The Synthesis and Assembly of Functional High and Low Light LH2 Antenna Complexes from Rhodopseudomonas palustris in Rhodobacter sphaeroides*

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Photosynthetic bacteria respond to lowered light intensity by increasing the level of the peripheral light-harvesting (LH2) complex. Several species possess an additional mechanism, responding to variations in light conditions by making different types of LH2 complex. However, the study of these complexes in isolation and in the native membrane environment has not been possible. Therefore two LH2 gene pairs from Rhodopseudomonas palustris, associated, respectively, with high light (pucBA) and low light (pucBA) growth conditions, were expressed in Rhodobacter sphaeroides. The high light LH2 complex PucBA was synthesized at appreciable levels in R. sphaeroides, had near-infrared absorption bands at ~800–855 nm, and was able to transfer energy efficiently to the native LH1 complex. In contrast, the low light complex PucBA was found at comparatively low levels, had absorption bands at ~797–830 nm, and did not transfer energy to the native LH1 complex efficiently. These observations are discussed in the light of site-directed studies on the R. sphaeroides LH2 complex, and the recently elucidated Rhodopseudomonas acidophila 10050 LH2 structure. Potentially important residues for energy transfer between LH2 and LH1 complexes are identified, as well as some of the factors that influence stability and assembly of LH2 complexes, such as the N-terminal sequences of their protein subunits and their carotenoid binding sites.

In photosynthesis, light energy is absorbed by carotenoid and (bacterio)chlorophyll molecules, which are found in the light-harvesting (LH1) complexes, and is transferred via the different pigments to the photochemical reaction center (RC) with high efficiency (see, for example, van Grondelle et al. (1994)). The best characterized light-harvesting system from a structural, spectroscopic, and genetic standpoint is that of the purple bacteria, and in general it consists of two types of antenna complex LH2 (B800–855 nm, and did not transfer energy to the native LH1 complex; RC, reaction center; BChl, bacteriochlorophyll; PCR, polymorphic chain reaction; WT, wild type; R., Rhodobacter; Rv., Rubrivivax; Rps., Rhodopseudomonas.

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1 The abbreviations used are: LH, light harvesting; LL, low light; HL, high light; RC, reaction center; BChl, bacteriochlorophyll; PCR, polymorphic chain reaction; WT, wild type; R., Rhodobacter; Rv., Rubrivivax; Rps., Rhodopseudomonas.

**Experimental Procedures**

Media, Antibiotics, and Growth Conditions—Escherichia coli strains were grown in Luria broth. R. sphaeroides strains were grown under semiaerobic/dark conditions in M22 medium (Hunter and Turner, 1988) supplemented with 0.1% casamino acids for growth in liquid culture. For E. coli, tetracycline was added at a concentration of 10 μg/ml. For R. sphaeroides, antibiotic concentrations were tetracycline, 1 μg/ml; neomycin, 20 μg/ml; and streptomycin, 5 μg/ml.

**Photosynthetic Growth Conditions for Rps. palustris Strain 2.1.6—**

The medium used for the growth of Rps. palustris was the RVY-based medium described in Elder et al. (1993). The terms "low light" (LL) and "high light" (HL) used throughout this work are equivalent to 0.1-0.2 watt m⁻² and 100-200 watt m⁻², respectively.

**Bacterial Strains and Plasmids—**The bacterial strains used in this work include E. coli strain S17-1 (thi pro hsdR− hsdS+ recA RP4-2 (T-C mku::Tn7); Simon et al. (1983). R. sphaeroides strains DD13 (genomic deletion of both pucBA and pufBAMX; insertion of SmR and KmR genes, respectively) and DBCl1 (genomic deletion of pucBA genes and replacement by a of SmR cassette leaving only the native LH1-RC...
Fig. 1. Sequence alignments for the LH2α and β polypeptides coded by the genes used in this study (see text). The polypeptides are aligned around conserved histidine residues; the protein sequences for two of the R. palustris (RP) LH2 complexes shown, PucBAα and PucBAβ, are those given in Tadros et al. (1993). The sequence identity values of the chosen R. palustris LH2α and LH2β protein subunits to the equivalent subunits in the LH2 of R. sphaeroides (RS) were, respectively, PucBAα - 46%; PucBAβ - 57% PucBAα - 44% and PucBAβ - 46%. Similarly for the novel translated protein sequence from the Rps. palustris DEP100 LH2 genes, the PucBDEP100-subunit has a 93% identity to the PucBα-subunit from R. palustris strain 2.1.6 but only a 74% identity to the PucBα-subunit, while the PucBDEP100-subunit has a 90% identity to the PucBα-subunit but only a 76% identity to the PucBα-subunit. Residues referred to in the text are numbered and shown in bold.

