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Biochimica et Biophysica Acta 1230 (1995) 147–154

BBA Biochimica et Biophysica Acta

Spectroscopic properties of the light-harvesting complexes from *Rhodospirillum molischianum*

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Received 14 November 1994; revised 10 March 1995; accepted 20 March 1995

Abstract

Spectroscopic properties, including low-temperature absorbance, linear and circular dichroism and site-selection fluorescence of the antenna complexes from *Rhodospirillum molischianum* have been determined. The unique 'LH1-like' character of the amino acid sequence from LH2 of this bacterium is reflected in the circular dichroism of the B850 band of this complex. The wavelength dependence of the polarization of the LH2 complex shows an unusual shape that is attributed to the octameric state of this complex. The complete amino acid sequence for the LH1 α-polypeptide and most of the β-polypeptides are presented. These conform to the general features of other LH1 polypeptides. This result, in combination with spectroscopic data for LH1 imply that the organisation of the core in this bacterium is not much different from that in other purple non-sulphur bacteria.

Keywords: Antenna complex; Light harvesting; LH1; LH2; Circular dichroism; Transmembrane helix; Bacterial photosynthesis; *(Rh. molischianum)*; *(Rb. sphaeroides)*

1. Introduction

Antenna complexes are the major component of the photosynthetic apparatus of purple non-sulphur bacteria [1]. Their function is to absorb light and transport the excitation energy to the photosynthetic reaction center that ultimately converts it into an electrochemical gradient (recently reviewed in [2]). The structures and mechanisms that lead to the extreme efficiency of this energy transfer process are not fully understood, although the spectroscopic and biochemical properties of the antenna complexes have been studied intensively. Considerable effort [3–6] has very recently led to the elucidation of the molecular structure of a light-harvesting complex from *Rps. acidophila* [4], which will stimulate interest in the spectroscopy of these complexes.

In general, two types of membrane-resident antenna complex occur in purple non-sulphur bacteria, that are distinguished on the basis of their absorbance properties and proximity to the reaction center [7]. The core antenna (LH1) is present in all known species, and located in the direct vicinity of the reaction center. It occurs in a fixed ratio of 24 to 32 BChl/RC to the reaction center, independent of growth conditions [8]. In the bacteriochlorophyll *a* (BChl *a*) containing species it exhibits a single near infra-red (NIR) absorbance maximum located between 870 and 895 nm. The peripheral antenna (LH2) does not occur in all species and is present in a variable amount with respect to the photosynthetic reaction center. The peripheral antenna usually has two NIR absorbance maxima, that are located around 800 and 850 nm in *Rhodobacter sphaeroides*.

The antenna complexes have been the subject of detailed spectroscopic studies like circular dichroism (CD), linear dichroism (LD) and resonance Raman spectroscopy,

Abbreviations: LH1, light-harvesting complex 1; LH2, light-harvesting complex 2; RC, reaction center; BChl, bacteriochlorophyll; CD, circular dichroism, LD, linear dichroism; NIR, Near infrared; DSM, Deutsche Sammlung für Microorganismen; LDAO, lauryl-*N*,*N*-dimethylamine *N*-oxide.

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SSDI 0005-2728(95)00046-1

that probe the interaction between the bacteriochlorophyll chromophores and the protein environment. These steady-state techniques have shown that the antenna complexes function as a large, well organised pool of pigment-protein complexes that are interconnected to enable ultra-fast, highly efficient energy transfer [2]. The energy transfer process itself has been studied with fluorescence as well as time-resolved spectroscopic techniques with pico- and femto-second time resolution. Recent measurements have demonstrated that the elementary energy transfer step occurs in at most a few 100 femtoseconds [9,10]. Moreover, it was concluded that, even at room temperature, the absorption bands of the light-harvesting antenna are strongly inhomogeneously broadened (i.e., a distribution of energy levels exists within the individual absorbance bands) which gives rise to fast spectral equilibration of the excitation density.

Recently, much progress has been made with the purification and crystallization of the light-harvesting complexes from the sparsely studied species *Rs. molischianum* [5]. Besides the potential resolution of their molecular structure, the antenna complexes from *Rs. molischianum* have other intriguing properties that merit a better description of their spectroscopic properties. On the basis of equilibrium sedimentation experiments it was concluded that the purified LH2 from *Rs. molischianum* DSM-119 (strain No. 119 from the Deutsche Sammlung für Mikroorganismen, Braunschweig) occurs as an octamer of the $\alpha\beta$-heterodimer [11], whereas most LH2 complexes from other species are thought to be hexamers or dodecamers of the $\alpha\beta$-heterodimer [12,13]. Furthermore, In the primary structure of the α- and especially of the β-polypeptides from the LH2 of this species several motifs occur that have thus far been observed only in core antenna polypeptides [14]. This unexpected similarity was further substantiated by resonance Raman spectroscopy, showing that indeed the mode of pigment binding in the LH2 of *Rs. molischianum* DSM-119 strain is like that of *Rb. sphaeroides* LH1 when considering the interactions with the 2-acetyl and 9-keto groups of the B850 pigments.

