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Properties of Mutant Reaction Centers of *Rhodobacter sphaeroides* with Substitutions of Histidine L153, the Axial Mg^{2+} Ligand of Bacteriochlorophyll B_A

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Abstract—Mutant reaction centers (RC) from *Rhodobacter sphaeroides* have been studied in which histidine L153, the axial ligand of the central Mg atom of bacteriochlorophyll B_A molecule, was substituted by cysteine, methionine, tyrosine, or leucine. None of the mutations resulted in conversion of the bacteriochlorophyll B_A to a bacteriopheophytin molecule. Isolated H(L153)C and H(L153)M RCs demonstrated spectral properties similar to those of the wild-type RC, indicating the ability of cysteine and methionine to serve as stable axial ligands of the Mg atom of bacteriochlorophyll B_A. Because of instability of mutant H(L153)L and H(L153)Y RCs, their properties were studied without isolation of these complexes from the photosynthetic membranes. The most prominent effect of the mutations was observed with substitution of histidine by tyrosine. According to the spectral data and the results of pigment analysis, the B_A molecule is missing in the H(L153)Y RC. Nevertheless, being associated with the photosynthetic membrane, this RC can accomplish photochemical charge separation with quantum yield of approximately 7% of that characteristic of the wild-type RC. Possible pathways of the primary electron transport in the H(L153)Y RC in absence of photochemically active chromophore are discussed.

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Key words: bacterial photosynthesis, reaction center, photochemical charge separation, site-directed mutagenesis, bacteriochlorophyll, *Rhodobacter sphaeroides*

Photosynthetic reaction center (RC) is a transmembrane pigment–protein complex that converts solar energy into the energy of separated charges. The mechanism of photochemical charge separation in the RC has remained a key problem of photosynthesis for several decades. One convenient object for studies is purple bacteria. According to data on 3-D structure of the *Rhodobacter sphaeroides* RC, this pigment–protein complex is composed of three protein subunits (L, M, and H) and 10 cofactors (four molecules of bacteriochlorophyll (BChl), two molecules of bacteriopheophytin (BPheo), two ubiquinone molecules (Q), one carotene molecule, and a non-heme iron atom) [1]. Two BChl molecules form a dimer (P) executing the function of primary electron donor. The cofactors form two pathways of electron transport. Despite their symmetrical positions in the protein, the major electron transport occurs via one, so-called A-chain—from P through the monomeric BChl (B_A) and BPheo (H_A) to the ubiquinone molecules (Q_A and Q_B).

The role of monomeric BChl B_A, located between the molecules of the special pair and BPheo H_A, in the primary electron transport process has been a subject for discussion since the 3-D structure of bacterial RC was determined. Some authors assigned B_A to the role of a bridge in the super-exchange mechanism of electron transport from P to H_A [2, 3], whereas others reported that the electron transport from P* goes via the B_A molecule, and the first stage of charge separation is the formation of state $P^+B_A^-$ [4-8]. The role of B_A as the primary electron acceptor was decisively confirmed in experiments

Abbreviations: B_A, monomeric bacteriochlorophyll, the primary electron acceptor; B_B, monomeric bacteriochlorophyll of the inactive chain of cofactors; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; H_A, bacteriopheophytin of the active chain of electron transport cofactors; P, photoactive bacteriochlorophyll dimer, the primary electron donor; RC, reaction center; Φ_A, BPheo located in place of B_A.

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using femtosecond spectroscopy [9, 10]. It is presently established that both direction and efficacy of photochemical reaction in RC depends largely on interaction of cofactors involved in the electron transport process with each other and with their protein environment [11]. An important contribution to research on this subject belongs to studies using site-directed mutagenesis allowing single amino acid substitutions in the RC protein subunit structure. Of special interest are amino acid residues forming ligands to magnesium atoms of bacteriochlorophylls. According to known data on RC, in most cases these ligands are histidine residues, but other liganding variants can also exist. Studies on RCs of purple bacteria suggest the loss of the Mg atom and substitution of BChl by BPheo when the liganding histidine is substituted by leucine or phenylalanine in the vicinity of bacteriochlorophylls P_A, P_B, and B_B [12-15]. A success was achieved in preparation of *Blastochloris viridis* RC with BPheo instead of B_A [7], but efforts to substitute B_A by BPheo in RCs of *Rb. sphaeroides* and *Rb. capsulatus* remained unsuccessful until the present. High instability of mutant RCs with substitutions of histidine L153 by amino acids unable to direct liganding of magnesium significantly hampered examination of these RCs [16, 17]. Nonetheless, it was supposed that no substitution of BChl by BPheo occurs in such mutant RCs [17]. Because of the important role of the B_A molecule as the primary electron acceptor at the initial stage of photochemical charge separation, the problem on specificity of this tetrapyrrole position in the *Rb. sphaeroides* RC structure remains relevant.

