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EXPERIMENTAL ARTICLES

Role of Bacteriochlorophyll in Stabilization of the Structure of the Core and Peripheral Light-Harvesting Complexes from Purple Photosynthetic Bacteria

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Abstract—Pheophytinization of bacteriochlorophyll (BChl) at low pH was investigated in the core (LH1) and peripheral (LH2) light-harvesting complexes, as well as in the ensemble of the reaction center (RC) with the LH1 complex. The stages in disintegration of the native BChl forms in the LH1 complex and in its ensemble with RC were revealed. They were observed as emergence of the absorption band of monomeric BChl and an increase in its intensity, followed by its transformation into the band of monomeric bacteriopheophytin (BPh) and then into the band of aggregated BPh. Unlike the LH1 complex, in the case of the LH2 complex, monomeric BChl was never detected as an intermediate product. While the spectra revealed formation of monomeric BPh, its accumulation did not occur, since its aggregation is very rapid compared to that in the LH1 complex and in the RC–LH1 ensemble. PAAG electrophoresis revealed that pheophytinization of BChl in the LH2 complex was accompanied by disruption of the stable cylindrical structure of this complex with emergence of characteristic fragments consisting of α and β peptides and bearing monomeric BPh, as well as of the α peptide aggregates bearing BPh aggregates. Unlike the LH2 complex, BChl pheophytinization in the LH1 complex did not result in its fragmentation. This is an indication of different types of structural stabilization in the LH1 and LH2 complexes. In the LH2 complex, coordination of bacteriochlorophyll Mg^{2+} by conservative histidine residues of the α and β polypeptides is the main factor responsible for the maintenance of its cylindrical structure. Stability of the LH1 complex is probably based primarily on the highly specific hydrophobic interactions between the surfaces of individual polypeptide chains, since the presence of hydrogen bonds results in autonomy of each $\alpha\beta BChl_2$ subunit, rather than in stabilization of the LH1 complex as a whole.

Keywords: absorption spectra, electrophoresis, bacterial photosynthesis, light-harvesting complexes (LHC), *Allochromatium minutissimum, Rhodospirillum rubrum*

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Processes of transformation of light energy into the energy of separated charges occur in photosynthetic reaction centers (RC). However, without light-harvesting complexes (LHC), photosynthesis would have been ineffective. LHCs increase light collection and coordinate the processes of quantum collection with their "processing" in RCs. All species of purple bacteria contain a core LHC (LH1), which directs the energy of excitation onto RC. Most purple bacteria species also contain peripheral LHCs LH2 transferring the excitation energy onto RC via the LH1 complex. By now, the LH1 and LH2 complexes have been studied in sufficient detail from structural, functional, and spectral points of view. For some LH2 complexes, complete spatial structure and mutual position of bacteriochlorophyll (BChl), carotenoids, and peptides have been established [1, 2]. The structure of LH1 complexes has not yet been solved at high resolution [3]. However, since the peptides of LH1 and LH2 complexes are known to be homologous, and the complexes are arranged in a universal block-wise manner, it was possible to build reasonable structure models of the core complexes using the structures of the peripheral ones [4, 5]. Both complexes comprise subunits formed by heterodimers of α- and β-polypeptide chains and the associated BChl and carotenoid molecules [6, 7]. These subunits aggregate, forming cylindrical structures stabilized by pigment–pigment interactions between porphyrin rings of BChl, a coordination bond between Mg ions and the peptides, as well as by hydrogen bonds, van der Waals, and hydrophobic interactions between the peptides and polyene chains of carotenoids and phytols of BChls. The α- and β-polypeptide chains contain 40 to 70 amino acid residues and have a three-domain structure. In each of the polypeptide chains, the polar N- and C-terminal regions are located on the cytoplasmic and periplasmic sides of the membrane, respectively, and the central hydrophobic region forms an α-helix which spans the membrane perpendicularly to its surface and con-

