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FTIR Spectroscopy of the Reaction Center of *Chloroflexus aurantiacus*: Photooxidation of the Primary Electron Donor

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Abstract—Photochemical oxidation of the primary electron donor P in reaction centers (RCs) of the filamentous anoxygenic phototrophic bacterium *Chloroflexus* (*C.*) *aurantiacus* was examined by light-induced Fourier transform infrared (FTIR) difference spectroscopy at 95 K in the spectral range of 4000-1200 cm^{-1}. The light-induced $P^+Q_A^-/PQ_A$ IR spectrum of *C. aurantiacus* RCs is compared to the well-characterized FTIR difference spectrum of P photooxidation in the purple bacterium *Rhodobacter* (*R.*) *sphaeroides* R-26 RCs. The presence in the $P^+Q_A^-/PQ_A$ FTIR spectrum of *C. aurantiacus* RCs of specific low-energy electronic transitions at ~2650 and ~2200 cm^{-1}, as well as of associated vibrational (phase-phonon) bands at 1567, 1481, and 1294-1285 cm^{-1}, indicates that the radical cation P^+ in these RCs has dimeric structure, with the positive charge distributed between the two coupled bacteriochlorophyll *a* molecules. The intensity of the P^+ absorbance band at ~1250 nm (upon chemical oxidation of P at room temperature) in *C. aurantiacus* RCs is approximately 1.5 times lower than that in *R. sphaeroides* R-26 RCs. This fact, together with the decreased intensity of the absorbance band at ~2650 cm^{-1}, is interpreted in terms of the weaker coupling of bacteriochlorophylls in the P^+ dimer in *C. aurantiacus* compared to *R. sphaeroides* R-26. In accordance with the previous (pre)resonance Raman data, FTIR measurements in the carbonyl stretching region show that in *C. aurantiacus* RCs (i) the 13^1-keto C=O groups of P_A and P_B molecules constituting the P dimer are not involved in hydrogen bonding in either neutral or photooxidized state of P and (ii) the 3^1-acetyl C=O group of P_B forms a hydrogen bond (probably with tyrosine M187) absorbing at 1635 cm^{-1}. Differential signals at 1757(+)/1749(–) and 1741(+)/1733(–) cm^{-1} in the FTIR spectrum of *C. aurantiacus* RCs are attributed to the 13^3-ester C=O groups of P in different environments.

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Photochemical reaction centers (RCs) of photosynthetic bacteria are transmembrane pigment–protein complexes where photon absorption induces a series of rapid electron transfer reactions resulting in spatial separation and stabilization of the charges [1]. The key element involved in the charge separation process is the primary electron donor P, the singlet excited state (P*) of which transfers the electron to the primary acceptor during a few picoseconds, triggering all subsequent photosynthetic reactions [1]. At the same time, P is transformed into a radical cation (P^+) and later becomes a part of all successively formed charge-separated states with the final localization of electron on the molecules of quinone acceptors Q_A and Q_B [1]. The study of the properties of P in its neutral and radical cation states is one of the central problems of elucidation of the molecular mechanisms of highly efficient primary charge separation in bacterial photosynthesis.

A promising approach to investigation of the mechanisms of primary charge separation is the comparative study of RCs of the natural strains of photosynthetic bacteria. In this respect, the RCs of the filamentous anoxygenic phototrophic bacterium *Chloroflexus* (*C.*) *aurantiacus* draw great attention [2]. Showing relatively distant evolutionary relationship to the purple photosynthetic

Abbreviations: ΔA, change in absorbance; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; E_m, midpoint redox potential; FTIR spectroscopy, Fourier transform infrared spectroscopy; HOMO, highest occupied molecular orbital; P, primary electron donor, a dimer of BChl molecules; P_A and P_B, BChl molecules comprising P; Q_A, primary quinone acceptor; RC, reaction center.

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bacteria, *C. aurantiacus* contains reaction centers similar to the RCs of the purple bacterium *Rhodobacter* (*R.*) *sphaeroides* in the type of pigments (bacteriochlorophyll *a* (BChl) and bacteriopheophytin *a* (BPheo)), general organization of cofactors, and primary photochemistry [2]. However, there are substantial differences in protein and cofactor compositions between these RCs. In particular, the *C. aurantiacus* RC consists of two protein subunits (L and M) and contains 3 BChl and 3 BPheo [2], while the *R. sphaeroides* RC includes three subunits (L, M and H) carrying 4 BChl and 2 BPheo [1]. A few functionally important amino acids typical of purple bacteria RCs are absent in the amino acid sequences of the *C. aurantiacus* RC [4-6].