In the present work, we have studied the properties of *Rb. sphaeroides* RCs with substitutions of histidine L153 by cysteine, methionine, leucine, or tyrosine. Both cysteine and methionine can form a coordination bond with the magnesium atom of BChl and so were used for the substitution as positive control. The substitution of histidine by tyrosine was made owing to a supposition that an OH-group can participate in either direct or indirect magnesium liganding in BChl [16]. Leucine was chosen because it, when substituting the liganding histidine, leads to appearance of BPheo instead of BChl in the case of the above mentioned mutations in the vicinity of P and B molecules [7, 12-14]. We used the methods of optical spectroscopy, recording of action spectra, as well as a method for estimation of pigment composition of unstable RCs by means of fluorescence emission spectra. We have demonstrated that the RC mutants H(L153)M and H(L153)C can be isolated from membranes, and their properties do not differ essentially from those of wild-type RC. Properties of unstable RC mutants H(L153)L and H(L153)Y were examined without their isolation from membranes. In chromatophores, the H(L153)L RC demonstrated the same photochemical activity as did the wild-type RC. The substitution of histidine L153 by tyrosine led to significant changes in the properties of the RC mutant H(L153)Y. Using a series of methods, we have shown that these changes are due to the loss of the B_A molecule from the RC. The results are discussed in connection with current ideas concerning the pathways of primary electron transport in RCs.

MATERIALS AND METHODS

The genetic system for site-directed RC mutagenesis was composed of the *Rb. sphaeroides* strain DD13 [18] devoid of light-collecting antennae and a complementing plasmid [19]. Mutagenesis was carried out using PCR by the method of overlapping DNA sequences as described earlier [20]. The culture medium for recombinant *Rb. sphaeroides* strains contained tetracycline (1 μg/ml), kanamycin (5 μg/ml), and streptomycin (5 μg/ml). The cells were grown in the dark under semi-aerobic conditions as described earlier [21].

Chromatophores were isolated by ultrasonication of cell suspension using a Soniprep 150 device (Sanyo) followed by two centrifugations: at 20,000*g* (r_m = 9 cm) and 200,000*g* (r_m = 10 cm) for 60 min each, at 4°C. The reaction centers were isolated by the method described earlier [22].

The data on the 3-D structure of *Rb. sphaeroides* RC were acquired from the database (Protein Data Bank, 1OGV.pdb [8, 23]). Amino acid substitutions were simulated using the Swiss-PDB-viewer software.

Absorption spectra of chromatophores were recorded at room temperature on a Shimadzu UV-1601PC spectrophotometer in samples containing 50% glycerol and 5 mM sodium ascorbate. Spectra of photoinduced absorption changes (ΔA) in the millisecond range were recorded on a single-beam differential spectrophotometer equipped with a phosphoroscope [24]. A weak monochromatic light was used in the measuring channel of the device. Action spectra in photoinhibition of respiration coupled with photosynthetic electron transport in RC were recorded by means of polarographic analysis of photoinduced variations in the rate of O_2 exchange in a thin near-surface layer of a platinum microelectrode [25].

Pigments were extracted from lyophilized membranes by acetone–methanol (7 : 2 v/v) mixture. We averaged 0.1 g of chromatophore specimen per ml of the solution, resuspended for 1 min on a vortexer and centrifuged for 5 min at 13,400 rpm. Supernatant samples with absorption less than 1 at 480 nm (absorption maximum of carotenoids) were used for experiments. Fluorescence and fluorescence excitation spectra of BPheo and BChl were recorded on a Hitachi 850 spectrofluorimeter (Japan).

RESULTS

We selected four *Rb. sphaeroides* mutants with RCs in which histidine L153 was substituted by cysteine,