In this study we present a full characterization of the spectroscopic properties of the antenna complexes from *Rs. molischianum* (strains DSM-119 and DSM-120). We studied complexes from two similar strains that are distinguished by the fact that one (DSM-120) makes a B800-820 complex when grown under the low-light conditions similar to that of *Rps. acidophila* [15]. A detailed comparison with the extensively studied antenna complexes of *Rb. sphaeroides* shows that the similarities between LH2 of *Rs. molischianum* DSM-119 and LH1 is only partially reflected in the spectroscopic properties of the LH2 complexes of this strain. The amino acid sequences of constituent polypeptides from the core antenna and their spectroscopic properties are very similar to the better-known core complexes from *Rs. rubrum* [16] and *Rb. sphaeroides* [17].

2. Materials and methods

Rs. molischianum strains DSM-119 and DSM-120 were grown and LH2 purified from these cells as in [14]. Purified LH2 complexes were stored at −20° C in 20 mM Tris-HCl buffer (pH 8.5) containing 0.1% LDAO. Core complexes were purified by means of sucrose density gradients and preparative isoelectrofocusing as in [13] and were stored in 20 mM Tris buffer with 0.1% dodecyl maltoside to keep the complexes solubilized. B800-820 complexes with different 800/820 ratios were purified by preparative isoelectrofocusing as in [14]. At least six different fractions could be separated this way.

The complete description of the cloning and partial sequencing of the genes coding for the The α- and β-polypeptides from the core complexes is described in [14]. Briefly, the peptides were separated on a reversed-phase HPLC column (Vydac C18) and N-terminal sequences of the purified polypeptides and fragments thereof were determined by automated Edman degradation. Further amino acid sequences were obtained by nucleotide sequencing of PCR fragments of the bacterial chromosome. To obtain these fragments, oligodesoxynucleotides were used that were deduced from the chemically determined N-terminal sequence of the β-polypeptide and a C-terminal *o*-iodobenzoic-acid-cleaved fragment of the α-polypeptide. A 300 basepair fragment obtained by this method was cloned and sequenced on both strands. The fragment contained the complete gene for the α-polypeptide and nearly all of the β-polypeptide.

For low-temperature spectroscopy, the samples contained 65% glycerol (w/v). Absorbance, linear and circular dichroism spectra were measured on a locally constructed instrument described in [18]. Low-temperature fluorescence spectroscopy was measured in a 90° setup described in [19]. A titanium-sapphire laser (Coherent model 890) pumped by an argon ion laser (Coherent Innova 310) was used to produce NIR excitation light. The laser beam was diverged to a diameter of 5 mm and the total energy was always kept below 10 mW. No time-dependent changes of fluorescence signals were observed, indicating that these precautions were sufficient to prevent the accumulation of photo-damage in the samples.

3. Results

3.1. Amino acid sequence of LH1

Reversed-phase HPLC chromatography of purified LH1 yielded 4 separate peaks, two of which contained highly purified α- and β-polypeptides. Two fractions that contained the β-polypeptide were N-terminally blocked and could not be sequenced directly, similarly to polypeptides from other core complexes [20]. Small cleavage fragments of these fractions obtained by treatment with pepsin or

α MWKIWTLYDPRRTLSGLFTFLTVlGLLIHFLLLSTDRFNWLDGAREAHNV *Rs. molischianum DSM-119*
β ...SGLSESEAQEFHGIFVTSFISFIVVAIVAHFLAWKWRPWLPGVKGYA *Rs. molischianum DSM-119*

Fig. 1. Amino acid sequences for α- and β-polypeptides from LH1 from Rs. molischianum DSM-119. Sequences that where initially obtained from peptide sequencing have been underlined. The identity of the first few (~ 7) N-terminal residues from the β- and the two C-terminal amino acids of the α-polypeptides could not be determined unequivocally from amino acid sequencing. However, both sequences presented are in agreement with the total mass as determined from electrospray mass spectrometry. The bold histidine residues most likely are the ligands to the central magnesium of the bacteriochlorophyll pigments.

iodosobenzoic-acid showed that these fractions were derived from the β-polypeptides. The N-terminal sequences were used to identify and clone a gene-fragment coding for the α-polypeptide and nearly all of the β-polypeptide. The amino acid sequences based on the DNA sequence, of the LH1 α- and β-polypeptides from *Rs. molischianum* DSM-119 are presented in Fig. 1.