Data of optical and EPR spectroscopies demonstrate the dimeric nature of the primary electron donor in *C. aurantiacus* RCs [2, 7-9]. However, in contrast to RCs of purple bacteria with known atomic-resolution crystal structure [3], an X-ray structural model for *C. aurantiacus* RCs has not been described in the literature [10, 11]. In this context, spectral methods are among the basic approaches to elucidation of the structural organization and properties of P in *C. aurantiacus* RCs.

Light-induced Fourier transform infrared (FTIR) difference spectroscopy is extremely sensitive to the molecular changes accompanying charge separation, providing information about the structure and interactions of RC cofactors [12]. It is particularly interesting that this method allows recording very low-energy electronic transitions in the infrared region of the spectrum typical of pigment dimers [13]. Previously, difference FTIR spectroscopy has been intensively used in the study of photooxidation of P in RCs of *R. sphaeroides* and other photosynthetic bacteria [13-17]. However, this method has not been used for studying the light-induced formation of P^+ in *C. aurantiacus* RCs, except for work [13] showing the presence of a hole transfer band in photooxidized P around 2600 cm^{-1} in the films of chromatophores.

In the present work, the light-induced FTIR spectrum of photooxidation of P to the radical cation P^+ has been studied in the isolated *C. aurantiacus* RCs in the frequency range of 4000-1200 cm^{-1} at a cryogenic (95 K) temperature. The spectrum obtained was compared to the analogous spectrum of *R. sphaeroides* R-26 RCs, many bands of which had been previously identified by model experiments, the data of X-ray structure analysis, and point mutagenesis [12-14]. This was the basis for obtaining new evidence favoring the dimeric structure of P^+ in *C. aurantiacus* RCs and characterizing its electronic and molecular properties.

MATERIALS AND METHODS

Reaction centers of *C. aurantiacus* and *R. sphaeroides* R-26 were isolated by treating the membranes with detergent *N*,*N*-dimethyldodecylamine-*N*-oxide (LDAO) followed by purification by ion-exchange chromatography on DEAE cellulose [18-20]. The LDAO detergent was replaced by Triton X-100 or *n*-dodecyl-β-D-maltoside using a 30 kDa membrane (Millipore, USA) in an ultra concentration cell under argon gas pressure. The *C. aurantiacus* RCs were suspended in 50 mM Tris-HCl buffer (pH 8.5) containing 0.1% Triton X-100. The *R. sphaeroides* R-26 RCs were suspended in 10 mM Tris-HCl buffer (pH 8.0) containing 0.1% *n*-dodecyl-β-D-maltoside. The samples for IR measurements (thin, partially dehydrated RC films contained between two CaF_2 plates) were obtained as described [21].

Light-induced (light-minus-dark) $P^+Q_A^-/PQ_A$ FTIR difference spectra were recorded with an IFS66v/s spectrometer (Bruker, Germany) with a DTGS detector and a KBr beam splitter. Spectral resolution was 4 cm^{-1}. Low-temperature (95 K) measurements were made in an optical cryostat with a temperature controller (Specac, Great Britain). Samples were illuminated with actinic light at 1100 nm ≥ λ ≥ 720 nm (~2 mW/cm^2). Illumination cycles were repeated hundreds of times to reach the necessary signal/noise ratio. The time delay between the cycles was sufficient for nearly complete relaxation of the $P^+Q_A^-$ state.

Absorption spectra of RCs in the films at 95 K in the region of 700-1000 nm were recorded with an UV-1601 PC spectrophotometer (Shimadzu, Japan). The sample preparation and optical cryostat were the same as those used for IR measurements. Difference (light-minus-dark) $P^+Q_A^-/PQ_A$ spectra were obtained as follows: the absorption spectra measured before illumination were subtracted from the spectra obtained during illumination of the samples with actinic light at 600 nm ≥ λ ≥ 350 nm. The photodetector was protected from actinic light with a filter with λ ≥ 680 nm.

Difference (chemically oxidized-minus-neutral RCs) P^+/P spectra in the region of 650-1350 nm were obtained with an UV-3101 PC spectrophotometer (Shimadzu) using RCs suspended in buffer solutions (see above) with the addition of 1 mM potassium ferricyanide. Measurements were made at room temperature in a quartz cuvette with an optical path length of 1 cm.

