Simultaneous functioning of different light-harvesting complexes – a strategy of adaptation of purple bacterium *Rhodopseudomonas palustris* to low illumination conditions

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Novel peripheral light-harvesting (LH) complex designated as LL LH2 was isolated along with LH4 complex from Rhodopseudomonas palustris cells grown under low light intensity (LL). FPLC-MS/MS allowed to reveal PucABd and PucBabc apoproteins in LL LH2 complex, which is different from previously described LH4 complex containing PucABd, PucABa and PucBb (Brotosudarmo et al., 2011). The main carotenoids in LL LH2 complex were rhodopin and 3,4-didehydrorhodopin. Three-dimensional modeling demonstrated which amino acid residues of all the β-subunits could interact with carotenoids (Car) and bacteriochlorophyll a (BChl a). Analysis of amino acid sequences of α-subunits of both LL complexes showed presence of different C-terminal motifs, IESSVNVG in αa subunit and IESSIKAV in αd subunit, in the same positions of C-termini, which could reflect different retention force of LL LH2 and LH4 on hydroxyl apatite, facilitating successful isolation of these complexes. Differences of these LL complexes in protein and carotenoid composition, in efficiency of energy transfer from Car to BChl a, which is two times lower in LL LH2 than in LH4, allow to assign it to a novel type of light-harvesting complex in Rhodopseudomonas palustris.

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13 ABSTRACT

14   Novel peripheral light-harvesting (LH) complex designated as LL LH2 was isolated

15 along with LH4 complex from *Rhodopseudomonas palustris* cells grown under low light

16 intensity (LL). FPLC-MS/MS allowed to reveal PucABd and PucBabc apoproteins in LL LH2

17 complex, which is different from previously described LH4 complex containing PucABd,

18 PucABa and PucBb (Brotosudarmo et al., 2011). The main carotenoids in LL LH2 complex were

19 rhodopin and 3,4-didehydrorhodopin. Three-dimensional modeling demonstrated which amino

20 acid residues of all the β-subunits could interact with carotenoids (Car) and bacteriochlorophyll *a*

21 (BChl *a*). Analysis of amino acid sequences of α-subunits of both LL complexes showed

22 presence of different C-terminal motifs, IESSVNVG in αa subunit and IESSIKAV in αd subunit,

23 in the same positions of C-termini, which could reflect different retention force of LL LH2 and

24 LH4 on hydroxyl apatite, facilitating successful isolation of these complexes. Differences of

25 these LL complexes in protein and carotenoid composition, in efficiency of energy transfer from

26 Car to BChl *a*, which is two times lower in LL LH2 than in LH4, allow to assign it to a novel

27 type of light-harvesting complex in *Rhodopseudomonas palustris*.

28 Key words: bacteriochlorophyll, light-harvesting complex, *puc*
29 operon, purple photosynthetic bacteria, *Rhodopseudomonas*
30 *palustris*, three-dimensional modeling

31 INTRODUCTION

32   Studies of molecular basics of formation and assembly of light-harvesting (LH) pigment-

33 protein complexes during adaptation of phototrophic purple bacterium *Rhodopseudomonas*

34 *(Rps.) palustris* to low light intensities have not led to complete understanding of this process yet

35   (Cogdell, Gall & Köhler, 2006). This is due to presence of at least five Puc_{abcde} genes encoding

36   $αβ_{abcde}$-apoproteins of LH-complexes (Evans, Hawthornthwaite & Cogdell, 1990; Tharia et al.,

37   1999; Hartigan et al., 2002) and complicated regulation of the expression of each gene by a

38   certain bacteriophytochrome. The number of bacteriophytochromes is equal to the number of

39   light-harvesting complex apoprotein genes (Vierstra & Davis, 2000; Evans et al., 2005; Fixen,

40   Oda & Harwood, 2019). Proteins of LH-complexes are non-covalently bound to

41   bacteriochlorophyll a (BChl a) and carotenoids (Car), and their interactions determine the ability

42   of the complexes to absorb photons in different spectral areas and to direct the resulting energy

43   to the reaction centers (RCs). Energy transfer is followed by its conversion into the chemical

44   energy (Lüer et al., 2012). Formation of certain type of LH complex depends on illumination

45   intensity. Under high illumination, phylogenetically close phototrophic purple bacteria

46   *Rhodoblastus (Rbl.) acidophilus* (formerly known as *Rhodopseudomonas (Rps.) acidophila)* and

47   *Rps. palustris* form LH2 complexes B800-850 with the main absorption maxima at 850 nm.

48   These complexes are located peripherally in relation to core complex (LH1-RC) (Evans,

49   Hawthornthwaite & Cogdell, 1990; Gall et al., 1999). Low illumination, or low light (LL) leads

50   to formation of other forms of LH2 complex, LH3 (B800-820) in *Rbl. acidophilus* and LH 4

51   (B800) in *Rps. palustris,* with the main absorption maxima at 800 nm (Moulisová et al., 2009;

52   Kotecha, Georgiou & Papiz, 2013; Montemayor, Rivera & Jang, 2018). All the types of

53   peripheral LH complexes consist of short (50-60 amino acid residues), very hydrophobic αβ-

54   polypeptides encoded by different gene pairs: $PucAB_{ab}$ for LH2, $PucAB_c$ for LH3, $PucAB_d$ for

55   LH4 (Taniguchi et al., 2014). The available X-ray crystallography data (Papiz et al., 2003;

56   Brotosudarmo et al., 2011), as well as data of single-molecule spectroscopy and molecular sieve

57   chromatography (Brotosudarmo et al., 2009) showed that all the peripheral LH-complexes of

58   these bacteria are nonameric ring-shaped structures made of symmetrically repeated basic

59   building blocks, protomers. Each protomer is composed of one α-subunit strongly interacting

60   with two BChl a molecules and of one β-subunit bound to one monomeric BChl a. The BChl a

61   molecules of α-subunits form the inner ring, BChl a molecules of β-subunits form the outer B800

62   ring. Each protomer also includes one carotenoid molecule, which interacts with polypeptides

63   and BChl a and stabilizes the structure of the complexes (Lang & Hunter, 1994; Hashimoto,

64   Uragami & Cogdell, 2016).

65   These two bacteria have identical quaternary structure of LH-complexes and almost the

66   same set of genes for the synthesis of αβ-chains, but they adapt differently to LL illumination

67   conditions. When shifting from HL to LL conditions, gradual substitution of LH2 (B800-850)

68   with LH3 (B800-820) occurs in *Rbl. acidophilus,* and the spectral changes are related to changes

69   of two amino acids in α_{ab}-subunits, while β-subunits remain unchanged. Amino acid residues α

70   Tyr44 and α Trp45 of LH2 complex that bind to acetyl group of BChl *a* via hydrogen bonding,

71   are substituted with α Phe44 and α Leu45 in LH3, that cannot form hydrogen bonds, which

72   causes hypsochromic shift in LH3 by 30 nm. Instead of these residues, the C3-acetyl group of

73   αBChla B820 molecules is bound via hydrogen bond to α Tyr41, which is substituted in

74   equivalent position of HL LH2 with Phe (Taniguchi et al., 2014).

75   Quite a different situation is observed in LL conditions in *Rps. palustris*. In this

76   bacterium, LH4 complex is formed of heterogenic polypeptides. Apart from $\alpha\beta_d$ subunits specific

77   for LH4 complex (B800), it includes also polypeptides of LH2 complex, $\alpha\beta_a$ and β_b. Hence, there

78   is always an absorption maximum at 850 nm in LH4 complex in addition to 800 nm, and the 800

79   to 850 nm ratio increases upon gradual decrease of illumination from 200 to 10 lx

80   (Brotosudarmo et al., 2011).xAbsence of absorption maximum at 850 nm in LH4 complex was

81   demonstrated only for a *Rps. palustris* mutant strain with four gene pair deletions, that had only

82   PucD genes of all the genes of biosynthesis of light-harvesting complexes (Southall et al., 2018).

83   The data on gradual changes of polypeptide composition of LH4 complex in this range of

84   illumination intensity (200-10 lx) are absent in the literature.

85   Another complex was isolated from *Rps. palustris* under illumination conditions of 50 lx,

86   along with the LH4 complex, and it was designated as LL LH2 (Serdyuk, Smolygina &

87   Ashikhmin, 2020). It differed from the LH4 and LH2 complexes of this bacterium by the

88   absorbance in the carotenoid region of the absorption spectrum (400–550 nm), by the ratio of

89   maxima at 800/850 nm, and by the different retention strength of the complexes on

90   hydroxylapatite (HA), with which the complexes were isolated. However, the LH4 and LL LH2

91   complexes slightly differed in the composition of carotenoids determined by the HPLC method

92   and in the efficiency of energy transfer from Car to BChl *a*. The obtained data did not give an

93   idea of the reason of significant differences between the two types of LL complexes, which

94   required new experiments.

