Deletion of a B800−850 Light-Harvesting Complex in *Rhodospirillum molischianum* DSM119 Leads to "Revertants" Expressing a B800−820 Complex: Insights into Pigment Binding[†]

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ABSTRACT: A B800−850 light-harvesting complex (also called LH2) deficient strain of *Rhodospirillum molischianum* was constructed by replacing a portion of the LH2 gene cluster by a kanamycin resistance gene cartridge. The LH2 deficient strain was characterized spectroscopically and by Southern blot analysis. Surprisingly, pseudorevertants were obtained which express a B800−820 complex which could not be observed in the wild type. This B800−820 complex was isolated and characterized. It consists of an α- and a β-subunit with 56 and 45 amino acid residues, respectively. The amino acid sequences of both subunits are extremely similar to those of the corresponding B800−850 complex. Resonance Raman spectroscopy shows that in the B800−820 complex the two 2-acetylcarbonyl groups of the bacteriochlorophyll *a* (BChl a) molecules absorbing at 820 nm are free from hydrogen bond interactions, whereas one of the two 2-acetylcarbonyl groups of the pair of BChl a molecules absorbing at 850 nm of the B800−850 complex is involved in hydrogen bonds. These different protein−pigment interactions are due to the replacement of αTrp43 in the B800−850 complex by a Phe in the B800−820 complex. Comparison of the amino acid sequences of the B800−850 and B800−820 complexes of *Rs. molischianum* and *Rhodopseudomonas acidophila* reveals a conserved motif comprised of three amino acid residues. Molecular modeling using the known LH2 structure of *Rps. acidophila* Ac10050 indicates that this motif might be important for the precise structural arrangement of the native complex and fine tuning of its spectroscopic properties.

The purple non-sulfur bacteria can grow either chemoheterotrophically in the dark or photoheterotrophically in the light under anaerobic conditions. When growing phototrophically, two major protein−pigment complexes are found in the photosynthetic membranes, namely the reaction center (RC)[1] and light-harvesting (LH) antenna complexes. All purple non-sulfur bacteria express a core antenna complex (LH1), absorbing at 870 nm or longer wavelengths, which is tightly bound to the RC in a fixed stoichiometry. Additionally, most species also express a peripheral antenna complex (LH2) absorbing at 800 and 850 nm, the so-called B800−850 complex. However, some species synthesize a second peripheral antenna complex, B800−820. A range of peripheral antenna complexes from several species of purple non-sulfur bacteria has been isolated and characterized. They all contain an α- and a β-subunit comprising 45−60 amino acid residues each. The native complexes are oligomers of α,β-heterodimers with bound bacteriochlorophyll and carotenoid molecules. In contrast to that of the core antenna complexes, the expression of the peripheral antenna complexes is subjected to a more complex regulation. All peripheral antenna complexes are synthesized in varying stoichiometry with respect to the RC, depending on the environmental conditions like light intensity, temperature, and oxygen, concentration (Aagaard & Sistrom, 1972). Some species, like *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, express one single type of B800−850 complex only. In others, such as *Rhodopseudomonas palustris* strain 2.1.6, the expression of two different types of B800−850 complexes with different α- and β-polypeptides is observed, depending on the light intensity (Evans et al., 1990; Hayashi et al., 1982). A further degree of complexity is found in a different strain of *Rps. palustris*, 1e5 (Tadros et al., 1993; Tadros & Waterkamp, 1989). The genome of this organism contains five LH2 gene clusters that are all transcribed under high light conditions. Under low light conditions, however, only three of them are transcribed. A completely different expression pattern that responds to changes in the growth conditions is observed in *Rhodopseudomonas acidophila* strains Ac7050 and Ac7750 (Gardiner et al., 1993). At high light intensities, only a B800−850 complex is expressed. At lower light intensities, this B800−850 complex is replaced successively by a B800−820 complex. When in addition the temperature is decreased at low light intensities, only a B800−820 complex is synthesized.

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[1] Abbreviations: BChl a, bacteriochlorophyll *a*; FT, Fourier transform; HPLC, high-performance liquid chromatography; LH1, core light-harvesting complex; LH2, peripheral light-harvesting complex; PAGE, polyacrylamide gel electrophoresis; RC, reaction center; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid.

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Table 1: Bacterial Strains and Plasmids

strain/plasmid	relevant characteristic(s)	source/reference
E. coli		
JM83	F$^-$, ara, L (lac-proAB), rpsL, ϕ80dlacZΔM15	Yanisch-Perron et al., 1985
S17-I	recA,pro$^-$,res$^-$,mod$^+$,Tp^r,Sm^r,RP4-2-Tc::Mu-Km::Tn7	Simon et al., 1983
Rs. molischianum		
DSM119	wild type	DSM, Braunschweig, FRG
plasmids		
p9/11	pBluescript II KS derivate + 2300 bp of *Rs. molischianum* DNA	Germeroth et al., 1993
pDVL	pSup202SacB derivate + 2300 bp *Rs. molischianum* DNA	this study
pRK404	RK2 derivate-mob$^+$,Tc^r	Ditta et al., 1985
pRKL	pRK404 derivate + *sac* R-*sac* B cartridge	this study
pSup202	pBR325-mob$^+$,Ap^r,Cm^r,Tc^r	Simon et al., 1983
pSup202SacB	pSup202 derivate + *sac* R-*sac* B cartridge	this study
pUM24Cm	pUC derivate + *sac* R-*sac* B cartridge	Ried & Collmer, 1987

The structure of the B800−850 complex from *Rps. acidophila* Ac10050 has recently been determined by X-ray crystallography at a resolution of 2.5 Å (McDermott et al., 1995). The native complex consists of nine α,β-heterodimers which bind nine bacteriochlorophyll *a* (BChl a) molecules absorbing at 800 nm and 18 absorbing at 850 nm. The nine α- and β-subunits form two concentric cylinders. The 18 BChl a molecules absorbing at 850 nm are located between the inner and the outer cylinder, forming a continous overlapping ring. The Mg atoms of the nine BChl a molecules absorbing at 800 nm are liganded by the N-terminal formylmethionines of the α-subunits.

