Consequences for the Organization of Reaction Center-Light Harvesting Antenna 1 (LH1) Core Complexes of Rhodobacter sphaeroides Arising from Deletion of Amino Acid Residues from the C Terminus of the LH1 α Polypeptide*

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Peter McGlynn, Willem H. J. Westerhuis, Michael R. J.onest, and C. Neil Hunter§

From the Robert Hill Institute for Photosynthesis and Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield, S10 2UH, United Kingdom

The light harvesting antenna 1 (LH1) complex of Rhodobacter sphaeroides is intimately associated with the reaction center (RC) as part of the reaction center RC-LH1 core complex. The pufA gene has been modified such that between 5 and 16 amino acid residues were progressively deleted from the C terminus of the LH1 α polypeptide. The two largest deletions produced strains which were deficient in LH1. The remaining four deletion mutants exhibited significant reductions in the average level of LH1 per reaction center. Analysis of detergent-solubilized cores on sucrose gradients showed that the mutant strains had a sizeable population of antenna-deficient reaction centers in addition to core complexes with a reduced ratio of LH1:RC. The decrease in the ratio of LH1:RC in core complexes of the mutant strains was accompanied by a progressive blue shift of the absorbance maximum of LH1, which we attribute to the reduced aggregation state of LH1 in the smaller cores. The PufX polypeptide was not required for photosynthetic growth in mutants with reduced core sizes. We conclude that the level of LH1 in the bacterial membrane, and the aggregation state of LH1 in core complexes, are both influenced by the C terminus of the α polypeptide, and we discuss possible models for the organization of the core complex in Rb. sphaeroides.

The photosynthetic bacterium Rhodobacter sphaeroides has a relatively simple membrane-bound photosystem comprising a single type of reaction center (RC)1 and two types of light harvesting complex, LH2, is present in variable amounts according to the incident light intensity whereas the core light harvesting complex, LH1, is present in a fixed stoichiometry to the RC (1, 2). In the simple “lake” model (3) light energy captured by LH2 migrates via LH1 to a dimer of bacteriochlorophyll (Bchl) within the RC, initiating the transfer of an electron through the RC from the periplasmic side to the cytoplasmic side of the membrane. The products of this transmembrane electron transfer, reduced ubiquinone and an oxidized Bchl dimer, trigger a cycle of electron transfer reactions involving the intramembrane pool of ubiquinone, the cytochrome bc complex and a soluble cytochrome c that results in the translocation of protons from the cytoplasmic side of the membrane to the periplasmic side, generating a proton electrochemical gradient.

All bacterial light harvesting complexes studied to date contain two small hydrophobic polypeptides designated α and β in a 1:1 ratio, both of which have a single membrane-spanning helix (4). Liganded to these polypeptides are the light harvesting pigments which, in the case of Rb. sphaeroides, are molecules of Bchl a. Carotenoids also act as light harvesting pigments but have an additional photoprotective role. In the LH2 complex of Rb. sphaeroides each pair of α and β subunits is associated with three molecules of Bchl, two of which absorb at 850 nm and the third at 800 nm. Within the LH1 complex there are only two Bchl molecules per pair of α and β subunits, and these absorb maximally at 877 nm. Historically, the structure and function of the bacterial antenna has been studied mainly through the application of biochemical and spectroscopic techniques (5). However, the first prospects for carrying out an examination of the relation between structure and mechanism based on detailed structural information have recently appeared with the publication of a high resolution structure for the LH2 complex from Rhodopseudomonas acidophila strain 10050 (6). The complex consists of a cylinder of 9 αβ heterodimers, with the Bchl a molecules arranged in 18-member (850 nm Bchls) and 9-member (800 nm Bchls) rings. It is thought that this arrangement allows the excited state of any Bchl a molecule to become rapidly delocalized over the entire ring, allowing efficient transfer of energy to an adjacent complex. Parallels with the LH2 structure have also been drawn from low resolution structural information on the LH1 complex from Rhodospirillum rubrum (7), which has been proposed to have 16 αβ units that are also arranged in a cylinder, with the organization of the 880-nm Bchls being analogous to that of the 850-nm Bchls of LH2. The exact spatial relationship of the LH2, LH1, and RC complexes of the bacterial photosystem is still uncertain, although it has been noted that there is sufficient space within the ring of the R. rubrum LH1 complex for a single RC complex (7). However, whether or not the RC resides within a ring of LH1 αβ units remains to be proven.