95   The aim of this work was to confirm the earlier suggestion about LL LH2 as a new type

96   of complex (Serdyuk et al. 2020), which is formed along with LH4. The main task was to study

97   the protein composition of LL LH2 as the main factor determining the type of complex (Tadros

98   and Watercamp, 1989; Papiz et al., 2003; Brotosudarmo et al., 2011). For this purpose, a

99   minimum illumination of 10 lux was used, at which the ratio of 800/850 nm in the absorption

100   spectrum of the LH4 complex was still unchanged (Brotosudarmo et al, 2011); the LL LH2

101   complex obtained on HA was additionally purified by HPLC on columns with a weak ion

102   exchanger DEAE-TOYOPEARL 650 S and mild detergent dodecyl maltoside (DDM), which did

103   not change its protein content. Other goals were to study the composition of Car, to calculate the

104   efficiency of energy transfer from Car to BChl a, and to determine of the sites of interaction of β-

105   subunits with pigments on three-dimensional models.
106

107   **Materials and methods**

108   The object of the study was purple phototrophic non-sulfur bacterium *Rps. palustris*,

109   strain KM MGU 286 (le5 strain, collection of Microbiology Chair of Freiburg University,

110   Germany (Lehrstuhl fur Mikrobiologie, Freiburg, BRD)), that was kindly granted by

111   Microbiology chair of Lomonosov Moscow State University. The bacteria were cultivated

112   anaerobically on Hutner's medium (Hutner, 1946) with ammonia succinate as nitrogen source at

113   pH 6.8, illumination 10 lx and t =30°C. *Rps. palustris* cells were grown in glass flat vessels 0.5 L

114   in volume, 2 cm thick, for 16 days. The vials were turned around twice a day for uniform

115   illumination. After the onset of the stationary growth phase with a constant cell density $OD_{850} =$

116   0.7 cm^{-1}, the cells were precipitated by centrifugation at 10000 x g, washed with 10 mM Tris-

117   HCl buffer solution and precipitated again. The cells were then resuspended in 10 mM buffer at a

118   ratio of 1:8 (weight/volume), the thin glass with cells was placed on ice and sonicated at 22 kHz,

119   8 times for 90 sec at 3 min intervals to prevent overheating. After sedimentation of the cell

120   debris, chromatophores were isolated from the supernatant on ultracentrifuge at 144000g. The

121   chromatophores were incubated in 20 mM Tris-HCl solution with 2% Triton X-100 for 40 min

122   and placed onto a column with hydroxyapatite (HA) that was prepared according to the method

123   used in earlier works (Siegelman, Wieczorek & Turner, 1965; Serdyuk, Smolygina & Khristin,

124   2018) and equilibrated with 10 mM Tris-HCl buffer solution, pH 7.8. Then the column was

125   washed with the same buffer solution to eliminate the residual detergent (Triton X-100), and to

126   elute the compounds that were bound weaker than the LH-complexes. LH4 complex was eluted

127   with 20 mM Tris-HCl buffer solution. After washing the complex with 20 mM sodium

128   phosphate buffer solution until complete loss of eluate colour, LL LH2 complex was eluted with

129   20 mM sodium phosphate buffer supplied with 0.25% Triton X-100. Then the eluates of

130   complexes with HA were additionally purified by chromatography on DEAE-TOYOPEARL 650

131   S column (Bol'shakov et al., 2016). For this step, linear gradient ranging from 0.1 to 0.3 M NaCl

132   was used; LL LH2 complex was eluted with 0.14 M NaCl suppplied with 0.02% DDM, and LH4

133   complex was eluted with 0.17 M NaCl suppplied with 0.02% DDM.

134   Pigment composition of LH complexes was analyzed by HPLC method (Bol'shakov et

135   al., 2016). HPLC appliance (Shimadzu, Japan) consisted of (1) LC-10ADVP pump with FCV-

136   10ALVP module, (2) diode matrix-based detector SPD-M20A, and (3) thermostat CTO-20 AC.

137   Carotenoids were separated on reverse-phase column 4.6 × 250 mm (Agilent Zorbax SB-C18,

138   Agilent Technologies, Santa-Clara, California, USA) at 22 °C. The carotenoids were identified

139   by retention time and absorption spectra. Quantitative determination of each carotenoid was

140   carried out by comparing peak area from 360 to 800 nm with the sum of all the carotenoid peaks

141   taken as 100%, and calculating the result in LC-solution program (Shimadzu, Kyoto, Japan)

142   using molar extinction coefficients (Britton, 1996).

143   Absorption spectra were recorded on spectrophotometers Cary 50 (Agilent Technologies,

144   USA) and Hitachi 557 (Hitachi, Japan). Emission and excitation spectra of fluorescence were

145   recorded on spectrofluorimeter Cary Eclipse (Agilent Technologies, USA). Energy transfer

146   efficiency from Car to BChl a was determined at the maximum absorption of carotenoids for

147   each complex according to the method described in the work (Niedzwiedzki et al., 2011) by

148   comparing the fluorescence excitation spectra with absorption spectra transformed into 1-T

149   spectra, where T is transmittance coefficient. The spectra were normalized by the peak of BChl

150   a Qx-transition at 590 nm. All the spectral measurements were carried out at room temperature.

151   Proteomic analysis of LL LH2 complex was carried out using chromatographic HPLC-

152   system Ultimate 3000 RSLCnano (Thermo Scientific, USA) coupled with mass spectrometer Q-

153   Exactive HF (Thermo Scientific, USA). The accuracy of mass measurements comprised 70

154   ppm. LL LH2 peptides separated by one-dimensional SDS-PAGE by Laemmli (Laemmli,

155   1970), in 15% acrylamide gel with 0.3% bis-acrylamide and 0.1% SDS, were used as samples

156   for mass-spectrometry. Bands with molecular weights from 10 kDa to the front line were excised

157   and subjected to trypsinolysis. LL LH2 complexes in 20 mM Tris-HCl buffer obtained by

158   aforementioned chromatographic methods were also studied by mass spectrometry.

159   All the mass spectrometry measurements were carried out using equipment of the

160   "Human Proteome" Core Facility Centre (Institute of Biomedical Chemistry).

161   Models of LL LH2 were built by molecular replacement method in YASARA Structure

162   (Krieger et al., 2012). For this purpose, alignments of alpha-subunits of LH2 complexes from

163   *Rps. palustris* (Uniprot ID: P35101) and *Rbl. acidophilus* (sequences from 3D structures, PDB

164   IDs: 1NKZ, 2FKW (Papiz et al., 2003; Cherezov et al., 2006) were built in ClustalOmega

165   (Sievers et al., 2011). Then the residues in the PDB files were substituted with the corresponding

166   residues of alpha subunit of LH4 complex from *Rps. palustris*. The obtained PDB-file served as

167   a source for all the following manipulations. Then, the residues of beta-subunits were substituted

168   with the corresponding residues of beta subunits LH2A-beta (βa), LH2B-beta (βb), LH3-beta

169   (βc), LH4-beta (βd) (Uniprot IDs: P35106, P35107, P35108, P35109). The bacteriochlorophyll

170   ligands were conserved as they are in the initial files (complexes from *Rbl. acidophilus*), while

171   rhodopin glucoside was substituted with rhodopin according to the experimental data on

172   carotenoid composition.

173   **RESULTS**

174   LL LH2 complex was successfully isolated in our work due to the used technique of

175   primary isolation of LH4 and LL LH2 complexes on hydroxyl apatite (HA) column. The earlier

176   experiments using the ion-exchange sorbents (Tadros & Waterkamp, 1989; Hartigan et al., 2002;

177   Taniguchi et al., 2014) did not include sorption chromatography based on different hydrogen

178   bonding ability. HA is a mixed-type sorbent. Its phosphate groups play a role of cation

179   exchanger, whereas hydroxyl groups retain the separated compounds via formation of hydrogen

180   bonds (Misra, 1988).

181   Spectral characteristics of the isolated LL LH2 and LH4 complexes were studied by

182   optical spectrophotometry. The most significant differences in their absorption spectra were

183   observed in the bands of weakly interacting β-bound monomeric BChl *a* molecules. The

184   corresponding absorption maximum at about 800 nm in the LH4 complex is the main one. It is

185 very narrow, which is typical for this type of complexes (Tharia et al., 1999; Hartigan et al.,
186 2002; Brotosudarmo et al., 2011; Southall et al., 2018) (Figure 1). In LL LH2 complex, this
187 maximum had a split band with maxima at 797 and about 800 nm (Fig. 1 (Evans,
188 Hawthornthwaite & Cogdell, 1990; Gall et al., 1999). This complex also differed from LH4
189 complex by prevalence of absorption maximum at 850 nm, so its absorption spectrum was more
190 in line with the LH2 complex (Evans, Hawthornthwaite & Cogdell, 1990; Gall et al., 1999;
191 Cogdell, Gall & Köhler, 2006). Previously, it was shown (Kotecha, Georgiou & Papiz, 2013)
192 that the ratio of the B850/B800 absorption bands in HL complexes can vary depending on
193 illumination and *Rps. palustris* strain. However, the most significant characteristic determining
194 the type of complex is its protein composition.