3.2. Absorbance, circular and linear dichroism

Fig. 2 shows the low-temperature (77 K) absorbance and CD spectra of the chromatophores (top panels), RC-LH1 complexes (middle panels), and the LH2 (B800-850) complexes (bottom panels) from *Rs. molischianum* DSM-119 and DSM-120. The chromatophores of the *Rs. molischianum* DSM-119 strain have an absorbance spectrum (top left panel) that is typical for purple non-sulphur bacteria like *Rb. sphaeroides* that contain LH1 (B880) and LH2 (B800-850) complexes. The absorbance spectrum of the *Rs. molischianum* DSM-120 strain, however, shows that it produces a third type of antenna complex (B800-820) much like the *Rhodopseudomonas (Rps.) acidophila* strain 7050. As for *Rps. acidophila* the presence of this complex is induced by low light conditions [15]. The CD spectra of the chromatophores are dominated by the bands from LH2, the most abundant pigment-protein complex present (see below). A small contribution of the core antenna can be observed on the long wavelength side of the B850 band.

The low-temperature (77 K) absorbance spectra of the RC-LH1 core preparations from both strains are not very different from those of the well studied BChl *a* containing species *Rb. sphaeroides* and *Rs. rubrum* [16,17,21]. The

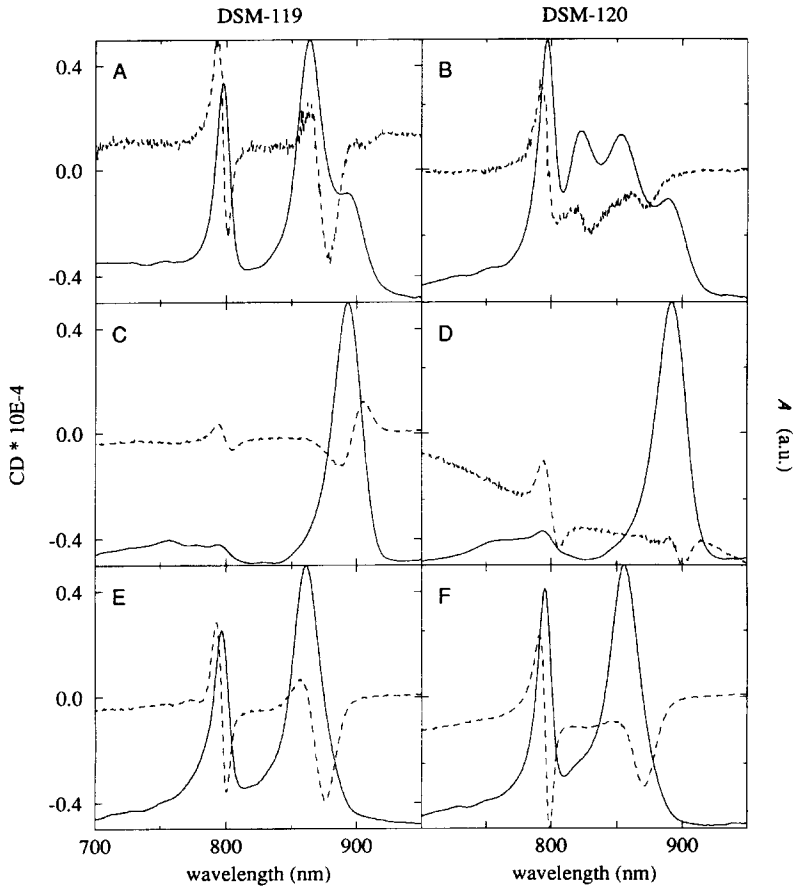

Fig. 2. Low-temperature (77 K) Absorbance (solid lines) and CD (dashed lines) of chromatophores (top panels), core-complexes (middle panels) and LH2 (B800-850) complexes (bottom panels) of *Rhodospirillum molischianum* DSM-119 (left side) and *Rhodospirillum molischianum* DSM-120 (right side).

single NIR absorbance band of the core antenna is located at 893 nm, relatively red compared to other species and has a width (FWHM) of 26 nm. Judging from the ratio between the RC band at 800 and the main antenna band at 880 nm there are between 20 and 30 BChl *a* pigments per RC in each of the core particles, which is similar to what has been observed for other core particles and in good agreement with recent results from electron-micrographic studies of these complexes [13]. The NIR-CD of the core complex from the DSM-120 strain is composed of a single negative band of moderate intensity with its extreme at 870 nm. The intensity of this signal is similar to values reported previously for the core antenna of *Rb. sphaeroides* [22]. It resembles the CD spectrum of LH1 from *Rs. rubrum* in chromatophores and that of the chromatophores of the LH1-only mutant M2192 from *Rb. sphaeroides*. The CD signal from the core complexes of the DSM-119 strain is different. It is composed of a double band that has a zero crossing around 890 nm, slightly to the red of the absorbance maximum. This signal is therefore more reminiscent of the conservative CD of the B850 of LH2 [21], and similar to the signal that is induced by detergent treatment of the core-antenna (Visschers, R.W., unpublished data). Similar CD spectra have also been observed in several site-specific mutants of LH1 of *Rb. sphaeroides* in which residues close to the BChl *a* binding site were modified (Olsen, J.D. and Somsen, O.J.G., personal communication). The CD signal from the monomer BChl *a* cofactors from the reaction center can be observed around 800 nm.