Functional Rps. palustris LH2 Complexes in R. sphaeroides

RESULTS

Fig. 1 shows the sequence alignments for the LH2 α and β polypeptides encoded by the genes used in this study; the polypeptides are aligned around the conserved histidine residues which coordinate the B850 pigments (McDermott et al., 1995). The two LH2 complexes chosen for the present study, encoded by the genes pucBAα and pucBAβ, are described in Tadros and Waterkamp (1989) and Tadros et al. (1993) for R. palustris strain 165; the protein sequences of these complexes (PucBAα and PucBAβ) match those given in the Ph.D. thesis of Evans (1989) for Rps. palustris strain French, but with a different nomenclature. In this latter work PucBAα is named α1, and PucBAβ is named α2. (Protein sequences for the LH2 complexes of Rps. palustris strain French are also given in Brunisholz and Zuber (1992).)

The pucBAα and pucBAβ genes from the Rps. palustris strain 2.1.6 were isolated using PCR, cloned into the pRKCB1 expression plasmid, and sequenced to check their authenticity (see “Experimental Procedures”); after conjugative transfer into R. sphaeroides these genes were recovered and sequenced. Translation of the gene pairs gave no differences from the PucBAα and PucBAβ protein sequences published by Tadros et al. (1993) (Fig. 1). Using the same PCR oligonucleotides, we attempted to isolate analogous LH2 genes from Rps. palustris strain DEP100; only the oligonucleotides designed to the pucBAα gene gave a positive PCR fragment (data not shown); those designed to the pucBAβ gene did not.

The room temperature absorbance spectra of membranes prepared from LH2-only transconjugant strains are shown in Fig. 2, and they clearly demonstrate the presence of the Rps. palustris LH2 complexes in the R. sphaeroides background strain DD13. The spectra are scaled to reflect the level of LH2 complex per amount of cellular membrane as quantified by total membrane protein, and it is clear that although the LH2 complex arising from the gene pair pucBAα (Fig. 2b) is present at levels comparable to that of WT R. sphaeroides (Fig. 2a), that arising from the gene pair pucBAβ (Fig. 2b) is present at low levels. The spectrum of the PucBAα LH2 membranes (Figs. 2c and 3c) also showed an attenuated band at 800 nm relative to that seen in whole cell samples (data not shown); the B800 band is labile, and pigment is apparently lost during the membrane preparation. Furthermore, the absorbance properties of the heterologously synthesized LH2 complexes differed from those of WT R. sphaeroides LH2 (λmax = 800, 850 nm; Fig. 2a). The LH2 complex from the pucBAα gene pair showed absorbance peaks in the near infrared at 800 and 855 nm (Fig. 2b), while that from the pucBAβ gene pair showed equivalent peaks at approximately 797 and 830 nm (Fig. 2c).

In order to measure the ability of the 800-nm absorbing pigments to transfer energy to the B850 pigments within the heterologously synthesized Rps. palustris PucBAα and PucBAβ LH2 complexes, excitation spectra in the near infrared region of the spectrum were measured (Fig. 3). For both Rps. palustris complexes the matching profiles of the absorption and emission
sion of the PucBAa and PucBAd LH2 complex was monitored at 77 K on membranes from strains containing the het-
erologously synthesized RC. For the Rps. palustris strain DD13 containing (i) heterologously synthesized Rps. palustris LH2 PucBAa; and (ii) heterologously synthesized Rps. palustris LH2 PucBAa, The near infrared regions of the PucBAa and PucBAa spectra are shown in greater detail in Fig. 3, a and c.