195

196 **Figure 1.** Absorption spectra of LL LH2 complex (solid line) and LH4 complex (dashed line)
197 from *Rps. palustris*

198   Differences in absorption spectra were also observed in positions of maxima around 370
199 nm (Soret-band) and 590 nm reflecting the contribution of three BChl *a* molecules of both B800
200 and B850 ring-shaped aggregates. Based on the analysis of the absorption spectra, it could be
201 assumed that LL LH2 has a heterogeneous peptide composition. It could be similar to the LH4
202 complex, but not identical to it.

203   Analysis of peptide samples obtained during SDS-electrophoresis of LL LH2 complex
204 (see Materials and Methods) by HPLC-MS/MS spectrometry revealed βa sp|P35106| and βb
205 sp|P35107| subunits of B-800-850 with real molecular weight of 5.3 and 5.7 kDa (Fig.2, bands
206 1 and 2, respectively). Search of proteins corresponding to other peptide mass peaks led to
207 reliable identification (18 peptide fragments, sequence coverage 83%) of uncharacterized
208 protein of *Rps. palustris* (UniProt ID: Q6N9P5, molecular weight 10.8 kDa) (Fig. 2, band 3).

209

210 **Figure 2.** SDS electrophoresis of LL LH2 complex. Left track – LL LH2; Right track –
211 molecular weight markers. The oval indicates the excision of peptides along the gel for their
212 study by HPLC MS/MS. 1-3, separate peptide bands that are clearly visible by naked eye.
213 Three more polypeptides, αd (P35104), βd (P35109) and βc (P35108), were detected in LL
214 LH2 complex by tandem LC-mass-spectrometry. Amino acid sequences of all peptides found in
215 complex LL LH2 are presented below (Table 1).

216

217   Thus, peptide composition in LL LH2 complex and LH4 complex of *Rps. palustris* strain

218 2.1.6 ($\alpha\beta_a$, $\alpha\beta_d$ and β_b) known from the literature (Brotosudarmo et al., 2011) were similar in

219 "excessive" variability of β-subunits, but LL LH2 differed from LH4 complex by the absence

220 of α_a subunit and presence of additional β_c subunit.

221   Currently, there is no definite data on presence of βcd subunits in all the types of LH-

222 complexes. These subunits were identified even in HL LH2 complex of *Rps. palustris*, but the

223 question whether they are an impurity or a component of the complex is not discussed in the

224 papers (Zurdo, Fernandez-Cabrera & Ramirez, 1993; Tharia et al., 1999). Current data on

225 polypeptide composition of HL and LL complexes from *Rps. palustris* are summarized in Table

226 2 (Brotosudarmo et al., 2011) with our data added.

227

228   Differences in polypeptide composition could explain different retention degree of LL

229 LH2 and LH4 complexes on hydroxyl apatite. Different C-terminal regions of alpha-subunits

230 could be the determinants of different sorption activity (Fig. 3).

231

232 **Figure 3.** Multiple alignment of alpha-subunits of *Rps. palustris* light-harvesting complexes. The

233 sequence differences in the C-terminal motif are underlined. The proteins are designated by their

234 Uniprot IDs: P35101, αa; P35102, αb; P35103, αc; P35104, αd. The colours used are from the

235 standard ClustalOmega colour code: red, hydrophobic; green, polar neutral; magenta, positively

236 charged; blue, negatively charged.

237   The difference in C-terminal sequence is presence of IESSIKAV motif in αd in the

238 position where αa subunit has IESSVNVG motif. The most differing amino acids are lysine K57

239 (positively charged amino acid, side chain pKa = 10.53) and asparagine N57 (neutral polar

240 amino acid with amide side chain). Besides, side chains of other residues could shield potential

241 sorbent binding sites (side chain of S, N or K; carboxyl group of the C-terminus) differently. It

242 should be noted, that, taken together, amino acids I, A, V in αd are more hydrophobic, than V, V,

243 G in αa. All this could lead to the differences in elution ability shown upon isolation of LL LH2

244 and LH4: LH4-complex was eluted by 20 mM Tris-HCl, whereas LL LH2 was eluted by 20 mM

245 sodium phosphate with 0.25% Triton-X100. In this case, phosphate ions apparently interacted

246 with lysine residue better than chloride ions, tearing it off the phosphate groups of hydroxyl

247   apatite, while the added detergent presumably prevented the aggregation of hydrophobic residues
248   of the C-termini of alpha-subunits between each other and with other hydrophobic components
249   retained at the start (at the top of the column).

250   Another important characteristic of LH complexes is composition and content of
251   carotenoids present. The composition of carotenoids in the LH4 and LL LH2 complexes isolated
252   from *Rps. palustris* grown under very low light conditions (10 lux) was studied. A distinctive
253   feature of LL LH2 was the different level of carotenoid biosynthesis from the LH4 complex
254   (Table 3). As can be seen from Table 3, significant differences are observed in the ratio of all
255   carotenoids in the two LL complexes, with the exception of anhydrorhodovibrin, which is
256   present in them in an equal percentage.

257

258   In the LH4 complex, the main carotenoids were lycopene and rhodopin and in a typical
259   ratio, as described in the literature (Gall et al., 2005; Brotosudarmo et al., 2015; Hashimoto,
260   Uragami & Cogdell, 2016). The LL LH2 complex contained almost an order of magnitude less
261   lycopene, and 1.5 times more rhodopin. Both LL complexes differed from the classical LH4
262   (Brotosudarmo et al., 2009, 2015) by rather high content of 3,4-didehydrorhodopin, the LL LH2
263   complex was distinguished by a relatively high level of spirilloxanthin, which was absent in the
264   LH4 complex. The detailed data of HPLC analysis are submitted as Supplementary file 1.

265

266   Apparently, selective distribution of carotenoids in LH4 and LL LH2 complexes affected
267   the energy transfer efficiency from Car to BChl *a*. Light-harvesting complexes are excited at
268   wavelength corresponding to carotenoid absorbance, and energy transfer to bacteriochlorophyll
269   is detected by monitoring of its fluorescence in red or near-infrared spectral area. The analysis of
270   excitation spectra of LH complex fluorescence in *Rps. palustris* showed that the efficiency of
271   energy transfer from Car to BChl in LL LH2 is significantly lower than in LH4 complex (Fig. 4).

272

273   **Figure 4.** Fluorescence excitation spectra (the dashed lines) and the absorption spectra (in the
274   form of 1-T, solid line) of the LH2 complexes from *Rps. palustris*. The spectra were normalized
275   by BChl *a* Qx-transition peak at 590 nm. The emission wavelengths for the fluorescence
276   excitation spectra of LL LH2 and LH4 were 864 and 861 nm, respectively. The percentages

277 written in the figure correspond to energy transfer efficiency from carotenoids to BChl in these

278 complexes.

279   This process could be affected by such factors as carotenoid orientation in relation to the

280 adjacent BChl molecules, structure of polypeptides composing the LH complexes and structure

281 of carotenoids themselves (Papagiannakis et al., 2003; Cong et al., 2008; Frank & Polívka, 2009;

282 Chi et al., 2015; Ashikhmin et al., 2017).

283   It is a common belief that the major carotenoid stabilizing the structure of peripheral LH

284 complexes in *Rbl. acidophilus* and *Rps. palustris* is rhodopin glucoside. Based on the

285 experimental data for LH3 and LH2-complexes of *Rbl. acidophilus*, structural modeling of LL

286 LH2 complex with this carotenoid was carried out, but the glycosyl residues were deleted (Fig.

287 5), taking into account our data on presence of rhodopin instead of rhodopin glucoside in LL

288 LH2 complex (see Table 2).

289

290   **Figure 5.** Models of LL LH2 complex based on 1NKZ template (LH2 complex of *Rbl.*

291 *acidophilus*). Rhodopin is colored red; bacteriochlorophyll is colored via conventional element

292 scheme – carbon, cyan; nitrogen, blue; oxygen, red; magnesium, yellow; alpha subunits are

293 yellow, beta subunits are magenta. A – hexameric view; B – close view of a single alpha-beta

294 dimer with two rhodopin molecules and three bacteriochlorophyll molecules.

295   To study the mutual relation between composition of peptides and chromophores and LL

296 LH2 functioning, we performed multiple alignment of β-subunit sequences detected in LL LH2

297 complex, and demonstrated interaction of various amino acid residues with Car and BChl *a* on

298 three-dimensional models. It is known that the specific subunit of LH4 complex is βd, and the

299 differences between primary sequences of β-subunits are slight (Fig. 6). To reveal the impact of

300 certain amino acid residues on the differences in spectral properties and energy transfer

301 efficiency, their positions in relation to chromophores were analyzed.

302

303 **Figure 6.** Multiple alignment of beta-subunits of peripheral LH-complexes in *Rps. palustris*. The

304 proteins are designated according to their UniProt IDs: P35106, βa; P35107, βb; P35108, βc;

305 P35109, βd. Standard ClustalOmega coloring scheme was used. Positions of variable amino

306 acids of beta-subunits are underlined.

