subunit B820 in detergent solution is assumed to be essentially an *αβ* heterodimer similar in structure to one of the two pairs that occur in the LH2 structure. As shown in Figure 2B, in one of these *αβ* pairs, the Bchl molecules overlap each other at pyrrole rings I whereas in the other pair they overlap at rings III and V of each Bchl (Figure 2A). If the first pair were found in the B820 structure, it could be stabilized by cross-hydrogen bonding between Trp 11 of the α-polypeptide and the C3 carbonyl of Bchl coordinated to the side chain of His 0 of the *β*-polypeptide and also between Trp 9 of the *β*-polypeptide and the C3 carbonyl of Bchl coordinated to the side chain of His 0 of the α-polypeptide. From measurements of association constants, each of these interactions could contribute 3.5 kcal/mol of stabilization energy (*34*). Although in the structure of LH2 the side chains of these two Trp are hydrogen bonded to the C3 carbonyl of Bchl coordinated to the same polypeptide in which the Trp is found, their switch to cross-hydrogen bonding in B820 would only require small structural changes which could be facilitated in detergent solution. Alternatively, the structure of B820 could consist of the *αβ* heterodimer in which rings III and V of each Bchl overlap. In this case, cross-hydrogen bonding involving the N3 position of the side chain of His 0 from one polypeptide and the $C13^1$ carbonyl of Bchl (see Figure 2C for atom numbering in Bchl) coordinated to His 0 of the other polypeptide (Figure 2D) could afford enough interaction energy to stabilize B820. Such cross-hydrogen bonding has been suggested as a stabilizing interaction in LH2 of *Phaeospirillum* (formerly *Rhodospirillum*) *molischianum*, although the bond distances and angles are not ideal (*16*). With only a small repositioning of the two Bchl molecules, these parameters could be much closer to ideal for B820 in detergent solution and contribute significantly to the energy of stabilization.

To test which of these two structures would best represent B820 and also to estimate interaction energies involving His 0, we have chemically synthesized two analogues of the *Rb. sphaeroides* LH1 *β*-polypeptide. In one of these, the complete native amino acid sequence was reproduced except His 0 was replaced by a Gly. Reconstitution experiments using this analogue with the native LH1 α-polypeptide offer the opportunity to estimate the stabilization energy for His interaction. The other chemically synthesized polypeptide represents the highest resolution type of a structure−function question that one might imagine. A single hydrogen atom was replaced by a methyl group at the N3 position of the side chain of His 0 of the LH1 *β*-polypeptide so that this side chain could not participate in hydrogen bonding. If the B820 structure is the one with pyrrole rings I overlapping, replacing His 0 by 3-methylhistidine should have no significant effect on subunit formation (Figure 2B and the middle pair of Bchl in Figure 2D), whereas if the B820

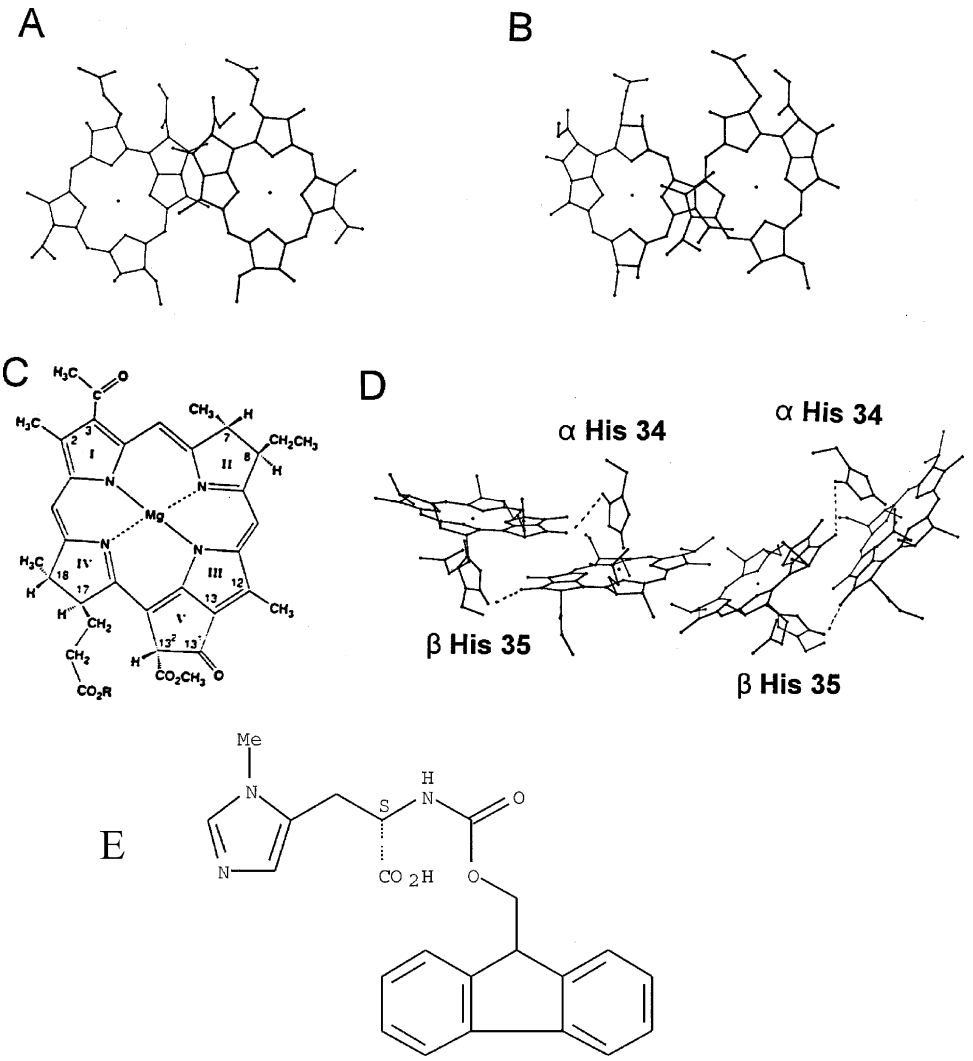


FIGURE 2: The two Bchl pairs that alternate in the cyclical structure of LH2 of *Ps. molischianum* (*16*) are shown in which the two Bchl overlap at pyrrole ring III and ring V (A) and that in which the two Bchl overlap at pyrrole rings I (B). (C) Structure of Bchl. (D) One-quarter of the ring structure of *Ps. molischianum* LH2 (*16*) showing Bchl and the coordinated His side chains. (E) Structure of Fmoc-protected 3-methylhistidine [L-histidine, *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-3-methyl-(9C1); CAS registry number 252049-16-4] which was used in the chemical synthesis of the analogue of the *Rb. sphaeroides* LH1 β-polypeptide as a substitute for His 0.

structure has rings III and V overlapping (Figure 2A and the first and second, or the third and fourth, pair of Bhl in Figure 2D), then B820 would not be formed.

MATERIALS AND METHODS

n-Octyl β-D-glucopyranoside (OG) was obtained from the Sigma Chemical Co. All HPLC solvents were of HPLC grade. Hexafluoroacetone trihydrate (HFA) was purchased from Aldrich Chemical Co. Fmoc-derivatized amino acids were purchased from Novabiochem except for Fmoc-3-methylhistidine, which was purchased from Bachem. Resins used in peptide synthesis were obtained from Applied Biosystems except for the resin containing Fmoc-Phe, which was a gift from the Macromoleclar Structure Facility of Michigan State University. Dichloromethane and dimethylformamide were Burdick & Jackson HPLC grade purchased from VWR. Diisopropylethylamine was purchased from Applied Biosystems. Trifluoroacetic acid was of sequencing grade obtained from Aldrich Chemical Co. Pybop and Hobt were purchased from Novabiochem. Bacteriochlorophyll *a* (Bchl) containing geranylgeraniol as the esterifying alcohol was isolated from the G-9 carotenoid-less mutant of *Rs. rubrum* following the procedures of Berger et al. (*38*) and Michalski et al. (*39*).

Isolation of Native Polypeptides. Rs. rubrum (strain S1) wild-type cultures were grown, and the α- and β-polypeptides of the LH1 complex were isolated by procedures previously described (*27*, *40*−*42*). Cultures of the PUC705-BA mutant [B800−850(−)] of *Rb. sphaeroides* (*43*) were grown and the α- and β-polypeptides of the LH1 complex isolated as previously reported (*36*, *44*).

The polypeptides were purified to homogeneity by reverse-phase HPLC either on a Waters 501 solvent delivery system interfaced to an NEC Powermate SX Plus microcomputer or on a Waters Breeze system with a Rheodyne manual injector and a Waters 2487 UV detector. Perkin-Elmer HCODS C18 columns (150 × 4.6 mm) were used for all purifications. The solvents and solvent gradients used for separating the α- and β-polypeptides have been described (*36*). The appropriate peaks were collected, lyophilized to dryness, and stored at −20 °C.