**Fig. 2.** Room temperature absorption spectra of membranes prepared from LH1, RC, R. sphaeroides strain DD13 containing a, R. sphaeroides LH2; b, heterologously synthesized Rps. palustris LH2 PucBAa; and c, heterologously synthesized Rps. palustris LH2 PucBAa. The near infrared regions of membranes prepared from LH1 and RC; a, Rps. palustris LH2 PucBAa and b, Rps. palustris LH2 PucBAa. The arrow in b indicates the position of the 800-nm band that is present in the (1 → T) spectrum but missing from the excitation spectrum.

**Fig. 3.** Room temperature absorption (a and c, solid line), fluorescence emission (a and c, dashed line), and fluorescence excitation spectra (b and d, solid line) in the near infrared region of membranes prepared from LH1, RC, Rps. palustris LH2 PucBAa (a and b) and (ii) heterologously synthesized Rps. palustris LH2 PucBAa (c and d). The fluorescence emission of the PucBAa and PucBAa LH2 complexes was monitored at a λ<sub>em</sub> of 900 and 860 nm, respectively, to produce the excitation spectra shown in b and d. The spectrum of the PucBAa LH2 membranes shows an attenuated band at 800 nm relative to that seen in cell samples (data not shown); the B800 band is slightly labile and pigment is apparently lost during the membrane preparation.

spectra suggest that the complexes are able to transfer energy from their B800 to their B850 pigments.

The ability of the peripheral LH2 antenna to transfer energy to the core LH1 complex was monitored by exciting the LH2 complex at various wavelengths, from 750 to 900 nm, while monitoring any fluorescence emitted from the LH1 complex at 910 nm. Fig. 4 shows the results of such an excitation experiment at 77 K on membranes from strains containing the heterologously synthesized Rps. palustris PucBAa and PucBAa LH2 complexes, both in the presence of R. sphaeroides LH1 and RC. For the Rps. palustris PucBAa LH2 complex the excitation spectrum profile is closely matched by the (1 → %) transmission profile (1 → T) (Fig. 4a), and the extent of the alignment between the two spectra suggests that this LH2 complex is able to transfer energy to the R. sphaeroides LH1 complex. A lack of transfer energy from the foreign LH2 species to the native LH1 complex would have resulted in the absence of a peak in the excitation spectrum at 800 nm, for example. In contrast, Fig. 4b shows the excitation and (1 → T) spectra from the Rps. palustris PucBAa LH2 complex; there are several regions of difference between them, most noticeably in the 800-nm region (indicated by an arrow). The absence of a peak at 800 nm in the excitation spectrum comparable to that in the (1 → T) spectrum indicates that there is a reduced contribution from the B800 bacteriochlorophyll (BchI) of the Rps. palustris PucBAa LH2 complex to the 77 K fluorescence emitted from the R. sphaeroides LH1 complex at 910 nm, compared to the results from the PucBAa LH2 complex (Fig. 4a). This suggests that energy transfer from the B800 absorbance band to the LH1 complex is less efficient in the PucBAa LH2 complex than in the PucBAa LH2 complex, and since it has already been established that B800 to B850 transfer in the PucBAa LH2 is efficient (see Fig. 3 above), it seems probable that it is B850 to LH1 transfer that is affected.