307   We showed presence of all the four variants of beta-subunits in LL LH2 complexes,

308   which is different from LH4 complex lacking βc subunit according to data of Tharia and

309   colleagues 1999; the fraction of αβa peptides in LH4 complex is significantly lower, than the

310   fraction of αβd peptides. That is why we examined differences between βa and βd and between

311   βc and βd when comparing LL LH2 and LH4 complexes. The figures below (7-10) show

312   superpositions of LH4 complex with LL LH2 complex including βa and βc subunits. The

313   distances between chromophores and corresponding amino acid residues were of special interest.

314   According to Onofrio, 2014, hydrophobic contacts in range from 3.8 to 9.5 Å between aliphatic

315   carbon atoms are the most widespread in protein structures (Onofrio et al., 2014). The

316   differences between valine V11 and isoleucine I15 were observed right in this range (Fig. 7).

317

318   **Figure 7.** Differences of xisoleucine residue I15 and valine residue V11 (specific for βa

319   subunit) in interaction with rhodopin. A – position of I15 (specific for βbcd subunits); B –

320   position of V11 (specific for βa subunit). The residues are coloured magenta. Protein subunits

321   are coloured gray. Rhodopin is coloured red. Bacteriochlorophyll is coloured via standard

322   element scheme (cyan, carbon; blue, nitrogen; red, oxygen; yellow, magnesium). The yellow

323   arrows show contacts with the chromophore for valine and isoleucine (distance 6.477 Å common

324   for valine and isoleucine; distance 6.546 Å unique for isoleucine).

325   As it can be seen, the presence of valine residue instead of isoleucine could weaken

326   interaction of the βa subunit with rhodopin due to the absence of hydrophobic contact specific

327   for isoleucine residue (via distal atom of sec-butyl side chain).

328   Substitution of alanine A16 in LH4 to glutamate E12 in LL LH2 has most probably no

329   effect on the spectral properties and energy transfer, because these residues do not interact with

330   chromophores.

331   Even more important difference than presence of valine instead of isoleucine is presence

332   of threonine or alanine on the position of serine. Hydroxyl group of serine residue does not

333   participate in formation of hydrogen bonds (neither with alpha-subunit nor with chromophores).

334   Presence of additional methyl group in threonine makes this amino acid position more involved

335   into interaction with both bacteriochlorophyll B850 and rhodopin (Fig. 8).

336

337   **Figure 8.** Serine and threonine residues and the difference in their interaction with

338   chromophores. Protein subunits are coloured gray. Rhodopin is coloured red, bacteriochlorophyll

339   B850 is coloured according to the standard element colouring scheme (carbon, cyan; nitrogen,

340   blue; oxygen, red; magnesium, yellow).The residues of interest are coloured magenta. A - serine

341   residue S29 specific for βd subunit; B - Yellow arrows show the contacts of threonine methyl

342   group with bacteriochlorophyll (distance 3.955 Å) and rhodopin (3.866 Å).

343   Presence of glycine residue G29 in place of valine V33 could also play a significant role.

344   Valine residue interacts with both rhodopin and bacteriochlorophyll. Meanwhile, in the built

345   model, interaction with bacteriochlorophyll takes place at extremely short distance, which could

346   lead to van der Waals repulsion (Fig. 9).

347

348   **Figure 9.** Valine residue V33 of LH4 complex and its interaction with chromophores

349   (rhodopin, coloured red, and bacteriochlorophyll, coloured via standard element coloring

350   scheme). A – valine residue V33. Yellow arrows shows interaction with rhodopin (longer arrow,

351   distance 6.958 Å)and possible van der Waals repulsion from bacteriochlorophyll (shorter arrow,

352   distance 2.370 Å). B – glycine residue G29 in the corresponding position.

353

354   The van der Waals repulsion of bacteriochlorophyll could lead to somewhat different

355   geometry of chromophores in the complexes containing subunits βc and βd, and this, in turn,

356   could lead to lower efficiency of energy transfer between rhodopin and bacteriochlorophyll. The

357   mechanism of this phenomenon requires more thorough examination, but now we could suppose

358   a conformational transition of bacteriochlorophyll causing the change of slope angle of

359   tetrapyrrol plane in relation to rhodopin axis.

360   The residue unique for beta-subunit βc is shown in Fig. 10.

361

362   **Figure 10.** A - Leucine residue L21 specific for βabd subunits; B - Isoleucine residue I21

363   specific for βc subunit. and Bacteriochlorophyll is colored according to standard element

364   scheme. Yellow arrows show a contact common for isoleucine and leucine residues with

365   rhodopin (upper arrow on panel A and the only arrow on panel B, distance 5.155 Å) and a

366   contact unique for leucine residue (lower arrow on panel A, distance 6.599 Å).

367   Isoleucine could interact with rhodopin weaker than leucine, which could be reflected in

368   spectral properties and/or energy transfer efficiency.

369   Thus, the mapped differences in amino acid sequences could explain the different spectral

370   properties and energy transfer efficiency.

371   **DISCUSSION**

372

373   Light, the main energy source in purple bacteria, is absorbed primarily by light-harvesting

374   LH2 complexes and then transferred to LH1-RC complex with further conversion of energy into

375   chemical bond energy in chromatophores (Fejes et al., 2003; Scheuring et al., 2006). Variability

376   of peptide and carotenoid composition in light-harvesting complexes makes them unique systems

377   of adaptation of phototrophic purple bacteria to different illumination conditions.

378   The results of the current work provide more reliable evidence for the existence of the LL

379   LH2 complex along with the LH4 complex, than the earlier study (Serdyuk, Smolygina &

380   Ashikhmin, 2020). Data on the composition of proteins and carotenoids of the LL LH2 complex

381   were obtained, which distinguish it from all the other known LH complexes (Zhou, Zhang &

382   Zhang, 2014; Lopez-Romero et al., 2020). Three-dimensional modeling showed which amino

383   acid residues of all βabcd subunits found in this complex can interact with Car rhodopin and

384   BChl a; the difference between LH 4 and LL LH2 complexes in the efficiency of energy transfer

385   from Car to BChl a was shown.

386   According to the literature, single-molecule spectroscopy of LH4 complexes in the

387   narrow LL illumination range from 200 lx to 5.5 lx showed gradual increase of 800/850

388   absorption ratio and constriction of Qy band of B850. This was observed to 10 lx value, while

389   further decrease of illumination led to the increase of Qy 800/850 ratio and broadening of the

390   peak at 850 nm. 10 lx was a margin of characteristic spectral changes upon the decrease of

391   illumination (Brotosudarmo et al., 2009).

392   Interestingly, in our earlier work (Serdyuk, Smoligina, Ashikhmin, 2020), the LH4 and

393   LL LH2 complexes from *Rps. palustris* grown under 50 lux illumination had almost the same

394   ratio of carotenoids. In the present study, these complexes differed significantly in this

395   parameter. The inconsistency of the data could be explained by the insufficiently careful

396   separation of the complexes into HA in the first study, but this assumption is refuted by

397   comparable data on Car for LH4 complexes in the earlier and present work. Most likely, these

398   differences in Car are associated with the use of different illumination in two studies (50 lx and

399   10 lx) and, to some extent, with additional purification of LL LH2 in this study by HPLC. The

400   composition of the proteins from LL LH2 complex was not investigated in our earlier work

401   (Serdyuk, Smolygina & Ashikhmin, 2020). The successful isolation of LL LH2 complex was

402   possible due to the used technique of primary isolation of LH4 and LL LH2 complexes on HA

403   column. The validity of this statement was confirmed by the analysis of the amino acid

404   sequences of the α-subunits of both LL-complexes, which showed possible determinants of

405   different retention force for LL LH2 and LH4 on hydroxylapatite as different C-terminal motifs

406   in the αd-subunit. Multiple alignment of the alpha-subunits (see Results, Fig. 2) showed that the

407   principal difference between αa and αd subunits is located at the C-termini. Moreover, it should

408   be taken into account that the isolated complexes are membrane-bound. Hence, the major part of

409   the polypeptide chain is shielded from the interaction with sorbents by lipids. That is why the

410   molecular reason for different retention force of LL LH2 and LH4 could be the difference in the

411   C-termini of α-subunits. In the previous studies (Tadros & Waterkamp, 1989; Hartigan et al.,

412   2002; Taniguchi et al., 2014) application of ion exchange sorbents did not include sorption

413   chromatography based on different abilities to form hydrogen bonds. HA is a mixed-type

414   sorbent, its phosphate groups play a role of cation exchange sorbent (Itoh, Yoshimoto &

415   Yamamoto, 2018), whereas hydroxyl groups retain the separated proteins via hydrogen bonding

416   (Misra, 1988).

417   Differences in the polypeptide composition of LL LH2 and other complexes are the main

418   features of its individuality and evidence of the biosynthesis of a new type of peripheral light-

419   harvesting complex under LL conditions. Mass spectrometry of proteins of the LL LH2 complex

420   showed their difference from the composition of LH4 complex (αβa, αβd, and only βb)

421   demonstrated earlier (Brotosudarmo et al., 2011). In this work, an even greater excess of the

422   number of β-subunits in relation to alpha subunits was shown, namely the presence of βc, and the

423   absence of αa-subunits. The observed absence of αb-subunits in LH4 complex in the work of

424   Brotosudarmo and colleagues (Brotosudarmo et al., 2011) and absence of αab subunits in LL

425   LH2 in our work may be explained by the loss of their functional importance at LL illumination

426   conditions. The ability to shift from B800–850 to B800 could increase the efficiency of RC

427   functioning, as shown in LL-adapted complex B800–820 (Deinum et al., 1991), where B800–

428   820 was an effective barrier against reverse energy transfer from LH1/RC. Such energy transfer

429 from LH1/RC to HL LH2 complex occurs under high illumination to prevent oxidation of RC

430 (Freiberg et al., 1996).