A number of studies using a range of bacterial species have shown that the N termini of both the α and β polypeptides of the LH1 complex play essential roles in the stable expression of the complex in the membrane (8–11). In contrast, the C ter-
nus of the LH1 complex has not been studied in detail. Gogel et al. (12) used dansyl chloride to modify the Rs. rubrum LH1 \( \alpha \kappa^{-110} \) residue with no effect upon the absorbance of LH1; electron transport activity in treated membranes was significantly reduced but due to the nonspecific nature of dansyl chloride this could not be unambiguously ascribed to the modified LH1 \( \alpha \) polypeptide. A more recent in vitro study (11) using Rs. rubrum LH1 polypeptides demonstrated that an apparently wild type LH1 complex could be formed from an intact \( \beta \) polypeptide plus an \( \alpha \) polypeptide which lacked 10 amino acids from the C terminus, ending at residue \( \epsilon^{-13} \). This study raised questions concerning the exact role of the C terminus of the LH1 \( \alpha \) polypeptide, since its removal appeared to have little impact upon the stable assembly of the LH1 complex in detergent solution. Comparison of a number of LH polypeptides from a variety of species of photosynthetic bacteria had previously shown that there was reduced homology at the C termini as compared with the N termini or the membrane spanning helices (13), which supported the lack of any specific role in formation or stability of the LH1 complex in vitro. However, an examination of the structure of the Rps. acidophila LH2 complex reveals that the interaction between polypeptides in LH2 is in part mediated by hydrogen bonds and hydrophobic interactions between residues in the C terminus of the LH2 \( \alpha \) and \( \beta \) polypeptides and between adjacent \( \alpha \) polypeptide C termini (6). Also, the \( \alpha W^{-11} \) residue of the Rb. sphaeroides LH1 complex has been shown to form a hydrogen bond to the 2-acetyl carboxyl group of one of the LH1 Bchls (14).

In the present study, we have examined the role of the C terminus of the \( \alpha \) polypeptide in the assembly of the RC-LH1 core complex through the introduction of six mutations into the 3' end of the pufA gene of Rb. sphaeroides that alter specific codons to a stop codon, resulting in the formation of \( \alpha \) polypeptides that are truncated by between 5 and 16 amino acids at the C terminus. To simplify the spectroscopic analysis of the altered complexes, the experiments were performed in an LH2-deficient background by expressing a plasmid-borne copy of the puf operon harboring the mutated pufA gene in the double-deletion strain DD13. This strain harbors genomic deletion/insertion mutations in the pufQBALMX (RC-LH1) and pucBAC (LH2) operons, and is devoid of pigment-protein complexes. Our results demonstrate that truncation of the LH1 \( \alpha \) polypeptide has a major impact on the levels and aggregation states of LH1 associated with RCs. They shed new light on the organization of the core complex and on the influence of antenna oligomer size on the spectroscopic properties of LH1 Bchls. We have also examined the effect of the absence of PufX on the capacity for photosynthetic growth and on the organization of the core unit in these truncation mutants.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, Gene Transfer, Growth Conditions, and Membrane Preparation**—With the exception of those described in detail below or specified in Table 1, all bacterial strains and plasmids were as described in Ref. 15. The transfer of mobilizable plasmids from Escherichia coli to Rb. sphaeroides was performed as described in Ref. 16. Growth conditions were as described in Ref. 15. Preparation of intracytoplasmic membranes was as described in Ref. 17.

**Construction of Mutated pufA Genes**—Two methods were used to introduce stop codons toward the 3' end of the pufA gene. In the first, the synthetic codon \( \alpha W^{-11} \) was introduced between a HindIII site immediately downstream of the stop codon for pufA (18). The mutation was introduced using a mismatch oligonucleotide according to the procedure supplied with the oligonucleotide. The mutantgenized BmH1-Xbal site was then introduced in the place of the corresponding restriction fragment in the expression plasmids pRKEH10 (20) and pRKEH10X (21) to form the plasmids pRKEH+10 and pRKEH+10X, respectively.

The remaining five pufA mutants were constructed using the polymerase chain reaction with the template in all cases being the plasmid pSELBLHX (see above). For all of the mutagenic changes, one of the pair of oligonucleotides was designed to be homologous to the 3' end of the pufA gene and encompassed the engineered HindIII site located between the pufB and pufA genes (see above). The second oligonucleotide for each PCR was designed to have a common sequence at the 5' end that encompassed the engineered NruI site immediately downstream of the pufA stop codon followed by tandem stop codons at a position 3' to the NruI site in the \( \alpha \) polypeptide.

Appendix to the 3' end of this common sequence were sequences of 17 bases that were homologous to the appropriate region of the pufA gene. The 5' end of this homologous sequence started with the codon of the amino acid that would form the C-terminal residue of each mutated \( \alpha \) polypeptide. The HindIII-NruI fragments encompassing the wild-type pufA gene in plasmid pRKEH18 (18) was then replaced by the HindIII-NruI fragments produced by the mutagenic PCR, and the resulting mutant constructs were shuttled as EcorI-XbaI fragments into the expression vectors pRKEH10 (20) and pRKEH10X (21) to form a series of expression plasmids for the mutated puf operon with and without the pufX gene, respectively (Table I). The nomenclature for the mutant strains (see Fig. 1) follows the numbering system described in Ref. 12 by Hunter et al. (21) and Olsen and Hunter (13), designating the histidine that forms the axial ligand to the LH1 Bchl as residue H0, with residues toward the C terminus having positive numbers.