In order to investigate this phenomenon further, emission spectra were recorded at 77 K from the same two samples (Rps. palustris PucBAa and PucBAa LH2) after excitation into both the Bchl Q<sub>e</sub> band at 590 nm and into the Bchl B800 Q<sub>e</sub> band at 790 nm (Figs. 5a and 5b; for each sample the absorption spectrum is shown on the left and the two emission spectra on the right of the figure). Excitation into the Bchl Q<sub>e</sub> band at 590 nm results in both the LH2 and LH1 complexes receiving excitation energy, while excitation into the Bchl B800 Q<sub>e</sub> band at 790 nm leads to most of the excitation energy going solely to the B800 band of the LH2 complex. In this way it was hoped to distinguish the contribution which direct LH1 excitation makes to LH1 emission from that due to indirect LH1 excita-
of a mutant R. sphaeroides LH2 complex, namely one in which the Tyr\(^{13}\) residue is changed to a Phe; one of the reasons for picking this particular mutant LH2 is that it has absorption peaks at 800 and 839 nm at 77 K, providing a blue-shifted B850 peak for comparison with the 830-nm peak in the PucBA\(_d\) LH2 complex. The absorption and emission spectra for the Tyr\(^{13}\) \rightarrow Phe LH2 complex (LH1 and RC are also present) are shown in Fig. 5c; the experimental conditions were identical to those used in obtained Fig. 5, a and b. The absorption spectrum of the Tyr\(^{13}\) \rightarrow Phe LH2 complex shows a pronounced B850 peak at \(\sim 839\) nm at 77 K compared to the situation for the PucBA\(_d\) LH2 complex in which the 830-nm peak (shown by the arrow in Fig. 5b) is barely visible. It is therefore significant that while only a small emission shoulder is seen at \(\sim 860\) nm for the Tyr\(^{13}\) \rightarrow Phe LH2 complex on excitation into its B800 band (Fig. 5c, upper emission trace), there is in contrast a pronounced emission peak observed at \(\sim 855\) nm for the PucBA\(_d\) LH2 complex (Fig. 5b, upper emission trace).

**DISCUSSION**

One reason for choosing Rps. palustris, extending previous work on Rps. acidophila (Fowler et al., 1995), was its ability to modify the cellular absorbance spectrum according to the incident light intensity. However, since several gene pairs are present and transcribed in Rps. palustris, it was thought prudent to identify the LH2 gene/protein pucBA/pucBA pair most associated with LL growth conditions. According to the work of Tadros et al. (1993), the three LH2 beta genes transcribed under LL conditions for Rps. palustris strain 1e5 are pucBA\(_a\), pucBA\(_d\), and pucBA\(_e\), while Evans (1989) gives as a rough estimate of the stoichiometries of the polypeptides retrieved from Rps. palustris cells (strain French) grown under LL conditions \(a_2:b_2:c_2=2:6:1:1\). The protein sequence given in this latter work for \(a_2\), the polypeptide with the highest apparent level in Evans (1989), is the same as that of PucBA\(_d\) in Tadros et al. (1993). These two sets of data convinced us that the pucBA\(_d\) gene pair would be one of those associated with LL growth conditions.

With respect to identifying a single gene pair most associated with HL growth conditions, the situation is less clear. Tadros et al. (1993) concluded that all five LH2 beta genes, pucBA\(_a\), pucBA\(_e\), pucBA\(_d\), pucBA\(_a\), and pucBA\(_e\), are transcribed under HL conditions for Rps. palustris strain 1e5, while the work by Evans and co-workers gives as HL polypeptide stoichiometries \(a_1:b_1:c_1=2:2:1:1\) for Rps. palustris strain French. However, according to Tadros and co-workers only three of the LH2 complexes, PucBA\(_a\), PucBA\(_d\), and PucBA\(_e\), are transcribed under LL conditions, and so for the purposes of the present work we assumed that either PucBA\(_a\) or PucBA\(_d\) LH2 complex was most associated with HL growth conditions. On comparing protein sequences, it became apparent that there was no equivalent to the PucBA\(_d\) complex mentioned in Evans (1989), but it can be seen that the sequence for PucBA\(_a\) is identical to that of the LH subunit \(a_1\) given in the Evans thesis. Therefore, since it shows a relatively high proportion of the total in the protein analysis of membranes from HL grown cells (Evans, 1989), and since according to Tadros and co-workers (1993) pucBA\(_d\) gives a stronger Northern blot signal in preparations from high light-grown cells, it was decided to investigate the gene pair pucBA\(_a\), on the assumption that it encoded an LH2 more associated with HL growth conditions.

