

EXPERIMENTAL
ARTICLES

Assembly of LH2 Light-Harvesting Complexes in *Rhodopseudomonas palustris* Cells Illuminated by Blue and Red Light

Z. K. Makhneva, Yu. E. Erokhin, and A. A. Moskalenko¹

Institute of Basic Biological Problems, Russian Academy of Sciences

Received April 3, 2007; in final form October 22, 2007

Abstract—We investigated the formation of the B800-850 complex in cells of the bacterium *Rhodopseudomonas palustris* AB illuminated by red and blue light under anaerobic growth conditions. Under red illumination, the B800-850 complex was assembled with a reduced absorption band at 850 nm. The results of re-electrophoresis of the B800-850 complex and oxidation in the presence of potassium iridate suggest its heterogeneity. It may be a mixture of two complexes (B800 and B800-850). The B800-850 complex lacks the capacity for conformational transitions if assembled under blue illumination. Accordingly, the light-harvesting complex assembled in the blue light contains polypeptides that are not synthesized under normal conditions or at increased or decreased light intensities. The mechanism of regulation of the synthesis of the polypeptides of light-harvesting the B800-850 complex and its dependence on the spectral composition of the light is discussed.

Key words: photosynthetic bacteria, LH2 complexes (B800-850), assembly, blue light, red light, spectroscopy, electrophoresis, re-electrophoresis.

DOI: 10.1134/S0026261708030144

Purple photosynthetic bacteria contain the simplest known light-harvesting system, which typically consists of two complexes, denoted as B800-850 (or B800-830) and B880, based on their absorption bands in the near IR wavelength range. Both complexes are assembled in vivo from two types of low molecular weight polypeptides with masses below 10 kDa (referred to as the α and β type), bacteriochlorophyll, and carotenoids [1, 2]. It has been shown that the polypeptides of the B800-850 complex form two rings containing 8–9 polypeptides each. Bacteriochlorophyll dimers absorbing at 850 nm and carotenoids are located inside this annular structure surrounded by hydrophobic polypeptide columns. The bacteriochlorophyll monomer (with an absorption band at 800 nm) is arranged on the outer surface of the annular structure. The B880 complex, which consists of 16 polypeptide pairs [3, 4], displays a similar structure.

The complexes differ in terms of their interaction with the reaction center (RC). Complex B880 encloses RC, forming a coherent entity (the core complex, B880-RC), while the B800-850 complex is located on the periphery of this system [1, 2]. The B800-850 complex is therefore also termed the peripheral complex. In some cases, the B800-850 complex does not interact with the B880-RC system and is loosely arranged in the

membrane, forming autonomous clusters [2]. Some bacteria, e. g., *Rhodopseudomonas palustris*, respond to light intensity changes during cultivation by altering the spectral properties of the B800-850 complex. At high light intensities (60–300 W/m²), a normal complex (with the 850/800 ratio not less than 1.05) is assembled. Upon decreasing light intensity to 20 W/cm², the absorption band at 850 nm is reduced in the complex (the 850/800 ratio is 0.45) [5–9]. Cells cultivated at low light intensities accumulate more lycopene and less rhodovibrin in comparison to the cells grown at high light intensities [6].

Recently, increased attention has been paid to gene expression regulation and to the synthesis and assembly of cell structures. New blue and red light photoreceptor types have been revealed. Their common feature is that their intracellular concentrations are very low. This prevents their spectrophotometric detection, particularly in the presence of the pigments that are characteristic of photosynthetic bacteria. Photoreceptors of the first type are flavin-binding proteins with BLUF domains (proteins AppA, RSP4060, and RSP1261 of *Rhodobacter sphaeroides*, protein YcgF of *Escherichia coli*, and protein Slr1694 of *Synechocystis* PCC6803) [10–16] or photoactive PYP proteins [17]. Proteins with BLUF domains enable bacteria to avoid the effects of extremely high, deleterious blue light intensities [15]. Such proteins do not occur in all bacteria, and they are

¹ Corresponding author; e-mail: andrey-mos@issp.serpukhov.su

only efficient under semiaerobic conditions (the gas mixture composition is 89% N₂, 10% O₂, and 1% CO₂) [11, 17]. Under these conditions, the expression of the *puf* and *puc* operons that encode the proteins of pigment-protein complexes is suppressed by blue light [10]. At lower oxygen concentrations in the environment, the expression of the *puf* and *puc* operons is not affected by blue light [10]. PYP proteins absorb light in the blue range; they are present in the cell either as separate proteins or as domains of larger proteins. Bacteriophytochromes are photoreceptors of the second type. They have been detected in a number of photosynthetic bacteria (*Rps. palustris*, *Rs. rubrum*, and *Rb. sphaeroides*). They resemble phytochromes of higher plants [17–19]. Unlike the alterations in the complexes caused by light intensity changes during the cultivation of bacteria [5–9], the impact of light of different spectral composition on the formation of these complexes under anaerobic conditions has not yet been investigated. Only one paper [12] has dealt with the effect of illumination with far red light on formation of the light-harvesting system in *Rps. palustris* cells during their cultivation at various oxygen concentrations. It was established that low amounts of light-harvesting complexes were assembled in *Rps. palustris* CEA001 at oxygen concentrations exceeding 8%. Polypeptide synthesis and the complex assembly were drastically stimulated by far red light that is absorbed by the bacteriophytochrome.

Of special interest was to test the impact of light with different spectral composition on the assembly of bulk complexes in anaerobically grown bacteria, because the efficiency of assembling the light-harvesting complexes of photosynthetic bacteria is maximum under these conditions. The complexes from *Rps. palustris* AB are convenient systems for such studies for two reasons. First, their spectral properties may vary depending on the light intensity used for cultivation [5–9]. Second, complexes of this type are characterized by conformational changes that result in 20–30 nm blue shifts in the long-wave absorption band under the influence of solvents, detergents, and salts [5, 20]. Hence it is fairly easy to find out, with the help of these two tests, whether the peripheral light-harvesting complexes isolated from anaerobically grown bacteria under illumination with blue or red light differ from those in the control system.