RESULTS

Figure 1a shows the typical low-temperature (95 K) electronic absorption spectra of *C. aurantiacus* and *R. sphaeroides* R-26 RCs in the thin, partially dehydrated films used in this work for IR measurements. These spectra are in good agreement with the absorption spectra obtained previously for *C. aurantiacus* and *R. sphaeroides* R-26 RCs at cryogenic temperatures [18, 19, 22-24]. In the absorption spectrum of *R. sphaeroides* R-26 RCs, the long-wavelength band at 885 nm observed in the region of

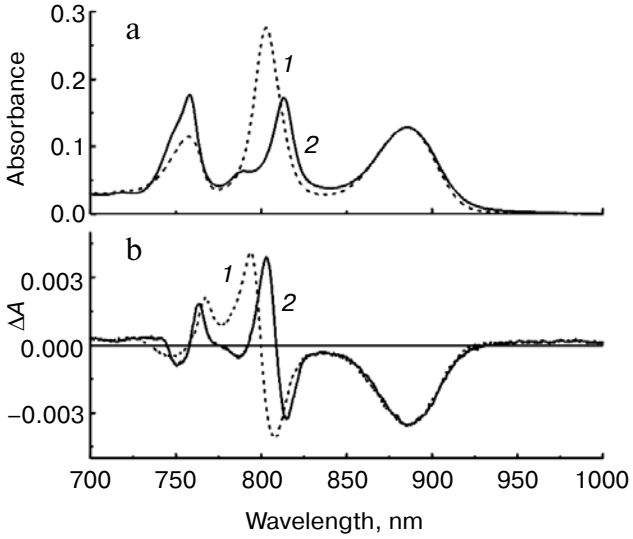

Fig. 1. a) Low-temperature (95 K) electronic absorption spectra of RCs of *R. sphaeroides* R-26 (*1*) and *C. aurantiacus* (*2*) in partially dehydrated films in the region of 700-1000 nm. The spectra are normalized at the P band at 885 nm. b) Low-temperature (95 K) light-induced (light-minus-dark) $P^+Q_A^-/PQ_A$ difference spectra of RCs of *R. sphaeroides* R-26 (*1*) and *C. aurantiacus* (*2*) in partially dehydrated films whose absorption spectra are shown in Fig. 1a. The difference spectra are normalized at the negative band at 885 nm. The results of nine light/dark cycles are averaged for each difference spectrum. Vertical scales in panels (a) and (b) apply to the spectra of *C. aurantiacus* RCs.

Q_y optical transitions (700-1000 nm) is assigned to the low-energy exciton transition of the primary electron donor P dimer. The bands at 803 and 758 nm were attributed to the absorption of two monomeric BChl molecules (with potential contribution of the high-energy exciton transition of the P dimer) and two BPheo molecules, respectively. The band at 885 nm in the spectrum of *C. aurantiacus* RCs was also attributed to the P dimer [2]. A single molecule of monomeric BChl in *C. aurantiacus* RCs absorbs at 813 nm; the band at 758 nm is attributed to the absorption of three BPheo molecules. The *C. aurantiacus* RC is also characterized by a relatively weak band at ~790 nm [18], the nature of which has not yet been determined [23, 25, 26].

Low-temperature (95 K) difference (light-minus-dark) spectra presented in Fig. 1b show that *C. aurantiacus* and *R. sphaeroides* R-26 RCs retain the ability for charge separation with formation of the $P^+Q_A^-$ state under conditions of partial dehydration[1]. In the region of Q_y transitions, the $P^+Q_A^-/PQ_A$ spectra of both RCs reflect mainly the effects associated with the photooxidation of the primary electron donor, including the bleaching of the P absorption band at 885 nm and spectral changes in the absorption region of monomeric BChl and BPheo molecules. The *C. aurantiacus* RCs spectrum also has a negative band corresponding to the bleaching of the transition at ~790 nm. The presented difference spectra correspond to the reversible changes in absorption and are in good agreement with the previously published low-temperature spectra of P photooxidation in *C. aurantiacus* and *R. sphaeroides* R-26 RCs [19, 23].

Figure 2 shows the low-temperature light-induced $P^+Q_A^-/PQ_A$ FTIR difference spectra for *C. aurantiacus* and *R. sphaeroides* R-26 RCs measured at 95 K in the region of 4000-1200 cm^{-1}. The positive and negative bands in these spectra reflect $P^+Q_A^-$ formation and PQ_A disappearance, respectively. The characteristic feature of the $P^+Q_A^-/PQ_A$ difference FTIR spectrum of *R. sphaeroides* RCs is the presence of a broad positive band at ~2650 cm^{-1} with a low-frequency shoulder at ~2200 cm^{-1} (Fig. 2, *1*; [13, 14]). IR bands in this spectral region have been previously attributed to the low-energy electronic transitions with the involvement of the highest occupied molecular orbitals of P^+ photooxidized dimer [13, 28]. The band at ~2650 cm^{-1} with a shoulder at ~2200 cm^{-1} is observed also in the $P^+Q_A^-/PQ_A$ spectrum of *C. aurantiacus* RCs (Fig. 2, *2*).