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tains conservative His residues—the Mg^{2+}-binding ligands in BChl molecules. In the membrane, α-helices of α-peptides form a tightly packed inner cylinder which is stabilized by interactions between the helices; α-helices of β-peptides form a looser outer cylinder, in which the distances between the polypeptides do not allow for direct β–β interactions. Between the helical regions of α- and β-polypeptides, direct interaction occurs only at the N- and C-termini. Inside the helical regions submerged into the membrane, interaction between them is mediated through the molecules of pigments and bound water. Porphyrin rings of BChl molecules are located inside the membrane, perpendicular to its surface and closer to the periplasmic side between the inner and outer cylinders formed by α- and β-polypeptides, and thus form a single exciton ensemble with absorption peaks at 850 and 880 nm for the LH2 and LH1 complexes, respectively. The central Mg^{2+} ions of these BChl molecules are ligated by conservative histidine residues of the α- and β-polypeptides. LH2 complexes also contain BChl molecules, absorbing light at 800 nm and located between the chains of β-polypeptides, closer to the cytoplasmic side of the membrane, so that their porphyrin rings are parallel to the membrane. Their central Mg^{2+} ions are ligated by N-terminal carboxylated methionines of α-peptides [6]. Recent calculations showed that the observed differences in the degree of oligomerization in LH2 complexes were determined, to a considerable extent, by the differences in the angles of interaction between the surfaces of neighboring subunits in the transmembrane domain [8]. The ring of LH2 complex of *Rhodopseudomonas acidophila* (*Rhodoblastus acidophilus*) contains nine subunits [1], while the LH2 complex of *Phaeospirillum molischianum* has eight subunits [2]. However, in the cells of various purple bacteria the number of subunits in LH2 complexes may vary considerably [6, 9–11]. The theoretical calculations proposed previously [8] are considered completely compliant with the observed heterogeneity, since the energy required for modification of the oligomer structure of an LH2 complex is low [12]. In many species of purple bacteria, cylindrical structure of LH1 complexes formed by repetition of 12–16 subunits is complicated by the presence of an additional component, the so-called PufX protein. Such LH1 complexes often form dimers [7]. However, in some purple bacteria, LH1 complex contains no PufX protein. For example, the cylindrical structure of the LH1 complex of *Rhodospirillum* (*Rsp.*) *rubrum* is formed by repetition of 16 subunits [13]. In contrast to LH2 complexes, the inner volume of LH1 complex cylinders is filled with RC—one RC per each cavity. Little is presently known concerning the assembly of LHCs and their ensembles with RCs in vivo. It is considered that the assembly is controlled by specialized enzymatic structures whose genes are localized in the cluster of photosynthesis genes and the *puc* operon. However, it is not known how exactly these factors are involved in the assembly of photosynthetic units of purple bacteria. The issue remains an important subject for the nearest future [12]. Directed mutagenesis is a powerful tool to study LHCs formation [14]. Another approach relies on construction of the functional model LHCs with components being intentionally interchanged during the experiments on self-assembly in vitro [15]. The ability of the central Mg^{2+} ion of (B)Chl to interact with amino acid residues through coordination bonding has long been considered the key factor in (B)Chl protein assembly. For example, it has been demonstrated that the *Rhodobacter sphaeroides* mutant with the α His and β His residues, which are the ligands of bacteriochlorophyll B850 Mg^{2+} in α and β peptides, substituted with Asn contains no LH2 complex [16]. Research on pheophytinization of BChl native forms and aggregation of the generated bacteriopheophytin (Bph), together with analysis of the fragments formed in the course of these processes, showed that impairment of the coordination bonds between Mg^{2+} and the histidine residues destroyed the initial ordered macrostructure of the LH2 complex in *Allochromatium* (*Alc.*) *minutissimum* [17].

It was of interest to compare the stability of such an LH2 structure with a more elongated, loose structure of the LH1 complex comprising 16 subunits, taking into account that the universal backbone of both structures is built of ring aggregates of BChl molecules involved in a strong exciton interaction. While it appears impossible to isolate an LH1 complex from *Alc. minutissimum*, LH1 complex from a nonsulfur purple bacterium *Rsp. rubrum* may be suitable for the task, since it forms a closed ring-shaped structure [13] similar to that of the LH2 complex. As in our previous study [17], we chose pheophytinization of BChls at low pH values as an influencing factor.

MATERIALS AND METHODS

Bacteria *Alc. minutissimum* and *Rsp. rubrum* (strain 1R) of the Collection of Microorganisms (Moscow State University) were grown on modified Larsen [18] and Ormerod [19] media, respectively, at 30°C and constant illumination (light intensity of 90 W/m^2). Cell biomass was collected at the beginning of the stationary phase.

Chromatophores were isolated by centrifugation after sonication on an UZG13-0.1/22 ultrasound generator at 22 kHz [20]. The pellets were resuspended in 0.05 M Tris-HCl buffer, pH 8.0.