The \(\alpha\beta\) protein pairs shown in Fig. 1 were thought to be good candidates for heterologous synthesis in R. sphaeroides, because they show some significant homology to the native proteins. One potential problem was that the N terminus of the PucBA\(_a\) subunit was considerably shortened compared to that of the R. sphaeroides \(\beta\)-subunit, but previous success with
another shortened β-subunit, that encoded by the silent puc<sup>c</sup>BA gene from Rps. acidiphila strain 7050 (Fowler et al., 1995), has demonstrated that such subunits can assemble a functional complex.

As a control experiment, we also investigated a different strain of Rps. palustris called DEP100 (Elder et al., 1993) which, whether grown under HL or LL conditions, gave the same absorption spectrum. Unlike the Rps. palustris strain 2.1.6 used in this work, only a spectrum resembling HL grown Rps. palustris was seen with strain DEP100, whatever the light conditions (data not shown). Using the PCR oligonucleotides designed for the isolation of the genes puc<sub>B</sub>BAd and puc<sub>B</sub>Ad, we attempted to isolate analogous LH2 genes from Rps. palustris strain DEP100. Only the oligonucleotides designed to the puc<sub>B</sub>Ad gene gave a positive PCR fragment (data not shown), leading us to suppose that an equivalent to the puc<sub>B</sub>Ad gene was not present in DEP100; this preliminary observation might explain why DEP100 shows only a HL absorption spectrum. A comparison of the protein sequence identity values shown in Fig. 1 shows that the PucBAd<sub>DEP100</sub> LH2 and the PucBA<sub>Ad</sub> LH2 are very similar, and this suggests that these LH2 complexes have similar roles in the two Rps. palustris strains.

The differences in the near infrared absorption bands seen for the two heterologously synthesized Rps. palustris LH2 complexes PucBA<sub>a</sub> and PucBA<sub>b</sub> (Fig. 2) can probably be explained in the light of their different protein sequences. Previous work has established that the two residues at positions 13 and 14 on the α-subunits of the LH2 complexes are important in determining the final absorbance properties of the B850 band (Fowler et al., 1992). In R. sphaeroides the residues at these positions are both Tyr, residues that seem to be hydrogen-bonded to the 2-acetyl carbonyl groups of the B850 Bchl pigments (Fowler et al., 1994); alteration of these residues to ones with less capacity for hydrogen bond formation, such as Phe and Leu, can lead to a blue shift in the B850 maximum of up to 24 nm (Fowler et al., 1994). Similarly, an examination of the crystal structure of the LH2 complex from Rps. acidiphila (McDermott et al., 1995) shows that the equivalent residues in this complex at positions 13 and 14 (Tyr and Trp) also form hydrogen bonds to the B850 Bchl pigment. At positions +13 and +14 on the α-subunit of the Rps. palustris PucBA<sub>b</sub> LH2 complex, these residues are Tyr and Thr (Fig. 1), both of which amino acids are capable of forming a hydrogen bond with a 2-acetyl carbonyl Bchl group, and this probably explains the pseudo-WT absorbance spectrum seen for this complex. The 5-nm red shift in the B850 band in the PucBA<sub>a</sub> complex might arise from subtle differences in the B850 binding sites of the LH2 complex, akin to those previously postulated for the LH1 complex under similar circumstances (Olsen et al., 1994). In contrast, at positions 13 and 14 on the α-subunit of the Rps. palustris LH2 protein PucBA<sub>b</sub>, one finds the residues Phe and Met (Fig. 1), neither of which are likely to form a hydrogen bond to the 2-acetyl carbonyl Bchl groups, a property which probably explains the blue shift in the B850 band for this complex of ~20 nm.