The structure of the LH2 provides much insight into the mode of the pigment binding. However, the precise protein−pigment interactions which are responsible for the specific absorbance characteristics of the antenna complexes remain to be determined. Brunisholz and Zuber (1988) proposed that the interaction of conserved aromatic residues is responsible for the observed red shift of the bound BChl a in the antenna complexes with respect to the absorption maximum of monomeric BChl a. Fowler et al. (1992) found a progressive blue shift of the B850 BChl a molecules from the *Rb. sphaeroides* B800−850 complex when mutating the highly conserved residues αTyr44-Tyr45 to Phe-Tyr (838 nm) and to Phe-Leu (826 nm), respectively. It is more likely that it is the hydrogen bond capability of the tyrosine residues and not the aromatic character which caused the blue shift. Fourier transform (FT) resonance Raman studies demonstrated that the blue shift in the mutants is accompanied by a loss of the hydrogen bonds to the two 2-acetylcarbonyl groups of the 850 nm-absorbing BChl a pair (Fowler et al., 1994). However, an absorbance spectrum corresponding to that of an authentic B800−820 complex was not observed, suggesting that more parameters contribute.

A similar blue shift was observed in a LH1 mutant. Olsen et al. (1994) changed the highly conserved αTrp43 in the LH1 of *Rb. sphaeroides* to Phe. The absorption maximum of this mutant shifted from 876 to 853 nm. FT resonance Raman studies confirmed a loss of a hydrogen bond to the 2-acetylcarbonyl group of the 876 nm-absorbing BChl a. Thus, site-directed mutagenesis demonstrated that the removal of one hydrogen bond to BChl a leads to a shift in the absorption maxima of more than 20 nm.

The B800−850 complex of *Rhodospirillum molischianum* DSM119 is unusual. Its amino acid sequence and its resonance Raman spectrum are partly similar to those of B870 complexes, while the absorbance characteristics are similar to those of other B800−850 complexes (Germeroth et al., 1993).

In this report, we describe the deletion of the LH2 gene cluster of *Rs. molischianum*. Unexpectedly, one out of 10^8 of the deletion mutants expresses a B800−820 complex that is not expressed in the wild type. This B800−820 complex was isolated and characterized biochemically and spectroscopically. The amino acid sequences of the α- and the β-subunit of this B800−820 complex are extremely similar to those of the B800−850 complex. Thus, the *Rs. molischianum* B800−820 complex offers the possibility to determine which amino acid residues are involved in the blue shift of its absorbance maximum compared to that of the corresponding B800−820 complex. The sequence data, resonance Raman spectroscopy, and model building indicate that differences in hydrogen bonding either directly or indirectly by causing slight structural rearrangements are responsible for the blue shift of B800−820 complexes.

EXPERIMENTAL PROCEDURES

Media, Strains, Plasmids, and Growth Conditions. *Rs. molischianum* DSM119 was grown photoheterotrophically in M27 medium at 28 °C as described previously (Germeroth et al., 1993). The *Escherichia coli* strains JM83 and S17-I were grown in LB medium at 37 °C. Antibiotics were added where appropriate in the following concentrations: *E. coli*, ampicillin (100 μg/mL), tetracycline (20 μg/mL), kanamycin (50 μg/mL), streptomycin (50 μg/mL), chloramphenicol (50 μg/mL); *Rs. molischianum*, tetracycline (10 μg/mL), kanamycin (20 μg/mL), chloramphenicol (20 μg/mL). Bacterial strains and plasmids are listed in Table 1.

Materials. All restriction endonucleases and nucleic acid-modifying enzymes were obtained from New England BioLabs (Beverly, MA) or Gibco BRL (Eggenstein, FRG). All antibiotics were purchased from Sigma (München, FRG). Fractogel TSK HW-55(S) was obtained from Merck (Darmstadt, FRG) and LDAO (30% solution) from Fluka-Chemie (Neu-Ulm, FRG). All other chemicals were of analytical grade and were purchased from local distributors.

DNA Isolation and Manipulation. Standard recombinant DNA techniques were performed according to Maniatis et al. (1989), if not indicated otherwise.

Genomic DNA was prepared as described by Laussermair and Oesterhelt (1992). Southern blot analysis was performed with chromosomal DNA transferred to a nylon membrane (Biodyne A, PALL, Portsmouth, U.K.) by capillary actions. For labeling of probes and detection of DNA, the nonradioactive DNA-labeling and detection kit from Boehringer-Mannheim (Mannheim, FRG) was used.

The *E. coli* strain S17-I was used to mobilize plasmid DNA into *Rs. molischianum* as described elsewhere (Davies

et al., 1988). The addition of phage T4D as described in that protocol was omitted. When the expression of the *sac* B gene was required, the bacteria were plated on selective medium supplemented with 5% sucrose.

Isolation and Purification of the B800−820 Complex. The α- and β-subunits of the B800−820 complex from *Rs. molischianum* were isolated and purified according to the protocol of Germeroth et al. (1993) except that the solubilized antenna complex was subjected to an additional anion-exchange chromatography step (MonoQ 10/10, Pharmacia) prior to molecular sieve chromatography.