In Fig. 3, the $P^+Q_A^-/PQ_A$ FTIR difference spectra of *C. aurantiacus* and *R. sphaeroides* R-26 RCs are shown in more detail in the range of 1800-1200 cm^{-1}. In this region, the spectrum of *R. sphaeroides* R-26 RC includes three intense bands at 1548, 1478, and 1296-1284 cm^{-1}

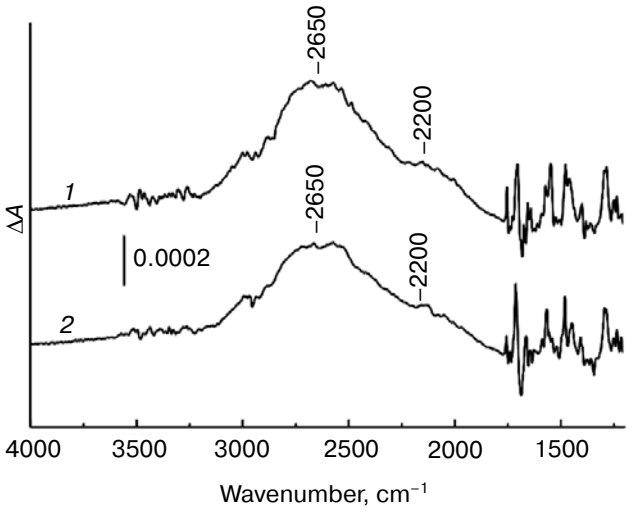

Fig. 2. Low-temperature (95 K) light-induced (light-minus-dark) $P^+Q_A^-/PQ_A$ FTIR difference spectra of RCs of *R. sphaeroides* R-26 (*1*) and *C. aurantiacus* (*2*) in partially dehydrated films in the region of 4000-1200 cm^{-1}. A total of 10,000 interferograms were averaged for each RC using three separate samples. Spectral resolution was 4 cm^{-1}.

[1] Electron transfer from the primary quinone Q_A to the secondary quinone Q_B in *R. sphaeroides* RCs is blocked at low temperatures [27]; Q_B is removed from the structure of *C. aurantiacus* RCs during their isolation and purification [2].

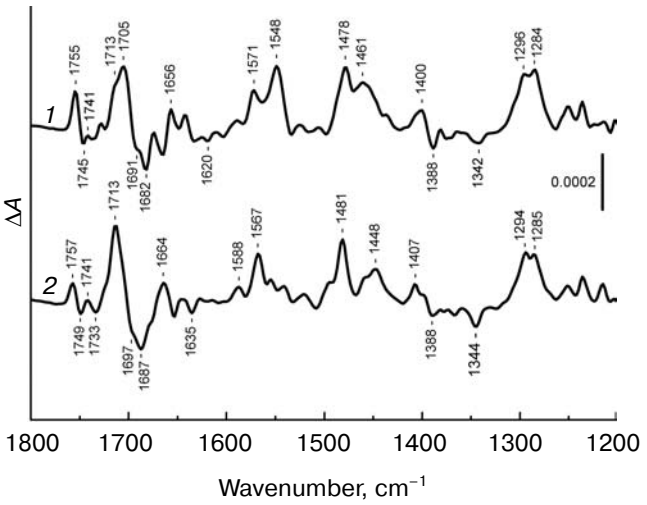

Fig. 3. Low-temperature (95 K) light-induced (light-minus-dark) $P^+Q_A^-/PQ_A$ FTIR difference spectra of RCs of *R. sphaeroides* R-26 (*1*) and *C. aurantiacus* (*2*) in partially dehydrated films in the region of 1800-1200 cm^{-1}: the low-frequency region of the spectra presented in Fig. 2 is given on a magnified scale.

(Fig. 3, *1*; [13, 14]) attributed to the vibrational (phase-phonon) modes [28]. The spectrum of *C. aurantiacus* RCs (Fig. 3, *2*) contains a band at 1294-1285 cm^{-1} close in form and position to the band at 1296-1284 cm^{-1} in the spectrum of *R. sphaeroides* R-26 RCs and intense bands at 1567 and 1481 cm^{-1}, which can also be attributed to the phase-phonon modes. It is expected that the $P^+Q_A^-/PQ_A$ spectra also contain the contributions of quinone modes; however, the intensive bands of P and P^+ dominate over the signals associated with Q_A and Q_A^- [14, 29].

Detailed comparison of FTIR spectra (Fig. 2) shows that the amplitude of the band at ~2650 cm^{-1} in the spectrum of *C. aurantiacus* RCs is less compared to the analogous band observed for *R. sphaeroides* R-26 RCs (approximately 1.3-fold). A slight decrease in intensity is observed also for the band at 1294-1285 cm^{-1} (Fig. 3). Taking into account that the rate constants of $P^+Q_A^-$ recombination in the given RCs are similar at cryogenic temperatures [22, 30], the difference in band intensities in the FTIR spectra may indicate the existence of differences in the electronic structure of P^+ between the two RCs.