The goal of the present work was to investigate the assembly of peripheral light-harvesting complexes in *Rps. palustris* AB cells grown under illumination with blue and red light.

MATERIALS AND METHODS

Rhodospseudomonas palustris AB cells were grown as described in [5]. To cultivate the cells under blue illumination (within the 430–570 nm range), we used a combination of SZS-22 and ZhS-12 filters. To cultivate them under red illumination (>600 nm), a KS-10 light

filter was used. All these filters were manufactured in Russia. A 300 W tungsten lamp with a mirror reflector was employed as the light source. A 5-cm water heat filter was placed between the lamp and the color filters. The light flux intensity was 70, 20, and 20 W/m² for illumination with white, blue, and red light, respectively. For comparison, the cells were also grown in low-intensity white light (with a light flux of 20 W/m²). The membrane isolation method was described in detail in [5, 20]. The complexes were isolated by preparative electrophoresis in 7% polyacrylamide gel with a length of 45 mm in tubes according to the protocol developed by us in [21]. The zone of the B800-850 complex in the gel moves 12–15 mm long in this setup. The re-electrophoresis of the B800-850 complex was carried out to assess their heterogeneity, using the same setup, with the gel length increased to 75 mm; the electrophoresis was terminated as soon as the zone of the complex moved 45–55 mm.

To extract carotenoids, 1 ml of the suspension of bacterial membranes with an optical density of 40–50 units at 850 nm was added to 10 ml of an acetone-methanol mixture (7 : 2) with continuous stirring. The extract obtained was sequentially supplemented with 2–4 ml of petroleum-ether and 20–25 of water; the mixture was stirred. The pigments extracted were located in petroleum-ether, in the upper layer of the mixture. They were collected with a pipette, placed in an ampoule and desiccated under nitrogen flow. The pigment film obtained was dissolved in acetone-methanol mixture (7 : 2). The extract (25 µl) was applied to a column.

The pigments were analyzed using high pressure liquid chromatography (HPLC) as described in [22, 23] on a Spherisorb ODS2 5 µm 4.6 × 250 mm column (Waters, United Kingdom). The HPLC setup included an LC 10ADvp pump with an FCV 10Alvp module, which enabled adjusting the solvent gradient at low pressure, and a detector equipped with an SPD-M20A diode matrix (Shimadzu, Japan). The solvent flow rate was 1.0 ml/min. The column was equilibrated with the mixture consisting of 77% acetonitrile-water (9 : 1) and 23% ethyl acetate. The mixture was passed through the column for the first 3 min. Subsequently, the mixture was linearly replaced with ethyl acetate for 37 min; pure ethyl acetate was thereupon passed through the column for 3 min. Carotenoid concentration was calculated using the respective absorbance coefficients and the area of the fraction obtained within the 415–550 nm range, using the LC-solution software package (Shimadzu, Japan) as described in [22, 23].

Absorption spectra were recorded with an UV-160 spectrophotometer (Shimadzu, Japan). A part of the spectra was directly converted into digital form in the course of measurement by means of an EL24L digital converter (L-Kard, Russia) using the PowerGraph software package (www.powergraph.ru). The spectra were digitized manually using the Graph2Digit 0.52b software package (developed by V. Plisko, website

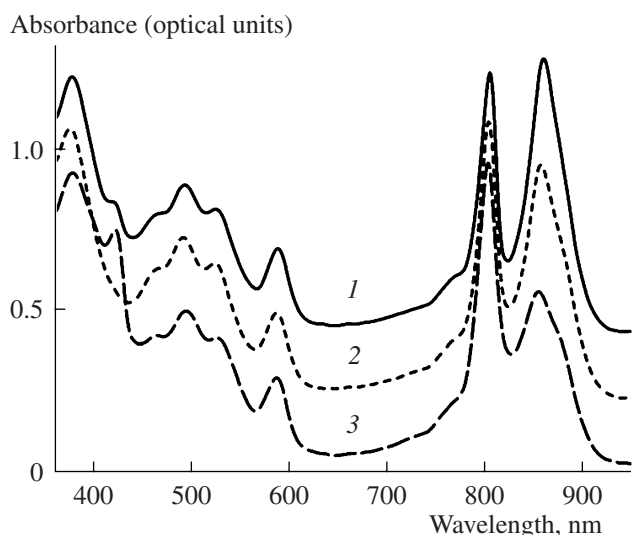


Fig. 1. Absorption spectra of the membranes isolated from *Rps. palustris* AB cells grown under illumination with white (1), blue (2), and red (3) light.

plsoft.narod.ru) in the “negative” mode with a step length of 2 (1 nm = 2 pixels) as described earlier [23]. To improve the images of the spectra, they were shifted by 0.2 to 0.5 optical density units relative to one another (Figs. 1, 4–6).

This study used Tris (Sigma, United States), dodecyl maltoside (Anatrace, United States), and potassium iridate (Aldrich, United States). Other reagents employed were produced in Russia (chemically pure).