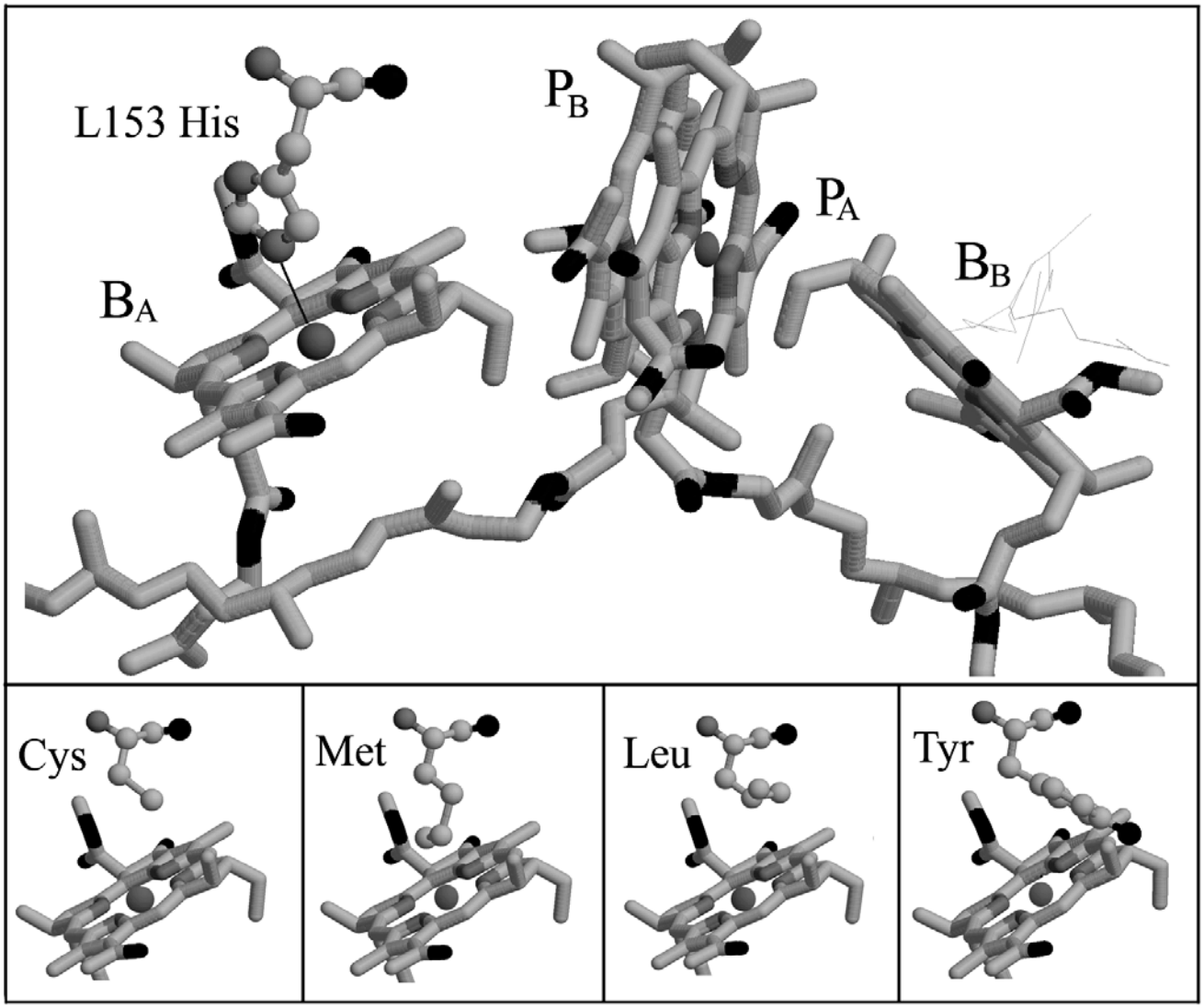

Fig. 1. Location of amino acid residues at position L153 relative to the nearest chromophore molecules in intact (His) (X-ray structure data) and mutant (Cys, Met, Leu, and Tyr) (computer simulation) reaction centers of *Rb. sphaeroides*.

methionine, leucine, and tyrosine, respectively (Fig. 1). The stable RCs were isolated from the *Rb. sphaeroides* mutants H(L153)C and H(L153)M. Their absorption spectra are shown in Fig. 2. The absorption band with maximum at 868 nm in the wild-type RC spectrum (spectrum *1*) corresponds to the low-energy Q_y-transition of BChl dimer (Q_y P), the band with maximum at 805 nm corresponds to both the Q_y-transition of two monomeric BChl and high-energy transition of dimer molecules (Q_y B), and the band with maximum at 758 nm corresponds to the Q_y-transition of two BPheo molecules (Q_y H). The band ratio Q_y P/Q_y B in this spectrum is 2.2. In the Q_x-area of the spectrum, the absorption bands of four BChl molecules with maximum at 599 nm and two BPheo molecules with maximum at 532 nm are observed.

Similar short-wavelength shifts and broadening of BChl absorption bands are observed in the absorption spectra of mutant RCs H(L153)C (spectrum *2*) and H(L153)M (spectrum *3*) normalized to the Q_y H band. The maximum of the Q_y-transition of BChl monomers is shifted to 803 nm in the spectrum of H(L153)C RC and to 802 nm in the spectrum of H(L153)M RC due to the shift of the B_A absorption band. The absorption band corresponding to the Q_x-transition of BChl molecules broadens in the spectrum of H(L153)C RC and splits into two bands, 600 and 580 nm, in the spectrum of H(L153)M RC.

The spectra of photoinduced changes in absorption of these RC mutants coincide with the corresponding wild-type RC spectrum in the area 700-950 nm, both in shape and amplitude (data not shown). Pigment analysis

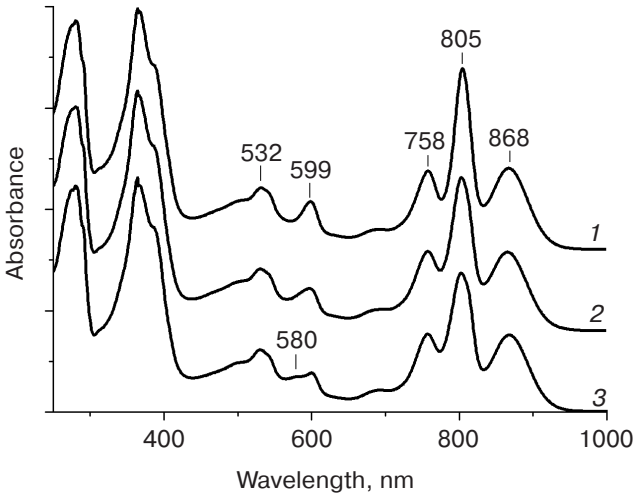

Fig. 2. Absorption spectra of wild-type (*1*) and H(L153)C (*2*) and H(L153)M (*3*) mutant reaction centers.

have shown that the BChl/BPheo ratio is 2 ± 0.1, both in RC mutants H(L153)C and H(L153)M and wild-type RC.