For the resequencing of pufA DNA from Rb. sphaeroides strains harboring mutant plasmids, 1 ml of culture of the required strain was pelleted by centrifugation at 13,000 rpm for 1 min at room temperature in a microcentrifuge and the cell pellet was resuspended in 100 \( \mu \)l of water. This cell suspension was boiled for 2 min and cell debris was removed by centrifugation at 13,000 rpm for 5 min at room temperature. The region of plasmid DNA encompassing pufBA was then amplified from the supernatant by PCR using two oligonucleotides that were homologous to sequences immediately upstream of pufB and downstream of pufA. The latter of these two oligonucleotides was then used for sequencing of the PCR product.

**Preparation of Solubilized RC-LH1 Complexes**—RC-LH1 complexes were extracted from intracytoplasmic membranes with 15 mM \( n \)-octyl-\( \beta \)-glucopyranoside and 15 mM deoxycholate and subsequently separated on 10-40% (w/v) sucrose gradients as described in detail in Ref. 22. However, centrifugation was performed for 4 h rather than 22 h in order to achieve better separation of RC and RH-LH1 complexes.

**SDS-Polyacrylamide Gel Electrophoresis**—Protein samples were separated on a 16.5% polyacrylamide gel according to the method of Schagger and von Jagow (23) and stained with Serva blue G. An amount of each intracytoplasmic membrane sample was loaded which contained 10 pmol of RC, estimated as described below. The exception was the total protein for which 5 \( \mu \)g of total protein was loaded.

**Spectroscopy**—Absorbance spectroscopy of membrane samples was performed on a Guided Wave Model 260 fiber optic spectrophotometer (Guided Wave Inc., El Dorado Hills, CA). This spectrophotometer was also used to obtain absorbance spectra on individual Rb. sphaeroides colonies from agar plates. Absorbance measurements on acetone/methanol extracts of membrane preparations for the purpose of estimating Bchl concentration were performed on a Beckman DU 640 spectrophotometer (Beckman Instruments UK, High Wycombe, UK). Low temperature absorbance spectra were obtained using the fiber optic spectrophotometer and a DN 1704 liquid nitrogen cryostat (Oxford Instruments Limited, Oxford, UK).

**Fluorescence Emission Spectra**—Fluorescence emission spectra of membrane samples were obtained using a Fluoromax spectrofluorometer (SPEX Industries Inc., NJ). The sample absorbance at room temperature was adjusted to 0.1 cm\(^{-1}\) at 885 nm in 60% glycerol (w/v), 10 mM Tris, pH 8.0. To promote accumulation of RCs in the photooxidized state, potassium ferricyanide was added to a final concentration of 100 \( \mu \)M, and the sample was illuminated with a tungsten light source (530 nm) and a RG630 filter for 10 s immediately prior to cooling. Fluorescence was detected at 90°C to the incident light at a resolution of 5 nm using 590 nm excitation.

**Calculation of RC Concentration and the Number of LH1 Bchls per RC**—RC concentrations were calculated from room temperature absorbance spectra acquired with the Guided Wave spectrophotometer, using the method described in Ref. 15 and an extinction coefficient of 805 nm of 312 pm\(^{-1}\) cm\(^{-1}\) for photooxidized reaction centers. The number of LH1 Bchls/RC was determined as described previously (15).
This method, in which the concentration of antenna Bchl is estimated from acetone/methanol extracts of intracytoplasmic membranes, circumvents inaccuracies that would arise from possible changes in the extinction coefficient of the Qy absorbance band in the mutant strains. Using this procedure extinction coefficients of 123, 124, and 111 nm cm⁻¹ were determined for strains RCLH12, RCLH +21, and RCLH +13, respectively. These were used in turn to estimate the number of LH1 Bchls per RC in sucrose gradient fractions of detergent-solubilized membranes which were too dilute for accurate estimates of Bchl concentrations by acetone/methanol extraction.

The percentage of free RCs in detergent-solubilized membranes was estimated by dividing the total estimated amount of LH1 Bchl in a sucrose gradient by the mean core size for that gradient to give the percentage of RCs associated with LH1. This figure was then used together with the total estimated amount of RCs in the gradient to give the percentage of RCs not associated with LH1.