Chemical Syntheses of Polypeptides. Peptides were synthesized under ultrapure nitrogen or argon gas on a Milligen 9600 peptide synthesizer using 9-(fluorenylmethoxycarbo-

nyl)benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafuorophosphate (Fmoc-BOP) chemistry as previously described (*33*, *45*). The identities of the synthesized polypeptides were confirmed by mass spectral analysis (Macromolecular Structure Facility, Michigan State University).

Verification of the Fmoc-3-methylhistidine Structure. Because substantial confusion exists in the literature regarding the naming of the nitrogen atoms in the histidine side chain, and because the experimental results using the synthetic polypeptide incorporating this modified amino acid constitute an important part of the basis for assignment of the structure of the B820 subunit, we felt it was important to verify that the structure of the Fmoc-3-methylhistidine used in our chemical synthesis is that shown in Figure 2E. This was accomplished by comparing NMR spectra of 3-methyl-L-histidine (Sigma-Aldrich Chemical Co., product no. M9005) and 1-methyl-L-histidine (Sigma-Aldrich Chemical Co., product no. 67520) with that of Fmoc-3-methylhistidine [L-histidine, *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-3-methyl-(9C1) (Bachem, CAS Registry No. 252049-16-4)]. A ROESY spectrum will unambiguously verify the position of the methyl group; if histidine is methylated at position 1, one should observe two equivalent ROEs between the methyl group and the two aromatic protons on the imidazole ring. If histidine is methylated at position 3, one should observe a single ROE between the methyl group and only one of the aromatic protons. The 2-D ROESY of the Fmoc derivative used in our chemical syntheses (Figure 2E) shows a single ROE between the methyl group and one of the aromatic protons, verifying that this material is methylated at the 3-position. The 2-D spectra of the 3-methylhistidine compound and the Fmoc derivative were essentially identical except for the additional peaks due to the Fmoc-protecting group.

Endoproteinase Arg-C Digestion. Endoproteinase Arg-C was purchased from Sigma-Aldrich Chemical Co. The procedure followed for proteolytic cleavage of the *Rs. rubrum* LH1 α-polypeptide was essentially that given by Sigma in the Product Information Bulletin (LKB6/01). The α-polypeptide (0.51 mg) was dissolved in 3 μL of HFA to which 0.23 mL of Tris-HCl buffer, pH 8.5, containing 4.5% OG was added. To this solution was slowly added 0.24 mL of Tris-HCl buffer, pH 8.5 (containing no OG), with mixing. The enzyme (10 μg) was activated in 50 μL of 1 mM HCl and added to the solution containing the α-polypeptide. The reaction mixture was incubated for 10 h at 37 °C in a shaker water bath. At the end of this incubation, the reaction mixture was freeze-dried and stored at −20 °C until subjected to HPLC purification.

Reconstitution Assay. The reconstitution procedures used here were described previously (*29*, *33*, *36*). In general, 0.03−0.10 mg of each polypeptide [that had previously been purified by HPLC, partitioned from hexafluoroacetone (HFA), and dried under vacuum] was dissolved in 5−10 μL of HFA. To this solution was added 0.50 mL of 4.5% (w/v) *n*-octyl β-D-glucopyranoside (OG) in 50 mM potassium phosphate buffer, pH 7.5, followed by 2.0 mL of 50 mM potassium phosphate buffer to bring the OG concentration to 0.90%. Bchl was then added to the sample by injecting 5−20 μL of an acetone solution to produce an absorbance of about 0.1 cm^{-1} at 777 or 820 nm and the absorption spectrum recorded. The sample was then diluted to 0.75% OG and again to 0.66% OG to optimize subunit formation. After each dilution, an absorption spectrum was recorded. For LH1 formation, the sample was then placed on ice for 1 h, an absorption spectrum was recorded, and the sample was kept at 4−8 °C overnight, after which a final absorption spectrum was taken. For evaluation of LH1 stability, the samples after 1 h on ice and after chilling overnight were brought to room temperature and the absorption spectra recorded over time to follow the conversion of LH1 to B820 and/or Bchl. Prior to reconstitution, protein concentrations were determined by dissolving the protein in HFA, 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 (*26*). Absorption spectra were recorded using either a Shimadzu UV-160 or a Cary 50 spectrophotometer.

Estimation of Association Constants. Association constants were calculated for the equilibria between the fully dissociated components (Bchl, α- and β-polypeptides) and the subunit complex. On the basis of determinations of stoichiometry (*46*), gel filtration experiments, and various spectroscopic analyses (*28*, *47*, *48*), 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, for complexes containing both α- and β-polypeptides

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

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

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

The concentrations of the subunit complex and uncomplexed Bchl were determined by deconvolution of the absorption spectrum in the Q_Y region (*46*). 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 two Bchl per complex (*49*). Use of the above equilibrium expressions assume that the α- and β-polypeptides exist either as free polypeptides or as B820 under the reconstitution conditions used. On the basis of measurement of K_{assoc} for Bchl binding to the *Rs. rubrum* LH1 α-polypeptide (*50*), this is probably a valid assumption for measurements at 0.90% OG. On the other hand, samples at 0.75% and 0.66% OG likely contain both free α- and β-polypeptides and also their Bchl-bound forms (α·Bchl and β·Bchl) in addition to B820 (*50*).

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 greater than 98% purity, control reconstitution assays (native polypeptides and Bchl) were always conducted in parallel to 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

LH1 β-His 0 → Gly. The full-length LH1 β-polypeptide of *Rb. sphaeroides* contains 48 amino acids. We have previously chemically synthesized analogues of the *Rb. sphaeroides* LH1 β-polypeptide containing 17, 32, 38, and 41 amino acids, all of which contained the native amino acid sequence to the C-terminus but lacked portions of the N-terminus (*33*, *34*). Although all of these would form B820-type complexes, only the 41mer exhibited LH1-forming properties characteristic of the native LH1 β-polypeptide. Through the use of reconstitution assays with polypeptides from different species (*29*), chemically synthesized analogues (*33*, *34*), and selected mutants (*32*, *35*), the roles of specific amino acid side chains in stabilizing B820 and LH1 have been evaluated. However, the interaction energy for His 0 has not yet been determined because the isolated LH1 β-polypeptide of selected His 0 mutants failed to form B820- or LH1-type complexes in reconstitution assays (*32*). We have attempted to address this question by chemical synthesis of two LH1 β-polypeptide analogues. The rationale for preparation of the first of these was that by substituting Gly for His 0, the lack of a side chain would allow flexibility and not interfere with other reactions around Bchl. Furthermore, addition of imidazole to reconstitution mixtures involving this analogue might provide a coordinating ligand to Bchl which would occupy the space of the normal His side chain and provide hydrogen-bonding interactions. Such a combination of a His → Gly mutation with subsequent supplementation by the addition of imidazole was successfully employed in studies of myoglobin (*51*, *52*) and the bacterial RC (*53*). If the His → Gly analogue showed reconstitution activity, with or without imidazole, this system could be used to measure several interaction energies involving the His side chain.

The complete LH1 β-polypeptide of *Rb. sphaeroides* was chemically synthesized except a Gly was inserted at position 0 instead of His. The synthesis went smoothly, and it was possible to isolate the 48mer using HPLC (Figure 3E). The 22.32 min peak was collected and a portion rerun on HPLC, yielding a single peak with the same retention time (data not shown) indicating the purity of the synthetic polypeptide. A mass of 5380 was determined by mass spectral analysis, which agrees well with the mass calculated for the 48mer (5377).

Several systems were tested to evaluate whether this polypeptide would react with the native LH1 α-polypeptide of *Rb. sphaeroides* to form B820- and/or LH1-type complexes. These assays included (1) the analogue 48mer plus Bchl, (2) the analogue 48mer plus native *Rb. sphaeroides* LH1 α-polypeptide plus Bchl, (3) the analogue 48mer plus *Rs. rubrum* α-polypeptide plus Bchl, (4) the analogue 48mer plus several concentrations of imidazole plus Bchl, and (5) the analogue 48mer plus native *Rb. sphaeroides* LH1 α-polypeptide plus several concentrations of imidazole plus Bchl. Several concentrations of polypeptide were also tested. In control experiments with native LH1 α- and β-polypeptides and Bchl, imidazole did not inhibit B820 and LH1 formation until very high concentrations (higher than 10 mM) were reached. In all cases tested with the His → Gly 48mer, neither a B820- nor LH1-type complex was observed.