Due to instability of the RC mutants H(L153)L and H(L153)Y in the presence of detergent, the properties of these RCs were examined without their isolation from membranes. To do this, we used recombinant *Rb. sphaeroides* strains whose chromatophores contain RC as the sole photosynthetic complex. We have shown that mutant RCs H(L153)L and H(L153)Y, when associated with chromatophores, possess photochemical activity. The spectra reflecting the photoinduced changes in absorption of the membrane-bound wild-type RC and its mutants H(L153)L and H(L153)Y are shown in Fig. 3. The band of special pair P molecule photobleaching with maximum at about 870 nm, a Z-shaped structure with the center at about 804 nm reflecting the electrochromic short-wavelength shift of the monomer BChl absorption band in the charge field of $P^+Q_A^-$ [26, 27] and the bleaching band of high-energy P transition [28, 29], and S-shaped structure reflecting the long-wavelength shift of the BPheo absorption band are present in the differential (light-minus-dark) spectrum of the wild-type chromatophores. The differential spectrum of the H(L153)L RC resembles that of wild-type RC, and there were no significant shift of P bleaching band maximum and short-wavelength shift of the B band, although the amplitude of the H(L153)L RC signal in the spectrum was twofold lower than that of wild-type RC signal. In a photoinduced differential spectrum of H(L153)Y RC, the bleaching band of the dimer P is shifted to the short-wavelength area (to 843 nm), and the Z-shaped structure is shifted to the long-wavelength area (to 811 nm). The amplitude of the signal in the differential spectrum of this RC mutant was one order lower than that in the differential spectrum of the wild-type RC, which might be associated with either reduced efficacy of electron transport or decrease in amount of active RCs in chromatophores of this mutant.

Further examination of the H(L153)L RC has shown their instability not only during isolation, but also in association with chromatophores. The H(L153)L chromatophores demonstrated spectral heterogeneity. In particular, the ratio between maxima of the bands corresponding to the Q_y-transition of BPheo and Q_y-transition of BChl varied in different samples and changed upon freezing–thawing, thus hampering unambiguous interpretation of the spectra (data not shown).

Figure 4a shows the absorption spectra of chromatophores containing wild-type RC and RC H(L153)Y recorded at room temperature (spectra *1* and *2*, respectively) and their differential spectrum. The spectra were normalized to the absorption maximum of BPheo at 758 nm. The same absorption bands of chromophores are observed in the absorption spectra of both chromatophore-associated and isolated wild-type RC (Fig. 2), and the Q_y P/Q_y B ratio remained unchanged. In the absorption spectrum of antenna-less chromatophores of the H(L153)Y mutant (Fig. 4a, spectrum *2*) a short-wavelength shift of the absorption band of the primary electron donor is observed, whose maximum, according to the data of Gaussian decomposition of the spectrum, is shifted to 843 nm. This shift probably reflects an abatement of exciton interaction between molecules of the dimer, due to the change in their intermodal configuration. The absorption Q_y-band of monomeric BChl in this spectrum is shifted to the long-wavelength area to 812 nm and sub-

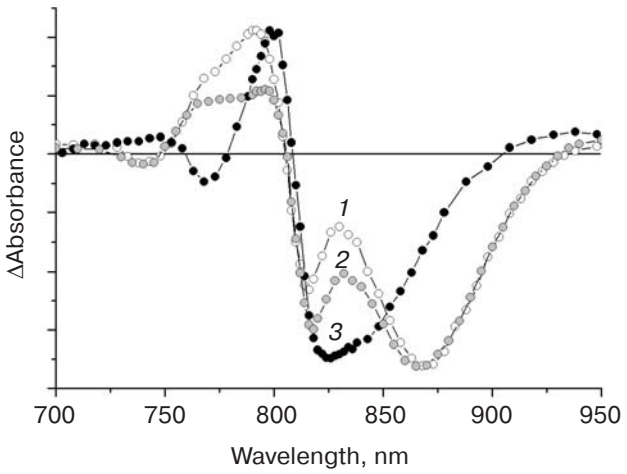

Fig. 3. Differential (light-minus-dark) absorption spectra (ΔA) of chromatophores of wild type (*1*, open circles) and H(L153)L (*2*, gray circles) and H(L153)Y (*3*, closed circles) mutants. Amplitude ΔA of spectrum *1* is drawn with factor 1, the amplitude ΔA of spectrum *2* is multiplied by factor 2, and the amplitude ΔA of the spectrum *3* is multiplied by factor 10.