### RESULTS

Construction of Mutant Strains in Which the LH1α Polypeptide Is Truncated at the C Terminus—In order to generate the transconjugant strains described in Table I, two series of expression plasmids were constructed both of which had the altered pufA gene together with wild-type copies of the pufQ, B, L, and M genes (Fig. 1). A wild-type copy of the pufX gene was present in the first series, but not in the second (X⁻ series). Both sets of plasmids were transferred into Rb. sphaeroides strain DD13 (19). All of the transconjugant strains bearing a truncation of the LH1α polypeptide showed a decrease in the level of LH1, as estimated from absorbance spectra of intact cells (data not shown). In some cases the level of LH1 dropped below the limits of detection.

Analysis of Intracytoplasmic Membranes from Mutant Strains—The transconjugant strains harboring the mutated pufA gene were grown to early stationary phase under reduced oxygen tension in the dark, and intracytoplasmic membranes were prepared. Membranes from a control strain (RCLH12) containing a plasmid encoding a wild type copy of the pufBA genes, and membranes from a “reaction center-only” strain (RCO2) which lacks an intact copy of both pufA and B (15, 20) were also prepared.

Fig. 2 shows the room temperature absorbance spectra of intracytoplasmic membranes from the mutant and control strains normalized on the basis of RC concentration. Membranes from control strain RCLH12 exhibited the characteristic wild-type LH1 absorbance at 877 nm and RC bands at 804 and 760 nm. In contrast, the spectrum of strain RCO2 had only the latter two peaks, characteristic of (photo)oxidized RCs, showing the complete absence of LH1. The 860-nm band of the RC Bchl special pair was bleached since the Guided Wave fiber optic spectrophotometer, used to acquire the spectra, irradiates the sample with white light. For each of the mutant strains the absorbance spectra showed reductions in the relative size of the 877-nm LH1 band (Fig. 2), indicating that the truncations in the LH1α polypeptide had led to reductions in the number of LH1 Bchls per RC, but that they had not significantly altered.

### TABLE I

**Bacterial strains and plasmids used in this report**

Note that RCLH12 and RCO2 are identical to strains RCLH11 and RCO1 (20) except that the former are in a wild type carotenoid background whereas the latter are in a neurosporene carotenoid background.

| Strain | Corresponding plasmid | Position of mutation to stop codon | Strain description |
|--------|------------------------|-----------------------------------|-------------------|
| PuF-containing series | | | |
| RCLH12 | pRKEH10 | V$^+$ 22 | RCLH1 control strain |
| RCLH +21 | pRKEH+21 | K$^+$ 10 | 5-residue truncation of LH1α |
| RCLH +17 | pRKEH+17 | I$^+$ 14 | 9-residue truncation of LH1α |
| RCLH +13 | pRKEH+13 | E$^+$ 13 | 13-residue truncation of LH1α |
| RCLH +12 | pRKEH+12 | L$^+$ 12 | 14-residue truncation of LH1α |
| RCLH +11 | pRKEH+11 | W$^+$ 11 | 15-residue truncation of LH1α |
| RCLH +10 | pRKEH+10 | | 16-residue truncation of LH1α |
| RCO2 | pRKEH10D | | RC-only control strain |
| PuF-deficient series | | | |
| RCLH12X | pRKEH10X | V$^+$ 22 | RCLH1 control strain |
| RCLH +21X | pRKEH+21X | K$^+$ 10 | 5-residue truncation of LH1α |
| RCLH +17X | pRKEH+17X | I$^+$ 14 | 9-residue truncation of LH1α |
| RCLH +13X | pRKEH+13X | L$^+$ 14 | 13-residue truncation of LH1α |
| RCLH +12X | pRKEH+12X | E$^+$ 13 | 14-residue truncation of LH1α |
| RCLH +11X | pRKEH+11X | L$^+$ 12 | 15-residue truncation of LH1α |
| RCLH +10X | pRKEH+10X | W$^+$ 11 | 16-residue truncation of LH1α |
| RCO2X | pRKEH10DX | | RC-only control strain |
| LHO1 | pRKEH1H | | LH1-only control strain |

**Fig. 1. Arrangement of the wild type puf operon and the nature of the truncation mutations.** A, represents the intact pufQBALMX operon present on plasmid pRKEH10 (20) and B, indicates the pufX-deficient version of this found in plasmid pRKEH10X (15). C shows the amino acid sequence of the LH1α polypeptide as encoded by the pufA gene, and the sequences of the polypeptides that arise from the six truncation mutations. The putative transmembrane helix is indicated by the horizontal bar, and the histidine residue that is thought to ligand the LH1 Bchls is shown by the arrow.