Because some stabilizing interactions in B820 and LH1 formation involve interaction between the N-terminal regions of these two polypeptides (*34*, *35*), it is of interest whether the His → Gly analogue could be competitive with the native LH1 β-polypeptide in forming B820 and LH1. This possibility was evaluated by examining the effects of the His → Gly analogue on reconstitution of B820 and LH1 using the native α- and β-polypeptides and Bchl. As can be seen in Figure 4, only a marginal inhibition of B820 and LH1 formation was observed. However, the rate of conversion of LH1 to B820 upon warming reconstituted LH1 formed after a 1 h chill was significantly increased (Figure 5, top). This suggests that the His → Gly analogue may be incorporated as a minor component into LH1-type complexes and destabilize them because the His → Gly polypeptide lacks a ligand to coordinate Bchl. Interestingly, however, when more time was allowed for LH1 formation (overnight cold), the LH1 was identical to the control in both extent of formation and its stability. This suggests that, given time, the native β-polypeptide replaces any His → Gly analogue initially incorporated into the oligomer.

LH1 β31mer His 0 → 3-Methylhistidine. On the basis of the above results, and earlier mutation experiments in which His 0 was replaced with Asn (*32*), it appears that the interaction energy associated with one His side chain is so large that we were unable to measure it in reconstitution experiments. To further address this question and to determine which of the two possible Bchl dimeric species exists in the B820 subunits, a LH1 β-polypeptide analogue was synthesized in which 3-methylhistidine (MeHis) was inserted in place of His 0. This analogue should not be hindered in providing a coordinating ligand to Bchl, but it would not be able to provide a hydrogen at the N3 position of the side chain for hydrogen-bonding interactions.

The polypeptide initially chemically synthesized was a 31mer of the *Rb. sphaeroides* LH1 β-polypeptide in which MeHis was substituted for His 0 (Figure 1). We have previously chemically synthesized such a 31mer with a native sequence as well as other 31mers with single amino acid substitutions (*33*, *34*). The shorter 31 amino acid polypeptide was chosen for initial synthesis rather than the entire 48 amino acid length of the LH1 β-polypeptide because it is a more facile synthesis with higher percent yield and the 31mer with the native amino acid sequence readily forms B820-type complexes (*33*), which is a key part of the reconstitution assay. The chemical synthesis proceeded smoothly, and the desired polypeptide was isolated by HPLC (Figure 3F). The two peaks at 44.98 and 45.97 min were collected separately, and each was submitted for mass spectral analysis. The material of each peak yielded a mass of 3679, which agrees well with the expected value of 3677. As is often the case with very hydrophobic membrane proteins, several peaks of the same material may be obtained on HPLC, probably indicating different states of oligomerization.

When the His → MeHis analogue was tested in the reconstitution assay for its ability to form B820- and LH1-type complexes, none were formed with or without a native α-polypeptide (Figure 6, top). On the basis of this result, the structure of B820 is assigned to that with rings III and V of each Bchl overlapping those of the other Bchl (Figure

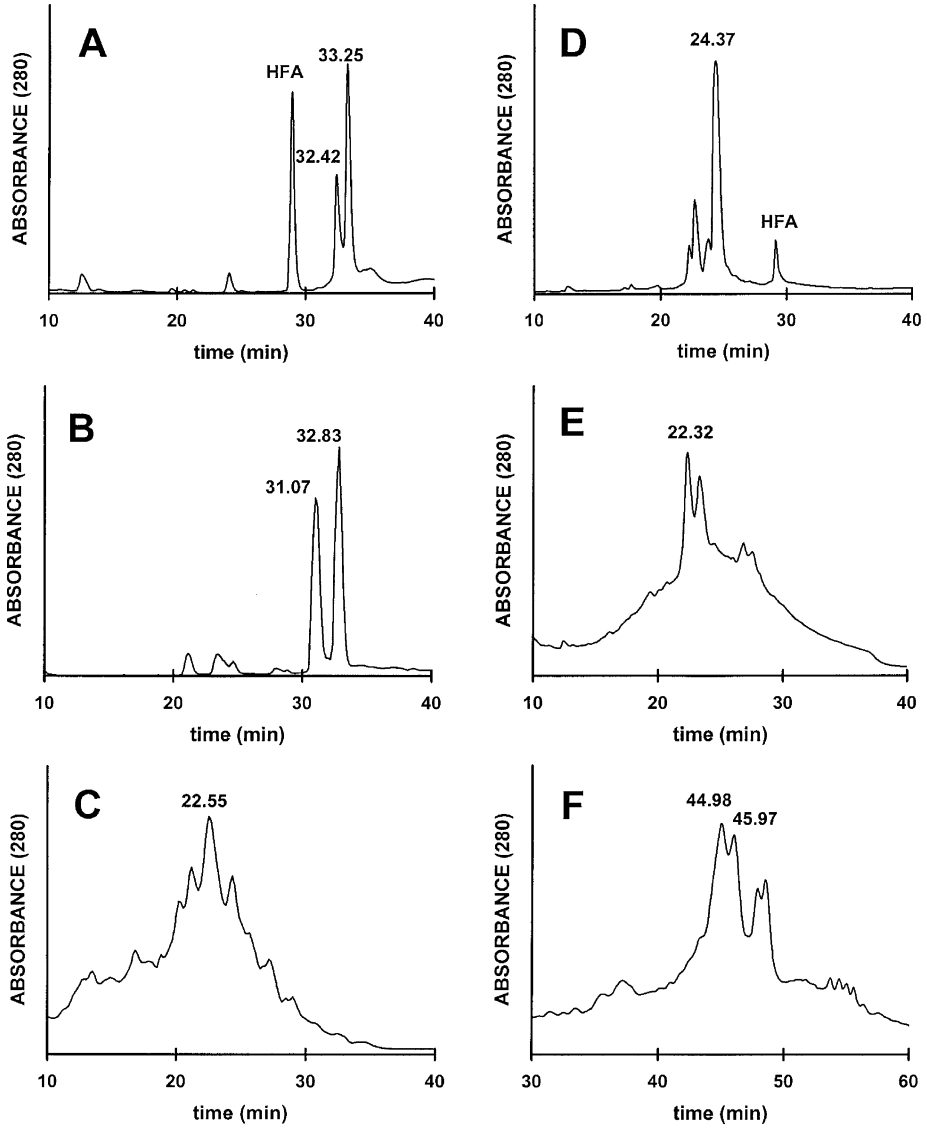


FIGURE 3: HPLC profiles of prepared polypeptides: (A) the native LH1 α-polypeptide of *Rs. rubrum*; (B) the products of the endoproteinase Arg-C digestion of the *Rs. rubrum* LH1 α-polypeptide; (C) the chemically synthesized 42mer reproducing most of the amino acid sequence of the *Rs. Rubrum* LH1 α-polypeptide; (D) the native LH1 *β*-polypeptide of *Rb. sphaeroides*; (E) the chemically synthesized analogue of the *Rb. sphaeroides* LH1 *β*-polypeptide containing Gly at position 0; (F) the chemically synthesized 31mer analogue of the *Rb. sphaeroides* LH1 α-polypeptide containing 3-methylhistidine at position 0. Those profiles with HFA marked are for a contaminant in the hexafluoroacetone (HFA) used to dissolve the samples. It serves as a convenient marker.

2A). It is important to note that Bchl alone at the concentrations used for the reconstitution assay partially forms a dimeric complex that absorbs at 846 nm (Figure 6, bottom). However, in the presence of an α-polypeptide, dimerization is prevented due to coordination of Bchl to His 0 of the α-polypeptide (*50*). Since the His → MeHis analogue also prevents the Bchl dimer from forming when present without an α-polypeptide, it is concluded that the His → MeHis analogue also binds Bchl.

LH1 β48mer His 0 → 3-Methylhistidine. Earlier results have indicated that the N-terminal regions of the α- and *β*-polypeptides of LH1 interact (*32*, *36*) and both LH2 crystal structures show a close association between the two polypeptides as the LH2 α-polypeptide crosses under the LH2 *β*-polypeptide near the N-terminus (*16*). Formation of heterodimeric B820- and LH1-type complexes are not observed when the α- or *β*-polypeptide is truncated by nine or more amino acids at the N-terminus (*36*). Thus, significant interactions between these two polypeptides would seem to occur in the N-terminal region, perhaps similar to those observed in the structures of the LH2 complexes of *Ps. molischianum* (*16*) and *Rhodoblastus* (formerly *Rhodopseudomonas*) *acidophila* (*15*). Since the His → MeHis 31mer does not react with Bchl to form B820- or LH1-type complexes, we next extended this polypeptide to 48 amino acids, reproducing the full amino acid sequence of the native LH1 *β*-polypeptide of *Rb. sphaeroides* except that His 0 is MeHis. A comparison of the reactivity of this 48mer with the His 0 → 3-methylhistidine to the 31mer with His 0 → 3-methylhistidine would test whether the N-terminus would provide enough interaction energy to form a heterodimeric B820-type complex and/or a heterologous LH1-type complex. Since the synthesis of the His → MeHis analogue went especially well, and since over half of the product was stored still on the resin, the chemical addition of 17 amino acids was undertaken.