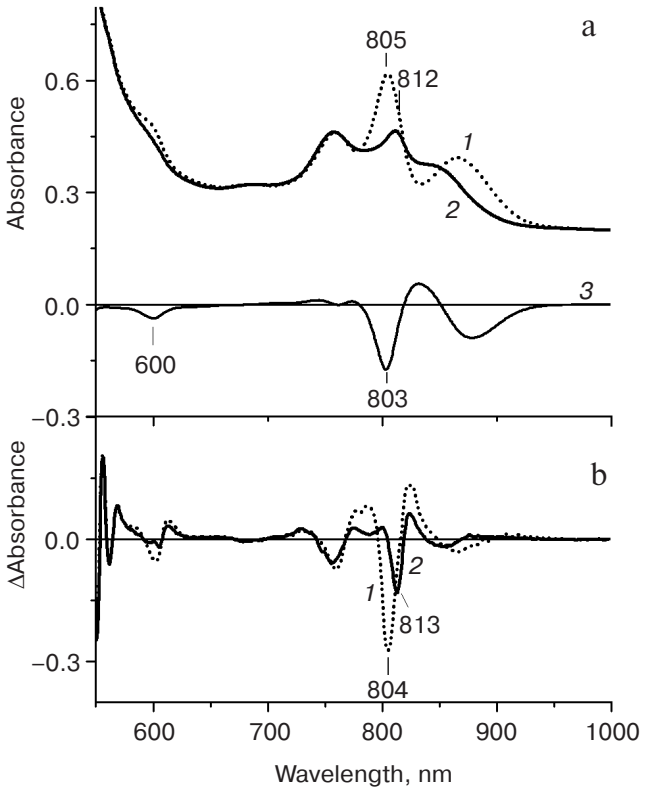

Fig. 4. a) Absorption spectra of *Rb. sphaeroides* chromatophores of wild type (*1*) and H(L153)Y mutant (*2*) normalized to 758 nm and differential spectrum (*3*). b) Second-order derivatives of chromatophore absorption spectra (notation is the same as in panel (a)).

stantially decreases in amplitude. Since this band is a superposition of absorption bands of two BChl molecules in the corresponding spectrum of wild-type RC, the cause of its shift may be either the shift of absorption maxima of both B_A and B_B, or shift of the absorption maximum of B_A without change in absorption of B_B, or decrease in dipole force of Q_y-transition of the B_A molecule. In the differential spectrum (Fig. 4) drawn by subtraction of spectrum *2* from spectrum *1* (spectrum *3*), the negative band with extremum at 803 nm and associated negative band at 600 nm reflect not only decrease in the dipole force of the Q_y-transition of B_A molecule, but also significant decrease of its absorption in the absorption spectrum of the H(L153)Y mutant. The amplitude of the negative band at 803 nm is comparable with the amplitude of BChl absorption band at 812 nm in the absorption spectrum of chromatophores. A Z-shaped structure in the long-wavelength area of the differential spectrum is observed reflecting the short-wavelength shift of the band of dimer P in the spectrum of the mutant. Besides, an S-shaped structure is visible reflecting the long-wavelength shift of the absorption Q_y-band of BPheo.

Figure 4b shows the plots of second-order derivatives of chromatophore absorption spectra in the area of absorbance of monomeric RC BChl. The second-order derivative of the wild-type RC spectrum (spectrum *1*) demonstrates the band of Q_y-transition of B_A and B_B molecules presented by two maxima: the major peak at 804 nm and weak shoulder at 813 nm. The second-order derivative of the H(L153)Y mutant chromatophore spectrum (spectrum *2*) demonstrates the band with maximum at 812 nm, whereas the short-wavelength band is absent. This indicates that absorbance of monomeric BChl B_A is not just reduced, but is completely missing in the spectrum of the H(L153)Y mutant.

The action spectra of chromatophores of wild-type (spectrum *1*) and H(L153)Y mutant (spectrum *2*) chromatophores in photoinhibition of respiration are presented in Fig. 5. In these spectra, one can see absorption bands of bacteriochlorin, which is capable of excitation energy transmission to the primary electron donor. The bands corresponding to the Q_y and Q_x-transitions of BChl and the Q_y-transition of BPheo are also present in the action spectrum of chromatophores. The Q_y P/Q_y B ratio in the action spectrum of wild-type RC was 1.9, whereas it was 2.2 in the absorption spectrum. In the action spectrum of H(L153)Y RC the band of dimer is shifted to the short-wavelength area, the amplitude of the Q_y B band is decreased, and its maximum is shifted to the long-wavelength area to 812 nm relative to the corresponding bands in the action spectrum of wild-type RC – on all four with the absorption band in Fig. 4a. This means that the Q_y B band corresponds to absorbance of only one molecule of monomeric BChl, namely B_B. The Q_y P/Q_y B ratio in the spectrum of the mutant falls to 1.5. Since the action spectra coincide with absorption spectra, one can conclude that the major portion of RC is functionally active in