**Fig. 2.** Room temperature absorbance spectra of membranes from mutant and control strains. Sample A, RCLH12, exhibits the characteristic wild-type LH1 absorbance at 877 nm and RC bands at 804 and 760 nm. Sample B, RCLH12X, shows a decrease in the level of LH1, as estimated from absorbance spectra of intact cells (data not shown). Sample C, RCO2, contains only RC bands at 804 and 760 nm.
the 82-amino acid polypeptide encoded by the pufX gene. In a recent publication (15), we reported that the removal of the pufX gene had led to a small increase in MLH1/RC, with the exception of strain RCLH +12 (Table I). This increase was consistent with an average increase in the size of the mutant core complexes of between one and two LH1 Bchls per RC. In those truncation mutants which did not express an LH1 complex (strains RCLH +11 and RCLH +10), removal of the PufX polypeptide did not restore an assembled LH1 complex. PufX did not affect the extinction coefficient for LH1 in the truncation mutants.

Visualization of the LH1 α and β polypeptides by SDS-polyacrylamide gel electrophoresis of intracytoplasmic membranes revealed no large differences in apparent molecular weight or amount of either polypeptide when comparing equivalent strains with and without the pufX gene (Fig. 3).

Low Temperature Absorbance and Fluorescence Spectroscopy—Room temperature absorption spectra of membranes from the mutants (Fig. 2) indicated that, in addition to a reduction in the MLH1/RC ratio, the position of the LH1 absorption maximum (λmax) was blue shifted for the larger truncations. Therefore absorption and fluorescence emission spectra were also recorded at 77 K. Whereas the λmax of strain RCLH +21 did not significantly differ from that of the control strain, blue shifts were apparent in both the absorption and the emission maxima for strains RCLH +17 (5 nm), RCLH +13 (5 nm), and RCLH +12 (7 nm) (Table II). Such spectral shifts could be the immediate result of small alterations in the structure of an αβ antennae unit, resulting in modified pigment-protein interactions. However, very similar blue shifts have previously been observed for detergent-solubilized wild-type LH1 complexes as the estimated aggregation state decreased from about αβ3 to αβ (24). Since in the present study these truncation-induced blue shifts are accompanied by apparent decreases in the core antenna levels, the simplest explanation would be that they again reflect reductions in the aggregation state of LH1.

Analysis of Detergent-solubilized Pigment-Protein Complexes from the Mutant Strains—The decreases in MLH1/RC observed in the truncation mutants could reflect a uniform reduction in the core antenna size. Alternatively, these membranes could contain a mixture of both antenna-deficient reaction centers and core complexes similar in size to those in the wild-type strain. Finally, an intermediate situation is possible with a range of core antenna sizes being present within a single membrane preparation. To investigate the change in the organization of the core complexes in the mutants in more detail, the pigment-protein complexes from strains RCLH +12, LH01, RCLH +21, RCLH +13, and RCLH +10 were extracted from intracytoplasmic membranes using n-octyl-β-D-glucopyranoside and deoxycholate and were separated by centrifugation on continuous sucrose gradients (see "Experimental Procedures").

The composition of the fractions was then investigated by room temperature absorbance spectroscopy (Fig. 4). As shown in panel d of Fig. 4, which describes data obtained with the LH1-deficient strain RCLH +10 (open triangles) and with an LH1-only strain LH01 (closed triangles) (19), such gradients can achieve an approximate separation of RCs and LH1 complexes, with the majority of "free" RCs migrating to between 0 and 12% sucrose, while the majority of LH1 forms a broad band between 10 and 30% sucrose. Gradients of this sort can therefore be used to investigate whether a significant population of
the RCs in the membrane are free from LH1.

Absorbance spectra of fractions from the sucrose gradients of the detergent-solubilized pigment-protein complexes from control strain RCLH12 indicated that the vast majority of RCs in this strain co-migrated with LH1 complexes, with very few free RCs being observed near the top of the gradient (Fig. 4a). In contrast, sucrose gradients containing solubilized complexes from strains RCLH121 (Fig. 4b) and RCLH113 (Fig. 4c) revealed a significant fraction of RCs that were not associated with LH1, suggesting that there was a significant population of free RCs in membranes from the truncation mutants. This antenna-free population was estimated to be 50% of the total RCs in strain RCLH121, 25% in strain RCLH113, and 2% in the control strain RCLH12. The average size of the core complexes (SLH1/RC) in the LH1-containing fractions from the sucrose gradient of the control strain was estimated to be ~29, close to the value for MLH1/RC of ~26 estimated on the basis of membrane spectra. In strain RCLH121, a value of ~20 was estimated for SLH1/RC, twice the value of MLH1/RC for this strain and consistent with the level of free RCs seen in the gradient fractions. For strain RCLH113, SLH1/RC was ~10, again consistent with the value of ~7 estimated for MLH1/RC taking into account a 25% population of free RCs.