This additional synthesis progressed well, and a prominent peak was obtained by HPLC (data not shown) which gave a

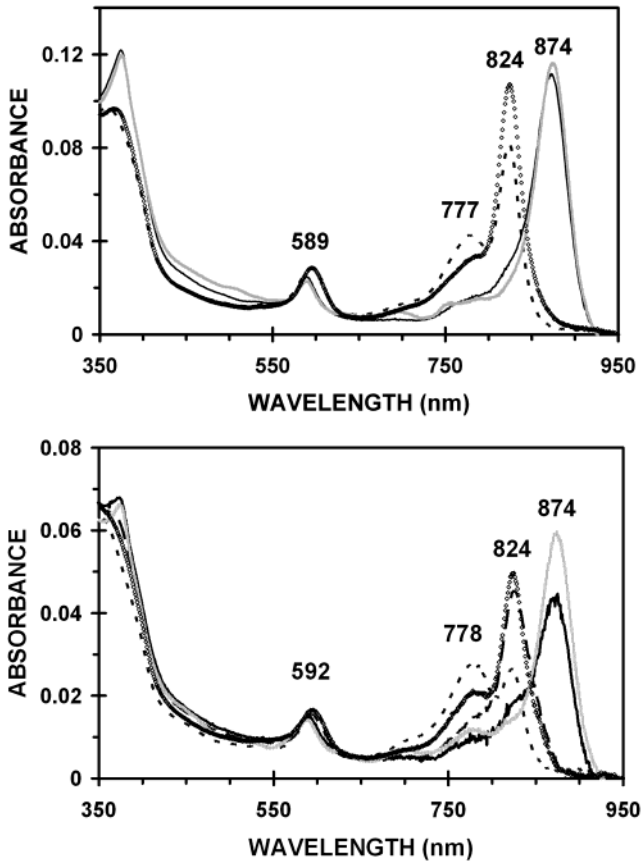


FIGURE 4: (Top) Reconstitution of B820 and LH1 using the native α- and *β*-polypeptides of *Rb. sphaeroides* and Bchl. Spectra were recorded at 0.90% OG (dashed curve), 0.75% OG (diamond symbols), 0.66% OG after chilling on ice for 1 h (solid black curve), and 0.66% OG after chilling overnight (gray curve). Concentrations of reactants were 3.1 *μ*M α-polypeptide, 2.2 *μ*M *β*-polypeptide, and 1.1 *μ*M Bchl, at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG. (Bottom) Same as (top) but with the LH1 *β*His 0 → Gly analogue additionally present (5.5 *μ*M). Spectra were recorded at 0.90% OG (dotted curve), 0.75% OG (diamond symbols), 0.66% OG (dashed curve), 0.66% OG after a 1 h chill (solid black curve), and 0.66% OG after being chilled overnight (solid gray curve).

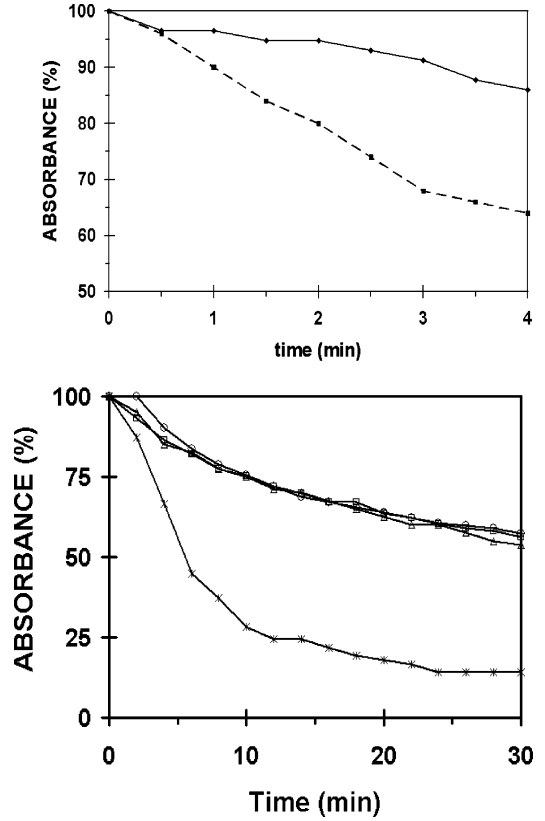


FIGURE 5: (Top) Rate of conversion of the LH1 complex to B820 upon placing the reconstitution mixture at room temperature after a 1 h chill on ice: solid line, control reconstitution shown in Figure 4 (top); dashed line, reconstitution system shown in Figure 4 (bottom). Absorbance was measured at 870 nm. (Bottom) Stability of the LH1-type complexes formed after a 1 h chill at 0 °C and then placed at room temperature: □, LH1 formed with the native α- and *β*-polypeptides of *Rs. rubrum* and Bchl; ○, LH1 formed with the *Rs. rubrum* LH1 α-polypeptide analogue containing 43 amino acids (42mer with the N-terminal Met added), native LH1 *β*-polypeptide, and Bchl; △, LH1 formed with the *Rs. rubrum* LH1 α-polypeptide with the sulfur of Met −28 oxidized to a sulfoxide, native LH1 *β*-polypeptide, and Bchl; *, LH1 formed with the chemically synthesized 42mer analogue of the *Rs. rubrum* LH1 α-polypeptide, native LH1 *β*-polypeptide, and Bchl. The absorbance was measured at 870 nm.

mass of 5459 by mass spectral analysis, in excellent agreement with the mass expected (5459). Once again, when tested in reconstitution experiments either alone or with a native *Rs. rubrum* LH1 α-polypeptide and Bchl, no B820- or LH1-type complexes were formed. Therefore, the presence of the complete N-terminal sequence of amino acids is not enough to overcome the modification of His 0. It is thus concluded that the substitution of a methyl group for the hydrogen atom at the 3-position of the imidazole side chain of histidine precludes oligomerization either because of the lack of a key hydrogen bond or because of steric interference, or both.

LH1 α-Polypeptide Analogues: Met Sulfoxide. Whereas the effects of several modifications in the N-terminal region of the LH1 *β*-polypeptide have been studied, more information is needed regarding the role of amino acids near the N-terminus of the α-polypeptide. In this regard, the N-terminal Met of *Rs. rubrum* LH1 α-polypeptide is easily oxidized to the sulfoxide, a modification that partially occurs during isolation of the native LH1 α-polypeptide (peak at 32.42 min of Figure 3A) (*27*). This oxidized polypeptide was prepared and, as reported by Wang et al. (*54*), was found to be less reactive with the native LH1 *β*-polypeptide to form a B820-type complex. A LH1-type complex was formed, but not as completely as with the native polypeptide (Figure 7, Table 1). The LH1-type complex formed was as stable as that of the native LH1 complex (Figure 5, bottom).

LH1 α-Polypeptide Analogues: 42mer. A 42 amino acid polypeptide which reproduced the native amino acid sequence of the *Rs. rubrum* LH1 α-polypeptide but did not contain the N-terminal Met residue or the last nine C-terminal amino acids was chemically synthesized. This 42mer synthesis proceeded smoothly, and the 22.55 min peak of the HPLC purification (Figure 3C) was collected for study. When this material was subjected to a second HPLC, a single peak with the same elution time as observed in the first HPLC was obtained. The mass spectral analysis of the protein in this peak gave a value of 5060, in excellent agreement with the expected mass of 5059.