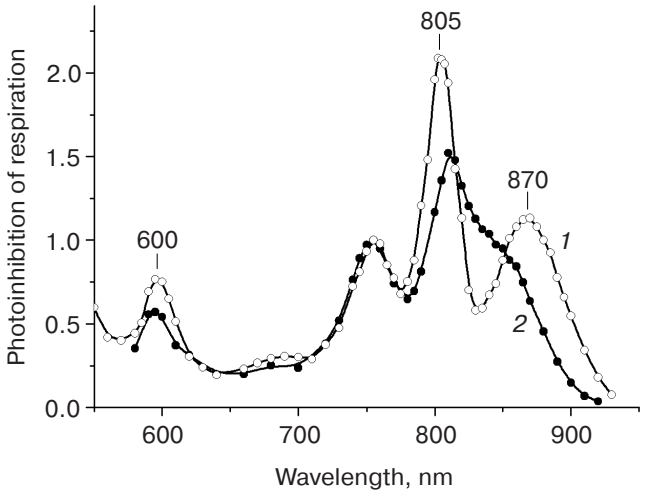

Fig. 5. Action spectra of wild-type (*1*, open circles) and H(L153)Y mutant (*2*, closed circles) chromatophores in photoinhibition of respiration. The amplitude of spectrum *2* is multiplied by factor 15.

chromatophores. Amplitude of the signal in the H(L153)Y specimen (Fig. 5, spectrum *2*) compared with the wild-type one is 7%, which suggests corresponding decrease in quantum yield of charge separation in the mutant RC. Suppose the quantum yield approaches one, and the time of charge separation (3 psec) is 100-fold shorter than the lifetime of excited state P* in the wild-type RC, then in the H(L153)Y RC the time of charge separation is about 4.3 nsec, which is more than three orders lower than that of the wild-type RC. The absence of B_A absorption band in the action spectrum of the H(L153)Y mutant indicates that energy transmission does not occur from this molecule to the primary electron donor P because of the absence of this molecule.

Thus, the presented data suggest that the monomeric BChl molecule of the electron transport chain of cofactors exhibits neither optical nor photochemical features in the H(L153)Y mutant reaction centers, and at the same time no additional bands appear in the mutant RC absorption spectrum. These data suggest that the substitution of histidine L153 by tyrosine disables the BChl B_A molecule from incorporating into the structure of the mutant RC. In this case it seems that pigment balance is disturbed in the RC with the mutation H(L153)Y. Examination of optical absorption spectra of acetone–methanol (7 : 2) extracts from antenna-less wild-type and H(L153)Y mutant chromatophores revealed a shift of the bacteriochlorin absorption band maximum to the short-wavelength area in the absorption spectrum of extract from chromatophores of the mutant (data not shown). This may suggest alteration of quantitative ratio between BChl and BPheo in mutant RCs because of either increase in number of BPheo or decrease in number of BChl. To determine the cause of altered BChl/BPheo ratio in mutant RC, we used the method of fluorescence detection after excitation in the maxima of the Q_x-transitions of these pigments.

Figure 6 shows the spectra of fluorescence (a) and fluorescence excitation (b) of BPheo (curve *1*) and BChl (curve *2*) in extract from wild-type (open symbols) and mutant H(L153)Y (closed symbols) chromatophores normalized to the bands of BPheo. According to the Bouguer–Lambert–Beer law, the band peaks of fluorescence and fluorescence excitation are directly propor-