The heterologously synthesized Rps. palustris LH2 complexes are obviously present in R. sphaeroides at vastly different levels, the PucBA<sub>b</sub> LH2 complex being found at much lower levels than the PucBA<sub>a</sub> LH2 complex, and it is not possible to make any categorical statements as to the reasons behind this difference at the moment. Two observations, however, may be of some relevance. First, the carotenoid content seen for photosynthetically grown WT Rps. palustris differs according to the light intensity; LL grown Rps. palustris contains proportionately more lycopene and less rhodovirgin than HL grown Rps. palustris (Evans, 1989). It is possible that the lack of appropriate carotenoid for the Rps. palustris complexes is likely to lessen their stability, since it is known that LH2 stability is dramatically influenced by the type of carotenoid present (Fowler et al., 1995; Lang and Hunter, 1996). Moreover, when foreign complexes were synthesized in R. sphaeroides, the native LH2 complex was present at reduced levels in the cell (Hunter et al., 1994). Since the R. sphaeroides carotenoids do not match those found in Rps. palustris, this is likely to affect the success in assembling these LH2 complexes. It is possible, therefore, that the PucBA<sub>b</sub> LH2 complex finds the sphaeroidene-dominated R. sphaeroides carotenoid background less suitable than does the PucBA<sub>a</sub> LH2 complex, leading to structural instability in the former LH2. Interestingly, examination of the LH2 structure of Rps. acidiphila (McDermott et al., 1995) shows that the β<sup>–11</sup> residue on the β-subunit (Thr<sup>–11</sup> in PucBA<sub>a</sub> and Ser<sup>–11</sup> in PucBA<sub>b</sub>; Fig. 1) lies very near to a carotenoid molecule, and that the residue in the equivalent position in the β-subunit of R. sphaeroides is also a Thr, implying that a Thr residue at this site is more suitable for the carotenoids of R. sphaeroides. In our previous work (Fowler et al., 1995) a heterologously synthesized Rv. gelatinous LH2, which has a Thr residue at the β<sup>–11</sup> position, gave higher apparent expression levels than an LH2 (Puc<sup>c</sup>BA) from Rps. acidiphila strain 7050, which has an Ala residue at the β<sup>–11</sup> position, an observation that seems to strengthen the hypothesis. Interestingly, examination of the Rps. acidiphila strain 10050 LH2 structure indicates that the methyl group of the Thr residue in this complex lies close to the methyl group of a nearby carotenoid molecule; changing the Thr methyl to a hydrogen (as is seen with Ser) might alter the carotenoid binding site.

The second observation involves the differences in the N termini of the two Rps. palustris β-subunits; not only is PucBA<sub>b</sub> five residues longer than PucBA<sub>a</sub>, it shows greater sequence homology to the R. sphaeroides β-subunit (Fig. 1). Interestingly, a possible link between the level of heterologously synthesized LH2 and the β-subunit N-terminal character can also be seen from our previous work (Fowler et al., 1995) in which a Rv. gelatinous LH2, which has an N-terminal sequence that is quite similar to that of the R. sphaeroides β-subunit, gave higher expression levels than a LH2 (Puc<sup>c</sup>BA) from Rps. acidiphila strain 7050, whose N-terminal sequence is considerably shortened. To sum up, the levels of apparent expression of these heterologously synthesized LH2 complexes may be linked to the availability of suitable carotenoid binding sites and the length of the N termini of the LH2 β-subunits.