To investigate whether the larger core complexes seen in the sucrose gradients of strains RCLH121 and RCLH113 arose from a detergent-induced redistribution of LH1 among the RC population, 77 K absorption spectra were recorded for the fractions indicated by the arrows in Fig. 4 and the \( \lambda_{\text{max}} \) of LH1 determined (Table II). For the control strain RCLH12, SLH1/RC was very similar to MLH1/RC and there were very few free RCs, suggesting that no significant reorganization of
the core complexes had taken place. Therefore we attributed the observed 2-nm blue shift in $\lambda_{\text{max}}$ for sample RCLH12 to the detergent extraction of the complexes. The $\lambda_{\text{max}}$ was also constant across the RCLH12 gradient which suggested LH1 had a similar aggregation state throughout the gradient. The solubilized core complexes from strains RCLH+21 and RCLH+13 showed the detergent-induced 2-nm blue shift, but despite the significant difference between SLH1/RC and MLH1/RC in each of these strains, there were no further differences in the $\lambda_{\text{max}}$ when 77 K spectra of gradient fractions and membranes were compared (Table II). This suggested that there had been no large scale redistribution of LH1 among the RC population.

Detergent extraction of membranes from strains RCLH-12X, RCLH-21X, and RCLH-13X was also performed to establish whether a lack of PuF significantly altered the distribution of LH1 between the RCs in the membranes of the mutant strains. No significant differences were observed between the distribution profiles seen for the three PuF-deficient strains (data not shown) and the profiles shown in Fig. 4 (a-c) for their PuF-containing counterparts.

Photosynthetic Growth Characteristics of the LH1α Polypeptide Mutants—The biochemical and spectroscopic data reported above indicated that the mutants with a truncated LH1α polypeptide contained RCs unconnected to a light harvesting system and RCs associated with the mutant LH1 complexes in core complexes with reduced LH1/RC ratios. To investigate the phenotypic effects of this change in the organization of the photosystem of each mutant, their photosynthetic growth characteristics were assayed. As previously reported (15), strain RCLH12 grew photosynthetically whereas the PuF-deficient counterpart RCLH12X did not, but both strains RCO2 and RCO2X grew photosynthetically (Fig. 5). Thus the PuF polypeptide was only required for photosynthetic growth when a light harvesting system was present in the membrane. Strain RCLH12 displayed a lag phase of approximately 35–40 h before photosynthetic growth occurred and the culture reached a final absorbance at 680 nm of between 2.0 and 2.5, whereas strains RCO2 and RCO2X grew without a lag phase but reached a final absorbance at 680 nm of only 0.6–0.9. We have previously attributed this reduced final cell density in the antenna-deficient strains, and the lack of a well defined loga-

rithmic phase, to a progressive decrease in the rate of photosynthetic growth caused by increasing self-shading as the culture thickens (15).

With the sole exception of strain RCLH+11, all of the PuF-containing truncation mutants were capable of photosynthetic growth, but scrutiny of the growth curves for the mutant strains revealed a complex pattern. The mutant strains which expressed an LH1 complex, namely RCLH+21, RCLH+17, RCLH+13, and RCLH+12, all displayed a reproducible biphasic growth (Fig. 5, b-e). The initial phase occurred with no lag and proceeded for approximately 50 h whereupon a second phase became dominant and growth continued to an absorbance at 680 nm of between 2.0 and 2.5. The first phase of growth in the LH1-containing mutants was very similar to that seen during the first 50 h of growth of the RC-only strain RCO2 (Fig. 5h). The second phase of growth in the LH1-containing mutant strains, which became dominant at the end of the initial 50-h period, was similar to that seen after approximately 50 h in the control strain RCLH12, with in all cases the final absorbance of the culture rising to a value greater than 2.0. Thus the four LH1-containing truncation mutants appeared to display features of both "RC-only growth" and "RC+LH1 growth." To determine whether this was due to segregation within the photosynthetic cultures into cells containing only the RC complex and cells containing both the RC and the mutant LH1 complex, aliquots of the photosynthetic cultures from early stationary phase were taken and appropriate dilutions were spread onto agar plates of M22 medium containing kanamycin and tetracycline and then incubated under dark, aerobic conditions at 34°C. Absorbance spectra taken of at least 20 individual colonies from each of the above four strains all displayed peaks for both RC and LH1 complexes (data not shown), showing that segregation had not occurred during photosynthetic growth, and making it unlikely that the biphasic growth patterns could be explained by two spectroscopically distinct cell types within the cultures. The mutant strains which exhibited reproducible growth under photosynthetic conditions were also screened for the presence of suppression mutations within the puB and A genes. Cells grown under photosynthetic conditions to early stationary phase were harvested, the puB and A genes were amplified by PCR and the DNA products sequenced as described under "Experimental Procedures." In all cultures tested there were no secondary mutations within either the puB or A gene.