The 77 K absorbance spectra of purified LH2 from the two strains shown in Fig. 2 (bottom panels) display the typical LH2 bands at 800 and 850 nm. In the case of strain DSM-120, a very small contribution from B800-820 can still be observed at 820 nm. Absorbance maxima are located at 797 nm and 861 nm for strain DSM-119 and at 795 nm and 855 nm for strain 120 at 4.2 K. The B800 bands are asymmetric, since the red side is steeper than the blue side, similarly to LH2 from *Rb. sphaeroides* [23].

The circular dichroism spectra at 77 K of the purified LH2 complexes are not at all similar to that of *Rb. sphaeroides* LH2. In both strains the B800 band displays an intense conservative CD band that is completely absent in *Rb. sphaeroides* LH2 [21]. The presence of this band probably indicates a more pronounced coupling between the B800 pigments in these complexes (see Discussion). Interestingly, a similar CD signal has been observed for the LH2 of *Ectothiorhodospira halophila* (Ortiz de Zarate, I. and Somsen, O.J.G., personal communication), *Rps. palustris* [24] and *Chromatium tepidum* [25]. In this respect, it is of interest to note that the LH2 complexes of *Rs. molischianum* are thermostable in vitro up to 60° C. This suggests that this type of CD is found in the LH2 complexes that are structurally stable, since *Ectothiorodspira halophila* and *Chromatium tepidum* are both extremophilic. Furthermore, LH2 from *Ectothiorhodospira*

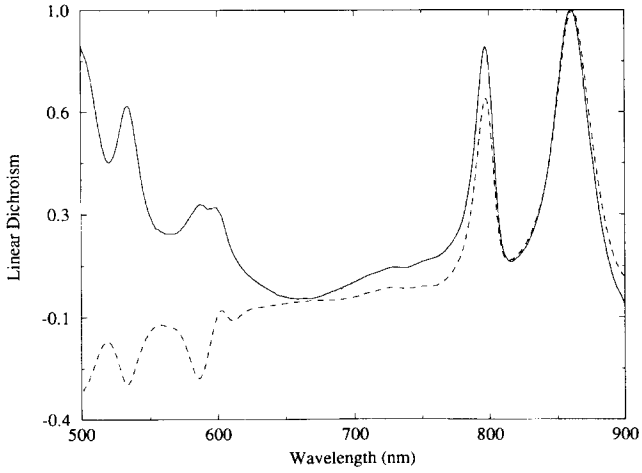

Fig. 3. 77 K absorbance (solid) and linear dichroism (dashed) of the LH2 complex from *Rs. molischianum* DSM-119. The absorbance spectrum is that of the particles in the actual acryl-amide gel, and therefore the ratio between 800 and 850 nm absorption is slightly different.

can occur in several forms modulated by ionic strength and detergent concentration [26]. The absorbance and CD properties of the LH2 from *Rs. molischianum* are most similar to those of the detergent-free LH2 complexes of *Ectothiorhodospira mobilis*.

The CD of the B850 band is also different from *Rb. sphaeroides*. Contrary to these complexes, the CD signal of the B850 band is not conservative, being nearly completely negative in the case of strain DSM-120, while in the DSM-119 strain an additional positive lobe is present on the blue side. To some extent, these spectra are typical for LH1 in *Rb. sphaeroides*, that is also completely negative in the case of M2192 chromatophores. This result, therefore, is in line with the observation that the resonance Raman as well as the primary structure of the LH2 is in several aspects homologous to LH1.

The 77 K LD spectra of the LH2 complex from strain DSM-119 (Fig. 3) demonstrate that the overall orientation of the Q_y dipoles of B800 and B850 pigments are nearly in the membrane plane, similarly as in *Rb. sphaeroides*. The Q_x absorbance band of LH2 at 77 K is split into two separate transitions at 592 and 601 nm. The LD of the band at 592 nm is negative whereas the band at 601 nm appears as a positive peak, superpositioned on the negative background. If we assign the stronger band at 592 to the Q_x transition of the B850 pigments, the Q_x of the B800 pigments would correspond to the 601 nm transition. In this interpretation, the B800 and B850 chromophores have an orientation in *Rs. molischianum* that is very similar to the same spectral species in *Rb. sphaeroides*., but the position of the Q_x transition from the B800 is located at 601 nm in *Rs. molischianum*, rather than at 580 nm as in *Rb. sphaeroides* [21]. The lycopene carotenoid of the LH2 complexes has a negative LD indicating that it has a predominant perpendicular orientation to the membrane plane, again similar to the orientation of the spheroidene

and spheroidenone carotenoids in LH1 and LH2 from *Rb. sphaeroides*.