431   Higher variability of β-subunits compared to α-subunits was also observed in another

432 work (Tharia et al., 1999), but the authors could not detect βc subunit in LH4 complex neither in

433 HL nor in LL samples. They supposed that its synthesis is mainly caused by other stimuli like

434 temperature, and it was not detected in their cultivation conditions (t =22^0 C). Other authors

435 detected βc-subunit in HL LH2 from *Rps. palustris*, but they did not demonstrate reliable proofs

436 of its presence (Tadros & Waterkamp, 1989) (See Results, Table 2). Other researchers supposed

437 that it is impossible to isolate LH3 and LH4 complexes from each other due to high identity

438 degree of primary sequence of their proteins (Brotosudarmo et al., 2011). The possibility of

439 existence of different LH4 complexes with various β-subunits in *Rps. palustris* might find

440 indirect proof in the work on single-molecule fluorescence of these complexes (Ilioaia et al.,

441 2018), where the presence of at least four one-ring "subpopulations" with high number of

442 conformational subvariants and configurations created by an unknown chaperone-like process

443 was shown. The authors suggest that addition of different types of PucB apoprotein in different

444 ratios into the B850 "rings" is a very effective strategy for increasing the light harvesting ability

445 under stressful LL illumination conditions (Ilioaia et al., 2018).

446   Absorption spectra of LH2 complexes in the near infrared (NIR) region depend on pigment-

447 protein interactions, which determine the energy levels of pigments, and on pigment-pigment

448 interactions, which determine exciton interactions (Taniguchi et al., 2014). Taking into account

449 the differences in the carotenoid region of the absorption spectra of the two LL complexes, as

450 well as the differences in the protein composition, we conducted a study of the carotenoid

451 composition. In the present study, the use of additional purification of LL LH2 and LH4

452 complexes on a DEAE-TOYOPEARL 650 S HPLC column and a change in the light regime of

453 cell growth (10 lx) revealed significant differences between their carotenoid ratios and levels of

454 energy transfer efficiency, distinguishing them from the same complexes obtained earlier (50 lx)

455 (Serdyuk, Smolygina & Ashikhmin, 2020). A distinctive feature of the LL LH2 complex is very

456 high content of rhodopin (66%), comparable to that of the HL LH2 complex (75%)

457 (Brotosudarmo et al., 2015) and high content of 3,4-didehydrorhodopin in both complexes,

458 which was not previously noted in the literature, and which is probably a characteristic feature of

459 carotenoid biosynthesis in the bacterium *Rps. palustris* 1e5. The presence of such a high amount

460   of rhodopin, its derivatives 3,4-didehydrorhodopin and spirilloxanthin, in the amount of 83% of

461   all other carotenoids of this complex, determines very low levelof lycopene (1.7%), which is an

462   intermediate product in the synthesis of these xanthophyllic carotenoids.

463   However, we did not observe the same differences in carotenoids and energy transfer

464   efficiency from carotenoids to BChl a in the two LL complexes in our earlier work. The

465   discrepancy between the results in our two works can be explained by different light conditions

466   (50 and 10 lx) for growing the cultures from which the complexes were obtained, or by the

467   absence of additional purification step of the complexes in the previous work.

468   Thus, rhodopin and 3,4-didehydrorhodopin are the main carotenoids in LH4 and LL

469   LH2 *Rps. palustris*, as shown in this work and in our previous one (Serdyuk, Smolygina &

470   Ashikhmin, 2020). Since lycopene is an intermediate in the biosynthesis of the above two

471   carotenoids, its content in the complexes depends on the need of cells for the two aforementioned

472   carotenoids, as was shown by us and other researchers (Brotosudarmo et al., 2011).. Apparently,

473   lycopene plays a role of source pool, and it is used for the synthesis of other carotenoids when

474   needed. Rhodopin and 3,4-didehydrorhodopin perform well-known functions specific for

475   carotenoids in LH complexes. The crystal structure of the LH2 complex shows that carotenoids

476   and B800 molecules interact closely (Papiz et al., 2003; Gall et al., 2005). Taking into account

477   the excessive variability of β-subunits compared to α-subunits in LH4 complex (Brotosudarmo et

478   al., 2011) and in LL LH2 complex shown in our work, we can suppose that this is the reason for

479   binding of different amounts and relations of carotenoids in these complexes.

480   In the present study, LL LH2 was found to be isolated together with uncharacterized

481   protein Q6N9P5. The functional role of this protein and its homologs has been unknown and

482   requires further examination. During the preparation of this manuscript, some interesting data on

483   cryo-electron microscopy of light-harvesting complexes were submitted to Protein Data Bank

484   database (PDB IDs: 7ZCU, 7ZDI, 7ZE8, 7ZE3). According to them, this protein forms a crown

485   around the cytosolic side of LH-complexes, and it could participate in arrangement of

486   bacteriochlorophyll molecules, performing a function of a scaffold aggregating six extra

487   bacteriochlorophyll molecules around the LH2 complex. Then, an article was published in PNAS

488   on these experimental data, where the authors proposed to designate this protein as gamma-

489   subunit of light-harvesting complexes (Qian et al., 2022).

490   **CONCLUSIONS**

491   It is known that different types of peripheral light-harvesting complexes known up to date

492   are characterized by different polypeptide composition, content and composition of carotenoids,

493   energy transfer efficiency from Car to BChl *a* . A new type of complex LL LL2, isolated by us

494   from *Rps. palustris*, 1e5 strain, differed from other known LH complexes in all of the above

495   physicochemical parameters.

496   The LL H2 complex differed from the LH4 complex by the presence of an additional βc

497   subunit, which is commonly specific for LH3 complex, and a low level (36% versus 48%) of the

498   efficiency of energy transfer from Car to BChl *a*, respectively. The main carotenoids were

499   rhodopin and 3,4-didehydrorhodopin, in a ratio of 43/32 in LH4 and 66/17 in LL LH2. To study

500   the mutual relation between composition of peptides and chromophores and LL LH2

501   functioning, we performed multiple alignment of β-subunit sequences detected in LL LH2

502   complex. Multiple alignment of sequences of β-subunits of the LL LH2 complex and its three-

503   dimensional modeling showed the interaction of isoleucine I21, specific for βc, and leucine

504   L17/L21, specific for the βabd subunit, with rhodopin. A weaker interaction of isoleucine than

505   leucine with rhodopin may affect the spectral properties of the LL LH2 complex and the

506   efficiency energy transfer.

507   High adaptability of *Rps. palustris* to various light conditions is associated not only with

508   its multiple *puc* genes responsible for the synthesis of LH complex proteins, but also with a

509   complex system of regulation of their biosynthesis by the same variety of bacteriophytochromes.

510   Previously, it was shown that when shifting from HL to LL conditions in *Rps. palustris*, unlike

511   *Rbl. acidophilus*, an LH4 complex is formed with a different composition of polypeptides than in

512   the LH2 complex. In this work, we have shown that another LL LH2 complex is formed along

513   with LH4 under very low illumination, with an even more complex protein structure.

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698

Data on polypeptide composition of LH2, LH4 and LL LH2 complexes of *Rps. palustris*.

The data sources (from the literature or from the present study) are listed in the first column

2   **Table 1.** Proteins detected in LL LH2 complex of *Rps. palustris* by mass-spectrometry.

Uniprot ID	Uniprot description	Raw sequence	Brief name
P35104	LHA4_RHOPA Light-harvesting protein B-800-850 alpha chain D	`MNQGRIWTVVKPTVGLPLLLGSVAIMVFLVHFAVL` `THTTWVAKFMNGKAAAIESSIKAV`	**αd**
P35106	LHB1_RHOPA Light-harvesting protein B-800-850 beta chain A	`MADKTLTGLTVEESEELHKHVIDGTRIFGAIAIVA` `HFLAYVYSPWLH`	**βa**
P35107	LHB1_RHOPA Light-harvesting protein B-800-850 beta chain A	`MADKTLTGLTVEESEELHKHVIDGTRIFGAIAIVA` `HFLAYVYSPWLH`	**βb**
P35108	LHB3_RHOPA Light-harvesting protein B-800-850 beta chain C	`MVDDSKKVWPTGLTIAESEEIHKHVIDGARIFVAI` `AIVAHFLAYVYSPWLH`	**βc**
P35109	LHB4_RHOPA Light-harvesting protein B-800-850 beta chainD	`MVDDPNKVWPTGLTIAESEELHKHVIDGSRIFVAI` `AIVAHFLAYVYSPWLH`	**βd**
Q6N9P5	Uncharacterized protein	`MSEEYKGHSGHPLILKQEGEYKGYSGEPLILKQEG` `EYKGYSGTPLILEQKGEYQSFSGTPLILKQEGEYR` `GFSGAPLILKQDGEYKSFSGYPLLLNI`	**γ(?)**

Carotenoid composition of LL LH2 and LH4 complexes from *Rps. palustris* cells grown at low light intensity.