When tested in reconstitution experiments with a native *Rs. rubrum* LH1 *β*-polypeptide and Bchl, a B820-type

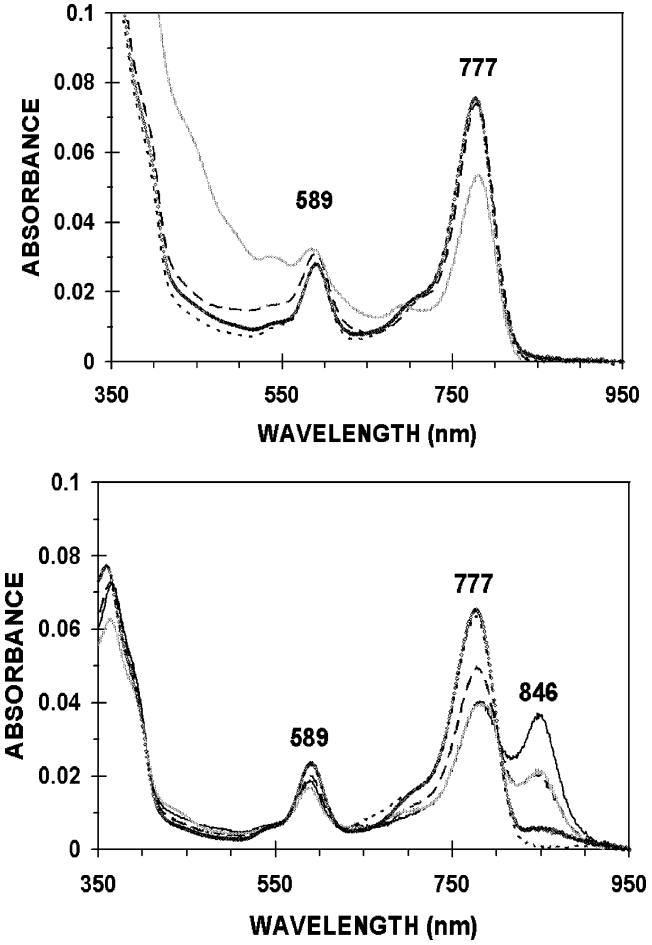


FIGURE 6: (Top) Reconstitution assay for B820 and LH1 formation using the LH1 β31mer His 0 → MeHis analogue of the *Rb. sphaeroides* LH1 β-polypeptide, native *Rb. sphaeroides* LH1 α-polypeptide, and Bchl. Spectra were recorded at 0.90% OG (dotted curve), 0.75% OG (solid black curve), 0.66% OG (dashed curve), and after chilling the 0.66% OG sample overnight at 6 °C (gray curve). Concentrations of reactants were 5.5 μM LH1 β31mer His 0 → MeHis analogue, 3.1 μM native LH1 α-polypeptide, and 1.6 μM Bchl at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG. (Bottom) Bchl association with no protein present under reconstitution conditions at 0.90% OG (diamonds), 0.75% OG (dotted curve), 0.66% OG (dashed curve), 0.66% OG after a 1 h chill (solid black curve), and 0.66% OG after chilling overnight (solid gray curve). The concentration of Bchl was 1.4 μM at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the Bchl concentration of the 0.66% OG sample.

complex was formed although the association constant was 10-fold smaller than that formed with the native *Rs. rubrum* LH1 α-polypeptide (Figure 8, top, and Table 1). Because the *Rs. rubrum* LH1 β-polypeptide will form a B820-type complex without the α-polypeptide (Table 1) (*27*), it is possible that a heterodimeric B820 complex is formed to a lesser extent than that of the homologous dimeric species and the B820-type complex is composed of a mixture of the two. Formation of a LH1-type complex was only partially observed after a 1 h chill, and most of that formed degraded on storing overnight in the cold (Figure 8, top). As with the native α-polypeptide of *Rs. rubrum* (*27*), the 42mer would not form red-shifted complexes with Bchl in the absence of a β-polypeptide. Because earlier experiments had shown that

Table 1: Energies of Interaction in B820-Type Complexes of LH1

system	K_{assoc} ($M^{-3} \times 10^{-16}$) 0.90%	K_{assoc} ($M^{-3} \times 10^{-16}$) 0.75%	$\Delta\Delta G^a$ (kcal/mol) 0.90%
Rs. rubrum native α + native β	25	≥300	
Rs. rubrum native β only	2.2	18	−1.6
Rs. rubrum native β + α sulfoxide	2.0	19	−1.6
Rs. rubrum native β + α 42mer	3.1	47	−1.4
Rs. rubrum native β + α Met 42mer	3.0	51	−1.4
Rs. rubrum native β + α Endo Arg-C	1.0	10	−2.0

[a] These values were calculated using $\Delta\Delta G = \Delta G_{native} - \Delta G_X$, where ΔG_{native} is the value for reconstitution of B820 at 0.90% OG using the native *Rs. rubrum* LH1 α- and β-polypeptides and Bchl, conducted in parallel for each experiment, and ΔG_X is the value obtained for the various reconstitutions at 0.90% OG using only a native LH1 β-polypeptide or the native LH1 β-polypeptide with analogues of the *Rs. rubrum* LH1 α-polypeptide and Bchl.

removal of 10 amino acids at the C-terminus of the *Rs. rubrum* LH1 α-polypeptide did not affect formation of B820 or LH1 (*36*), the N-terminal fMet which is missing in the 42mer is presumably important for stabilizing the B820 and LH1 complexes. It is interesting that the LH1-type complex formed is much less stable than native LH1 or the sulfoxide derivative of the native LH1 when warmed to room temperature (Figure 5, bottom). Perhaps this reflects an important role for the fMet in the native *Rs. rubrum* LH1 α-polypeptide.

LH1 α-Polypeptide Analogues: Addition of Met to the 42mer. Several methods were used in an attempt to modify the *Rs. rubrum* LH1 α-polypeptide by removal of the formyl group from the N-terminal Met residue. Because methanol is usually employed as a solvent (or included as a major solvent component) under acidic conditions, methyl esters of Glu, Asp, and the C-terminal carboxyl group were also formed. Also, the Met at the N-terminus was oxidized to the sulfoxide to variable extents. Thus, it proved difficult to selectively remove the formyl group without also modifying some other part of the polypeptide. To examine an α-polypeptide with an N-terminal Met that was not formylated, the chemically synthesized 42mer was lengthened by the addition of Met. This was accomplished using a portion of the synthetic polypeptide that was still attached to the resin with sensitive amino acids still protected. The 43 amino acid polypeptide product of this extension was isolated by HPLC and a portion submitted for mass spectral analysis. A mass of 5191 was found, in excellent agreement with the expected mass of 5191.

When this 43mer was examined in the reconstitution assay, a B820-type complex formed, very similar to that observed with the 42mer (Figure 8, bottom, and Table 1), but a LH1-type complex was formed to a much greater extent than that observed with the 42mer (Figure 8, top), and it was much more stable than that formed with the 42mer (Figure 5, bottom). These results indicate that addition of Met to the N-terminus of the 42mer did not seem to enhance the ability of this α-polypeptide analogue to form a B820-type complex, but it substantially improved its ability to form a more native-like LH1 complex.

LH1 α-Polypeptide Analogues: Endoproteinase Arg-C. To further evaluate the role of the N-terminal region of the *Rs. rubrum* LH1 α-polypeptide, the native polypeptide was digested with endoproteinase Arg-C. As can be seen in Figure