The absorption spectra of HL and LL photosynthetically grown Rps. palustris strain 2.1.6 cultures mentioned in the present work were extremely similar to those published previously (van Mourik et al., 1992; Tadros et al., 1993). In the HL grown Rps. palustris cells, the absorbance spectrum, which largely represents the LH2 complex, was essentially like that of the B800–850 LH2 complex from R. sphaeroides (see, for example, Fig. 2a), with the difference that the R. sphaeroides complex has a less intense B800 absorbance band. In contrast, in the LL grown Rps. palustris cells, the B850 band had a shoulder at the short wavelength side, and the B800 band was broadened at the long wavelength side; additionally, the B850 band was less intense relative to the B800 band. It is possible, in view of the result obtained for the heterologously synthesized Rps. palustris PucBA<sub>b</sub> LH2 complex (see earlier), that the B850 short wavelength shoulder and the B800 long wavelength broadening seen for the LL Rps. palustris LH2 are in fact due to an underlying PucBA<sub>b</sub> LH2 absorbance peak at ~830 nm. However, it was reported in van Mourik et al. (1992) that attempts to isolate a separate B800–830 complex from LL Rps. palustris.
palustris membranes were not successful. Interestingly, in the same work a room temperature difference spectrum between the spectra for HL and LL grown Rps. palustris cells showed a peak at ~800 nm with a shoulder at 820–830 nm (van Mourik et al., 1992) (Fig. 2a). This result when taken with the results in the present study suggest that the inability to purify a B800–830 complex from LL Rps. palustris may have been due to technical problems, possibly due to an inherent instability in the complex. The existence of a LL B800–830 LH2 complex in Rps. palustris is in line with observations for other purple bacteria such as Rps. acidophila 7050, in which LL growth conditions lead to the expression of an B800–820 LH2 complex with a blue-shifted B850 band (Cogdell et al., 1983). Finally, it is interesting to speculate that the decreased B850: B800 peak ratio seen in LL-grown Rps. palustris (relative to HL) may be due to the attenuation and blue shifting of the B850 band of some of the LH2 complexes present, rather than to an increase in the B800 band intensity.

For the experiments probing energy transfer within the Rps. palustris LH2 complexes, the presence of an excitation shoulder at 800 nm (Fig. 3) demonstrates that this absorption band plays a role in the production of an emission associated with a B850-like bacteriochlorophyll; thus, it appears that in both these heterologously synthesized complexes (PucBAa and PucBAd), some energy is transferred from the B800 to the B850 pigments.

One possible clue to the relatively poor energy transfer to LH1 from the Rps. palustris PucBAd LH2 may lie in the differences between the Rps. palustris PucBAd β-subunit sequence, and the β-subunit sequences of both the Rps. palustris PucBAd LH2 complex and the R. sphaeroides LH2 complex (Fig. 1). The recent publication of a high resolution crystal structure for the Rps. acidophila 10050 LH2 (McDermott et al., 1995) has shown that the LH2 complex is arranged in a ring of 9 αβ dimers with the β-subunits on the "outside"; it is residues on the outside of the LH2 ring that might be expected to dictate interactions with neighboring light-harvesting assemblies such as LH1, including, perhaps, LH2 to LH1 energy transfer. An examination of the Rps. acidophila 10050 LH2 structure (McDermott et al., 1995) indicates which residues could be expected to be on the outside of the LH2 structure. When these observations are applied to the LH2 β-subunit sequences shown in Fig. 1, the most obvious difference is at residue β 7. In the Rps. palustris PucBAd residue PucBAd7 is a valine residue, while in both the Rps. palustris PucBAd LH2 complex and the R. sphaeroides LH2 complex it is a glycine residue. Following the recent characterization of the LH2 to LH1 energy transfer processes (Hess et al., 1995), it is hoped to clarify this point in future work on site-directed mutagenesis on both native and heterologously synthesized foreign LH2 complexes.

We have demonstrated that it is possible to express heterologously two novel gene pairs from Rps. palustris in R. sphaeroides, and that the complexes, although largely intact, show different functional properties as assessed by steady-state spectroscopy. The LH2 to LH1 energy transfer properties for the two complexes have been measured, and a tentative interpretation of the results in the light of LH2 structural information put forward. This provides some basis for the observations made on the individual expression of genes from a multigene LH2 family (Tadros et al., 1993), and therefore, represents the first opportunity to examine the properties of the complexes encoded by such genes in isolation from other LH2 complexes, experiments that were not possible with membranes from wild-type Rps. palustris or Rps. acidophila. In the future it will be interesting to combine time-resolved spectroscopic studies on these heterologously synthesized high light and low light Rps. palustris LH2 complexes with judicious site-directed mutagenesis experiments to elucidate the structural requirements underlying energy transfer between the light-harvesting complexes of purple bacteria.

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