Amino Acid Sequence Analysis. Protein sequencing was performed on an Applied Biosystems gasphase sequencer 477 A equipped with an on-line 120 APTH analyzer according to the instructions of the manufacturers.

Spectroscopy. Screening for LH2 deficient strains of *Rs. molischianum* was performed with a microscope-photometer (Zeiss Microscope UEM, Photometer 03) interfaced with a computer (HP 300 series) and controlled by the λ-Scan software package (Zeiss). Slit width and step width were 1 nm each. Absorption spectra were measured with a SLM Aminco DW-2000 spectrophotometer with an optical band width of 2 nm.

The Raman measurements were carried out at room temperature with CW excitation provided by an Ar^+ laser (Spectra Physics, model 2020-05). The excitation wavelength used was 363.8 nm (Ar^+ laser) with a laser power of 20 mW. The laser beam, polarized perpendicular to the scattering plane, was focused by a cylindrical lens with a 10 cm focal length onto the sample which was situated in a cylindrical quartz cuvette. The scattered light was recorded in backscattering geometry. It was collected by a camera objective (Astro objective, 75 mm, effective *f*/2.5) and imaged on the entrance slit of the spectrometer (Spex 1401) equipped with a CCD camera (Photometrics, SDS 9000) containing a SITE chip (TK 512 CB). The data were collected digitally with a computer, which also controlled the spectrometer.

RESULTS

Effect of sac B Expression in Rs. molischianum Cells. Deletion of the *Rs. molischianum* LH2 genes was accomplished using the conditionally lethal *sac* B gene from *Bacillus subtilis* (Ried & Collmer, 1987). The *sac* B gene codes for a 50 kDa levansucrase whose expression is induced by sucrose. Growth of *Rs. molischianum* wild type was not influenced by the addition of 5% sucrose to the medium.

After conjugational transfer of the vector pRKL, which was constructed by cloning the 2.6 kb *Pst*I restriction endonuclease *sac* R-*sac* B cartridge from pUM24Cm into the *Pst*I site of the broad host range plasmid pRK404, no transformants could be obtained on medium containing 5% sucrose, but about 10^7 colonies could be observed on medium without sucrose. This result indicates that the expression of the *sac* B gene is lethal in *Rs. molischianum.*

Construction of a LH2 Deletion Strain of Rs. molischianum. A vector for the deletion of the LH2 genes was obtained by replacing a 770 bp *Eco*47III/*Eco*NI restriction endonuclease fragment (converted to blunt ends) of the 2.3 kb *Bam*HI restriction endonuclease genomic fragment of *Rs. molischianum*, containing the LH2 gene cluster, with a 1.8 kb kanamycin resistance cartridge (see Figure 1). This

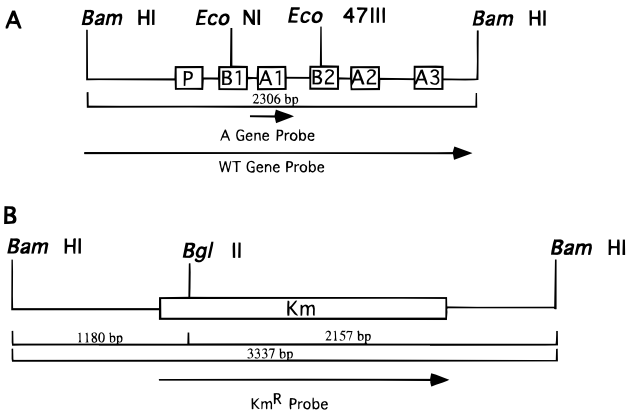

FIGURE 1: Modification of the *Rs. molischianum* LH2 gene cluster. The arrows indicate the positions of the probes used for the Southern blot analysis. The numbers show the size of the restriction endonuclease fragments. (A) The 2.3 kb *Bam*HI genomic fragment from plasmid p9/11 containing the promotor (P) and the structural genes B1 and B2 coding for the subunits β_1 and β_2 and A1−A3 coding for the subunits $\alpha_1-\alpha_3$. (B) The 3.3 kb fragment resulting from deletion of the 770 bp *Eco*47III/*Eco*NI fragment and replacement with the 1.8 kb Km resistance gene.

recombination cartridge was cloned into the *Bam*HI site of the suicide plasmid pSup202SacB in order to generate plasmid pDVL. The vector pSup202SacB was constructed by cloning the 2.6 kb *sac* R-*sac* B cartridge from pUM24Cm into the *Pst*I site of pSup202.

The plasmid pDVL was transferred into *Rs. molischianum* via conjugation. In order to select directly for double cross-over recombinants, the conjugation mixture was plated on medium containing 5% sucrose.

Single cross-over recombinants would be predicted to show the phenotype $Km^rCm^rLH2^+$, while double cross-over recombinants should show the phenotype $Km^rCm^sLH2^-$. Four of the 4.5×10^5 colonies obtained displayed a $Km^rCm^sLH2^-$phenotype, while all other transformants showed the phenotype predicted for single cross-over mutants (see Figure 3A,B).

Southern blot analysis of the genomic DNA of the LH2 deficient mutants confirmed that the chromosomal *puc* operon was interrupted by the Km resistance gene by a double cross-over recombination (see Figure 2). Hybridization with the Km probe demonstrated the integration of the 1.8 kb Km resistance gene and the absence of the 770 bp *Eco*47III/*Eco*NI fragment of the LH2 gene cluster, resulting in a 3.3 kb *Bam*HI and a 2.2 kb *Bam*HI/*Bgl*II restriction fragment, respectively. The predicted 1.2 kb *Bam*HI/*Bgl*II fragment was not detected by the Km probe because the overlap between the Km probe and this 1.2 kb fragment comprises only 110 bp (see Figure 1). This 1.2 kb *Bam*HI/*Bgl*II fragment was detected using the wild type gene probe. No hybridization signal was observed with the pSup probe, demonstrating that the suicide vector had not been integrated into the chromosome. Using the smaller A gene probe instead of the 2.3 kb wild type gene probe, two weaker additional bands at 2.8 and 8.0 kb for the digestion with *Bam*HI and at 1.8 and 3.5 kb for *Bam*HI/*Bgl*II digestion, respectively, are observed, indicating that the genomic DNA contains at least two gene clusters with sequence similarities to the deleted LH2 gene cluster.