Isolation of LH2 complex and the RC–LH1 ensemble from *Alc. minutissimum*, re-electrophoresis of the native complexes, and separation of their destruction products were performed according to the Matson's procedure [21] in a 7% PAAG as described previously in [17, 20] using Triton X-100 or β-dodecyl maltoside (β-DM) as detergents. The LH2 complex was also isolated separately by the same technique with

sodium dodecyl sulfate (SDS). Solubilization of chromatophores in Triton X-100 was performed at 4°C during 2 h at the detergent concentration of 3.3%. Solubilization of chromatophores in β-DM or SDS was performed at room temperature during 30–40 min at the detergent concentrations of 2.6 and 3.0%, respectively. Optical absorption of the chromatophores at the major maximum in the near-IR region of the spectrum was 40 U.

Elution of LHCs and products of their destruction was performed by mincing of the relevant gel bands and their maceration in 0.05 M Tris-HCl buffer, pH 8.0, upon stirring at 4°C during 24 h. After filtration through a paper filter, the complexes were concentrated using the UMP-20 (Vladipor, Russia) polysulfone amide membranes in Amicon-like cells.

The RC–LH1 ensemble of *Rsp. rubrum* was isolated by treatment of chromatophores with lauryldimethylamine oxide (LDAO) or Triton X-100 with subsequent centrifugation in sucrose gradient [22].

To isolate the LH1 complex, chromatophores of *Rsp. rubrum* were resuspended in 0.05 M sodium phosphate buffer, pH 7.0, followed by solubilization of the material in LDAO, dilution and fractional centrifugation, and chromatography on DEAE-cellulose [23].

A sample of LHC in the volume of 0.7–1.0 mL was titrated with small volumes (not exceeding 10 μL) of 0.1 N HCl using a microsyringe under vigorous stirring. During titration, the temperature of the sample did not exceed 25°C. The pH was controlled with a SevenEasy (Mettler Toledo, Switzerland) combined pH meter. The pH value obtained was stable for the duration of the treatment and measurement.

Analytical SDS-PAGE was performed in a Schägger and Jagow system [24] (10% separating gel with a 33 acrylamide–*N,N*'-methylene-bis-acrylamide ratio). The samples were mixed with equal volumes of the buffer, containing 0.125 M Tris-HCl, pH 6.8, 4% SDS, 0.2 M dithiothreitol, 20% glycerol, and 0.02% bromophenol blue, and heated on a boiling water bath for 90 s [17].

In the present work, Tris(hydroxymethyl)aminomethane (trizma base) from Sigma (United States), β-DM from Anatrace (United States), Triton X-100 from Merck (Germany), SDS from Serva (Germany), LDAO, acrylamide, methylene-bis-acrylamide, and glycine from Fluka (Switzerland), and trycine from AppliChem (Germany) were used.

Absorption spectra in solutions and gels were recorded on a Cary 50 Varian spectrophotometer. To register LHC spectra directly in gels, the bands were excised from the columns. To compensate for light scattering, clear gels of equal thickness were placed in the control cuvette.

RESULTS AND DISCUSSION

Spectral investigation of the effect of low pH values on LHC from *Rsp. rubrum* and *Alc. minutissimum*. Spectroscopic study of changes in LHCs under the effect of low pH values performed previously [17] and the present work demonstrated that, independent of the environment (chromatophores, LH1 and LH2 complexes and RC–LH1 ensembles which were isolated using different detergents), antenna components B800, B830, B850, and B880 were destroyed as a result of pheophytinization of BChl, and aggregated BPh was the final product of the process. Our results showed that the rate and completeness of aggregated BPh formation could be an indicator of compactness of the structure involved in BChl pheophytinization. For example, the band of the BPh monomer was absent from the spectra of chromatophores from *Alc. minutissimum* [17] and *Rsp. rubrum* (Fig. 1), in contrast to the isolated LH1 and LH2 complexes in detergent solutions, which reflects the compactness of LHC structure within the chromatophores, resulting in rapid aggregation of the monomeric BPh. We followed the behavior of 800- and 880-nm spectral forms at low pH values in the chromatophores of *Rsp. rubrum*. Since the internal cytoplasmic membrane of these bacteria contains no LH2 complexes, this could be done by following the absorption spectra. The 800-nm spectral form, belonging to RC, was completely destroyed after 5 h. As follows from the differential spectra (data not shown), the spectral form of BChl dimer in the RC 870 nm was destroyed after 3 h. The 880-nm spectral form belonging to LH1 complex did not change within the time period. It degraded very slowly, by 40% within 20 days (Fig. 1). This slowdown of pheophytinization can not be explained at the moment. It is clear that BChl molecules of the LH1 complex are available for protons since the neighboring BChl molecules of RC, absorbing at 800 and 870 nm, pheophytinize within time periods common to BChl molecules in LHCs. For LHC in detergent solutions at pH close to 2, these time periods are typically in the range of several minutes to several hours. In the chromatophore of *Alc. minutissimum*, a similar process of 880-nm spectral form decomposition proceeded by an order of magnitude faster than in the chromatophores of *Rsp. rubrum* [17], yet considerably slower than in LHCs in detergent solutions.