In the region of 1760-1620 cm^{-1}, the $P^+Q_A^-/PQ_A$ FTIR spectrum of *R. sphaeroides* R-26 RCs (Fig. 3, *1*) demonstrates signals associated mainly with the shifts of carbonyl modes of P_A and P_B molecules during photooxidation of P [14]. The bands at 1745 and 1755 cm^{-1} (differential signal at 1755(+)/1745(–)) were attributed to the absorption of at least one of the two 13^3-ester C=O groups of the primary electron donor in the P and P^+ states, respectively [14, 17]. The analogous but less intense differential signal in the $P^+Q_A^-/PQ_A$ spectrum of *C. aurantiacus* RCs is located at 1757(+)/1749(–) cm^{-1} (Fig. 3, *2*). However, this frequency region of the *C. aurantiacus* spectrum contains also another differential signal at 1741(+)/1733(–) cm^{-1}. The comparable amplitudes of signals observed for *C. aurantiacus* and the equal values of high-frequency shifts (8 cm^{-1}) suggest that both signals belong to the BChl molecules constituting P.

Stretching vibrations of the free 13^1-keto C=O groups of P_A and P_B molecules in the $P^+Q_A^-/PQ_A$ FTIR spectrum of *R. sphaeroides* R-26 RCs (Fig. 3, *1*) have been previously associated with the bands at 1691 and 1682 cm^{-1}, respectively, which shift during P^+ formation to 1713 and 1705 cm^{-1}, respectively, generating two superimposed differential signals [14]. The spectrum of *C. aurantiacus* RCs (Fig. 3, *2*) demonstrates negative bands at 1697 and 1687 cm^{-1}, which can also be attributed to the stretching modes of the 13^1-keto C=O groups of BChl molecules in the neutral state of the primary electron donor. However, absorption of these groups in the P^+ state in *C. aurantiacus* RCs is characterized by a single band with the maximum at 1713 cm^{-1}. This band is slightly asymmetric, apparently including the weak shoulder on its low-frequency slope.

The minor negative band at 1620 cm^{-1} observed in the $P^+Q_A^-/PQ_A$ spectrum of *R. sphaeroides* R-26 RCs (Fig. 3, *1*) is attributed to the 3^1-acetyl C=O group of the P_A molecule hydrogen bonded to histidine L168 [14]. Identification of the band of the free 3^1-acetyl group of the P_B molecule in the region of ~1650 cm^{-1} is difficult because of the contributions of the protein and quinone carbonyl modes [14]. In the context of these assignments, the negative band at 1635 cm^{-1} in the $P^+Q_A^-/PQ_A$ FTIR spectrum of *C. aurantiacus* RCs (Fig. 3, *2*) may belong to the 3^1-acetyl C=O group of BChl molecule, which constitutes P and is linked via a hydrogen bond to the RC protein.

The band at 1656 cm^{-1} in the $P^+Q_A^-/PQ_A$ FTIR spectrum of *R. sphaeroides* R-26 RCs (Fig. 3, *1*) seems to reflect the changes in vibrations of the peptide C=O groups in response to photooxidation of P [14, 15]. The analogous signal in the FTIR spectrum of *C. aurantiacus* RCs seems to be located at 1664 cm^{-1} (Fig. 3, *2*). The relatively low amplitude of absorption changes in the region of the protein modes shows that, similar to the situation observed for the purple bacteria [31], photooxidation of P in *C. aurantiacus* RCs does not result in significant changes in protein structure.

Figure 4 shows difference P^+/P spectra obtained for *R. sphaeroides* and *C. aurantiacus* RCs during chemical oxidation of P with potassium ferricyanide (1 mM) in a suspension at room temperature. Both spectra contain the well known absorption band of the radical cation P^+ at ~1250 nm (1247 nm for *R. sphaeroides* R-26 RCs and 1243 nm for *C. aurantiacus* RCs in Fig. 4) [7, 9, 16, 32, 33]. The presented spectra (Fig. 4) were measured in samples with equal absorption at 865 nm. With consideration of the closeness of extinction coefficients in this band for the two RCs [2, 34], this corresponds to compa-