RESULTS AND DISCUSSION

In absorption spectra of the membranes isolated from cells grown in the white light, bacteriochlorophyll complexes (B800-850 and B880) are characterized by absorption bands with maxima at 377, 589, 804, and 858 nm and a small shoulder at 876 nm (Fig. 1, spectrum 1). The three peaks in the 430–560 nm range are due to light absorption by the carotenoids, and the small peak at 421 nm was identified as the cytochrome peak. In the control membranes, the ratio between the intensities of the B800-850 complex-specific absorption bands at 858 and 804 nm was 1.03. The near-IR absorption spectrum of the membranes isolated from the cells grown under blue illumination (Fig. 1, spectrum 2) revealed almost no differences from that of the control membranes. Based on the intensity of the blue light flux, one could expect changes in the absorption spectrum of the membranes, similar to those caused by low light intensity [5, 6, 8]. However, we detected only a slight decrease in the longer-wave absorption band of the B800-850 complex (the 858/804 ratio decreased to 0.83); the cytochrome absorption band virtually disappeared. The most significant changes occurred in the membranes isolated from red light-grown cells (Fig. 1, spectrum 3). The absorption spectrum of these mem-

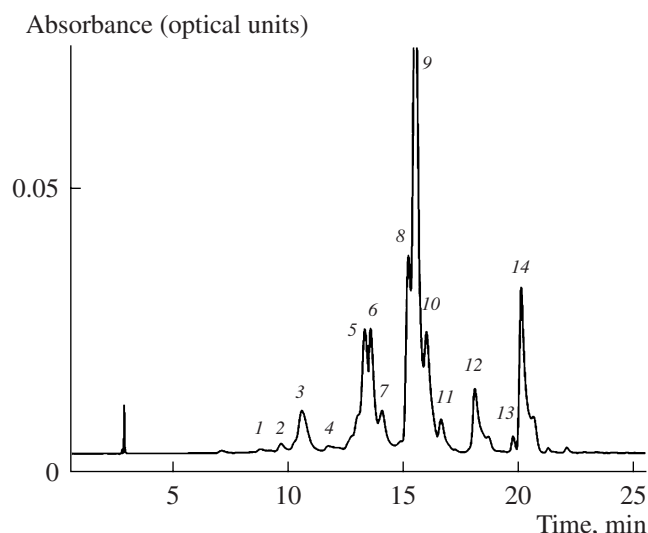


Fig. 2. HPLC separation of pigments from the membranes of *Rps. palustris* AB cells grown in blue light. Registration range was 415–550 nm. Peaks: 1, 2, 4, 6, bacteriochlorophyll; 3, rhodopin derivative; 5, rhodovibrin; 7, product of bacteriochlorophyll oxidation; 8, dedihydrorhodopin; 9, rhodopin; 10, spirilloxanthin; 11, anhydroporphyrin derivative; 12, anhydroporphyrin; 13, bacteriopheophytin; 14, lycopene.

branes revealed a significant decrease in the intensity of the longer-wave absorption band of the B800-850 complex (the 858/804 ratio was 0.55), which was accompanied by an increase in the intensity of the cytochrome absorption band. The absorption spectra of all the tested samples within the carotenoid absorption range were similar (the main absorption maximum was at 491 nm). The absorption spectra of the pigments extracted from the samples were also virtually identical. The chromatogram of the pigments from blue light-grown *Rps. palustris* cells is shown in Fig. 2. Similar results were obtained with the other two samples. By varying the spectral composition of the light used to illuminate *Rps. palustris* cells, we obtained membranes with the carotenoid composition presented in the table.

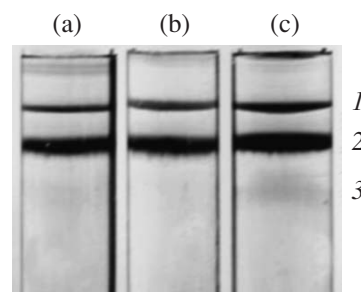


Fig. 3. Electrophoresis of the membranes isolated from *Rps. palustris* AB cells grown in white (a), blue (b), and red (c) light. Zones: 1, B880-RC system; 2, the B800-850 complex; 3, cytochrome.

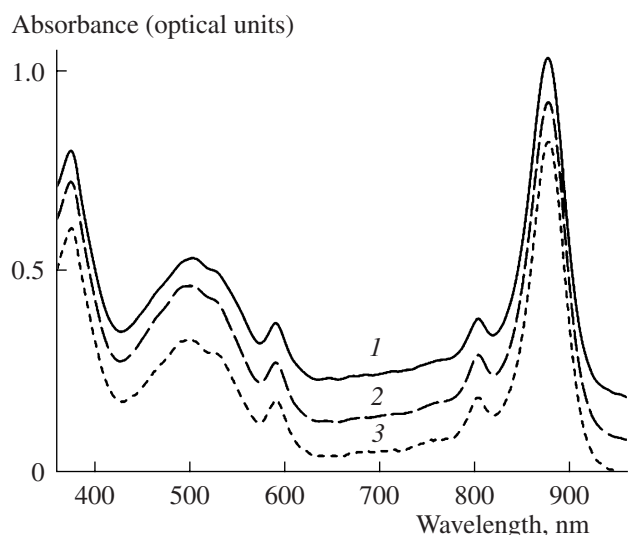


Fig. 4. Absorption spectra of the B880-RC systems isolated from *Rps. palustris* AB cells grown in white (1), blue (2), and red (3) light.

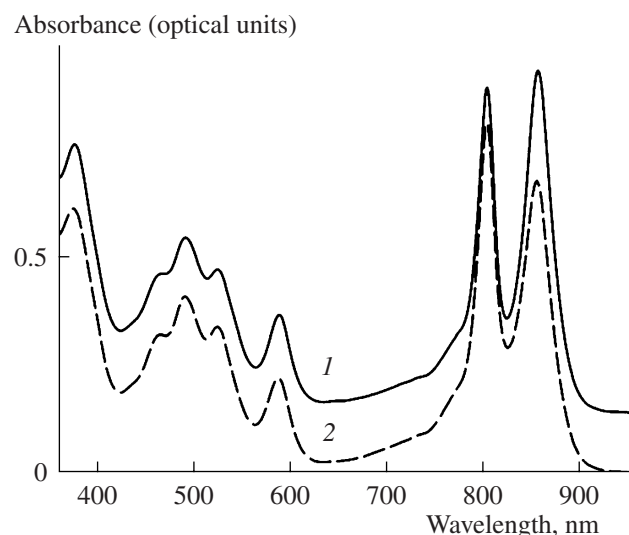


Fig. 6. Absorption spectra of the light-harvesting complexes (B800-850) from *Rps. palustris* AB cells grown in white (1) and blue (2) light.