Mutants RCLH+11 and RCLH+10 both had a RC-only absorbance spectrum (Fig. 2) but only strain RCLH+10 was capable of photosynthetic growth with kinetics similar to those displayed by the RC-only control strain RCO2. Mutant RCLH+11 also appeared to be capable of weak photosynthetic growth, but seemed to be impaired relative to strains RCO2 and RCLH+10. The reasons for this were not investigated further.

The puF gene appeared not to have any effect on the ability of the mutant strains to grow under photosynthetic conditions, with the exception of strain RCLH+21X (Fig. 5b) in which there was a reproducible inhibitory effect due to the absence of the PuF polypeptide. However, this inhibition was not as dramatic as that seen for the control strain RCLH12X which in the absence of secondary suppression mutations was entirely non-photosynthetic. In contrast, strain RCLH+21X was still capable of biphasic photosynthetic growth, but the second phase of growth became dominant after a longer period (75 h) than was seen for the other LH1-containing truncation mutants (~50 h). Therefore there was some correlation between the number of LH1 Bchl s per RC and the effect of PuF, since the only strains whose growth was sensitive to the absence of

Fig. 5. Photosynthetic growth of the control and mutant Rb. sphaeroides strains. Growth was monitored by the absorbance of cultures at 680 nm. The strains containing puF are shown by open circles and those lacking puF by filled circles. Strains are (a) RCLH12X, (b) RCLH+21X, (c) RCLH+17X, (d) RCLH+13X, (e) RCLH+12X, (f) RCLH+11X, (g) RCLH+10X, and (h) RCO2X.
PuF was RCLH12X−, which had normal core complexes, and strain RCLH+21X−, the truncation mutant with the largest core complexes.

**DISCUSSION**

The Consequences of Truncation of the LH1 α Polypeptide for Core Complex Assembly in Vivo—As described in the Introduction, in vitro studies of the assembly of the LH1 antenna complex in Rb. rubrum have shown that the C terminus region of the α polypeptide is not required for stable assembly of the LH1 complex (11). In order to examine the in vivo role of this region of the α polypeptide, six mutant versions of the Rb. sphaeroides pufA gene were constructed in which specific codons toward the 3′ end of the gene were altered to stop codons, resulting in the formation of LH1 α polypeptides with truncated C termini which was visualized as a progressive decrease in apparent molecular weight (Fig. 3). Room temperature absorbance spectra recorded for intracytoplasmic membranes from the mutants strains (Fig. 2) showed that the truncated forms of the LH1 α polypeptide all caused a reduction in the amount of LH1 complex per RC, the severity of reduction increasing as the number of amino acids deleted from the C termini increased (Table II). Data obtained with core complexes that had been solubilized in an appropriate combination of detergents and centrifuged on continuous sucrose gradients (Fig. 4) revealed that in mutants RCLH+21 and RCLH+13 the decrease in the overall level of LH1 observed in membranes from the strains resulted in core complexes with a reduced stoichiometry of LH1:RC, together with a pool of free RCs. For the reasons outlined under “Results” we believe that the data from the sucrose gradients provide an accurate reflection of the organization of the core complexes in membranes from the mutant strains. Furthermore, the similarities in the properties of strains RCLH+13, RCLH+12, and RCLH+17 lead us to believe that a similar arrangement of core complexes of reduced size was also present in the latter two strains.

Although at present little is known about the assembly pathway of the LH1 complex, topology studies have demonstrated that the C-terminal regions of both the α and the β polypeptides are located on the periplasmic side of the membrane (25). Thus it is conceivable that truncations of the C terminus of the α polypeptide would affect the process by which it is translocated across the bilayer, leading to a decrease in the level of the α polypeptide in the membrane, accompanied by a decrease in the stability of the β polypeptide. This was supported by SDS-polyacrylamide gel electrophoresis of mutant intracytoplasmic membranes in which the observed amounts of both the LH1 α and β polypeptides correlated with the decreases in amounts of LH1 Bchl (Fig. 3). The RC-only phenotype exhibited by the RCLH+10 and RCLH+11 mutants may have arisen from a further destabilization of the LH1 complex caused by disruption of the hydrogen bond that has been shown to exist between the LH1 αW11 residue and the 2-acetyl carboxyl group of one of the LH1 Bchls (14). While disruption of this hydrogen bond in an otherwise wild-type LH1 complex does not prevent assembly (14) the further destabilization arising from breaking of this hydrogen bond may have lead to a complete inhibition of LH1 assembly in mutants RCLH+10 and RCLH+11. Neither the α nor β polypeptide could be detected in membranes from these strains (Fig. 3).