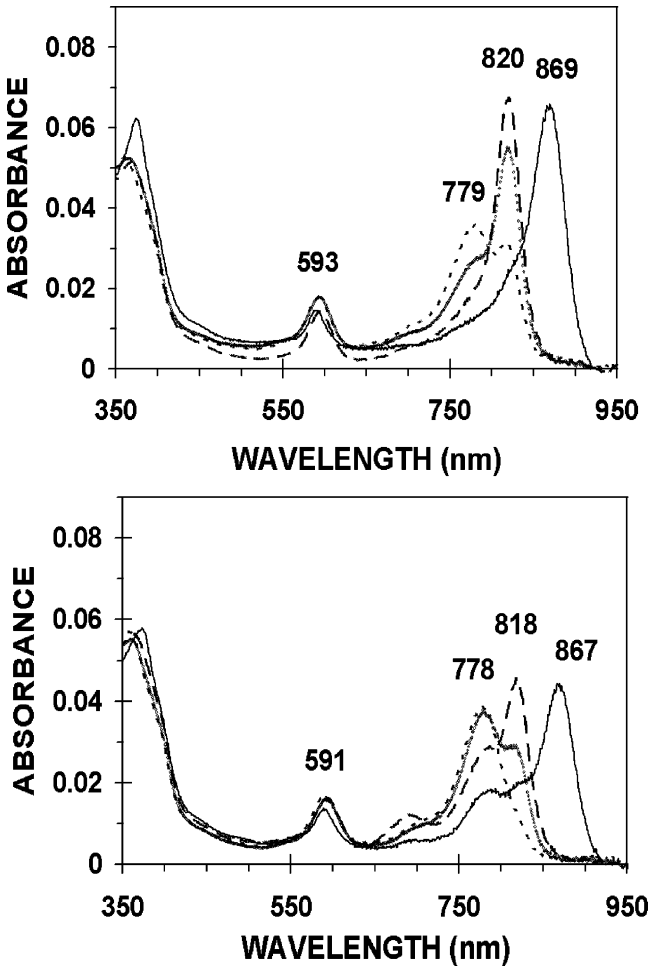


FIGURE 7: (Top) Reconstitution of B820 and LH1 using the native α- and β-polypeptides of *Rs. rubrum* and Bchl. Spectra were recorded at 0.90% OG (dotted curve), 0.75% OG (diamond symbols), 0.66% OG (dashed curve), and 0.66% OG after chilling on ice for 1 h (solid curve). The spectrum of the sample at 0.66% OG after cooling overnight (not shown) was very similar to that of the 1 h chill on ice. Concentrations of reactants were 1.5 μM α-polypeptide, 1.5 μM β-polypeptide, and 0.71 μM Bchl, at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG. (Bottom) Reconstitution assay for B820 and LH1 formation using the *Rs. rubrum* LH1 α-polypeptide with the sulfur of Met −28 oxidized to a sulfoxide, native *Rs. rubrum* LH1 β-polypeptide, and Bchl. Spectra were recorded at 0.90% OG (dots), 0.75% OG (diamonds), 0.66% OG (dashed curve), and 0.66% OG after a 1 h chill on ice (solid curve). The spectrum of the sample at 0.66% OG after cooling overnight (not shown) was very similar to that of the 1 h chill on ice. Concentration of reactants were 1.5 μM LH1 α-polypeptide analogue with Met −28 oxidized to the sulfoxide, 1.5 μM native LH1 β-polypeptide, and 0.80 μM Bchl, at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG.

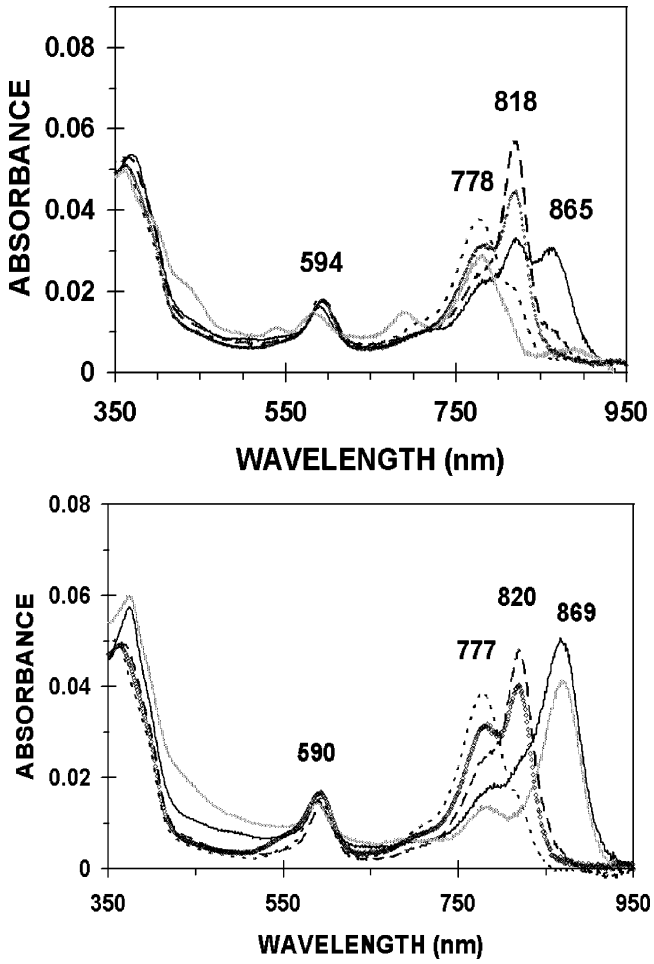


FIGURE 8: (Top) Reconstitution assay for B820 and LH1 formation using the chemically synthesized *Rs. rubrum* LH1 α-polypeptide analogue containing 42 amino acids, native *Rs. rubrum* LH1 β-polypeptide, and Bchl. Spectra were recorded at 0.90% OG (dots), 0.75% OG (diamonds), 0.66% OG (dashed curve), 0.66% OG after a 1 h chill on ice (solid black curve), and 0.66% OG after chilling overnight (solid gray curve). Concentrations of reactants were 1.8 μM chemically synthesized LH1 α-polypeptide analogue, 1.5 μM native LH1 β-polypeptide, and 0.75 μM Bchl, at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG. (Bottom) Reconstitution assay for B820 and LH1 formation using the chemically synthesized *Rs. rubrum* LH1 α-polypeptide analogue containing 43 amino acids (42mer with the N-terminal Met added), native *Rs. rubrum* LH1 β-polypeptide, and Bchl. Spectra were recorded at 0.90% OG (dots), 0.75% OG (diamonds), 0.66% OG (dashed curve), 0.66% OG after a 1 h chill on ice (solid black curve), and 0.66% OG after chilling overnight (solid gray curve). Concentration of reactants were 1.8 μM chemically synthesized LH1 α-polypeptide analogue, 1.5 μM native LH1 β-polypeptide, and 0.75 μM Bchl, at 0.66% OG. Spectra were recorded in 1 cm cuvettes, and the data at 0.90% and 0.75% OG were multiplied by appropriate factors to compare each spectrum at the polypeptide concentrations of 0.66% OG.

3B, even though there are three Arg in the polypeptide, one major product was formed (31.07 peak) and isolated. The N-terminal amino acid sequence of this polypeptide was found to be IXQLFD..., and the mass spectral analysis gave a value of 5608, both of which are consistent with the native polypeptide from which three N-terminal amino acids (MetTrpArg) were removed, which would have a calculated mass of 5607.

When this LH1 α-polypeptide analogue was tested for its ability to form a B820-type complex with a native *Rs. rubrum* LH1 β-polypeptide and Bchl, a complex was partially formed with a smaller association constant than that formed with a native *Rs. rubrum* LH1 α-polypeptide or the other LH1 α-polypeptide analogues (Table 1). As discussed above for the 42mer, the B820-type complex formed is probably that of a homologous β-polypeptide dimer, possibly with a very

small amount of heterologous dimer. A small amount of a LH1-type complex was formed after a 1 h chill (about 15% of that obtained with the native α-polypeptide) but was completely degraded on standing in the cold overnight (data not shown). Thus, this behavior is similar to that of the 42mer, but less LH1 was formed and it was much less stable. If higher concentrations of the analogue were used, more LH1-type complex could be formed, but it was also unstable on storage in the cold.

DISCUSSION

Structure of B820. The B820 subunit of *Ps. molischianum* LH2 and that of *Rs. rubrum* LH1 appear to have identical structures since their reconstitution behavior, absorption, and CD spectra are the same (*31*, *49*). Therefore, the *Ps. molischianum* LH2 crystal structure (*16*) should be a good guide for predicting the expected structural features of B820 prepared from either LH1 or LH2 polypeptides. On the basis of the high-resolution LH2 structure (*16*), one would expect the B820 subunit to consist of either αβ·2Bchl with rings III and V of each Bchl overlapping (Figure 2A and the left two or the right two Bchl in Figure 2D) or αβ·2Bchl with pyrrole rings I overlapping (Figure 2B and the center two Bchl in Figure 2D). From the results with the His 0 → MeHis analogue of the LH1 β-polypeptide, in which no B820 could be formed, the Bchl dimer in B820 must have a structure in which rings III and V of each Bchl overlap. This implies that cross-hydrogen bonding between the His 0 side chains and the other Bchl, which is blocked by the methyl groups, is an important stabilizing interaction. Such interaction is consistent with the *Ps. molischianum* LH2 structure in which such hydrogen bonding has been proposed for the αβ·2Bchl units whose Bchl overlap at pyrrole rings III and V (*16*). This structure assignment is also consistent with the NMR experiments of Wang et al. (*56*) in which it was concluded that the two Bchl adopt a parallel face-to-face conformation with partial overlap of the periphery of rings II, III, and V. It should be noted that Visschers et al. reported that although the $C13^1$ carbonyl groups of both Bchl in LH1 of *Rs. rubrum* appear to be involved in hydrogen bond interactions, a fraction of these groups becomes "free" from interaction in B820 (*55*). This result might suggest that only one of the His 0 of B820 is involved in cross-hydrogen bonding, which would also be consistent with our results.

In addition to the inability of the His 0 → MeHis analogue to cross-hydrogen bond, steric constraints imposed by the additional methyl group may also play an important role. The close and precise packing in the region of the Bchl may be disrupted by the methyl group on the MeHis analogue since neither a B820- nor a LH1-type complex could be formed, even when a native α-polypeptide and the full-length β-polypeptide His 0 → MeHis analogue were tested.