Expression of the B800−820 Complex. Three days after the LH2 deficient strain of *Rs. molischianum* was plated on solid medium containing Km, dark red colonies grew on the

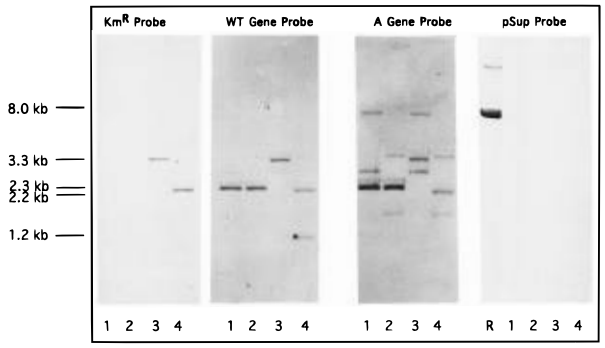

FIGURE 2: Southern blot analysis of the LH2 deficient strain confirming the replacement of a 770 bp fragment of the LH2 gene cluster with the 1.8 kb Km resistance gene. Lanes 1 and 2 contain digested genomic DNA of the wild type strain, while lanes 3 and 4 contain those from the deletion strain. The DNA was digested with *Bam*HI in lanes 1 and 3 and with *Bam*HI/*Bgl*II in lanes 2 and 4. Lane R contain the plasmid pSup202. As shown in Figure 1, the wild type gene probe was the 2.3 kb *Bam*HI genomic fragment containing the LH2 gene cluster; the Km probe was the 1.8 kb Km resistance gene. The A gene probe was a 139 bp *Sal*I/*Eco*47III fragment from p9/11, and the pSup probe was a 1545 bp *Pst*I/*Pvu*II fragment of pSup202. The position of the DNA markers is indicated on the left.

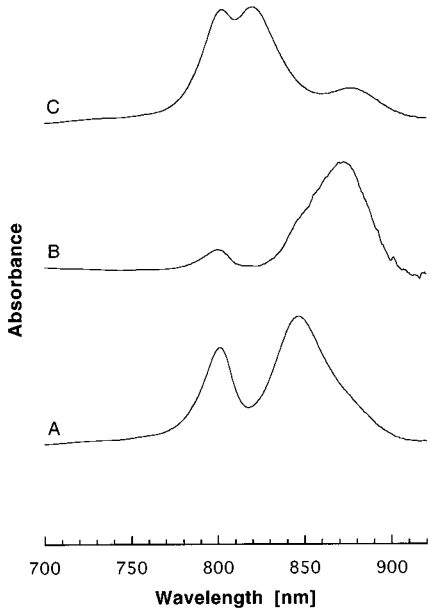

FIGURE 3: Near-infrared spectra of the *Rs. molischianum* wild type (A), the *Rs. molischianum* mutant strain deficient of the B800−850 complex (B), and the *Rs. molischianum* deletion strain expressing a B800−820 complex (C).

orange-colored LH2 deficient colonies. Spectroscopic characterization of these dark red colonies demonstrated that they synthesize a B800−820 complex (see Figure 3C). Southern blot analysis using the A gene probe is performed for both genomic DNA from a LH2 deletion strain expressing a B800−820 complex and those from a LH2 deletion strain not expressing a B800−820 complex. Both samples show the bands at the same positions, indicating that the expression of the B800−820 complex was not due to recombination events within the LH2 gene cluster (data not shown).

Purification of the Native B800−820 Complex and Isolation and Sequencing of the α- and β-Subunits. The B800−820 complex was purified by molecular sieve chromatography and by anion-exchange chromatography. The B800−820 complex elutes from the anion-exchange column in two

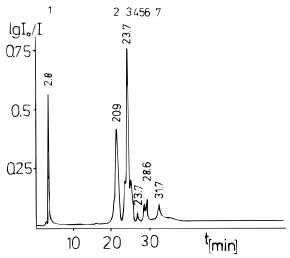

FIGURE 4: Elution profile of the *Rs. molischianum* B800−820 complex, separated by reversed-phase HPLC. The reversed-phase HPLC was performed using a Vydac C_{18} column with a continuous gradient from starting solvent (acetonitrile/water/TFA, 1:1:0.001 v:v:v) to elution solvent (2-propanol/TFA, 1:0.001 v:v) applied for 60 min at a flow rate of 2 mL/min. Peaks 2 and 3 correspond to the β- and the α-subunit as shown in Figure 5; peaks 4−7 contain pigments as shown by their spectra.