3.3. Fluorescence site selection

To further study the aggregation and energy transfer properties of the LH1 core and the purified LH2 antenna complexes we have measured the 4.2 K polarized emission spectra of these complexes upon wavelength selective excitation across the absorbance band [27]. At 4.2 K, narrow-band excitation in the center of the absorbance band yields a broad and featureless emission spectrum (Fig. 4) as was previously observed for *Rb. sphaeroides* LH1. The absence of any fine structure indicates a relatively strong electron-phonon coupling for the main NIR transition i.e. strong interactions between the electronic transitions in the pigments and low frequency protein vibrations [22,28]. The polarization of the emission peak is low (≈ 0.05) upon excitation in the main part of the band and therefore extensive energy-transfer between the pigments still occurs at low temperature. The polarization is constant over the major part of the emission band. When the excitation wavelength is shifted to the red an increase in the polarization is observed, caused by selective excitation of those pigments within one LH1-complex that are not capable of transferring their excitation energy to pigments at even lower energy. The contribution of these 'local traps' to the observed emission is even larger on the blue side of the emission band which explains why the polarization depends on the emission wavelength. A more quantitative explanation of this phenomenon will be given elsewhere (Monshouwer et al., unpublished data). As has been argued [27], the relative shift of the emission band as a function of the excitation wavelength and the shape of the polarization are a function of the number of interacting pigment molecules within the complex. The excitation wavelength dependence of the average polarization and of the emission maximum resemble those of LH1 *Rb. sphaeroides*, showing that the size of the core antenna is similar.