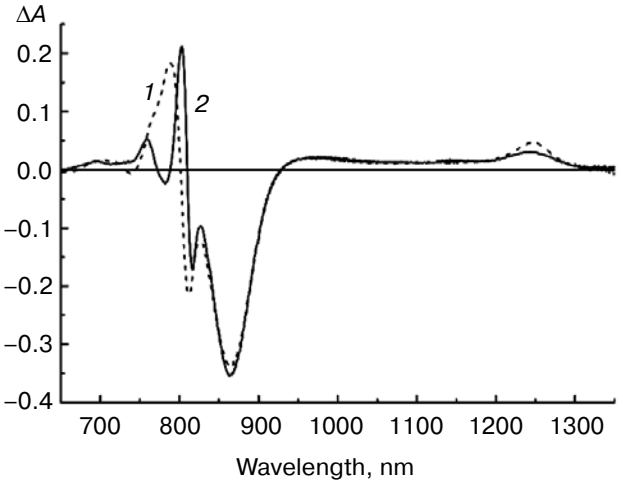

Fig. 4. Difference (oxidized-minus-neutral RCs) P^+/P spectra of RCs of *R. sphaeroides* R-26 (*1*) and *C. aurantiacus* (*2*) in the region of 650-1350 nm obtained during chemical oxidation of the primary electron donor with potassium ferricyanide (1 mM) in a suspension at room temperature.

rable RC concentration. The substantial result of these measurements is the fact that intensity of the band at 1243 nm in the P^+ spectrum of *C. aurantiacus* RCs is considerably (approximately 1.5-fold) lower compared to the band at 1247 nm in *R. sphaeroides* R-26 RCs.

DISCUSSION

The results show that the infrared spectra of $P^+Q_A^-$ formation in *C. aurantiacus* and *R. sphaeroides* R-26 RCs are characterized by well-defined general similarity but demonstrating, however, considerable differences in details (Figs. 2 and 3). Spectral similarity leads to the conclusion that the radical cations of the primary electron donor P^+ in *C. aurantiacus* and *R. sphaeroides* R-26 RCs have similar structural organization. However, the differences in position and/or intensity of infrared bands in the difference IR spectra demonstrate the existence of subtle differences in the molecular and electronic properties of P^+ in these RCs.

Dimeric structure of P^+ in *C. aurantiacus* RCs. One of the most marked common features of the $P^+Q_A^-/PQ_A$ FTIR spectra of *C. aurantiacus* and *R. sphaeroides* R-26 RCs is the presence of a broad absorption band at ~2650 cm^{-1} with a shoulder at ~2200 cm^{-1} (Fig. 2). Previous studies have shown that the band at ~2650 cm^{-1} belongs to the low-energy electronic transition associated with the transfer of positive charge (a "hole") from one half of the oxidized P^+ dimer to the other half [13, 28]. This transition is absent in the monomeric radical cation $BChl^+$ [14], being the unique characteristic of dimeric structure of the oxidized primary electron donor in the RCs of purple bacteria [13]. The shoulder at ~2200 cm^{-1} was attributed to the transition from the second highest occupied molecular orbital (HOMO-1) to the HOMO in the P_A^+ molecule carrying a positive charge in the radical cation state of the primary electron donor [28]. The HOMO-1 → HOMO transition is intensified in the photooxidized dimer due to coupling with the transition at ~2650 cm^{-1}, and its presence in the FTIR spectra also demonstrates the dimeric nature of P^+ [28].

Just like the intense bands observed in the FTIR spectrum of *R. sphaeroides* R-26 RCs at 1548, 1478, and 1296-1284 cm^{-1} (Fig. 3, *1*; [14]), the infrared bands in the spectrum of *C. aurantiacus* RCs at 1567, 1481, and 1294-1285 cm^{-1} (Fig. 3, *2*) can be attributed to the phase-phonon vibrational modes of the oxidized primary electron donor [28]. Such vibrational modes are formally symmetry-forbidden in monomeric BChl; however, they intensify due to the coupling with the hole-transfer transition, being indicators of the dimeric structure of P^+ [28]. Phase-phonon bands were not observed in the FTIR spectra of monomeric $BChl^+$ [35].

Thus, the presence of specific low-energy electronic transitions and marker vibrational bands in the light-induced FTIR spectrum of *C. aurantiacus* RCs (Figs. 2 and 3) typical of dimeric P^+ leads to the conclusion that the radical cation of the primary electron donor in this bacterium is a homodimer of coupled BChl *a* molecules. It is significant that both the bands at ~2650 and ~2200 cm^{-1} and the phase-phonon bands are not observed in the $P^+Q_A^-/PQ_A$ FTIR spectra of mutant RCs of purple bacteria containing a heterodimer of BChl and BPheo molecules, where the positive charge in the P^+ state is completely localized on a single BChl molecule [13, 36]. It denotes a distribution of the positive charge in the P^+ dimer in the *C. aurantiacus* RC between P_A and P_B molecules.