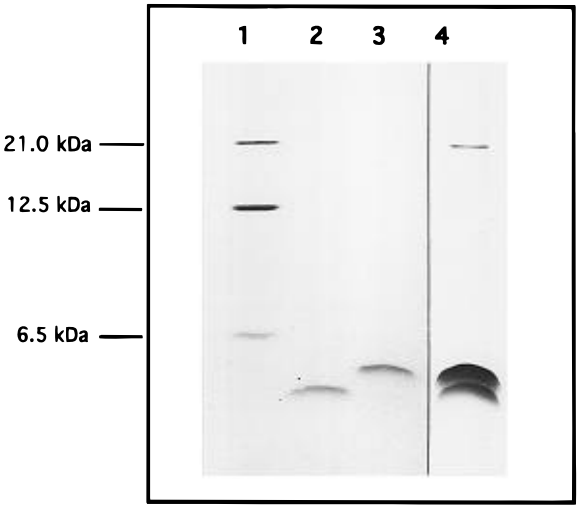

FIGURE 5: Tricine−SDS−PAGE of the α- and β-subunits from the *Rs. molischianum* B800−820 complex [separating gel, 16% T, 6% C; spacer gel, 10% T, 3% C; stacking gel, 4% T, 3% C according to Schägger and Jagow (1987)]: lane 1, standard proteins with molecular mass markers on the left; lane 2, β-subunit of the B800−820 complex (corresponding to peak 2 in Figure 5); lane 3, α-subunit of the B800−820 complex (corresponding to peak 3 in Figure 5); lane 4, B800−820 complex incubated at 100 °C for 5 min.

fractions which show identical absorption spectra and the same electrophoretic mobilities (data not shown).

The separation of the two subunits was achieved by reversed-phase HPLC. Seven peaks were observed (see Figure 4). Peaks 2 and 3 correspond to the β- and α-subunits, respectively, as shown by SDS−PAGE (see Figure 5). Peaks 4−7 correspond to pigments as shown by their spectroscopic properties (data not shown). The absorption spectrum and SDS−PAGE confirmed that peak 1 does not contain protein (data not shown) and might be an artifact due to the elution solvent at the beginning of the gradient.

The amino acid sequences of the α- and β-polypeptides were determined by automated Edman degradation (see Figure 7). The complete sequence of the β-subunit was determined by degradation of the intact chain. The N-terminal sequence of the α-subunit (up to position 31) was determined by sequencing the intact chain, the C-terminal sequence by sequence analysis of peptides obtained after partial digestion with pepsin.

Electrospray mass spectroscopy of both subunits yielded molecular masses of 6083 Da and 5116 Da, respectively,

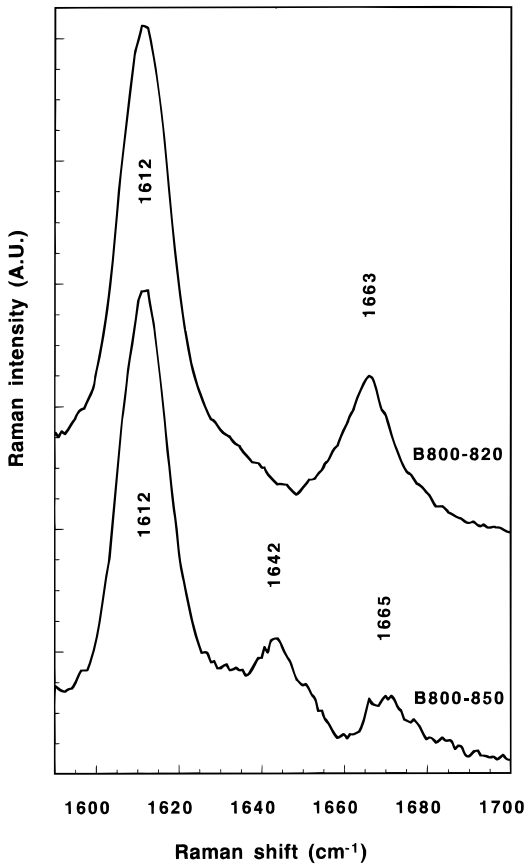

FIGURE 6: Resonance Raman spectra of the B800−820 and B800−850 complexes of *Rs. molischianum* recorded at room temperature with an excitation wavelength of 363.8 nm.

which are in agreement with the molecular masses calculated from the amino acid sequences. which are in agreement with the molecular masses calculated from the amino acid sequences.

Resonance Raman Spectroscopy. The high-frequency regions (1600−1700 cm^{-1}) of the resonance Raman spectrum obtained from both the B800−850 complex and the B800−820 complex are shown in Figure 6. This region of the resonance Raman spectrum is dominated by an intense band at either approximately 1600 or 1615 cm^{-1}. This band arises from the methine bridge-stretching mode of the BChl a and is sensitive to the coordination state of the central Mg ion (Cotton & Van Dyne, 1981). Its position at 1600 cm^{-1} indicates the existence of two axial ligands, whereas at 1615 cm^{-1}, it indicates the existence of only one external ligand. In the range from 1620 to 1700 cm^{-1}, bands are found resulting from the stretching modes of the carbonyl groups which are in conjugation with the delocalized tetrapyrrole system, namely, the 2-acetyl and 9-ketocarbonyl groups. When these groups do not interact with hydrogen bond donors, the corresponding bands are observed at 1660 and 1700 cm^{-1}, respectively. Upon formation of hydrogen bonds, these bands shift down up to 40 wavenumbers, depending on the strength of the hydrogen bonds formed (Robert & Lutz, 1985).