Rhodopin and dehydrorhodopin were the main carotenoids in all tested membranes, and their total ratio was 60–68%. As is apparent, the light composition did not influence carotenoid synthesis in *Rps. palustris* AB cells.

To obtain light-harvesting complexes, the membranes were solubilized using dodecyl maltoside to retain the native conformation of the B800-850 complexes [20]. The complexes were isolated electrophoretically in polyacrylamide gel. After electrophoresis, the gel contained three pigmented zones (Fig. 3).

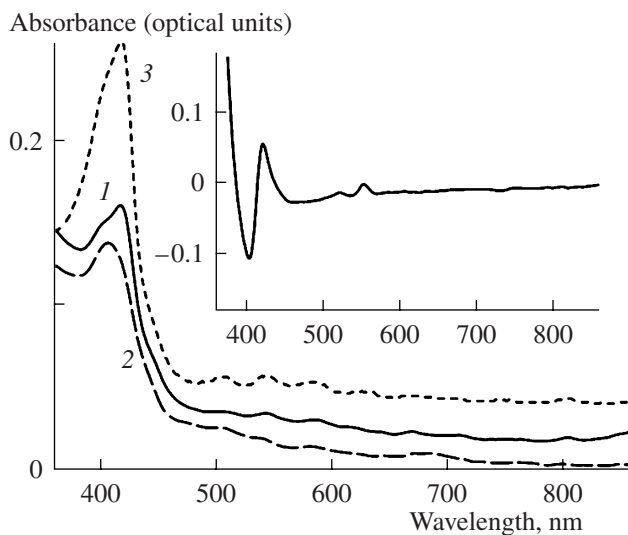


Fig. 5. Absorption spectra of the cytochromes isolated from *Rps. palustris* AB cells grown in white (1), blue (2), and red (3) light. Insert: the “oxidized-reduced” difference spectrum from red light-grown cells.

According to their absorption spectra, these zones were identified as the B880-RC system (upper zone), the B800-850 complex (middle zone), and the cytochrome (lower zone). The absorption spectra of the B880-RC systems in all tested samples were virtually identical. The main absorption band in the near-IR range was located at 876 nm (Fig. 4). These complexes are the most conserved part of the bacterial photosynthetic apparatus, and their *in vivo* assembly is influenced neither by light intensity [5–9] nor by the spectral composition of the light. The cytochrome absorption band at 420 nm is almost invisible in the absorption spectrum of the membranes from blue light-grown cells. Nevertheless, a minor cytochrome fraction was detected using electrophoresis (Fig. 5). The cytochromes were also identified using their difference spectra (Fig. 5, insert). A more detailed study of the cytochromes was beyond the scope of this work. Hence, the most active cytochrome synthesis occurred in red light-grown *Rps. palustris* AB cells. From these data it is evident that varying the spectral composition of the light produced a different effect than varying light intensity. Changing light intensity failed to cause significant changes in cytochrome synthesis in *Rps. palustris* AB cells [5]. The *Rps. palustris* CEA001 and CGA009 mutants did not reveal an increase in cytochrome synthesis either [12].

The absorption spectra of the B800-850 complexes that were isolated from the samples grown under illumination with white and blue light are shown in Fig. 6. The near-IR absorption bands are located at 804 and 856 nm in the control sample and at 804 and 855 nm in the “blue” complex, while the ratio between the band intensities is 1.05 and 0.85, respectively. The absorption spectrum of the B800-850 complex from red light-grown cells is shown in Fig. 7 (spectrum 1). The near-

IR absorption bands are located at 803 and 852 nm in this complex, while the ratio between the band intensities (852/803) is 0.47. The same ratio is characteristic of the B800-850 complex isolated from low intensity light-grown *Rps. palustris* AB cells (Fig. 7, spectrum 2). Complexes with the spectral properties similar to those of the “red” complex (with maxima at 802 and 805 nm and a 850/802 ratio of 0.45) were also isolated from low intensity light-grown *Rps. palustris* 2.1.6 cells [9]. When grown under illumination with red light, the CEA001 and CGA009 mutants of *Rps. palustris* did not undergo any changes in respect to the spectral properties of the B800-850 complexes [12].

Our studies concerning the possibility of conformational transitions in the complexes obtained were aimed at resolving the issue whether cultivation in the blue/red light changes the structure of the B800-850 complexes in *Rps. palustris* cells. We have earlier demonstrated that B800-850 complexes from 850 *Rps. palustris* grown at various light intensities and from *Alc. minutissimum* are capable of conformational transitions. They result in blue shift in the longer-wave absorption band from 858 to 830–840 nm upon treating the complexes or the membranes with low concentrations of solvents or nonionic detergents [5, 20]. The results of treating the B800-850 complex with the nonionic detergent Triton X-100 are shown in Fig. 8. The control and the “red” B800-850 complex reveal the conformational changes as described above. Unexpectedly, the “blue” B800-850 complex lacked the conformational changes. It is known that these changes occur only if the interactions among the polypeptides of the light-harvesting complex are influenced by external factors. Such alterations in protein–protein interactions result in changes in the pigment–pigment interactions in bacteriochloro-

Carotenoid composition (%) of the membranes of *Rps. palustris* AB cells illuminated with light of different spectral composition

Carotenoid	Control	Blue	Red
Rhodopin derivative	1.44	5.65	1.67
Demethylspirilloxanthin	3.12	–	–
Rhodovibrin	4.89	2.82	2.09
Dedihydrorhodopin	21.38	12.01	24.07
Rhodopin	36.94	44.39	42.18
Spirilloxanthin	8.14	10.97	6.02
Anhydrorhodovibrin derivative	0.78	2.49	0.51
Anhydrorhodovibrin	7.11	4.93	6.3
Lycopene	16.2	16.74	16.3
Neurosporene	–	–	0.86

phyll dimers that manifest themselves in a blue shift in the absorption band at 850 nm [5, 20, 24, 25]. These results were confirmed by studies on point mutations in the polypeptides of the light-harvesting complex (B800-850) from *Rb. sphaeroides* [26]. The single substitution of Phe for Tyr44 or the double substitution of Phe and Leu for Tyr44 and Tyr45 in the α -polypeptide of the B800-850 complex caused a blue shift in the longer-wave band of the absorption spectrum by 11 and 24 nm, respectively. These amino acid residues are located in the terminal COOH domain of the polypeptide, out of contact with the bacteriochlorophyll dimer. Hence, the lack of conformational changes in the