The carotenoids were detected by HPLC according to their retention times.

1   Table 2. Data on polypeptide composition of LH2, LH4 and LL LH2 complexes of *Rps.*

2   *palustris*.

Data source	LH2	LH4	LL LH2
(Tadros & Waterkamp, 1989)	PucABa, PucABb, PucABc, PucABd	-	-
(Tharia et al., 1999)	PucABa, PucABb, PucABd	PucABa and PucABd	-
(Brotosudarmo et al., 2011)	PucABa and PucABb	PucABa, PucABd, PucBb	-
Present study	-	-	Puc ABd, PucBabc

Carotenoid composition of LL LH2 and LH4 complexes from *Rps. palustris* cells grown at low light intensity.

1   **Table 3.** Carotenoid composition of LL LH2 and LH4 complexes from *Rps. palustris*

2   cells grown at low light intensity.

Carotenoid	Retention time, min	Content, mol %	
		LL LH2	**LH4**
Demethyl-spirilloxanthin	14.4	0.6	1.1
Rhodovibrin	15.2	0.3	2.2
3,4-didehydrorhodopin	19.1	17.4	32.5
Rhodopin	20.1	65.8	43.4
Spirilloxanthin	20.6	9.6	-
Anhydrorhodovibrin	26.1	4.6	4.6
Lycopene	31.4	1.7	16.2

Figure 1

Absorption spectra of LL LH2 and LH4 from *Rps. palustris*

LL LH2 absorption spectrum is shown in solid line, LH4 complex absorption spectrum is shown in dashed line

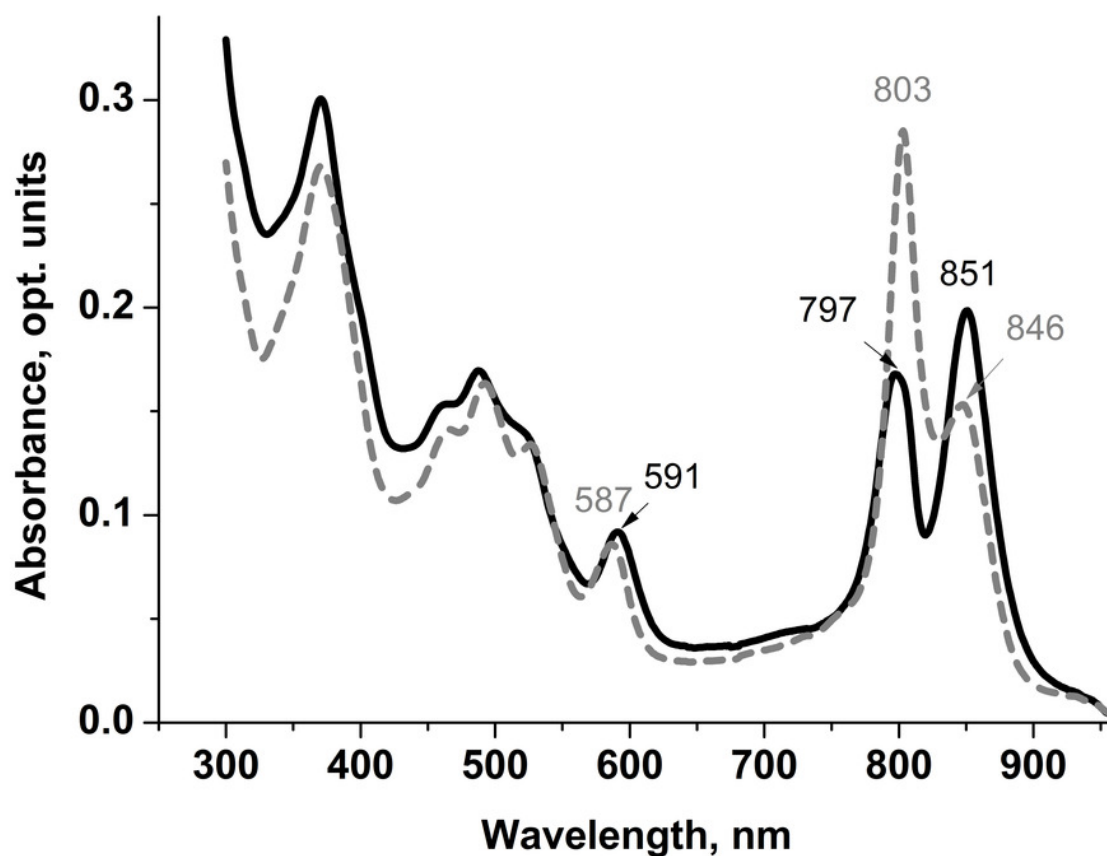



Figure 2

SDS electrophoresis of LL LH2 complex.

Left track – LL LH2; Right track – molecular weight markers. The oval indicates the excision of peptides along the gel for their study by HPLC MS/MS.

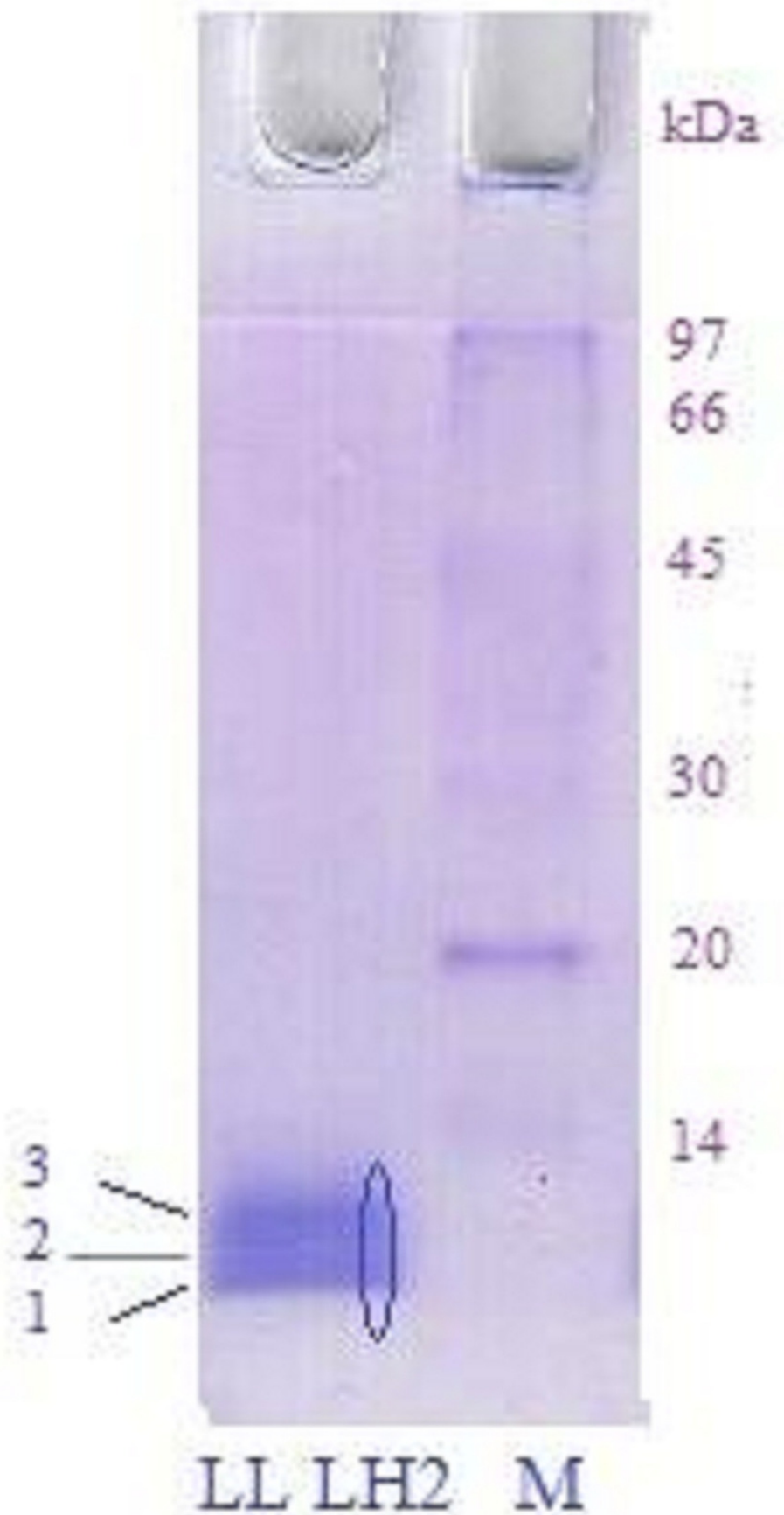



Figure 3

Multiple alignment of alpha-subunits of *Rps. palustris* light-harvesting complexes.