Native aggregated forms of 880-nm BChl in the LH1 complex and RC–LH1 ensemble isolated from *Rsp. rubrum* with Triton X-100, as well as those in the RC–LH1 ensemble from *Alc. minutissimum*, degraded stepwise (Fig. 2). While monomeric BChl absorption band with a peak at 878 nm decreased, the intensity of the monomeric BChl band with peak at 785 nm increased. Monomeric BChl was then transformed into monomeric BPh with absorption peak at 762 nm. Monomeric BPh, in turn, aggregated with formation and expansion of an absorption band at 841 nm. As follows form the absorption spectra, decomposition of

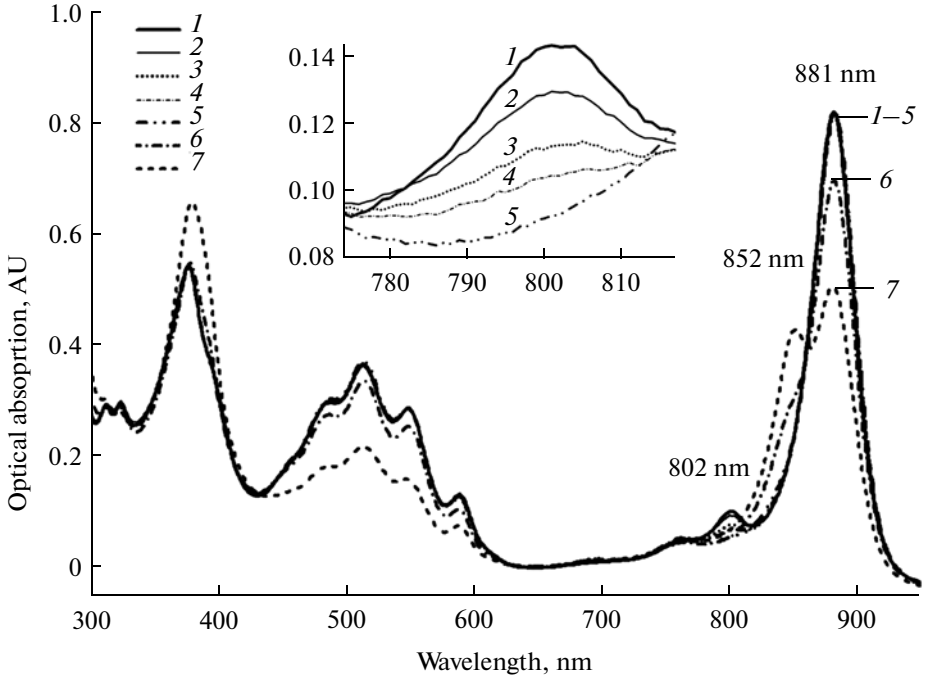

Fig. 1. Changes in absorption spectra of chromatophores from *Rsp. rubrum* after pH decrease to 2.0: 0 min (*1*), 20 min (*2*), 1 h (*3*), 2 h (*4*), 5 h (*5*), 12 days (*6*), and 20 days (*7*). In the inset, changes in the 802-nm band are preesnted.

the 878-nm form of native BChl and formation of the 762-nm BPh proceeded approximately within the same time limits, that is, the spectral band of the monomeric 785-nm BChl was a rather short-lived intermediate form of both the LH1 complex and the RC–LH1 ensemble. In the presence of oxygen, BChl molecules undergo spontaneous oxidation (dehydrogenation) [25, 26]. Since pheophytinization at low pH values was performed under intensive stirring, a small band corresponding to 3-acetylpheophytin absorbing at 685 nm was observed in the spectra of all studied samples.

The fact that aggregation of the generated BPh was much less pronounced in the spectra of LH1 complex, if compared to the RC–LH1 ensemble, was a specific feature of LH1 complex. However, the content of monomeric BPh formed in the process of LH1 complex destruction decreases considerably with time. This evidences a looser structure of the LH1 complex, if compared to that of the RC–LH1 ensemble, which leads to attenuation of pigment binding with the protein carrier and to relatively fast modifications of BPh under low pH values on one hand and slower aggregation of BPh, on the other.