The resonance Raman spectrum of the *Rs. molischianum* B800−820 complex shows two bands at 1612 and 1663 cm^{-1}. The band at 1612 cm^{-1} can be assigned to the methine bridge-stretching mode of the BChl a, indicating that the cental Mg ion is pentacoordinated. The band at 1663 cm^{-1} can be attributed to an unbound 2-acteylcarbonyl group or a bound 9-keto carbonyl group. The corresponding bands for the B800−850 complex lie at 1612, 1642, and 1665 cm^{-1}. The assignment of these bands was done by Germeroth et al. (1993). The band at 1642 cm^{-1} is attributed to a bound 2-acetylcarbonyl group and the band at 1665 cm^{-1} to a free 2-acteylcarbonyl group. The bands for the 2-acteylcarbonyl and the 9-ketocarbonyl groups of the 800 nm-absorbing BChl a are not detectable in the resonance Raman spectrum because of their intrinsic weakness under these conditions of excitation (Germeroth et al., 1993). Consequently, the band at 1663 cm^{-1} in the resonance Raman spectrum of the B800−820 complex can be assigned to an unbound 2-acteyl-carbonyl group. This demonstrates that the two 2-acetyl-carbonyl groups of the 820 nm-absorbing BChl a pair are free from hydrogen bond interactions. In contrast to this,

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                                  1   5   10   15   20   25   30   35   40   45   50   55
Rs. molischianum  B800-820 α      SNPKDDYKIWLVINPSTWLPVIWIVALLTAIAVHSFVLSVPGYNFLASAAAKTAAK
Rs. molischianum  B800-850 α      SNPKDDYKIWLVINPSTWLPVIWIVATVVAIAVHAAVLAAPGFNWIALGAAKSAAK
Rs. molischianum  B870 α             MWKIWTLYDPRRTLSGLFTFLTVLGLLIHFLLLSTDRFNWLDGAREAHNV
Rps. acidophila   Ac7050 B800-820 α  MNQGKIWTVVPPAFGLPLMLGAVAITALLVHAAVLTHTTWYAAFLQGGVKKAA
Rps. acidophila   Ac7750 B800-820 α  MNQGKIWTVVNPAVGLPLLLGSVAITALLVHLAVLTHTTWFPAFTQGGLKKAA
Rps. acidophila   Ac7050 puc 1 α     MNQGKIWTVVDPAVGIPLLLGSVAVTALLVHLAILQNTTWFPAFMQGGLKKAAAIVQVVG
Rps. acidophila   Ac7050 puc 2 α     MNQGKIWTVVNPAVGFPLLLGSVAITALLVHLAVLTHTTWFPAFMQGGLKKAAAIEHVVG
Rps. acidophila   Ac7050 puc 3 α     MNQGKIWTVVNPAIGLPLLLGSVAITALLVHLAVLTHTTWFPAYMQGGLKKAAAIEHVVG
Rps. acidophila   Ac7050 puc 4 α     MNQGKIWTVVNPSVGLPLLLGSVTVIAILVHLAVLSNTKWFPAYWQGGLKKAAAIETTIVG
Rps. acidophila   Ac7050 B800-850 α  MNQGKIWTVVNPSVGLPLLLGSVTVIAILVHAAVLSHTTWFPAYWQGGLKKAA
Rps. acidophila   Ac7750 B800-850 α  MNQGKIWTVVNPAIGIPALLGSVTVIAILVHLAILSHTTWFPAYWQGGVKKAA
Rps. palustris    B800-850 αa      MNQARIWTVVKPTVGLPLLLGSVTVIAILVHFAVLSHTTWFSKYWNGKAAAIESSVNVG
Rps. palustris    2.6.1. B800-850 α MNQGRIWTVVNPGVGLPLLLGSVTVIAILVHYAVLSNTTWFPKYWNGATVAAPAAA...
Rb. sphaeroides   B800-850 α       MTNGKIWLVVKPTVGVPLFLSAAFIASVVIHAAVLTTTTWLPAYYQGSAAVAAE
Rb. sphaeroides   B870 α          MSKFYKIWMIFDPRRVFVAQGVFLFLLAVMIHLILLSTPSYNWLEISAAKYNRVAVAE
Rb. capsulatus    B870 α          MSKFYKIWLVFDPRRVFVAQGVFLFLLAVLIHLILLSTPAFNWLTVATAKHGYVAAAQ
Rps. marina       B880 α             MWKVWLLFDPRRTLVALFTFLFVLALLIHFILLSTDRFNWMQGAPTAPAQTS

                                  1   5   10   15   20   25   30   35   40   45
Rs. molischianum  B800-820 β      AERSLSGLTEEEAVAVHDQFKTTFSAFILLAAVAHVLVWIWKPWF
Rs. molischianum  B800-850 β      AERSLSGLTEEEAIAVHDQFKTTFSAFIILAAVAHVLVWVWKPWF
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FIGURE 7: Amino acid sequences of the α- and *β*-subunits of the *Rs. molischianum* B800−820 complex compared to those of other core and peripheral antenna complexes of other non-sulfur purple bacteria. Conserved residues discussed in the text are marked bold.

one of the two 2-acetylcarbonyl groups of the 850 nm-absorbing BChl a pair is involved and the other one is not involved in intermolecular H bond interactions.

DISCUSSION

In this report, the deletion of a B800−850 complex gene cluster in *Rs. molischianum* DSM119 is described. To our surprise, frequently, pseudorevertants are obtained which synthesize a B800−820 complex. The expression of this complex could not be observed in the wild type. However, a B800−820 complex is formed in *Rs. molischianum* DSM120, depending on the temperature and/or light intensities (unpublished results).

In contrast to the construction of the corresponding LH2 deletion strain of *Rb. sphaeroides* (Burgess et al., 1989), all attempts to obtain an LH2 deletion strain of *Rs. molischianum* without using further selection pressure failed. First, we attempted to obtain a LH2 deletion strain by conjugational transfer of a suicide plasmid not coding for the *sac* B gene. All transformants obtained were single cross-over mutants which showed a $LH2^+$ phenotype. We tried to select for double cross-over mutants by exposing single cross-over mutants to light of wavelengths longer than 870 nm. Even under these conditions, where the growth rates of $LH2^+$ and $LH2^-$ strains should be equal, only single cross-over mutants could be observed. Only application of the technique of Ried et al. (1987) led to the deletion of the LH2 gene cluster. Four out of 4.5×0^5 transformants growing on a medium containing 5% sucrose after conjugational transfer of the deletion vector were phenotypically deficient in LH2. This corresponds to an efficiency of 9×10^{-4}%. This efficiency is significantly lower than the efficiency reported by Cai et al. (1990), who deleted the *nif* D gene in *Anabaena* sp. applying the same technique. They observed double cross-over mutants with an efficiency of 95%. In contrast to our experiments, the deletion of the *nif* D gene was achieved under conditions where this gene is not expressed.