Role of Polypeptide Interaction in Stabilizing B820 and LH1. Upon assignment of the Bchl pair of B820 to the structure with rings III and V overlapping, a first approximation of the relationship between the α- and β-polypeptides may be made. In the crystal structure of *Ps. molischianum* LH2, other than interaction with Bchl, the interaction between the α- and β-polypeptides that coordinate Bchl overlapping at rings III and V is at only one point in the C-terminal region but more extensively in the N-terminal region (Figure 9).

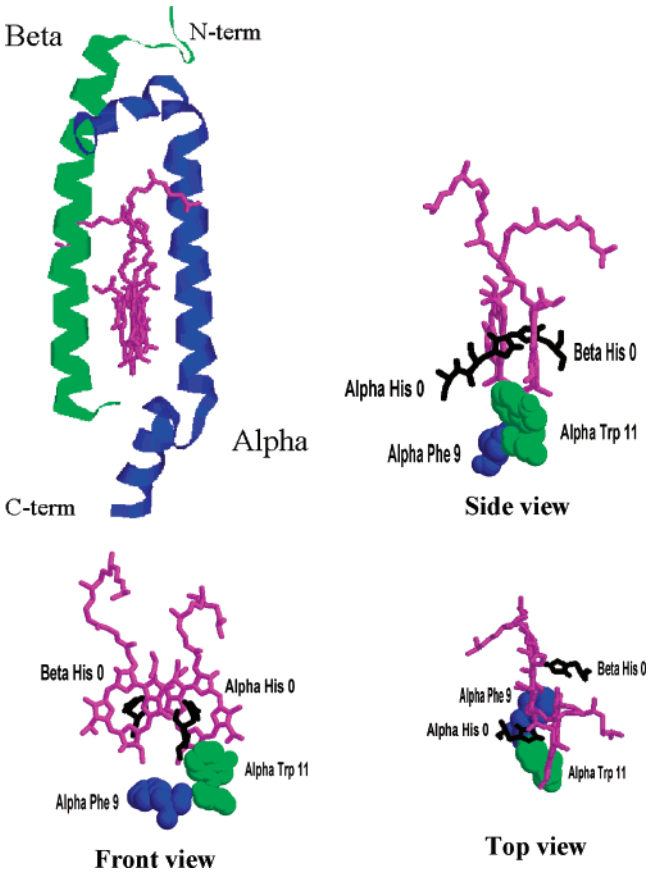
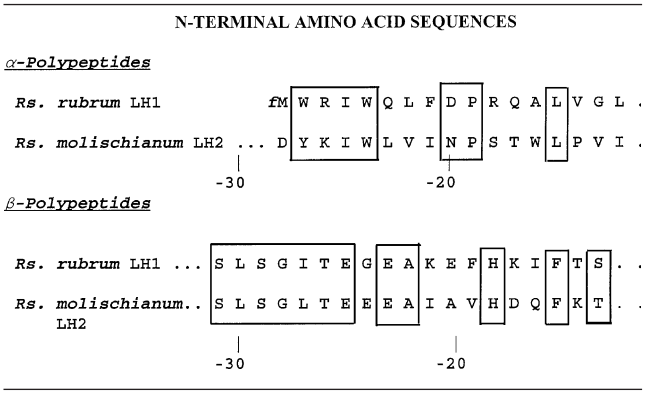


FIGURE 9: (Upper left) Structure of one of the two αβ·2Bchl repeating units of the *Ps. molischianum* LH2 structure (*16*) (with rings III and V of Bchl overlapping) showing that in the N-terminal region the α-polypeptide crosses under the β-polypeptide and runs alongside it in the opposite direction for about 9 amino acids. The cartoon also shows that the C-terminal end of the β-polypeptide has only a short segment and is restricted to one side of the Bchl plane. (Upper right, lower left, and lower right) The side, front, and top views, respectively, of the αβ·2Bchl repeating unit of the *Ps. molischianum* LH2 structure (*16*) showing Bchl, the coordinating His residues, and the two amino acids of the LH1 α-polypeptide whose side chains extend beyond the plane of the Bchl.

The point of closest approach in the C-terminal region is between hydrophobic surfaces of Gly 8 and Phe 9 of the LH2 α-polypeptide (using numbering comparable to Figure 1) and hydrophobic surfaces of Trp 6 and Lys 7 of the β-polypeptide. There are considerably more extensive interactions in the N-terminal region in which the α-polypeptide turns from the membrane-spanning segment, crosses under the β-polypeptide, and runs in the opposite direction adjacent to it for approximately 10 amino acids (Figure 9). Hydrophobic surfaces of the two polypeptides are in contact throughout this region, and some specific interactions may occur at the two ends of this stretch where, at one end, the side chain of His −18 of the LH2 β-polypeptide makes a close approach to Asp −29, Asp −28, Tyr −27, and Lys −26, which form a tight cluster in the LH2 α-polypeptide with Tyr −27 hydrogen bonded to the carboxyl group of Glu −25 of the LH2 β-polypeptide. At the other end of the interacting sequence, Ser −29 of the LH2 β-polypeptide makes a close approach to Asn −20 of the LH2 α-polypeptide. The corresponding positions in the LH1 polypeptides of *Rs. rubrum* at one end of the proposed interacting sequence are His −18 of the LH1 β-polypeptide and the N-terminal fMet −28, Trp −27, and Arg −26 of the LH1

Table 2: N-Terminal Amino Acid Sequences



α-polypeptide where Trp −27, by analogy, could hydrogen bond to Glu −25 of the LH1 *β*-polypeptide. At the other end of the interacting sequence are Ser −29 of the LH1 *β*-polypeptide and Asp −20 of the LH1 α-polypeptide (Table 2).

Although the N-terminal regions of LH2 α- and *β*-polypeptides have at least one known function different from that of the N-terminal regions of the LH1 α- and *β*-polypeptides, that of binding the so-called monomeric Bchl (*15*, *16*), there is a high amino acid sequence homology in the corresponding regions of the α- and *β*-polypeptides of these two complexes (Table 2). Especially interesting in this regard are the results of the reconstitution experiments reported here as well as earlier (*33*, *34*, *36*). In earlier experiments, LH1 *β*-polypeptides of *Rs. rubrum* and *Rb. sphaeroides* with 10 or more amino acids removed from the N-terminus failed to recognize their respective native α-polypeptides (*33*, *34*, *36*), and the *Rs. rubrum* LH1 α-polypeptide with 9 amino acids removed from the N-terminus failed to recognize the native *Rs. rubrum* *β*-polypeptide (*36*). Thus, the importance of these polypeptides in the region of the amino acid sequence from −18 to −29 is emphasized for formation of heterodimeric B820 and LH1 complexes. The experiments reported here further underscore the importance of the N-terminus of the *Rs. rubrum* α-polypeptide. Removal of three amino acids from the N-terminus of the *Rs. rubrum* LH1 α-polypeptide resulted in a polypeptide that either does not react with a native *β*-polypeptide or reacts very poorly, forming little LH1 complex, and that which does form rapidly degrades. Of the three amino acids at the N-terminus (fMetTrpArg), fMet appears to play an especially important role. Taken together, these observations suggest a special role for the N-terminal three amino acids of the LH1 α-polypeptide of *Rs. rubrum* which would be in the corresponding region to the LH2 tightly folded cluster of the LH2 α-polypeptide of *Ps. molischianum*, assuming similar folds and interactions occur in this part of the N-terminal regions of these LH1 and LH2 complexes. Because the structures of *Ps. molischianum* and *Rps. acidophila* LH2 show very similar folds and interactions in the N-terminal regions of their α- and *β*-polypeptides, residues −26 to −28 of the LH1 α-polypeptides and residues −18 and −25 of the LH1 *β*-polypeptides of other photosynthetic bacteria would be expected to also play an important role in LH1 stabilization.

To evaluate the role of the fMet, a methionine was attached to the N-terminus of the 42mer. The resulting polypeptide contained 43 amino acids and behaved similarly to the 42mer analogue in forming a B820-type subunit complex. Because the K_{assoc} for B820 formation is about the same as that for a native *Rs. rubrum* LH1 *β*-polypeptide alone, the B820 formed is probably a mixture of β_2·2Bchl and $\alpha\beta$·2Bchl. It would appear from these results that the formyl group on Met in the native *Rs. rubrum* α-polypeptide plays an important role in interacting with the *R. rubrum* *β*-polypeptide to form a B820-type complex.