In contrast to the LH1 complex, we could not observe monomeric BChl as an intermediate product in the case of the LH2 complex. In absorption spectra, monomeric BPh formation was observed, but it was not accumulated significantly in the environment of 0.1% β-DM, SDS, or Triton X-100, since BPh aggregation proceeded very rapidly, if compared to that in the LH1 complex and the RC–LH1 ensemble [17]. For example, as follows from Fig. 3, in spectra of the LH2 complex from *Alc. minutissimum* in 0.1% Triton X-100, the band of aggregated BPh at 850 nm of increasing intensity predominated, and the minor band of monomeric BPh at 755 nm indicated the presence of only minute amounts of this intermediate. This evidences the compactness of the structure of the LH2 complex in the detergent solutions leading to relatively fast aggregation of the monomeric BPh, similar to how it happens in chromatophores. When pheophytinization of the LH2 complex occurred in the environment of 0.1% LDAO, a considerable amount of monomeric BPh was accumulated, which made it possible to trace its localization in the protein fragments generated [17].

Electrophoretic separation and analysis of LHC degradation products. Earlier, it was demonstrated that pheophytinization of BChl and aggregation of BPh in the LH2 complex was accompanied by decomposition of its stable cylindrical structure [17]. As follows from Fig. 4, three bands were observed upon electrophoretic separation of the products of degradation of LH2 from *Alc. minutissimum* in 0.1% Triton X-100 in Matson's system: (1) BPh aggregates, (2) undecomposed LH2 complex, and (3) monomeric BPh. No freely migrating BChl, BPh, or their aggregates—that is, pigments free of protein carriers—were detected. A similar picture may be observed upon electrophoretic separation of the degradation products of the LH2 complex in 0.1% LDAO. In this case, the monomer

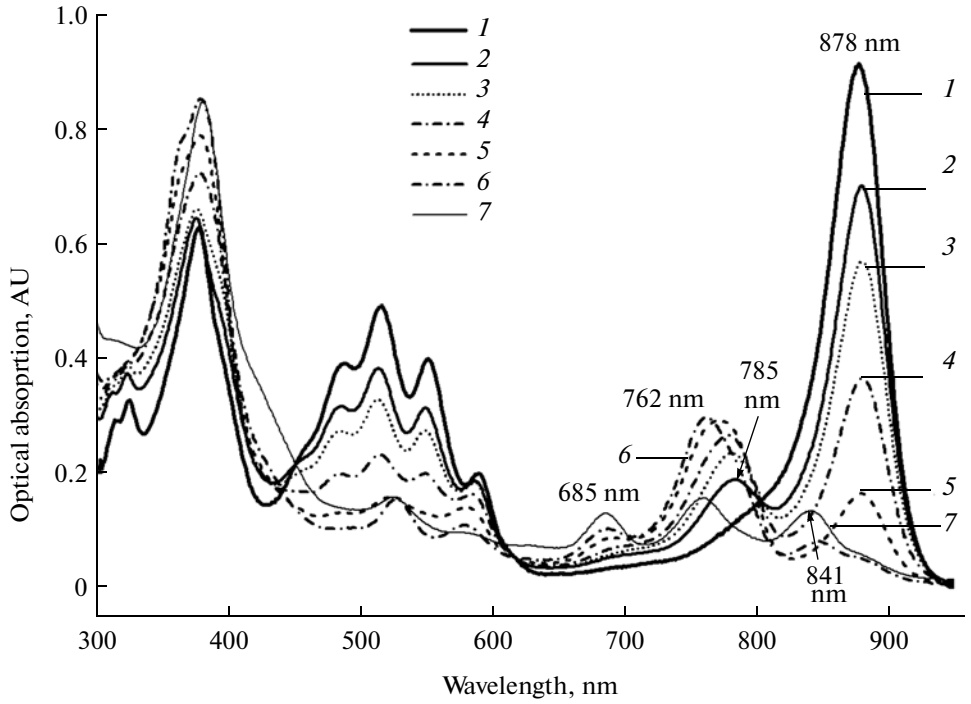

Fig. 2. Changes in absorption spectra characterizing the stepwise nature of 878-nm BChl decomposition in the LH1 complex of *Rsp. rubrum* in the presence of 0.1% Triton X-100 after pH decrease to 3.5: control, pH 8.0 (*1*), pH 3.5: 2 min (*2*), 5 min (*3*), 25 min (*4*), 1 h 15 min (*5*), 3 h 30 min (*6*), and 24 h (*7*).