The finding of P^+ dimeric structure is also in agreement with observation of the absorption band of the oxidized primary electron donor at 1243 nm in the P^+/P spectrum of *C. aurantiacus* RCs (Fig. 4, *2*; [7, 9]) similar in shape to the band at 1247 nm in *R. sphaeroides* R-26 RCs (Fig. 4, *1*; [32]). According to the literature data, the band at ~1250 nm is attributed to formation of a triplet excited state in the P^+ dimer [37]. Later, based on theoretical calculations, the absorption in this spectral region was attributed to the triplet-coupled transition localized on the BChl molecule P_B carrying no positive charge in the P^+ state [38]. This transition is spin-forbidden but intensifies in the presence of the other half of the dimer, P_A molecule, supposedly present in the radical cation state P_A^+. In accordance with this consideration, the electronic band at ~1250 nm is a characteristic feature of dimeric P^+, and its intensity depends strongly on the value of electronic coupling between the two halves of the dimer [38]. It is interesting that the minor short-wavelength shift and decrease in the P^+ band intensity at

~1250 nm was observed at 30 K for the mutant *R. sphaeroides* HL168F RCs relative to the wild type *R. sphaeroides* RCs [16]. An analogous amino acid substitution of His → Phe is expected also for the *C. aurantiacus* RC [4-6].

Electronic properties of P^+ in *C. aurantiacus* RCs. For explaining the optical and electronic properties of the primary electron donor in bacterial RCs, Breton et al. [13] and Plato et al. [39] proposed a molecular-orbital model that was used particularly for the analysis of influence of mutations on the physicochemical characteristics of P dimer [16, 17, 40]. According to this model, the HOMO energies of individual bacteriochlorophylls P_A and P_B are not equivalent to each other, differing by the value $\Delta\alpha$. The interaction between the orbitals of P_A and P_B molecules, the energy of which is characterized by resonance integral β_D, results in formation of two HOMOs of P dimer separated by an energy gap $\Delta E = (\Delta\alpha'^2 + 4\beta_D^2)^{1/2}$. In this expression, the energy difference $\Delta\alpha'$ includes both the electronic energy difference $\Delta\alpha$ and the contribution of electron-phonon coupling [41]. In the framework of this model, the hole-transfer band in the IR spectrum of P^+ is attributed to the electronic transition between HOMOs of the dimer [13]; the maximum of this band corresponds to energy difference ΔE. Since the maximums of hole-transfer bands in the FTIR spectra of both *C. aurantiacus* and *R. sphaeroides* R-26 RCs are located at ~2650 cm^{-1} (Fig. 2), it can be concluded that ΔE values are similar for these RCs.

The decrease in intensity of the P^+ absorption band in the region of 1250 nm (Fig. 4) indicates some weakening of electronic coupling between the two halves of the dimer [38], i.e. the decrease in β_D in *C. aurantiacus* RCs compared to *R. sphaeroides* R-26 RCs. This conclusion is in agreement also with observation of a minor drop in intensity of the electronic transition band at ~2650 cm^{-1} in the FTIR spectrum of *C. aurantiacus* RCs (Fig. 2) [38]. Taking into account conservation of the ΔE, the decrease in β_D for *C. aurantiacus* RCs suggests the respective increase in $\Delta\alpha'$. The possibility of increase in $\Delta\alpha'$ is in agreement with the presence of phenylalanine L207 and tyrosine M187 in the amino acid sequence of *C. aurantiacus* RCs, which are homologous to histidine L168 and phenylalanine M197 in *R. sphaeroides* R-26 RCs, respectively [4-6]. In contrast to histidine L168, phenylalanine L207 cannot form a hydrogen bond with the acetyl carbonyl of P_A [9]. It can be expected that (in the absence of other changes) it will destabilize the HOMO of P_A molecule, increasing $\Delta\alpha'$ and decreasing the midpoint redox potential (E_m) of P/P^+. Tyrosine residue M187 due to the hydrogen bond with the acetyl carbonyl of P_B molecule, on the contrary, will stabilize the HOMO of P_B, increasing both $\Delta\alpha'$ and E_m of P/P^+ in *C. aurantiacus* RCs.