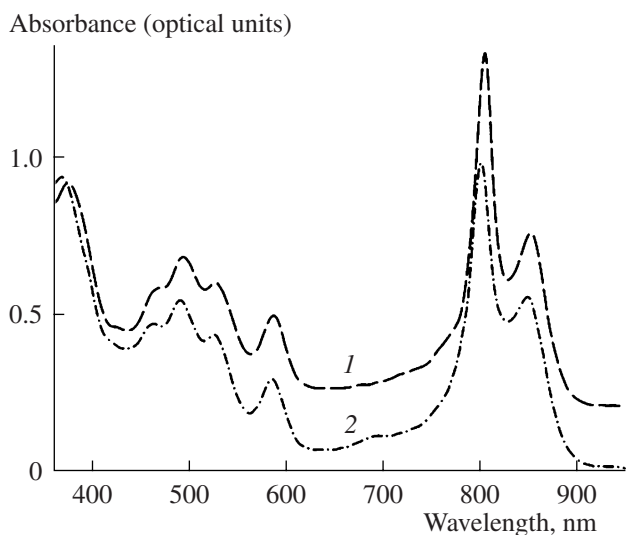


Fig. 7. Absorption spectra of the light-harvesting complexes (B800-850) from *Rps. palustris* AB cells grown in red light (1) and low-intensity light (2).

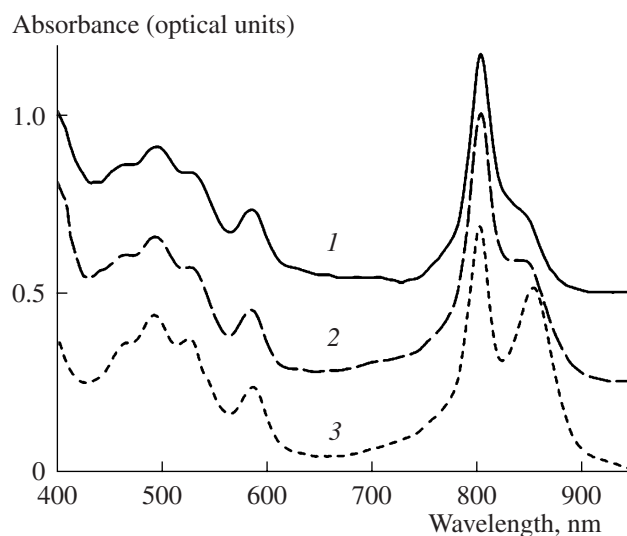


Fig. 8. Changes in the absorption spectra of the B800-850 complexes under the influence of the nonionic detergent Triton X-100: 1, complex from white light-grown cells; 2, complex from red light-grown cells; 3, complex from blue light-grown cells.

“blue” complex indicates that it has undergone significant changes in terms of protein–protein interactions. Since these can only be due to changes in the primary polypeptide structure, blue light-grown *Rps. palustris* AB cells presumably synthesize B800-850 complex proteins that are different from those formed under red/white illumination. These polypeptides may be encoded by genes that are inactive at changing light intensities [7, 12]. *Rps. palustris* cells contain the gene multifamily that code for the α - and β -polypeptides of the B800-850 complex [19]. However, not all of the genes are expressed at a detectable level. The question is to clarify which gene products are synthesized in *Rps. palustris* 2.1.6 cells at high and low light intensities. It was revealed [7] that the pairs of polypeptides (α and β) encoded by *PucBA_a* and *PucBA_d* genes are synthesized at high and low light intensities, respectively. Two amino acids are substituted in the amino acid sequences of both polypeptides in response to a light intensity change. It was established [8] that *Rps. palustris* cells grown at high (300 W/m²) or intermediate (60 W/m²) light intensities synthesize three polypeptide pairs ($\alpha\beta_a$, $\alpha\beta_b$, and $\alpha\beta_d$). Lowering the light intensity to 20 or 6 W/m² results in a lack of $\alpha\beta_b$ -polypeptides, whereas the level of $\alpha\beta_a$ -polypeptide synthesis drastically decreases. Up to 6 amino acid residues may be substituted in the α -polypeptides, based on the data available in the literature. Only six genes of the ten coding for the α and β -polypeptides of the B800-850 complex are expressed in *Rps. palustris* 2.1.6 cells grown in the white light at various light intensities [19]. Taking into account our results and the data reported in the literature, we suggest that blue light illumination results in the synthesis of the α - and β -polypeptides that are not synthesized in the white light, regardless of its intensity. These polypeptides are then assembled into B800-850 complexes that lack the capacity for conformational transitions. In order to test this suggestion, we separated the polypeptides of different complexes by electrophoresis with sodium dodecyl sulfate. However, we revealed no significant differences in terms of electrophoretic mobility or band intensity. Evidently, more efficient methods are required to separate the hydrophobic polypeptides of the B800-850 complex that only differ in several amino acid residues.