Most interestingly, a LH1-type complex was formed with the 43mer analogue almost as well as with the native LH1 α-polypeptide and much better than with the 42mer analogue lacking the N-terminal Met. In addition, the stability of the LH1-type complex formed was indistinguishable from that of reconstituted LH1 using native polypeptides (Figure 5, bottom). Furthermore, results with the native *Rs. rubrum* LH1 α-polypeptide with oxidized fMet (sulfoxide) parallel those with the Met-containing 43mer (cf. Figures 7, bottom, and 8, bottom), and its stability was comparable to that of LH1 reconstituted with native polypeptides (Figure 5, bottom). It is striking that LH1 formation in these reconstitution experiments changes incrementally from that of a very unstable and poorly formed species when the three N-terminal amino acids are absent, to a somewhat improved formation and stability when only the N-terminal fMet is missing, to an almost native complex when only the formyl group is missing. This would be exactly what one would expect if the N-terminal region of the α- and *β*-polypeptides of *Rs. rubrum* LH1 existed with the same fold and interactions as observed in *Ps. molischianum* LH2 α- and *β*-polypeptides (*16*).

Additional evidence for the importance of interactions at the N-terminal region of the α- and *β*-polypeptides has been presented recently by Arluison et al. (*57*). In their studies, LH1 and B820 of *Rs. rubrum* were exposed to partial digestion by trypsin and endoproteinase Glu-C. They also concluded that the N-terminal regions of the α- and *β*-polypeptides play a dominant role in heterodimer formation.

LH1 α-Polypeptide and Subunit Formation. Some of the experimental results obtained in this study have a bearing on another question of interest. That is, why is it that a B820-type complex can be formed by reconstitution using only a native LH1 *β*-polypeptide of *Rs. rubrum*, *Rb. sphaeroides*, *Rhodobacter capsulatus*, *Rhodopseudomonas viridis*, or *Ps. molischianum* or with the native LH2 *β*-polypeptide of *Ps. molischianum* and Bchl, but attempts to form such complexes with only the corresponding α-polypeptides and Bchl have been unsuccessful? Furthermore, LH1 *β*-polypeptides truncated at the N-terminus still readily form homodimeric B820-type complexes, but the LH1 α-polypeptide of *Rs. rubrum* truncated at the N-terminus still does not. One possibility would be that the C-terminal regions beyond the membrane-spanning segment of the α-polypeptide have a conformation that would cause steric restraints for dimer formation. In this regard, the chemically synthesized 42mer analogue of the *Rs. rubrum* LH1 α-polypeptide contained nine fewer amino acids at the C-terminus but also failed to form a B820- or LH1-type complex without a *β*-polypeptide. It may therefore be concluded that some part of the Bchl-binding region of the LH1 α-polypeptide may be responsible for the inability to form B820-type complexes.

Our understanding of the requirements for Bchl binding and specific dimer formation in B820 is that the polypeptide should have (1) a hydrophobic, α-helical segment of about 20 amino acids containing a His residue to provide a coordinating ligand to Bchl, (2) amino acids with small side chains in the region of Bchl binding to allow a close approach of Bchl to the polypeptide, and (3) a Trp residue strategically placed to further bind Bchl and establish an orientation that is conducive to oligomerization (*35*). The *β*-polypeptides all have the required hydrophobic α-helical region, a His at position 0, and a Trp at position 9 to provide the required binding. In this scenario, the α-polypeptides also have the required hydrophobic α-helical region of about 20 amino acids, a His at position 0, and a Trp that establishes the orientation of Bchl, but the latter is at position 11 rather than position 9. As a consequence of the difference in Trp locations, and the conformation of the amino acids in residues 4 to 11 which are involved in the termination of the membrane-spanning α-helical segments and the turn of the polypeptide to enable Trp 11 to hydrogen bond to Bchl, the extent to which each polypeptide extends toward the opposite face of the Bchl is different (*15*, *16*). In the case of the LH2 *β*-polypeptide of *Ps. molischianum* and *Rps. acidophila* the C-terminal ends of the polypeptides remain on one side of the Bchl plane (the side of the Bchl to which His 0 is coordinated; Figure 9) and do not extend to the other side. However, in the case of the LH2 α-polypeptide of *Ps. molischianum*, Phe 9 extends to the other side of the plane of the Bchl molecule to which its His 0 is coordinated (*16*) (Figure 9). Since dimer formation requires a specific van der Waals overlap distance at rings III and V with nearly parallel bacteriochlorin rings, presumably in order to allow cross-hydrogen bonding via His 0, steric hindrance in the α-polypeptide caused by Phe 9 encountering Trp 11 may be sufficient to prevent the necessary close approach for dimerization of two such molecules (Figure 9). A similar steric problem would also be encountered in the structure of the *Rps. acidophila* LH2 α-polypeptide, but in this case, it is the steric restrictions of Tyr 13 and Trp 9 that would prevent dimer formation (*15*). If the conformation of this polypeptide is the same as that of the *Ps. molischianum* LH2 α-polypeptide, formation of a homodimeric B820-type subunit would be prevented by steric restrictions involving Trp 11 and Phe 9. This explanation pointing to restrictions of steric interference is consistent with the inability of the LH1 *β*-polypeptide analogue containing MeHis at position 0 to form B820-type complexes. Both behaviors point to a highly specific and closely packed environment around Bchl in both B820 and LH1.

These considerations of the behavior of the LH1 α-polypeptide seem rational but leave one question unanswered. If a subunit cannot form with a LH1 α-polypeptide, then how do LH1 α-polypeptides truncated at the N-terminus, which also do not form B820-type complexes (*36*), form LH1-type (LH2-type) oligomeric complexes? A possibility is that the very large driving force caused by reducing the detergent concentration to below its cmc overcomes the steric restrictions and causes the C-terminal amino acids to adopt a different conformation compatible with oligomerization.

Even though a single polypeptide could be designed to bind Bchl and oligomerize to form complexes with LH1- and LH2-type spectra, it would appear that there is an advantage for photosynthetic bacteria to form LH1 and LH2 with $\alpha\beta$·2Bchl units rather than with α_2·2Bchl or β_2·2Bchl units. Perhaps a simple reason for this is that two different polypeptides offer more diversity of structure possible for important roles such as recognition of RC polypeptides and other photoreceptor components. Two distinct polypeptides would also facilitate oligomerization to form curved aggregates that are important for encircling, or partially encircling, the RC.

CONCLUSIONS

The reason the LH1 α-polypeptides do not form homodimeric B820-type complexes is attributed to steric restrictions between residues 9 to 13. No such steric restrictions appear to be present in LH1 *β*-polypeptides.

Reconstitution experiments with a chemically synthesized LH1 *β*-polypeptide analogue having MeHis at position 0 instead of His have provided the experimental data needed to assign to B820 one of the two possible $\alpha\beta$·2Bchl pairs that are observed in the crystal structure of *Ps. molischianum* LH2 (*16*), the one with rings III and V of Bchl overlapping. Consistent with the assigned structure, experimental evidence is provided that significant stabilizing interactions for both B820 and LH1 occur between the N-terminal regions of the α- and *β*-polypeptides of *Rs. rubrum*. On the basis of the results with the chemically synthesized polypeptides used in this study, along with earlier results with protease-modified polypeptides (*36*), mutants (*32*, *35*), and chemically synthesized polypeptides (*33*, *34*), the importance of a stretch of 9−13 amino acids at the N-terminal end of the α- and *β*-polypeptides is demonstrated. In the case of the *Rs. rubrum* LH1 α-polypeptide, fMet −28, Trp −27, and Arg −26 are involved in stabilizing interactions with the *Rs. rubrum* LH1 *β*-polypeptide most likely involving close interaction with the side chain of His −18 of the *β*-polypeptide. A progressive loss of interaction with the LH1 *β*-polypeptide was found as the first three N-terminal amino acids of the LH1 α-polypeptide were removed. The absence of fMet −28 resulted in a significant decrease in LH1 formation (Figure 8, top) and also in a decrease in the stability of the LH1 formed (Figure 5, bottom). In addition to the removal of fMet, removal of the Trp −27 and Arg −26 also resulted in a decrease in K_{assoc} for B820 formation and nearly eliminated the ability to form LH1. Trp (or Tyr) −27 and Arg (or Lys) −26 are conserved in the LH1 α-polypeptides and also present in some LH2 α-polypeptides (*58*). However, the N-terminal fMet in the *Rs. rubrum* LH1 α-polypeptide is not conserved so that its involvement in stabilizing LH1 formation may be unique to *Rs. rubrum*.

Taken together, the results provide evidence that the folding motif of the α- and *β*-polypeptides in the N-terminal region observed in crystal structures of LH2 is also present in LH1 and contributes significantly to stabilizing the complex. It would be predicted that, during development of the photosynthetic membrane, B820 would first form and then these subunits would associate to form LH1. However, if this occurs, the lifetime of B820 must be very short as this species has apparently not been observed in vivo. The reason for this may be related to the presence of carotenoid in the native system, which is known to dramatically drive the oligomerization toward LH1 formation (*59*).

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