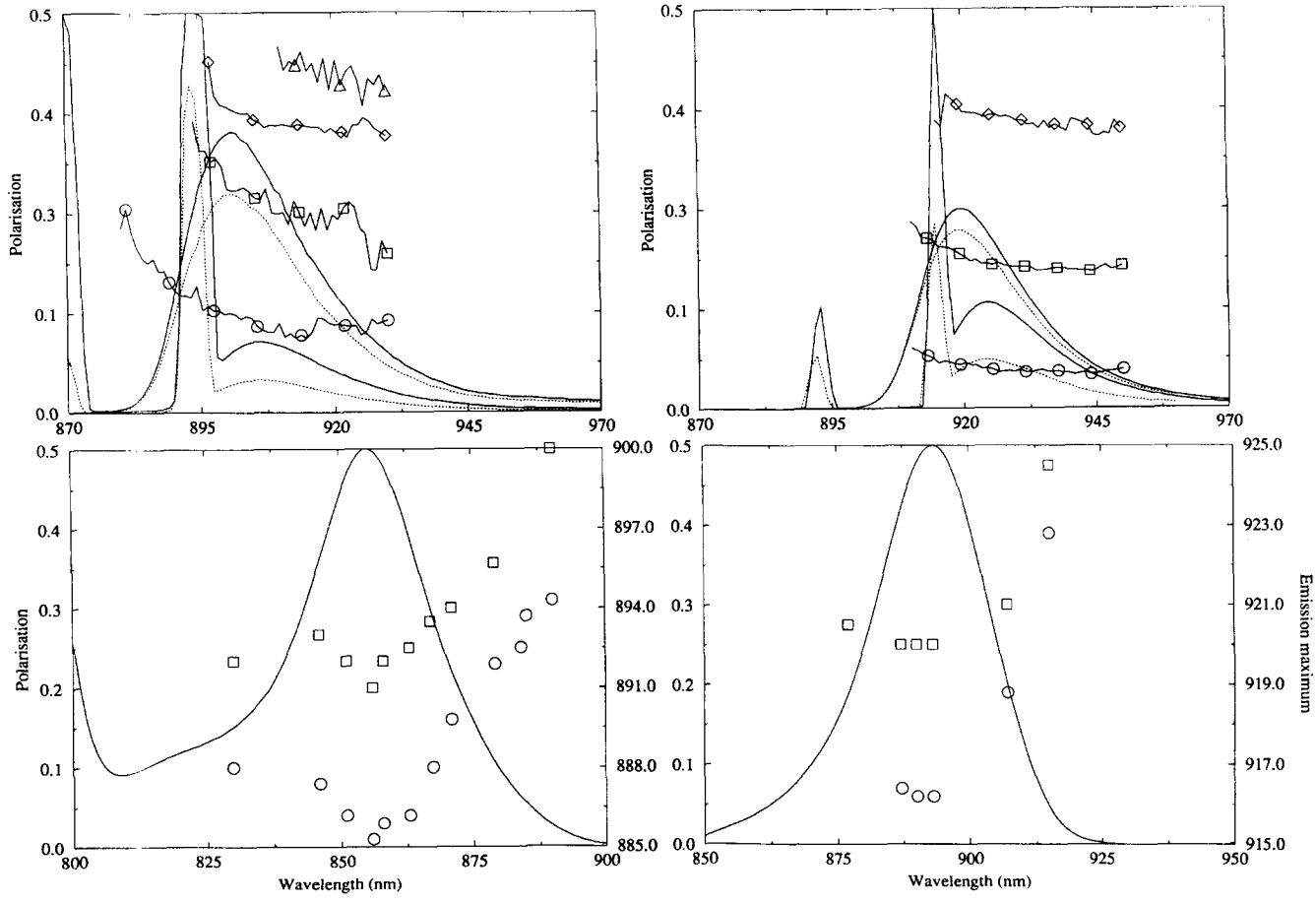

Fig. 4. Top panels: Polarized emission spectrum at 4.2 K (solid line: emission polarizer parallel to excition polarizer, dotted line: detection polarizer perpendicular to excitation polarizer). The resulting polarization at several excitation wavelengths are shown For LH2 (topleft panel): Circles: excitation at 870 nm, Squares: excitation at 890 nm, Diamonds: excitation at 894 nm, Triangles: excitation at 902 nm. For LH1 (topright panel): circles: excitation at 893 nm, Triangles: excitation at 907 nm, Diamonds: excitation at 915 nm. Bottom panels show the 77 K Absorbance (solid line), polarization (circles), and emission maxima (squares) at 4.2 K of the LH2 (bottom-left panel) and core complex (bottom right) from *Rs. molischianum*. The increase of the polarization at the red side is caused by trapping of excitations on low energy pigments in the antenna incapable of energy-transfer.

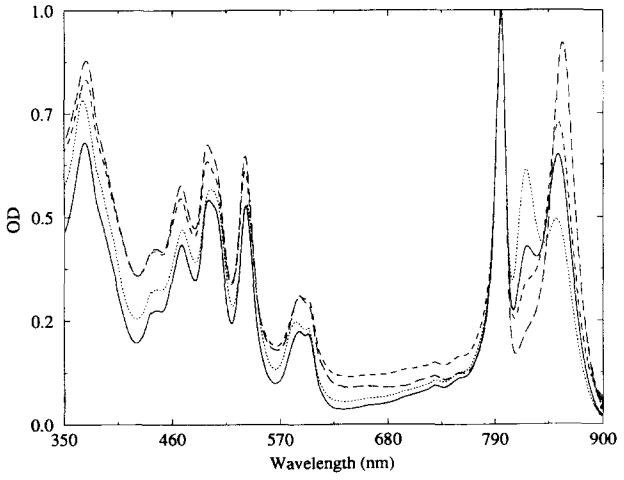

Fig. 5. 77 K absorbance spectra of 'mixed complexes' from *Rs. molischianum* DSM-120. The different samples were prepared by iso-elctrofocusing. The absorbance spectra have been normalized to $A = 1$ at the maximum of the 795 nm band. See text for details.

For the purified LH2 complexes from *Rs. molischianum* there is a more pronounced dependence of the shape, position and polarization of the emission band on the excitation wavelength. In the case of the LH2-120 which, in this respect, is nearly identical to that of LH2-119, we observed that the polarization strongly depends on the excitation and also on the emission wavelength. Tuning the excitation wavelength from blue to red, three distinct regions are observed. On the blue side of the absorbance maximum a polarization of 0.1 is measured, as is generally found for LH2 complexes [21]. Exciting a few nanometers blue from the absorbance maximum, a distinct decrease in the polarization is observed, with a minimum close to zero at 856 nm. Excitation to the red of this wavelength yields a nearly linear increase in the polarization to a maximum of 0.31 at 897 nm, which is limited only by the signal intensity.

The B800 → 850 transfer time of the 119 LH2 complex was estimated by exciting the complex at 4.2 K with the 488 nm line from a continuous argon-ion laser. Since no direct emission of the B800 band could be observed (data not shown), we can infer an upper limit to the ratio between the 850 and 800 emission of at least 1000, which indicates that the B800 lifetime must be less than 1 ps, if one assumes that the 850 lifetime is 1 ns.