If the B800-850 complex is assembled under red illumination, it resembles the complex isolated from cells grown at a low light intensity. Under these conditions, *Rps. palustris* 2.1.6 cells assemble two types, and not just one type, of peripheral light-harvesting complexes [8], i.e., the anomalous complex with a near-IR absorption band at 800 nm and the usual complex (B800-850). These complexes were isolated and characterized. We attempted to assess the heterogeneity of the B800-850 complex isolated from red light-grown cells using several methods. If the longer-wave band of the absorption spectrum of the control (“white”) and “red” B800-850 complex was standardized, the intensity of the absorption band at 800 nm was almost two

times higher in the “red” than in the control complex. To resolve the mixture of the complexes, we carried out re-electrophoresis of the isolated “red” B800-850 complex on a long gel column with dodecyl maltoside. The complex migrated as a single zone during re-electrophoresis. Monitoring the absorption spectra of the front and the tail part of this zone revealed that the ratio between the longer-wave band intensities in their spectra was approximately 1.25 (Fig. 9a). Similar results were earlier obtained by us by re-electrophoresis of the isolated B800-850 complex from *Thiorhodospira sibirica*. We originally considered this complex homogeneous and believed that the differences were due to the different conformational state of the electrophoretic fractions of the complex [27, 28]. However, we established later that this complex, in turn, includes complexes of two types (B800-830 and B800-850; Tikhonova et al., unpublished). Another approach to assessing the possible heterogeneity of this complex was based on the assumption that if it was heterogeneous, different stability of the partial complexes or different products resulting from their modification could result. We investigated the effect of sodium boron hydride, sodium dodecyl sulfate, and potassium iridate on the complex or the membranes containing it. Tests with the first two substances revealed no significant differences in the behavior of the complex in comparison to the control system. Bacteriochlorophyll molecules absorbing at 800 nm were predominantly oxidized in the “red” complex by the oxidant potassium iridate (Fig. 9b). This was its main difference from the control complex, where the bacteriochlorophyll dimer was the first to be oxidized. From these data we drew the preliminary conclusion that the “red” B800-850 complex that is assembled in *Rps. palustris* AB cells in the red light appears to be heterogeneous, like that of *Rps. palustris* 2.1.6 [8].

Summing up the results obtained and the data available in the literature [5, 8, 9], we infer that several kinds of regulatory processes related to the synthesis and assembly of the building blocks of the light-harvesting system can be in operation in *Rps. palustris* cells, depending on the intensity and the spectral composition of the light. White light with a normal or high intensity allows the cells to synthesize and assemble the standard set of complexes of the photosynthetic machinery with standard spectral properties [5]. Low-intensity white light causes the cell to assemble two types, not one type, of bulk light-harvesting complexes, i.e., the anomalous complex with an absorption band at 800 nm (within the near-IR range) and the usual complex (B800-850) [5, 8, 9]. In the red light, cytochrome synthesis was enhanced and a B800-850 complex with a reduced absorption band at 850 nm was assembled. Obviously, this complex was heterogeneous. As for illumination with blue light, the data obtained indicated that the B800-850 complex lacked the capacity for conformational changes.

The spectral properties of the B800-850-type light-harvesting complexes of cells grown in red light or low-intensity light are very similar in terms of the location of the absorption bands within the near IR zone and of the ratio between the band intensities. Presumably, the assembly of these complexes is triggered by the same photoreceptor type. In *Rps. palustris* cells, the photoreceptor role may be played by the phytochrome that absorbs in the red spectral range (>650 nm) [17]. Supposedly, this regulates the “photosynthetic biosynthesis pathways,” including the genes involved in the biosynthesis of bacteriochlorophyll, carotenoids, and reaction center proteins (*pufLM*) [29]. The *Rps. palustris* CEA001 strain lacked the light-harvesting system in the dark in the presence of oxygen; both the synthesis of the relevant polypeptides and the assembly of the complexes were drastically stimulated by red light [18]. We cultivated *Rps. palustris* AB cells in the red light anaerobically. In this system, only the assembly of the 800-850 complex was changed. No regulation of the biosynthesis of carotenoids (according to the HPLC data) and bacteriochlorophyll was observed. This strain of *Rps. palustris* was also characterized by a drastic increase in cytochrome synthesis in the red light. Accordingly, the *Rps. palustris* AB strain can regulate both the formation of the polypeptides of the bulk complex and its assembly and the cytochrome synthesis. If bacteriophytochrome controls the assembly of light-harvesting complexes at a low light intensity, there should be an additional mechanism that “turns it on” if light intensity decreases to a certain threshold value.

Under blue illumination, *Rps. palustris* AB cells assemble a B800-850 complex that lacks the capacity for conformational transitions. Cytochrome synthesis also decreases under these conditions. Light within the blue spectral range is absorbed by two types of photoreceptors: flavin-binding proteins (with BLUF domains) and PYP proteins [10–17]. Since PYP proteins have not been revealed in the cells of three *Rps. palustris* strains [17], they are presumably lacking in the tested strain *Rps. palustris* AB. Accordingly, an AppA type flavin-binding protein [10, 11] can be responsible for the blue light effect in our system. This type of photoreceptor only functions under semiaerobic conditions [10, 17], and the expression of the *puf* and *puc* genes that encode the photosynthetic apparatus proteins is blue light-independent at a low oxygen concentration [10]. The membrane absorption spectra of the tested control and blue light-grown cells were virtually identical, indicative of similar expression levels of the genes that code for light-harvesting complexes in these systems. Thus, the synthesis of individual proteins (such as the cytochrome) and the polypeptides that make up the light-harvesting complex (B800-850) is subject to regulation in *Rps. palustris* AB cells grown in red and blue light.