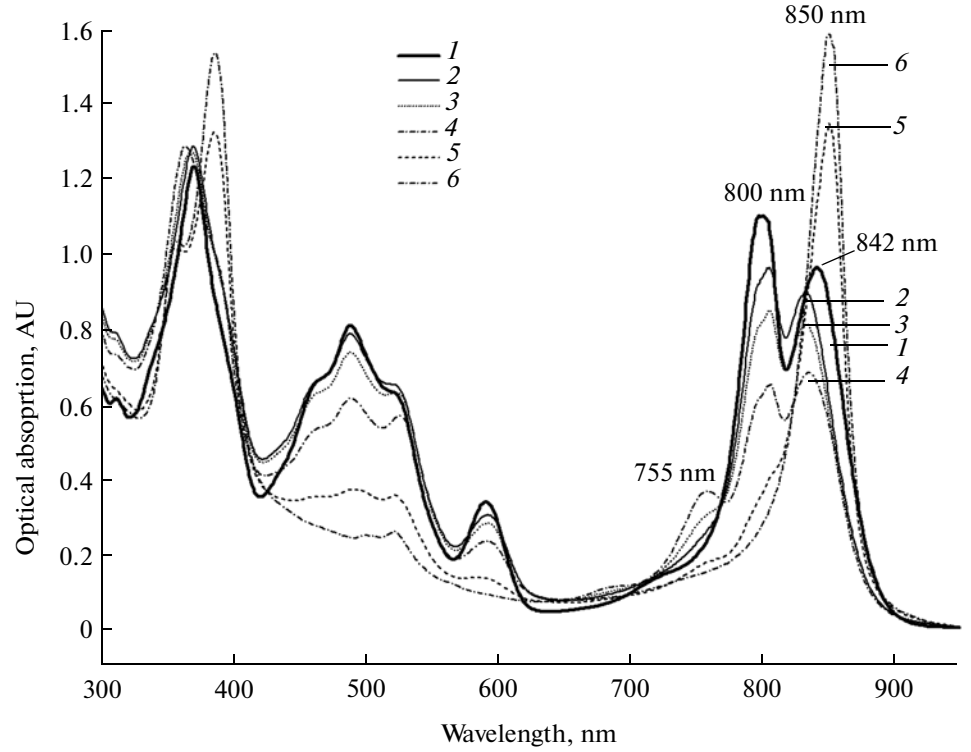

Fig. 3. Changes in absorption spectra of the LH2 complex from *Alc. minutissimum* in the presence of 0.1% Triton X-100 after pH decrease to 2.1: control, pH 8.0 (*1*), pH 2.1: 2 min (*2*), 15 min (*3*), 1 h (*4*), 4 h (*5*), and 12 h (*6*).