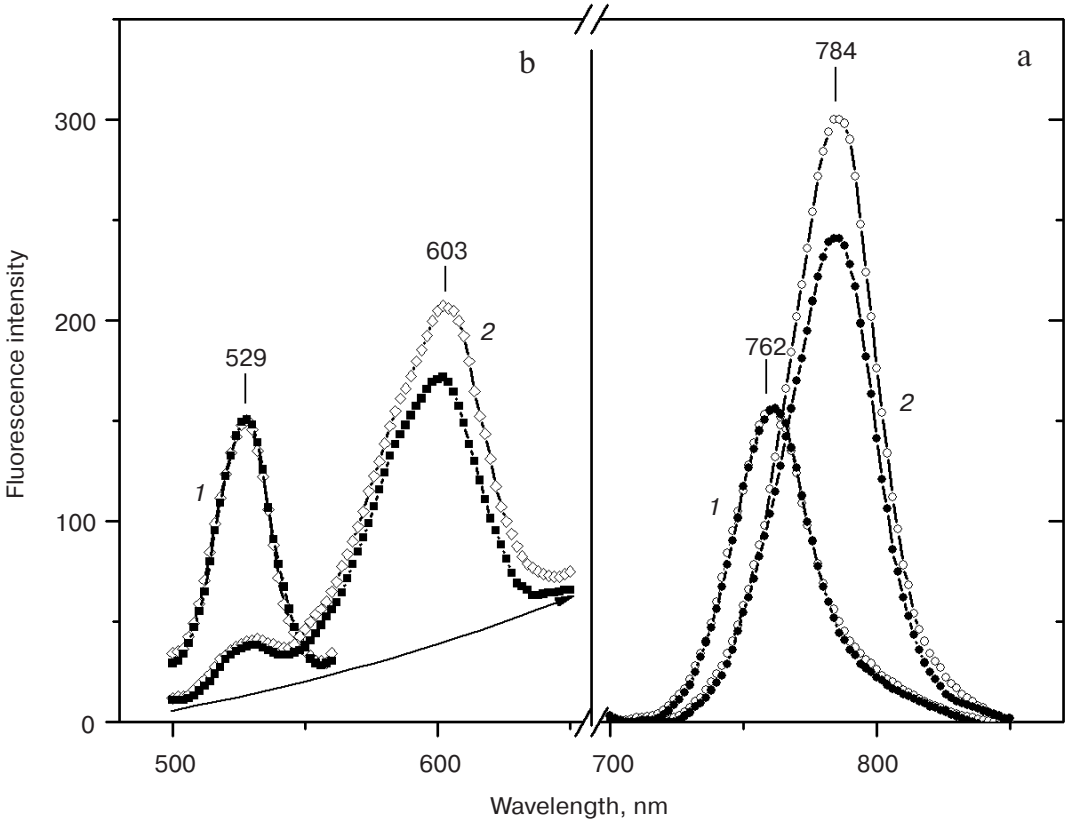

Fig. 6. a) Fluorescence spectra of BPheo (curve *1*) and BChl (curve *2*) in extract from wild-type (open symbols) and H(L153)Y mutant (closed symbols) chromatophores normalized to the band of BPheo with maximum at 762 nm. b) Fluorescence excitation spectra of BPheo (curve *1*) and BChl (curve *2*) in extract from wild-type (open symbols) and H(L153)Y mutant (closed symbols) chromatophores. The recording wavelength is 760 nm for BPheo and 790 nm for BChl. The spectra are normalized to the band of BPheo with maximum at 529 nm and multiplied by factor 2.

tional to amounts of BPheo and BChl in extracts when bacteriochlorin concentration is low. Figure 6 shows that the levels of BPheo are equal in extracts from the wild-type and H(L153)Y mutant chromatophores, whereas the level of BChl in extract from mutant chromatophores is lowered by 21 ± 2% according to the fluorescence spectrum and by 22 ± 3% according to the fluorescence excitation spectrum. The fluorescence and fluorescence excitation spectra of pigments in extracts from both H(L153)M and H(L153)C mutant chromatophores coincided with those of wild type (data not shown).

Comparison of the absorption spectra (Fig. 4a) and action spectra (Fig. 6) of chromatophores shows that the portion of free BPheo and BChl is insignificant in pigment extracts, so we suggest that in the wild-type fluorescence spectrum the band with maximum at 762 nm reflects the fluorescence of two BPheo molecules and the band with maximum at 784 nm reflects four BChl molecules per RC if the pigments are completely extracted from membranes. The amount of BChl in extract of H(L153)Y mutant chromatophores corresponds to about three BChl molecules per two BPheo molecules in RC with this mutation. A small deviation of observed decrease in fluorescence from the expected level (25%) seems to be associated with elevated level of carotenoids in extract from the H(L153)Y mutant chromatophores.

DISCUSSION

Histidine is not the only possible ligand of the central magnesium atom in the BChl molecule, although in most of the studied bacterial RCs it is the amino acid that is localized in corresponding position in the vicinity of the central magnesium atoms of all four BChl molecules [30]. In bacterial RCs histidine as the BChl magnesium ligand can be substituted by other amino acids such as Ser, Cys, Thr, Asp, and Asn [7, 16, 31]. The RC of *Thermosynechococcus elongates* photosystem II contains isoleucine and threonine near Mg atoms of monomeric BChl molecules [32].

According to the literature data, bacteriochlorophylls B_B, P_A, and P_B in RCs of purple bacteria *Blastochloris viridis*, *Rb. sphaeroides*, and *Rb. capsulatus* can be substituted by BPheo when the Mg-liganding histidine is substituted by leucine (by means of site-directed mutagenesis) [7, 12, 14, 33]. Among the three mentioned bacterial species, only *B. viridis* allows similar substitution of B_A by BPheo. The goal of substitution of B_A by BPheo in RCs of purple bacteria was investigation of the electron transport pathways in these mutant RCs. According to theoretical computation, the level of free energy of the state $P^+\Phi_A^-H_A$ is lower than that of the state $P^+\Phi_AH_A^-$, so electron transport via a common pathway is to be hampered in such mutant RCs [7]. In fact, the long-lived $P^+\Phi_A^-$ state was observed, and efficacy of primary electron transport significantly decreased in the *B. viridis* RC mutant H(L153)L [7]. Repeated attempts to obtain the analogous mutation of *Rb. sphaeroides* were unsuccessful for a long time, despite substantial homology between RCs of these bacteria. Extremely high instability of *Rb. capsulatus* RC mutants with substitution H(L153)L was noted, and in the case of H(L153)R mutation the authors presumed the absence of functionally active RCs in chromatophores of this bacterium [16]. Katilius and colleagues prepared a series of partially purified RC mutants with substitutions of histidine L153 and also noted that all the specimens but H(L153)S RC are unstable and heterogeneous [17]. They concluded that BChl B_A is not substituted by BPheo in RCs of the tested mutants.