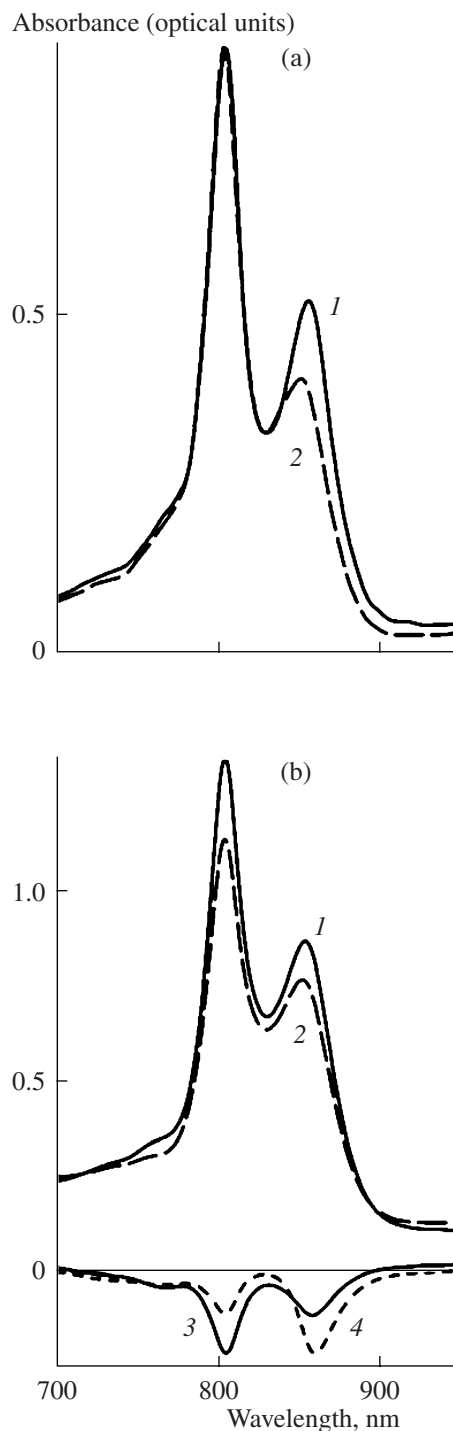


Fig. 9. Absorption spectra of the front (1) and tail (2) zone of the B800-850 complex isolated from *Rps. palustris* AB cells grown in red light after re-electrophoresis on long gel columns (a) and absorption spectra of the B800-850 complex isolated from *Rps. palustris* AB cells grown in red light before (1) and after (2) treating them with 0.4 mM potassium iridate; 3, 4, “control–0.4. potassium iridate” difference spectra of the B800-850 complex isolated from *Rps. palustris* AB cells grown in red light (3) and the B800-850 complex from the control cells (4).

ACKNOWLEDGMENTS

The authors wish to thank Z.A. Zhuravleva for cultivating the bacteria and assisting in isolation of the complexes.

This study was conducted under the "Principally New Carotenoid Function" program and supported by the Russian Federation budget and, in part, by the Russian Foundation for Basic Research (grant no. 06-04-48516).