Finally, we show the 77 K absorbance spectra for a number of what we call 'mixed complexes' from *Rs. molischianum DSM-120*. At room temperature these complexes appear to have different NIR absorbance maxima, but low-temperature (77 K) measurements reveal that the position of the NIR bands are not shifting. Instead, the different complexes have different ratios of 820 and 850 nm absorbing bacteriochlorophylls (see Fig. 5). At room temperature, these bands are broader and yield a single band that appears to shift as a function of the 850/820 ratio. These complexes could either exist as specific complexes containing B800, B820 and B850 pigments at the same time. Alternatively, it could be that, despite the the extensive purification procedure, we are dealing with mixtures of B800/820 and B800/850 complexes. However, excitation spectra of the complexes indicate that energy transfer occurs between the B820 and B850 pigments,

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N-terminal=><== membrane spanning ==><== C terminal

    10        20        30        40        50
  MWKIWTLYDPRRTLSGLFTFLTVlGLLIHFLLLSTDRFNWLDGAREAHNV      Rs. molischianum DSM-119

 MSKFYKIWMIFDPRRVFVAQGVFLFLLAVMIHLILLSTPSYNWLEISAAKYNRVAVAE  Rb sphaeroides  LH1-a
 MSKFYKIWLVFDPRRVFVAQGVFLFLLAVLIHLILLSTPAFNWLTVATAHGYVAAAQ  Rb. capsulatus  LH1-a
   MYKLWLLFDPRRALVALSAFLFVLALIIHFIALSTDRFNWLEGKPAVKAA  Rps. acidophila 7050  LH1-a
   MWRIWQLFDPRQALVGLATFLFVLALLIHFILLSTERFNWLEGASTKPVQTS      Rs. rubrum      LH1-a
ATEYRTASWKLWLILDPRRVLTALFVYLTVIALLIHFGLLSTDRLNWWEFQRGLPKAA      Rps. viridis     LH1-a
   MWRIWRLFDPMRAMVAQAVFLLGLAVLIHLMLLGTNKFNWLDGAKKAPVASA      Rc. gelatinosus  LH1-a
   MWKVWLLFDPRRTLVALFTFLFVLALLIHFILLSTDRFNWMQGAPTAPAQTS      Rps marina       LH1-a

    10        20        30        40        50
...SGLSESEAQEFHGIFVTSFISFIVVAIVAHFLAWKWRPWLPGVKGYA   Rs. molischianum DSM-119
    ||||   ||| |  |  ||  | |||||||   | ||||  ||  ||
ADKSDLGYTGLTDEQAQELHSVYMSGLWPFSAVAIVAHLAVYIWRPWF          Rb sphaeroides          LH1-b
MADKNDLSFTGLTDEQAQELHAVYMSGLSAFIAVAVLAHLAVMIWRPWF          Rb. capsulatus          LH1-b
  AEDRSSLSGVSDAEAKEFHALFVSSFMGFMVVAVLAHVLAWAWRPWIPGPKGWA   Rps. acidophila 705   LH1-b
  EVKQESLSGITEGEAKEFHKIFTSSILVFFGVAAFAHLLVWIWRPWVPGPNGYS   Rs. rubrum           LH1-b
  ADLKPSLTGLTEEEAKEFHGIFVTSTVLYLATAVIVHYLVWTAKPWIAPIPKGWV  Rps viridis         LH1-b
  AERKGSISGLTDDEAQEFHKFWVQGFVGFTAVAVVAHFLVWVWRPWL          Rc. gelatinosus         LH1-b
AEIDRPVSLSGLTEGEAREFHGVFMTSFMVFIAVAIVAHILAWMWRPWIPGPEGYA  Rps marina           LH1-b
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Fig. 6. Comparison of LH1 α- and β-polypeptide amino acid sequences. Amino acid sequences were taken from [29] (*Rb. sphaeroides*); [30] (*Rb. capsulatus*); [[20]] (*Rs. rubrum*); [31] (*Rps. viridis*); [32] (*Rps marina*); [[12]] (*Rc. gelatinosus* and *Rps. acidophila*).

since both peaks are observed in the excitation spectrum, when detecting in the 850 emission band. Therefore, the B820 and B850 pigments must be relatively close together, since otherwise no energy transfer could occur. A detailed description of these complexes will be presented elsewhere.

4. Discussion

A comparison between the amino acid sequence of *Rs. molischianum* DSM-119 α- and β-polypeptides is given in Fig. 6. The main features of the sequences are LH1-like, but in several instances, marked differences can also be observed.