It should be noted that the decrease in β_D must be accompanied by a short-wavelength shift Q_y of the P band in the electronic absorption spectrum of RCs [33, 42]. In this case, the increase in E_m of P/P^+ can be expected as well. However, it is known that Q_y of the P band in the absorption spectra of *C. aurantiacus* and *R. sphaeroides* R-26 RCs are similar to each other ([2]; Fig. 1), in spite of certain differences in the P band fine structure between the spectra of these RCs observed at helium temperatures [43]. The E_m of P/P^+ value in *C. aurantiacus* RCs was reduced by ~110 mV compared to *R. sphaeroides* RCs [19]. Probably, the decrease in β_D in *C. aurantiacus* RCs is too small to materially affect the spectral and electrochemical properties of P. The spectral position of the long-wavelength P band can depend on a number of factors including the formation of hydrogen bonds [44], orientation of 3^1-acetyl groups [42, 44], formation of charge transfer states [42], and electrostatic interactions [44, 45]. The absence of hydrogen bond to the 3^1-acetyl C=O group of P_A molecule seems to be the dominant factor determining the lower value of E_m of P/P^+ in *C. aurantiacus* RCs [9].

In the molecular-orbital representation, the ratio of the spin densities ρ_A/ρ_B (and positive charges) at P_A and P_B molecules is a function of $\Delta\alpha'/\beta_D$ [17, 39-41]. Electron nuclear double resonance (ENDOR) for *R. sphaeroides* RCs showed that ρ_A/ρ_B was 2.1 [46]. Based on the data of (pre)resonance Raman spectroscopy, in work [9] it was concluded that charge distribution in P^+ in *C. aurantiacus* RCs was more symmetric compared to *R. sphaeroides* RCs. However, the results of FTIR measurements discussed above suggest that the ratios of positive charge values of P_A and P_B molecules in the radical cation P^+ are comparable for these RCs, or charge distribution is slightly more asymmetric in *C. aurantiacus* RCs. For more detailed consideration of this problem, it would be very interesting to determine ρ_A/ρ_B using ENDOR. As far as we know, studies of this kind for *C. aurantiacus* RCs have not yet been described in the literature.

Molecular interactions of carbonyl groups of P and P^+ in *C. aurantiacus* RCs. The differences in the position and intensity of the bands of stretching vibrations of the C=O groups of BChl molecules observed between the $P^+Q_A^-/PQ_A$ FTIR spectra of *C. aurantiacus* and *R. sphaeroides* R-26 RCs in the region of 1760-1620 cm^{-1} (Fig. 3) reflect, to a considerable extent, the peculiarities of P and P^+ interactions with the nearest protein environment in the two RCs. The decrease in differential signal intensity at 1757(+)/1749(–) cm^{-1} and appearance of a marked signal at 1741(+)/1733(–) cm^{-1} in the FTIR spectrum of *C. aurantiacus* RCs (Fig. 3, *2*) show that in these RCs the 13^3-ester C=O groups of BChl molecules constituting the P dimer are more spectrally distinguishable than in *R. sphaeroides* R-26 RCs (Fig. 3, *1*; [14, 17]). This probably indicates the differences in the environment of these groups in *C. aurantiacus* RCs. Further investigations are needed to make a correlation between differential signals at 1757(+)/1749(–) and 1741(+)/1733(–) cm^{-1} and P_A^+/P_A and P_B^+/P_B.

The results of FTIR measurements in the absorption region of the 13^1-keto and 3^1-acetyl groups of *C. aurantiacus* RCs (1710-1620 cm^{-1}) (Fig. 3, *2*) can be compared with the data obtained by (pre)resonance Raman spectroscopy [9]. The Raman spectrum of *C. aurantiacus* RCs measured at room temperature contained a single band at 1696 cm^{-1} attributed to the two free 13^1-keto C=O groups in the neutral state of P; under chemical oxidation of P, there was a band at 1717 cm^{-1} [9]. The results of the present work are in agreement with the conclusion that keto groups of P_A and P_B molecules in *C. aurantiacus* RCs are not involved in formation of hydrogen bonds with the protein in either the neutral or photooxidized state of P [9]. It was previously supposed that leucine L170 and isoleucine M150 unable to donate the hydrogen bond are located in these RCs near the 13^1-keto carbonyl groups of P_A and P_B molecules, respectively [4-6, 9]. The fact that the bands of stretching vibrations of the 13^1-keto C=O groups of BChl molecules of the P dimer are resolved in the $P^+Q_A^-/PQ_A$ FTIR spectrum in *C. aurantiacus* RCs at 95 K, being located at 1697 and 1687 cm^{-1} (Fig. 3, *2*), probably indicates the differences in dielectric environments of these groups [47].

Based on the amino acid sequence of the M protein subunit of *C. aurantiacus* RCs [4-6], the negative band at 1635 cm^{-1} (Fig. 3, *2*) can be attributed to the hydrogen bonded 3^1-acetyl group of the P_B molecule, where the hydrogen bond donor is tyrosine positioned at M187. This assumption is in agreement with the data of Raman spectroscopy and with the model of the primary electron donor microenvironment in *C. aurantiacus* RCs based on these data [9].

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