In our work, we have constructed four mutant strains with substitutions of histidine by either Cys, Met, Leu, or Tyr in their RCs and shown that none of the mutations leads to appearance of BPheo in position B_A. The data of pigment analysis of isolated RCs H(L153)M and H(L153)C indicates unchanged pigment composition of these RCs. Hence, Met and Cys, like Ser [17], can serve as relatively stable ligands for the central magnesium atom of the B_A molecule in *Rb. sphaeroides* RC. We have also noted the splitting of BChl Q_x bands in absorption spectra of RC mutants H(L153)M and H(L153)C, which is more pronounced at low temperatures (data not published) and was earlier observed by other authors [7, 16, 17]. The splitting of this band is indicative of modification of electron transitions in the B_A molecule and, according to the present data, is associated with changes in the nature of the axial ligand [34, 35].

Since the RC mutants H(L153)Y and H(L153)L quickly degrade in the presence of detergent, we attempted examination of these RCs in their natural environment, namely, in association with photosynthetic membranes. To do this, we used antenna-less *Rb. sphaeroides* strains whose chromatophores contain RC as the sole bacteriochlorin–protein complex [18, 19, 36, 37]. We have shown that the chromatophore-associated RC mutant H(L153)L possesses photochemical activity comparable with that of wild-type RC. A comparison of differential (light-minus-dark) spectra (Fig. 3) with the same spectra of *B. viridis* RC mutant H(L153)L [7] confirms the assumption of invariability of pigment composition of *Rb. sphaeroides* RC with substitution H(L153)L [17].

It remains unclear why the substitution of B_A molecule by BPheo in *B. viridis* RC is possible when all attempts to do it failed in functionally and structurally similar RCs from *Rb. capsulatus* and *Rb. sphaeroides*. Also unanswered is the question how leucine, which has no free electron pair for coordination bond formation, can act as a ligand for the magnesium atom of a B_A molecule. In 1988 Feher and coworkers found that coordination of the central magnesium atoms of bacteriochlorophylls in A and B chains of *Rb. sphaeroides* RC is not symmetrical. According to their data, in *Rb. sphaeroides* RC the dis-

tance between histidine L153 and magnesium atom of B_A is not close enough for formation of stable coordination bond. It was supposed that in this case a water molecule can serve as the fifth coordination ligand [11]. Later the presence of bound water molecule HOH 55 was shown in the vicinity of P_B and P_A molecules in the bacterial RC structures and its important role was determined in the process of primary electron transport [9, 38, 39], including formation of hydrogen bonds connecting a series of amino acids near P_B and B_A. The known instability of RCs carrying mutations in the vicinity of histidine L153 seems to be associated with dissolution of potentially weak and possibly water molecule-involving interactions between the amino acid residue L153 and other sites of the L-subunit [16].

The most unexpected data were obtained when we examined the properties of membrane-associated H(L153)Y RC. According to the data of Bylina and coworkers [16], the pigment composition of *Rb. capsulatus* RC remained unchanged when His M182 was substituted by Ser and His L173 by Thr or Ser, thus suggesting the possible involvement of an OH-group of amino acids in either direct or indirect liganding of the magnesium atom of bacteriochlorophylls in RC. A substitution of histidine L153 by tyrosine, which also contains an OH-group, was introduced recently in *Rb. sphaeroides* RC [17]. According to this data, the spectral features of H(L153)Y RC in many ways coincided with those of another RC mutant, H(L153)V, despite noticeable difference in chemical properties of tyrosine and valine. Analysis of pigment composition of H(L153)Y has shown that the BChl/BPheo ratio varied in the range 1.6-1.8 in different fractions of partially purified complexes [17]. However, besides this fact, the authors did not find other peculiarities of this RC. The data we have presented on the properties of membrane-associated H(L153)Y RC fundamentally differ from those reported for isolated H(L153)Y RC [17]. Our data obtained using fluorescence detection and recording of absorption and action spectra indicate that the substitution of histidine by tyrosine at position L153 results in formation of photosynthetic RC complexes in *Rb. sphaeroides* membranes that do not contain the monomeric BChl molecule in the active chain of electron transport cofactors. Irreversible oxidation of the B_A molecule seems unlikely in this case, because no new bands appear in the absorption spectrum of the mutant RC.

A particular fact is that the RC mutant H(L153)Y is capable of charge separation in the light, although less effectively than is the wild-type RC. In the light of this data, a reasonable question arises on the pathway of primary electron transport in H(L153)Y RC devoid of natural closest acceptor of electron from P*. One can suppose that, since the electron transport in this RC is impossible via the common pathway including formation of the state with separated charges $P^+B_A^-$, it seems to occur via an alternative pathway. Possibly, the state $P^+H_A^-$ is formed by a mechanism of electron tunneling between P* and H_A. Additional studies are necessary to determine how the absence of B_A molecule influences mechanisms of photosynthetic charge separation in H(L153)Y RC.

Numerous studies suggest decided importance of B_A as a component of electron transport A-chain, and the BChl molecule is more energetically effective than is the BPheo molecule in this position [7, 17]. However, our data indicate the possibility of primary electron transport in H(L153)Y RC devoid of B_A molecule, but with much lower quantum yield than that in natural RC.

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