Multiple alignment of alpha-subunits of Rps. palustris light-harvesting complexes. The sequence differences in the C-terminal motif are underlined. The proteins are designated by their Uniprot IDs: P35101, αa; P35102, αb; P35103, αc; P35104, αd. The colours used are from the standard ClustalOmega colour code: red, hydrophobic (A, V, I, L, M. F, W, P); green, polar neutral (G, S, T, Q, N, Y, H); magenta, positively charged (R, K); blue, negatively charged (D, E).

```
P35101    MNQARIWTVVKPTVGLPLLLGSVTVIAILVHFAVLSHTTWFSKYWNGKAAAIESSVNVG-          59
P35102    MNQGRIWTVVNPGVGLPLLLGSVTVIAILVHYAVLSNTTWFPKYWNGATVAAPAAAPAPA          60
P35103    MNQGRIWTVVSPTVGLPLLLGSVAAIAFAVHFAVLENTSWVAAFMNGKSVAAAPAPAAPA          60
P35104    MNQGRIWTVVKPTVGLPLLLGSVAIMVFLVHFAVLTHTTWVAKFMNGKAAAIESSIKAV-          59
          ***.******.* **********: :.: **:*** :*:*.  : ** :.*   :  .

P35101    ------          59
P35102    APAAKK          66
P35103    APAKK-          65
P35104    ------          59
```

Figure 4

Fluorescence excitation spectra (the dotted lines) and the absorption spectra (in the form of 1-T, solid line) of the LH2 complexes from *Rps. palustris.*

The spectra were normalized by BChl *a* Qx-transition peak at 590 nm. The emission wavelengths for the fluorescence excitation spectra of LL LH2 and LH4 were 864 and 861 nm, respectively. The percentages written in the figure correspond to energy transfer efficiency from carotenoids to BChl in these complexes.

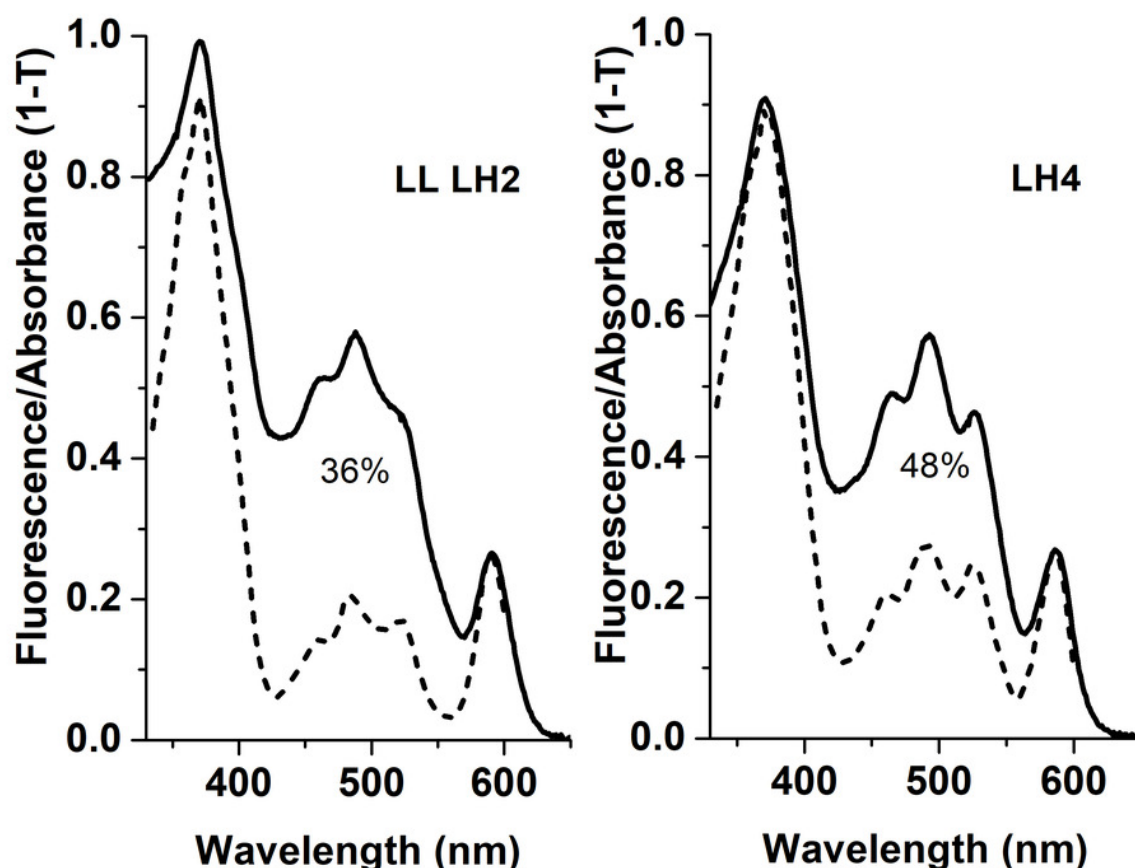



Figure 5

Models of LL LH2 complex based on 1NKZ template (LH2 complex of *Rbl. acidophilus*).

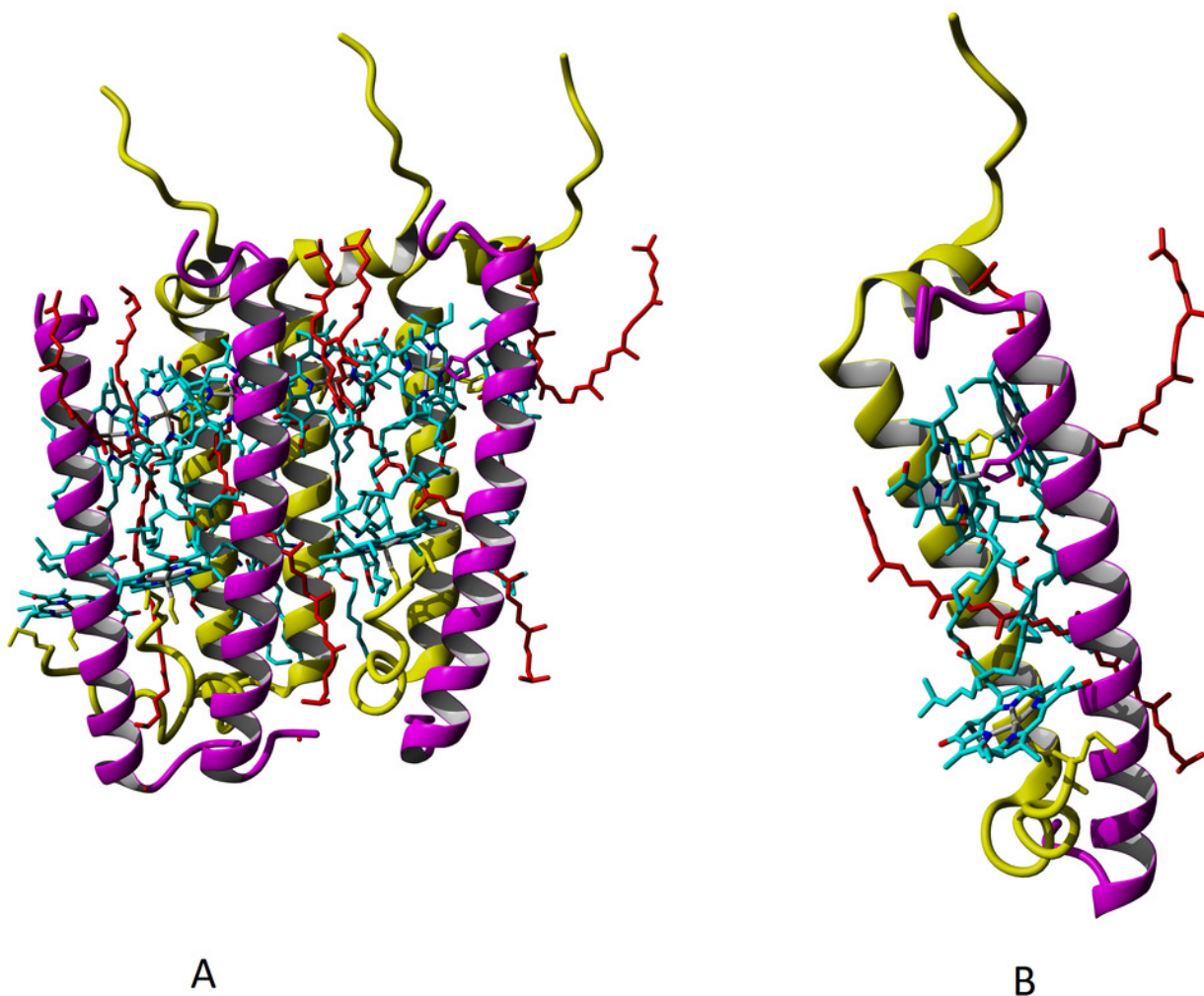
Rhodopin is colored red; bacteriochlorophyll is colored via conventional element scheme – carbon, cyan; nitrogen, blue; oxygen, red; magnesium, yellow; alpha subunits are yellow, beta subunits are magenta. A – hexameric view; B – close view of a single alpha-beta dimer with two rhodopin molecules and three bacteriochlorophyll molecules.