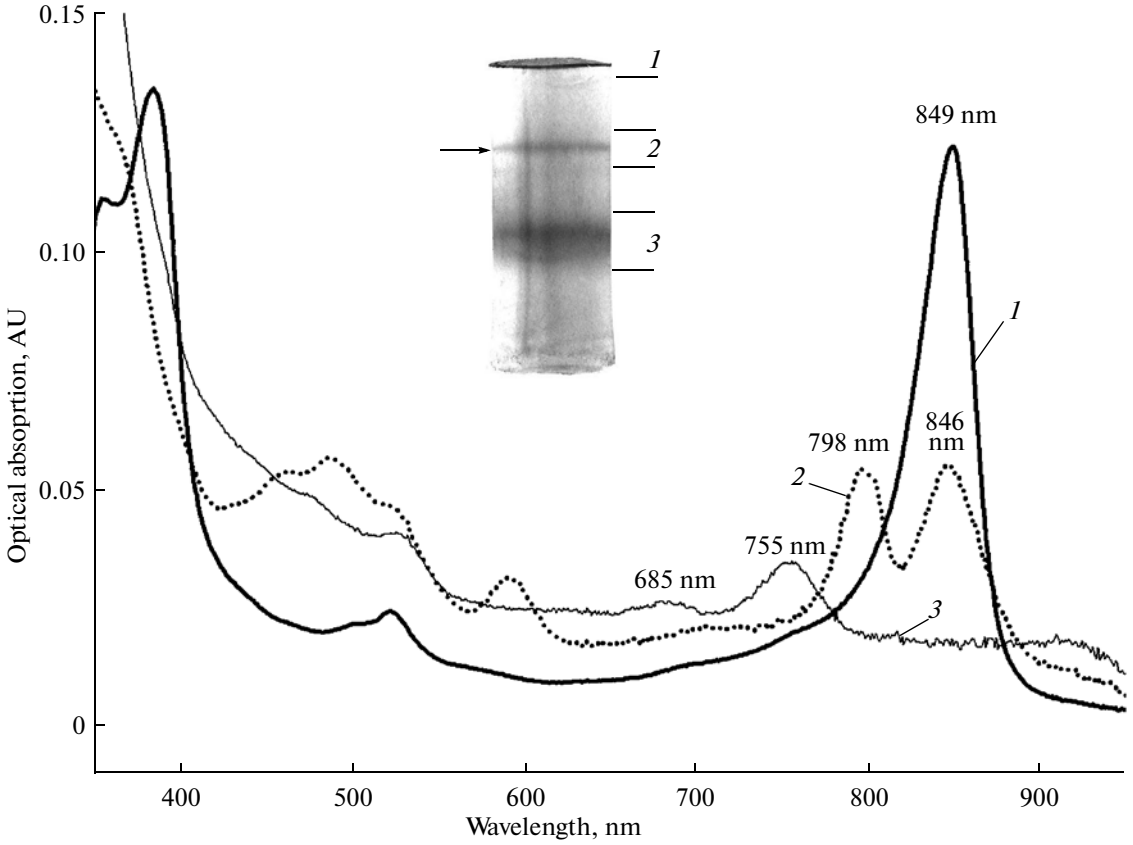

Fig. 4. Electrophoretic separation of the degradation products of the LH2 complex from *Alc. minutissimum* obtained in 0.1% Triton X-100 at pH 2.1 in 7% PAAG and their absorption spectra. The arrow indicates the position of the native LH2 complex upon re-electrophoresis in parallel with separation of the degradation products. Gels were stained with Coomassie R250.

BPh band was much more pronounced. On the contrary, in 0.1% SDS, aggregated BPh band predominated [17]. Figure 5 presents the results of denaturing SDS-PAGE in the Schrägger and Jagow system of the pigment–protein eluates obtained upon separation of decomposition products of the LH2 complex from *Alc. minutissimum* concentrated on Vladipor membranes in 7% PAAG in Matson's system. It can be seen that monomeric BPh is contained in protein associates comprising α and β polypeptides. Aggregated BPh binds mostly to associates of α-polypeptides.

Proceeding from the structural similarity of the LH2 and LH1 complexes, one could expect that pheophytinization of BChl in the latter one would also induce intense fragmentation of its cylindrical structures. As follows from Fig. 2, BChl in the LH1 complex from *Rsp. rubrum* undergoes complete pheophytinization within 3.5 h at pH 3.5. However, further

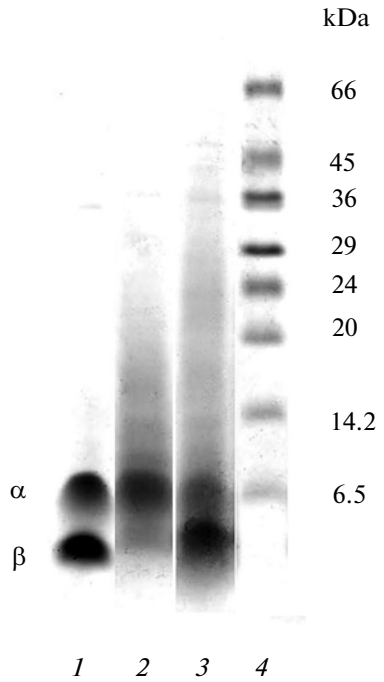

Fig. 5. Analytical SDS-PAGE in Schägger and Jagow system [24]: LH2 complex from *Alc. minutissimum* (lane *1*); eluates of the electrophoretic zones concentrated on the Vladipor membrane with spectra of aggregated (lane *2*) and monomeric (lane *3*) BPh, respectively; protein markers from Sigma (lane *4*).