The Relationship between the Aggregation State of LH1 and the Spectroscopic Properties of the Complex—The correlation observed between the Qy absorption maximum of LH1 and its apparent aggregation state in core complexes, isolated in sucrose gradients, had been observed previously for wild-type LH1 complexes isolated by lithium dodecyl sulfate-polyacrylamide gel electrophoresis (24). In the latter study, the position of the absorption maximum of LH1 remained the same as the estimated aggregation state decreased from about 30 coupled Bchls in an (αβ)13 arrangement to 22 coupled Bchls in an (αβ)11 complex, but gradually shifted to shorter wavelengths upon a further reduction in size with the smallest complex, (αβ)6, exhibiting a 5–6 nm blue shift. This dependence of the spectral properties of LH1 on its aggregation state can be reproduced using a model for the core antenna in which excitonically-coupled Bchls are arranged in a circular array (26), analogous to the organization of B850 Bchls in the Rps. acidophila LH2 complex as revealed by x-ray crystallography (6). In this model spectral blue shifts of relatively small complexes, regarded as fractions of a larger fully circular array, are the result of a reduction in the number of coupled Bchls. The spectral changes described in the present study, with no alteration in the absorption maximum of LH1 as the size of the core complex dropped from about 30 (RCLH12) to 20 (RCLH+21) Bchls per RC, while an additional decrease to 10 Bchls per RC (RCLH+13) produced a 5-nm blue shift, are in agreement with this model.

Consequences of Our Findings for Possible Structures for the RC/LH1 Core Complex—The principal finding from the work described in this article is that the RC-LH1 core complexes in the mutant strains can adopt a range of LH1:RC stoichiometries, but that in the control strain the cores have an essentially uniform structure, with approximately 29 LH1 Bchls associated with each RC (based on sucrose gradient data on strain RCLH+12). X-ray crystallography has revealed that the LH2 complex from Rps. acidophila consists of a nonamer of αβ heterodimers arranged in a concentric ring with all the α subunits on the inner face of the ring (6). In parallel with this, low resolution structural information on the LH1 complex of Rs. rubrum has led to the proposal that the core complex may be comprised of a RC surrounded by a 16-member ring of αβ heterodimers (7). Clearly, such a structure would place constraints upon the minimum number of αβ units that can be associated with each RC in a core complex, and is incompatible with the small cores observed by us in sucrose gradients prepared from our truncation mutants. If the structure put forward by Karrasch and co-workers (7) is appropriate for the wild-type core complex and, irrespective of the effects of mutagenesis, the essential structure of the LH1 complex is a ring of αβ units along the lines of the LH2 structure, then this leads to the unsatisfactory conclusion that the core complexes in the mutant strains must consist of RCs that are associated with the outer face of a (small) ring of LH1. This would require a major reorganization of the RC/LH1 system and the breakage of any specific molecular contacts between the RC and LH1. As an alternative, we would suggest that the truncations of the LH1 α polypeptide described in this report lead to a rearrangement in the structure of LH1, such as a change in the degree of curvature of the LH1 aggregate, that in turn leads to the formation of core complexes consisting of a RC together with a partial ring or “arc” of LH1. This arrangement would allow the RC to be in contact with the concave surface of the arc thereby permitting retention, in the mutant core complexes, of any specific interactions between the RC and the inner surface of the ring of LH1 that are present in normal core complexes, except of course interactions with the C terminus of the α polypeptide.

The Function of PuF—A number of studies have shown that the PuF protein is required for growth of antenna-containing strains under photosynthetic conditions (27, 15). The role of PuF may be to organize the supramolecular structure of the photosynthetic membrane such that ubiquinone/ubiquinol exchange can occur between the RC and the cytochrome bc3.
complex (28, 29). As reported previously (15), photosynthetic growth of the antenna-deficient strain RCO2 does not require the presence of the puF gene. Therefore, photosynthetic growth might be expected in truncation mutants containing a sizeable population of RCs not associated with a core antenna. In addition to demonstrating this, the photosynthetic growth curves determined for the PuF-deficient truncation mutants that had retained an LH1 complex (Fig. 5) also revealed a second phase of growth that was supported by RCs in core complexes of the mutants generated in this study only strain RCLH +21, with the smallest truncation of the α polypeptide, showed a sensitivity to the absence of PuF.

In a recent paper (15) we discussed the possibility that the small increase in the aggregation state of LH1 observed in the absence of the PuF polypeptide (RCLH12X) results in a physical blockage of the Qb site of the RC, preventing ubiquinone/ubiquinol exchange with the cytochrome bc1 complex (28, 29). From the results presented here, RC-LH1 core complexes with a reduced LH1:RC stoichiometry remain fully functional in the absence of PuF, thus supporting this hypothesis. The threshold level of LH1 per RC at which the requirement for PuF is lost is not known, but our results with strains RCLH12X and RCLH +21X suggest it lies somewhere between 29 and 20 Bchls per RC.

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