A   B

Figure 6

Multiple alignment of beta-subunits of peripheral LH-complexes in *Rps. palustris*.

Multiple alignment of beta-subunits of peripheral LH-complexes in Rps. palustris. The proteins are designated according to their UniProt IDs: P35106, βa; P35107, βb; P35108, βc; P35109, βd. Positions of variable amino acids of beta-subunits are underlined. The colours used are from the standard ClustalOmega colour code: red, hydrophobic (A, V, I, L, M. F, W, P); green, polar neutral (G, S, T, Q, N, Y, H); magenta, positively charged (R, K); blue, negatively charged (D, E).

```
P35106     ----MADKTLTGLTVEESEELHKHVIDGTRIFGAIAIVAHFLAYVYSPWLH     47
P35107     MADDPNKVWPTGLTIAESEELHKHVIDGTRIFGAIAIVAHFLAYVYSPWLH     51
P35108     MVDDSKKVWPTGLTIAESEEIHKHVIDGARIFVAIAIVAHFLAYVYSPWLH     51
P35109     MVDDPNKVWPTGLTIAESEELHKHVIDGSRIFVAIAIVAHFLAYVYSPWLH     51
              .       ****: ****:*******.*** ******************
```

Figure 7

Differences of valine residue V11 and isoleucine residue I15 in interaction with rhodopin.

Alpha subunits are coloured yellow, beta subunits are magenta. Rhodopin is coloured red. Bacteriochlorophyll is coloured via standard element scheme (cyan, carbon; blue, nitrogen; red, oxygen; yellow, magnesium). Valine resiude V11 (specific for βa subunit) is coloured magenta; isoleucine I15 (specific for βbcd subunits) is coloured orange. Black arrow shows hydrophobic contact common for valine and isoleucine (distance 6.477 Å); green arrow depicts a contact unique for isoleucine residue (distance 6.546 Å).

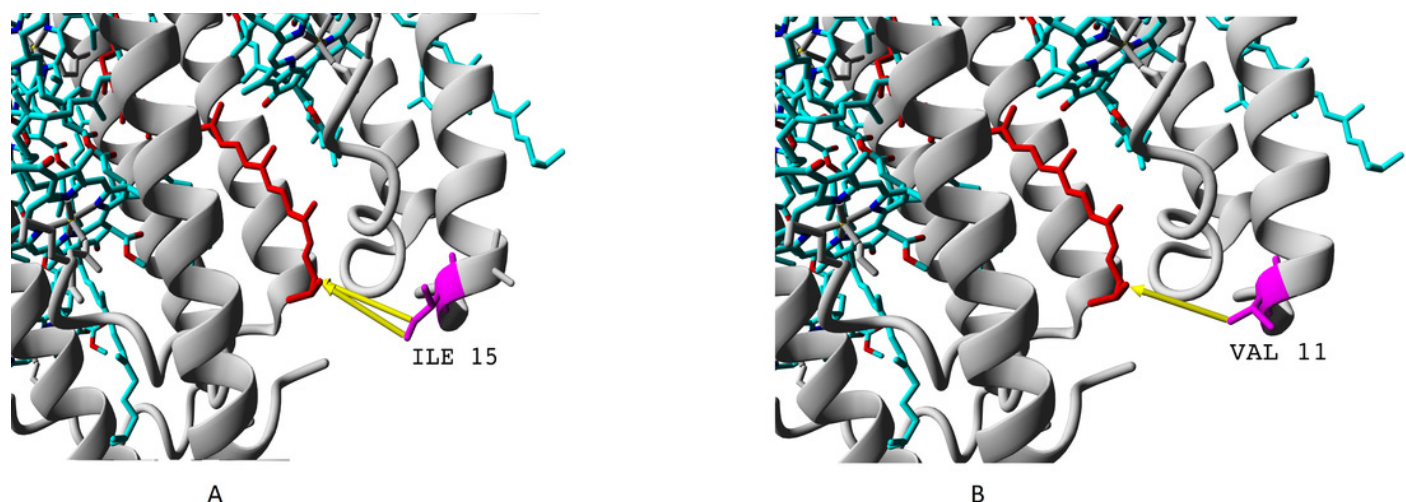



Figure 8

Serine and threonine residues and the difference in their interaction with chromophores.

Alpha subunits are coloured yellow, beta subunits are coloured magenta. Rhodopin is coloured red, bacteriochlorophyll B850 is coloured according to the standard element colouring scheme (carbon, cyan; nitrogen, blue; oxygen, red; magnesium, yellow). Threonine residue T25 specific for βab subunits is coloured orange; serine residue S29 specific for βd subunit is coloured magenta Green arrows show the contacts of threonine methyl group with bacteriochlorophyll (distance 3.955 Å) and rhodopin (3.866 Å).

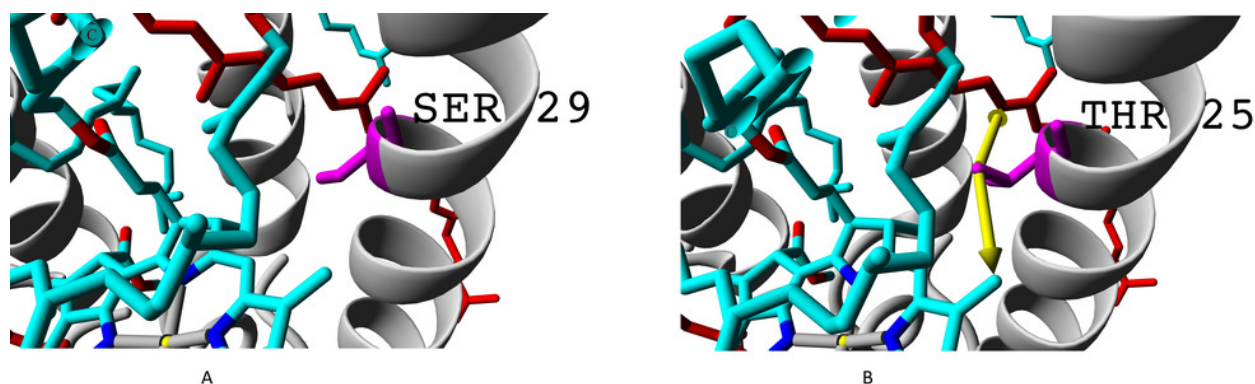



Figure 9

Valine residue V33 of LH4 complex and its interaction with chromophores (rhodopin, coloured red, and bacteriochlorophyll, coloured via standard element coloring scheme)

Green arrow shows interaction with rhodopin (distance 6.958 Å); black arrow shows possible van der Waals repulsion from bacteriochlorophyll (distance 2.370 Å).

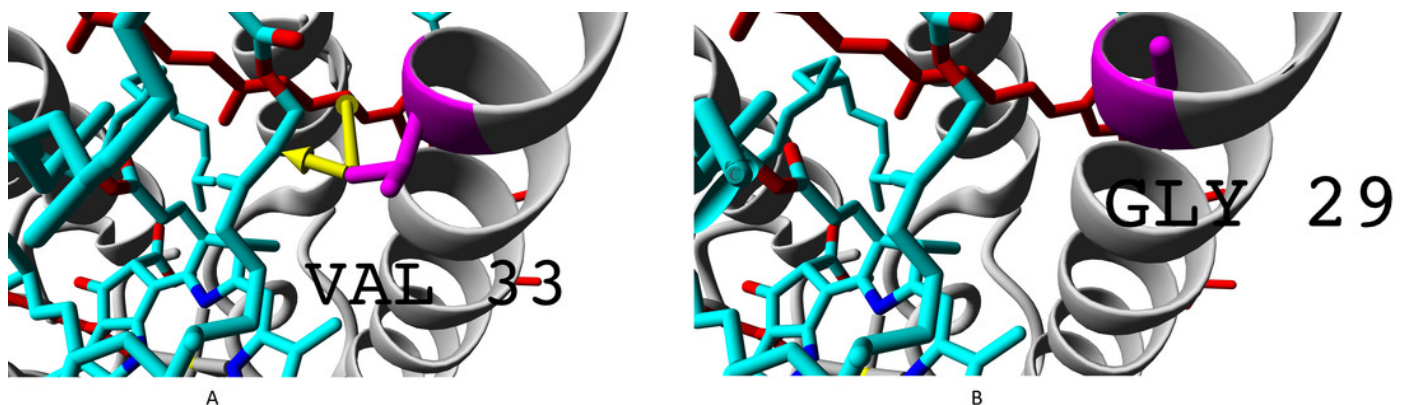



Figure 10

Isoleucine residue I21 (specific for βc subunit) and leucine residue L17/L21 (specific for βabd subunits), differences in interactions with chromophores (rhodopin, colored red).

Bacteriochlorophyll is colored according to standard element scheme. Black arrow shows a contact common for isoleucine and leucine residues with rhodopin (distance 5.155 Å); green arrow shows a contact unique for leucine residue (distance 6.599 Å).