The absence of the LH2 genes was shown by Southern blot anaysis and the absence of the B800−850 complex by optical spectroscopy. Southern blot analysis using a short hybridization probe indicated that the genome of *Rs. molischianum* contains at least two additional genomic fragments with sequence similarities to the 2.3 kb genomic fragment coding for the expressed wild type B800−850 complex. When grown phototrophically, about one out of 10^8 colonies of the B800−850 complex deficient strain produced a B800−820 complex. The expression of this B800−820 complex was not observed in wild type, either under different light or under different temperature conditions. The formation of a B800−820 complex which is not produced in the wild type under various growth conditions after deletion of the B800−850 complex has not been observed in other species of non-sulfur purple bacteria. Some bacteria like *Rb. sphaeroides* (Kiley et al., 1987) and *Rb. capsulatus* (Youvan et al., 1985) only express one kind of B800−850 complex. In contrast in *Rps. palustris* strain 1e5, five genes were found coding for a B800−850 complex (Tadros et al., 1993; Tadros & Waterkamp, 1989). These genes were alternatively expressed, depending on the environmental conditions, especially the light intensity. A different regulation of the expression of peripheral antenna complexes was found in *Rps. acidophila*. All known strains of this species were able to express a B800−850 as well as a B800−820 complex. Under high light conditions, only a B800−850 complex was expressed. When the light intensity was lowered, in addition to this B800−850 complex, a B800−820 complex was synthesized. When the light intensity was decreased, the B800−820 complex was expressed to a greater extent than the B800−850 complex. When the temperature was decreased (at low light intensities), only the expression of a B800−820 complex could be observed. Thus, the conditions for the expression of light-harvesting complexes are different among the various species of non-sulfur purple bacteria. Those of *Rs. molischianum* are most comparable to those of *Rps. acidophila*, but in contrast to the latter, *Rs. molischianum* DSM119 seems to have lost the capacity to express the B800−820 complex in the wild type. The exact mechanism responsible for the activation of the silent genes coding for the B800−820 complex in the deletion strain of *Rs. molischianum* has not been determined in this study. As judged from the results of both Southern blot analysis and PCR studies (unpublished data), a recombinational event can be excluded. The ratio of one colony of the deletion strain expressing a B800−820 complex per 10^8 suggests that the activation could be due to a single point mutation in the regulatory region of the gene cluster coding for the B800−820 complex.

The protein sequences of both the B800−820 and the B800−850 complex from *Rs. molischianum* display a high degree of similarity (see Figure 7). The β-subunit of the B800−820 complex even has the same length and, apart from three amino acid residues, the same amino acid sequence as the β-subunit of the corresponding B800−850 complex. All three amino acid residues are very similar in size and hydrophobicity, and thus, the β-subunits of both the B800−850 complex and the B800−820 complex are identical in charge distribution, hydrophobicity, and potential hydrogen bond formation. The amino acid sequences of α-subunits of both antenna complexes are slightly more different. Both are equal in length; their N-terminal amino acid sequences are identical up to position 26, and their C-terminal amino acid sequences are almost identical. Significant differences in the amino acid sequences of both complexes are formed in the α-subunit between positions 27 and 48 where 10 amino acid residues are exchanged. The high homology in the protein sequence of the B800−850 and the B800−820 complex of *Rs. molischianum* permits conclusions about the contribution of indicated amino acid residues to the specific absorption of the corresponding antenna complexes.

Resonance Raman spectroscopy demonstrates that in the B800−820 complex of *Rs. molischianum* the two 2-acetylcarbonyl groups of the 820 nm-absorbing BChl a pair are not involved in hydrogen bond interactions, whereas in the corresponding B800−850 complex, one of the two 2-acetylcarbonyl groups of the 850 nm-absorbing BChl a pair is involved in hydrogen bond interactions. The absence of any hydrogen bonds to the 2-acteylcarbonyl group of the 820 nm-absorbing BChl a can only be due to an amino acid residue acting as a hydrogen bond donor in the B800−850 complex that is replaced by an amino acid incapable of forming a hydrogen bond in the B800−820 complex. The only candidate is αTrp45 of the B800−850 complex which is replaced by Phe in the B800−820 complex.

Recent work with mutants from light-harvesting complexes of other non-sulfur purple bacteria showed that a loss of hydrogen bonds to the 2-acetylcarbonyl groups of the BChl

a leads to a blue shift in the absorption maxima. Fowler et al. (1992) constructed two double mutants of the *Rb. sphaeroides* B800−850 complex. They mutated αTyr44 and αTyr45 to Phe-Tyr and Phe-Leu, respectively, and observed a blue shift in the absorption maximum from 850 to 839 and 826 nm, respectively. FT resonance Raman studies on these mutants demonstrated the loss of one or two hydrogen bonds, respectively, to the 2-acetylcarbonyl group of the 850 nm-absorbing BChl a (Fowler et al., 1994).