The N-terminal part of the α-polypeptide from *Rs. molischianum* LH1 is typical for LH1. Especially the aspartic acid at position 14 is always found in LH1, whereas in the LH2 an uncharged residue is usual in this position. Also, the phenylalanine at position 25 is typical for LH1, since in LH2 there is usually an aliphatic residue in this position. In position 30 a glycine is found, instead of an alanine residue that is highly conserved in this position in nearly all other β-sequences. Although it has been suggested that the small alanine residue might be essential in creating the specificity of the B880 binding pocket versus B850, this is apparently not the case in *Rs. molischianum*. Brunisholz and Zuber [33] have suggested that there is a correlation between the intensity of the CD signal of the B880 band and the presence of a phenylalanine next to the conserved histidine in position 34 in the α-subunit. Complexes with a phenylalanine as well as the the motif PxPxxY in the β-subunit have a strong biphasic CD signal. The phenylalanine is present in *Rs. molischianum*, but the middle proline from the motif in the N-terminal part of the β-polypeptide appears to be missing in the *Rs. molischianum* sequence.

The intense conservative CD around the 800 nm band, the shift of the Q_x transition of B800 chromophore and the non-conservative CD of the B850 band appear to be the major differences between the spectroscopic properties of the light-harvesting complexes from *Rs. molischianum* and those from *Rb. sphaeroides*. It is not clear what causes the relatively blue position of the Q_x transition of the B800 chromophore, but the intense CD of the B800 band probably indicates a stronger interaction between those chromophores in LH2 complexes exhibiting this type of CD. Since the LD shows that the Q_y transition still resides in the plain of the membrane, this stronger interaction might be due to the B800 pigments being close to each other. However, it is difficult to attribute these differences to specific changes of the amino acid sequence of either the α- or the β-polypeptide.

An interesting correlation appears in the sensitivity of the B800 band to treatment with the detergent lithium dodecylsulfate (LDS), the strong 800 CD and the vibrational frequency of the 9-keto group as measured by resonance Raman spectroscopy. The B800 band of the B800-850 complex of *Rs. molischianum* is insensitive to this detergent, like *Rps. palustris*, whereas in the B800-850 complexes of *Rb. sphaeroides* and *Rps. acididophila* the B800 almost completely and reversibly dissapears upon treatment with low concentrations of this detergent. This susceptibility apparently correlates with the frequency of the vibration of the 9-keto carbonyl group in *Rs. molischianum* and *Rps. paustris* that is assumed to be part of a hydrogen-bridge in these complexes, while it is free from interactions in *Rb. sphaeroides* and *Rps. acidophila*. Interestingly, The B800 band of *Rps palustris* B800-850 complexes displays a fairly strong CD signal, like *Rs. molischianum*.

The absorbance properties of the LH1 and LH2 complexes are similar to those of *Rb. sphaeroides*. The anomalous amino acid sequence of the *Rs. molischianum* LH2 protein subunits, that have quite a large homology with LH1 polypeptides from other strains, is, however, reflected in the CD of the B850 band. Much like the resonance Raman data, the CD spectra point to a structural resemblance of the B850 binding-site of the *Rs. molischianum* LH2 and the B880 binding-site of *Rb. sphaeroides* LH1.

The observation of a drop of the polarization of LH2 around the absorbance maximum has not been reported before. Although reported here for the first time, it has also been observed in antenna complexes from other species (Somsen, O.J.G., personal communication). In our present model for inhomogenously broadened clusters, such a drop in the polarization could possibly be explained by small cluster sizes [27] and is possibly enhanced by the relative large angles between the dipoles in a C4 symmetrical configuration. Alternatively, using a model in which the individual clusters are more strongly coupled by exciton interactions, the dip could be explained by a relatively intense high exciton component which is preferentially excited in the wavelength region of the dip. This component, which must be perpendicular to the low exciton component, causes a decrease in the polarisation below 0.1. The increase in the polarisation in the blue part of the spectrum could in this case be ascribed to non-selective excitation of all the pigments through their overlapping vibrational modes on the high-energy side of the spectrum. In either case, the number of pigments that participate in energy transfer within the LH2 complex must be smaller than in *Rb. sphaeroides*, that probably contain 12 $\alpha\beta$-heterodimers [34]. This is in good agreement with the apparent molecular masses of 300 kDa of the *Rb. sphaeroides* LH2 vs. 180 kDa for the *Rs. molischianum* LH2 on gel-filtration columns.

It is firmly established that antenna complexes occur as oligomeric structures of the α- and β-polypeptides. It seems that most antenna complexes are composed of only one class of α- and β-polypeptides. Although for *Rps. acidophila* LH3 [12] and *Rps. palustris* LH2 [35] it has

been been shown that multiple copies of genes coding for α- and β-polypeptides exists, and in the case of *Rps. palustris* their gene products also occur simultaneously, it has not been established whether these multiple proteins occur mixed in individual light-harvesting complexes. The mixed complexes of *Rs. molischianum* would reflect a new class of antenna complexes in this respect, since they have a varying composition, and probably contain different α- and β-polypeptides. The presence of B820 and B850 bands within one complex also indicates that the in vivo red shift of the BChl pigments is influenced by the local protein environment.

Acknowledgements

R.M. and R.v.G. are supported by EC grant CT920796.

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