REFERENCES

- Erokhin, Yu.E. and Moskalenko, A.A., Characterization of the Proteins of the *Chromatium* Pigment-Lipoprotein Complexes (Number of Chains and Molecular Weights), *Dokl. Akad. Nauk SSSR*, 1973, vol. 212, pp. 495-498.
- Zuber, H. and Cogdell, R.J., Structure and Organization of Purple Bacterial Antenna Complexes, in *Anoxygenic Photosynthetic Bacteria*, Blankenship, R.E., Madigan, M.T., and Bauer, C.E., Eds., Dordrecht: Kluwer, 1995, pp. 315-348.
- Rozsak, A.W., Howard, T.D., Southall, J., Gardiner, A.T., Law, C.J., Isaacs, N.W., and Cogdell, R.J., Crystal Structure of the RC-LH1 Core Complex from *Rhodospseudomonas palustris*, *Science*, 2003, vol. 302, pp. 1969-1972.
- McDemott, G., Princ, S.M., Freer, A.A., Hawthornthwaite-Lawless, A.M., Papiz, M.Z., Cogdell, R.J., and Isaacs, N.W., Crystal Structure of an Integral Membrane Light-Harvesting Complex from Photosynthetic Bacteria, *Nature*, 1995, vol. 374, pp. 517-521.
- Prokhorenko, I.R., Makhneva, Z.K., and Erokhin, Yu.E., Effect of Different Light Intensities on the Organization of the *Rhodospseudomonas palustris* AB Photosynthetic Apparatus, *Biol. Membrany*, 1991, vol. 8, pp. 476-481.
- Evans, M.B., Hawthornthwaite, A.M., and Cogdell, R.J., Isolation and Characterization of the Different B800-850 Light-Harvesting Complexes from Low- and High-Light Grown Cells of *Rhodospseudomonas palustris*, Strain 2.1.6, *Biochim. Biophys. Acta*, 1990, pp. 71-76.
- Fowler, G.J. and Hunter, C.N., The Synthesis and Assembly of Functional High and Low Light LH2 Antenna Complexes from *Rhodospseudomonas palustris* in *Rhodobacter sphaeroides*, *J. Biol. Chem.*, 1996, vol. 271, pp. 13356-13361.
- Tharia, H.A., Nightingale, T.D., Papiz, M.Z., and Lawless, A.M., Characterization of Hydrophobic Peptides by Rp-HPLC from Different Spectral Forms of LH2 Isolated from *Rps. palustris*, *Photosynth. Res.*, 1999, vol. 61, pp. 157-167.
- Gall, A. and Robert, B., A Structural Characterisation of the Different Peripheral Light-Harvesting Complexes from High- and Low-Light Grown Cells from *Rhodospseudomonas palustris*, Strain 2.6.1, *Biochemistry*, 1999, vol. 38, pp. 5185-5190.
- Braatsch, S., Gomelsky, M., Kuphal, S., and Klug, G., A Single Flavoprotein, AppA, Integrates Both Redox and Light Signals in *Rhodobacter sphaeroides*, *Mol. Microbiol.*, 2002, vol. 45, pp. 827-836.
- Braatsch, S., Moskvin, O.V., Klug, G., and Gomelsky, M., Responses of the *Rhodobacter sphaeroides* Transcriptome To Blue Light Under Semiaerobic Conditions, *J. Bacteriol.*, 2004, vol. 186, pp. 7726-7735.
- Giraud, E., Zappa, S., Vuillet, L., Adriano, J.-M., Hannibal, L., Fardoux, J., Berthomieu, C., Bouyer, P., Pignol, D., and Vermeglio, A., A New Type of Bacteriophytochrome Acts in Tandem with a Classical Bacteriophytochrome To Control the Antennae Synthesis in *Rhodospseudomonas palustris*, *J. Biol. Chem.*, 2005, vol. 280, pp. 32389-32397.
- Anderson, S., Dragnea, V., Masuda, S., Ybe, J., Moffat, K., and Bauer, C., Structure of a Novel Photoreceptor, the BLUF Domain of AppA from *Rhodobacter sphaeroides*, *Biochemistry*, 2005, vol. 44, pp. 7998-8005.
- Jung, A., Domratcheva, T., Tarutina, M., Wu, Q., Ko, W.-H., Shoeman, R.L., Gomelsky, M., Gardner, K.H., and Schlichting, I. Structure of a bacterial BLUF photoreceptor: Insights into blue light-mediated signal transduction, *Proc. Natl. Acad. Sci. USA*, 2005, vol. 102, pp. 12350-12355.
- Kort, R., Crielgaard, W., Spudich, J.L., and Hellingerwerf, K.J., Color-Sensitive Motility and Methanol Release Responses in *Rhodobacter sphaeroides*, *J. Bacteriol.*, 2000, vol. 182, pp. 3017-3021.
- Jiang, Z.Y., Swem, L.R., Rushing, B.G., Devanathan, S., Tollin, G., and Bauer, C.E., Bacterial Photoreceptor with Similarity To Photoactive Yellow Protein and Plant Phytochromes, *Science*, 1999, vol. 285, pp. 406-409.
- Kyndt, J.A., Meyer, T.E., and Cusanovich, M.A., Photoactive Yellow Protein, Bacteriophytochrome, and Sensory Rhodopsin in Purple Phototrophic Bacteria, *Photochem. Photobiol. Sci.*, 2004, vol. 3, pp. 519-530.
- Giraud, E., Zappa, S., Jaubert, M., Hannibal, L., Fardoux, J., Adriano, J.-M., Bouyer, P., Genty, B., Pignol, D., and Vermeglio, A., Bacteriophytochrome and Regulation of the Synthesis of the Photosynthetic Apparatus in *Rhodospseudomonas Palustris*: Pitfalls of Using Laboratory Strains, *Photochem. Photobiol. Sci.*, 2004, vol. 3, pp. 587-591.
- Larimer, F.W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., Land, M.L., Pelletier, D.A., Beatty, J.T., Lang, A.S., Tobita, F.R., Gibson, J.L., Hanson, T.E., Bobst, C., Torres, J.L., Peres, C., Harrison, F.H., Gibson, J., and Harwood, C.S., Complete Genome Sequence of the Metabolically Versatile Photosynthetic Bacterium *Rhodospseudomonas palustris*, *Nature Biotechnol.*, 2004, vol. 22, pp. 55-61.
- Moskalenko, A.A., Kuznetsova, N.Yu., Erokhin, Yu.E., and Toropygina, O.A., Organization and Conformational Transitions in the B800-850 Complex from *Chromatium minutissimum*, *Biokhimiya*, 1996, vol. 61, pp. 429-439.
- Moskalenko, A.A. and Erokhin, Yu.E., Isolation of Pigment-Lipoprotein Complexes from Purple Bacteria by Preparative Electrophoresis in Polyacrylamide Gel, *Mikrobiologiya*, 1974, vol. 43, pp. 654-655.
- Moskalenko, A.A., Britton, G., Konnor, A., Iang, A., and Toropygina, O., Composition of Carotenoids in the Chromophores and Pigment-Protein Complexes Isolated from *Chromatium minutissimum* Cells Grown in the Presence of diphenylamine, *Biol. Membr.*, 1991, vol. 8, pp. 249-260.
- Makhneva Z. and Moskalenko, A., Pigment-Protein Complexes from the Now Sulfur Photosynthetic Bacterium *Ectothiorhodospinus mongolicum* Strain M9 with

- Normal and Inhibited Carotenoid Synthesis, *Biol. Membr.*, 2004, vol. 191.
24. Moskalenko, A.A., Pigment-Protein Complexes and Their Interaction in the Structures of Bacterial and Plant Photosynthetic Apparatus, *Doctoral (Biol.) Dissertation*, as a report, IPFS RAN. Pushchino, 1993.
 25. Razjivin, A.P., Moskalenko, A.A., and Novoderezhkin, V.I., A Model of Spectral Changes of the B800-850 Complex from *Chromatium minutissimum* Induced by Detergent Treatment, *IUBMB Life*, 2000, vol. 50, pp. 115–117.
 26. Fowler, G.J., Visschers, R.W., Grift, G.G., Grondelle, R., and Hunter, C.N., Genetically Modified Photosynthetic Antenna Complexes with Blueshifted Absorbance Bands, *Nature*, 1992, vol. 335, pp. 848–850.
 27. Moskalenko, A.A., Makhneva, Z.K., Zhuravleva, Z.A., and Erokhin, Yu.E., Some Spectral Characteristics of Pigment-Protein Complexes and Their Interaction in Membranes of *Thiorhodospira sibirica*, *Dokl. Akad. Nauk*, 2002, vol. 382, pp. 836–839 [*Dokl. Biochem. Biophys.* (Engl. Transl.), vol. 382, pp. 63–66].
 28. Pishchal'nikov, R.Yu., Moskalenko, A.A., and Razzhivin, A.P., Hypothesis on the Origin of Three Bands in Absorption Spectrum of the New Type LH2 Complex (B800-830-850) from *Thiorhodospira sibirica*, *Biol. Mem.*, 2003, vol. 20, pp. 386–394.
 29. Giraud, E., Fardoux, J., Fourier, N., Hannibal, L., Genty, B., Bouyer, P., Dreyfus, B., and Vermeglio, A., Bacteriophytochrome Controls Photosystem Synthesis in Anoxygenic Bacteria, *Nature*, 2002, vol. 417, pp. 202–205.