However, it is noteworthy that the amino acid sequence of the α-subunit of the *Rs. molischianum* B800−820 complex as well as that of the corresponding B800−850 complex is more similar to those of the B870 complexes of other species. Thus, as shown in Figure 7, all known B800−850 complexes, except that from *Rs. molischianum*, display a motif consisting of two aromatic amino acids capable of forming hydrogen bonds at position 44−45 of the α-subunit (*Rb. sphaeroides* numbering corresponding to positions 47 and 48 of *Rs. molischianum*). These two aromatic residues form hydrogen bonds to the two 2-acetylcarbonyl groups of the 850 nm-absorbing BChl a pair as shown by resonance Raman spectroscopy (Fowler et al., 1994; Sturgis et al., 1995) and as shown by the X-ray structure determination of the B800−850 complex from *Rps. acidophila* Ac10050 (McDermott et al., 1995). In the corresponding B800−820 complexes, these two aromatic residues are replaced by one aromatic residue, incapable of forming a hydrogen bond, and one aliphatic residue. Thus, the two 2-acetylcarbonyl groups of the 820 nm-absorbing BChl a pair of these complexes are free from H bond interactions (Sturgis et al., 1995). The C-terminal amino acid sequences of the α-subunits for both the B800−850 and the B800−820 complexes of *Rs. molischianum* do not display these conserved amino acid residues but are more similar to the amino acid sequences of the B870 complexes of other species. Moreover, only one 2-acetylcarbonyl group of the 850 nm-absorbing BChl a pair is involved in hydrogen bond interactions. This strongly suggests that the presence or absence of hydrogen bonds to the 2-acetylcarbonyl groups of BChl a is only one factor contributing to the shifts of the BChl a absorption maxima up to 30 nm relative to each other.

A sequence comparison of the α-subunits of the peripheral antenna complexes from *Rps. acidophila* and *Rs. molischianum* indicates a conserved three-amino acid motif located in positions 27−29 of *Rs. molischianum* (residues 24−26 in *Rps. acidophila*, respectively; see Figure 7). The *Rps. acidophila* sequences *puc* 1 to *puc* 4 were recently obtained by Gardiner (personal communication). The absorption characteristics of the encoded antenna complexes can be assigned from residues 44 and 45 as discussed above. Thus, *puc* 4 codes for a B800−850 complex, whereas *puc* 1, *puc* 2, and *puc* 3 code for B800−820 complexes. As seen from Figure 7, in both *Rs. molischianum* and *Rps. acidophila*, the complexes absorbing at 850 nm display the motif Thr followed by two hydrophobic residues, whereas complexes absorbing at 820 nm show the motif Thr preceded by two hydrophobic residues.

Model building using the coordinates of the recently published structure of the *Rps. acidophila* Ac10050 B800−850 complex (McDermott et al., 1995) shows that the replacement of residue αIle26 by Thr, simulating the corresponding motif of B800−820 complexes, might allow the formation of a hydrogen bond between αThr26 (corresponding to position 29 in *Rs. molischianum*) and the carbonyl oxygen of the methyl ester carbonyl group of ring V in the BChl a molecule (see Figure 8). However, the hydrogen bond would be a rather long and weak one without structural rearrangements. The importance of this methyl ester carbonyl group for the formation of native light-harvesting complexes was demonstrated by Loach et al. (1990). They established a method to form native LH1 by reassociating α- and β-polypeptides with free BChl a. They failed to obtain native LH1 complexes when using modified BChl a molecules where the methyl ester carbonyl group was replaced by either a hydrogen atom or a hydroxyl group. This methyl ester carbonyl oxygen is not in conjugation with the delocalized electron system of the tetrapyrrole ring, and thus, a hydrogen bonding to this carbonyl oxygen is not expected to influence directly the absorption maximum of the antenna complex. On the other hand, this potential hydrogen bond might be important for structural reasons. It is known from in vitro work that different aggregation states cause blue shifts in the absorption maxima of bound BChl a (Visschers et al., 1991). This result implies that the precise orientation and the distances between the BChl a molecules influence the absorbance characteristics of the light-harvesting complexes. Thus, the hydrogen bond in the motif discussed above for the B800−820 complex could cause a slight reorientation of the BChl a molecules absorbing then at 820 nm and contribute indirectly to the blue shift of absorption maxima.

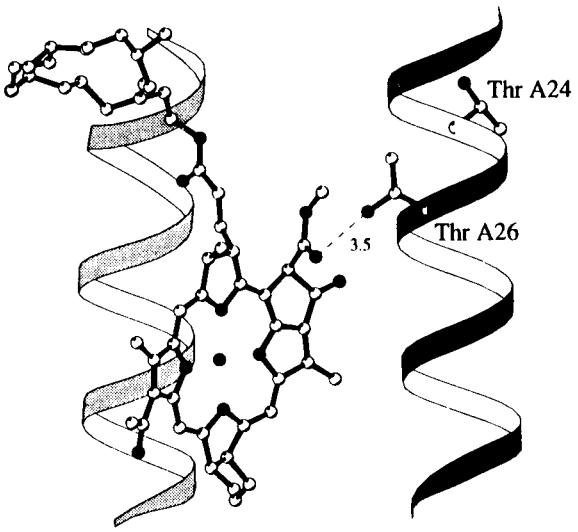

FIGURE 8: Partial model of the LH2 structure of *Rps. acidophila* Ac10050 where the residue Ala26 was exchanged with Thr (modeled with computer program O). The helix on the right represents a part of the backbone of the α-subunit and the helix on the left that of the β-subunit. Between the two helices, a 850 nm-absorbing bacteriochlorophyll *a* tetrapyrrole ring forming a hydrogen bonding to the residue αThr26 is shown. The length of the possible hydrogen bond between the donor and acceptor is indicated in angstroms. The coordinates of the LH2 complex from *Rps. acidophila* Ac10050 were obtained from R. J. Codgell.

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