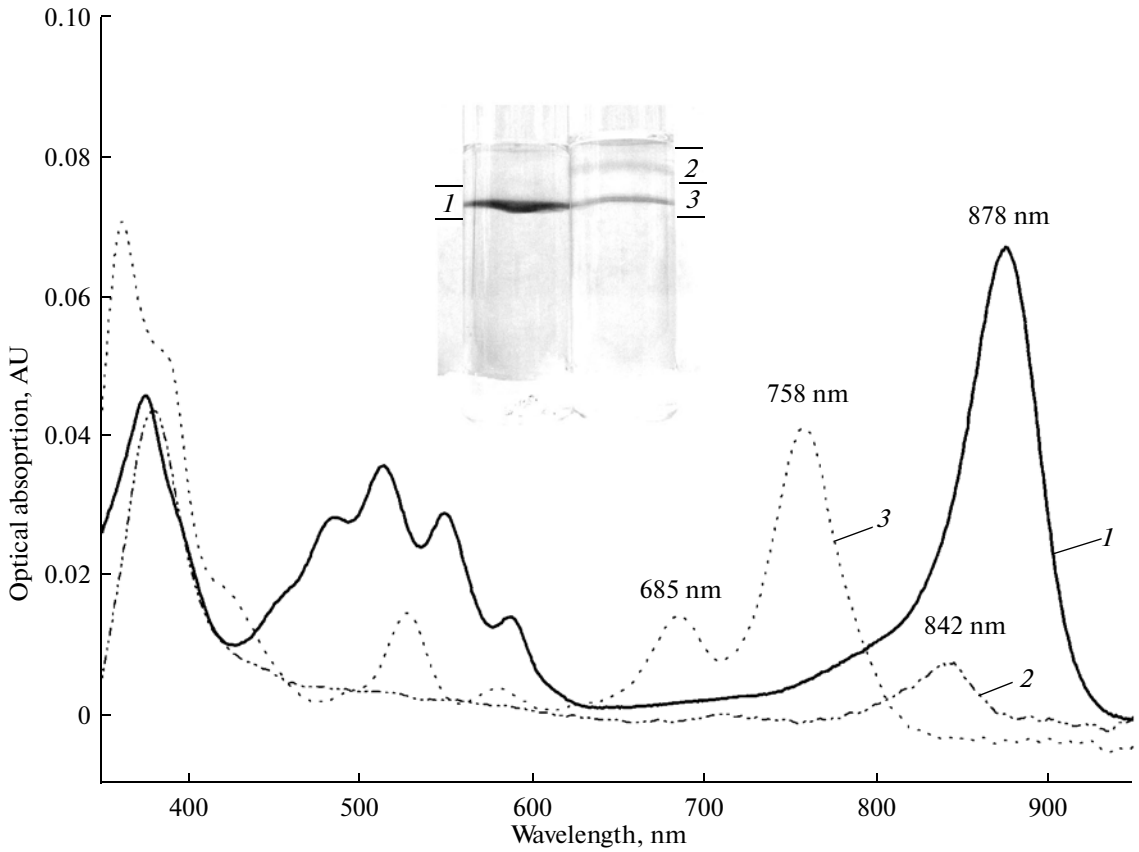

Fig. 6. Electrophoretic separation of decomposition products of the LH1 complex from *Rsp. rubrum* obtained in 0.1% Triton X-100 at pH 3.5 in 7% PAAG and their absorption spectra. Gels not stained with Coomassie R250 are presented in the figure because gel staining did not reveal new bands, and the band of region 2 of the aggregated BPh disappeared upon background washing off.

electrophoresis in Matson's system with Coomassie R250 staining did not reveal any protein bands in the obtained pheophytinized sample that would have evidenced fragmentation of the cylindrical structure of the LH1 complex (Fig. 6). The only protein band of the pheophytinized sample (region 3) with monomeric BPh and products of its oxidation revealed in the spectrum was identical in terms of electrophoretic mobility with the band of initial LH1 complex (region 1). Apart from region 3, there was a minor band (region 2) in the electrophoregram of the pheophytinized sample, with aggregated BPh revealed in its spectrum. The band was not stained with Coomassie R250, that is, in this case BPh aggregates were free from protein carriers, which was not the case upon pheophytinization and decomposition of the samples of LH2 complex [17]. The absence of ring fragmentation in LH1 complex upon pheophytinization of BChl agrees with the data evidencing that histidines liganding BChl in LH1 complexes may be substituted with other amino acid residues in contrast to BChl-B850 in LH2 complexes [16]. Functional binding of BChl in RC and LHC is known to require additional types of interactions involving peripheral substituents in the macrocycle, primarily hydrogen bonds of the oxo groups of BChl isocycle. However, the presence of these hydrogen bonds leads to the autonomy of each of $\alpha\beta BChl_2$ subunits in LH1 complex, but not to stabilization of the structure of the LH1 complex as a whole, as in the case of the LH2 complex [27]. Since it was found experimentally that LH1 complexes in the presence of detergent may reversibly dissociate into individual subunits and their associates [7], most likely, the forces stabilizing the cylindrical structure of LH1 complexes are specific hydrophobic interactions between the surfaces of individual subunits.

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