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Investigation of the Redox Interaction between Mn-Bicarbonate Complexes and Reaction Centers from *Rhodobacter sphaeroides* R-26, *Chromatium minutissimum*, and *Chloroflexus aurantiacus*

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Abstract—The change in the dark reduction rate of photooxidized reaction centers (RC) of type II from three anoxygenic bacteria (*Rhodobacter sphaeroides* R-26, *Chromatium minutissimum*, and *Chloroflexus aurantiacus*) having different redox potentials of the P^+/P pair and availability of RC for exogenous electron donors was investigated upon the addition of Mn^{2+} and HCO_3^-. It was found that the dark reduction of P_{870}^+ from *Rb. sphaeroides* R-26 is considerably accelerated upon the combined addition of 0.5 mM $MnCl_2$ and 30-75 mM $NaHCO_3$ (as a result of formation of "low-potential" complexes $[Mn(HCO_3)_2]$), while $MnCl_2$ and $NaHCO_3$ added separately had no such effect. The effect is not observed either in RC from *Cf. aurantiacus* (probably due to the low oxidation potential of the primary electron donor, P_{865}, which results in thermodynamic difficulties of the redox interaction between P_{865}^+ and Mn^{2+}) or in RC from *Ch. minutissimum* (apparently due to the presence of the RC-bound cytochrome preventing the direct interaction between P_{870}^+ and Mn^{2+}). The absence of acceleration of the dark reduction of P_{870}^+ in the RC of *Rb. sphaeroides* R-26 when Mn^{2+} and HCO_3^- were replaced by Mg^{2+} or Ca^{2+} and by formate, oxalate, or acetate, respectively, reveals the specificity of the Mn^{2+}-bicarbonate complexes for the redox interaction with P^+. The results of this work might be considered as experimental evidence for the hypothesis of the participation of Mn^{2+} complexes in the evolutionary origin of the inorganic core of the water oxidizing complex of photosystem II.

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Key words: Mn-bicarbonate complexes, reaction centers, purple bacteria, RC-bound cytochrome

Green plants and cyanobacteria use water as an electron donor for the primary reactions of photosynthesis. Molecular oxygen is formed as a result of photosynthetic oxidation of H_2O in the enzymatic water-oxidizing complex (WOC) of photosystem II (PSII) based on the so-called inorganic core with hypothetical stoichiometry of $Mn_4Ca_1O_xCl_y$ [1]. In four successive photoacts of PSII, the Mn cluster accumulates four oxidative equivalents (S_n-states, where n = 0-4), which promote initialization of the reaction of simultaneous oxidation of two water molecules with formation of O_2 [2].

In spite of considerable progress in the study of structural and functional organization of the Mn cluster of PSII, the question about the evolutionary origin of the WOC inorganic core is still under discussion. The comparison of structural peculiarities, the mechanisms of functioning of bacterial reaction centers (RC) and PSII RC, and comparison of the results of sequencing of the genes encoding RC proteins suggest that the type II RCs from the purple anoxygenic bacteria was an evolutionary precursor to the PSII RC [3]. In contrast to PSII, bacterial RC use different compounds such as Fe^{2+}, H_2S, S_x, etc. as substrates for reduction of the oxidized primary electron donor P^+. These compounds directly (or indirectly, via a cytochrome molecule) reduce the oxidized RC during a one-electron reaction. In addition, the midpoint redox potential (E_m) of the primary electron donor, the dimer of bacteriochlorophyll (P_{870}) molecules, in the purple bacteria is about 0.5 V [4-6], while the potential of H_2O oxidation to O_2 is 0.82 V (pH 7).

Recent data demonstrate the necessity of bicarbonate ions for the maximum activity of the donor side of PSII. Thus, for effective reactivation of electron transport [7-9] and oxygen release [9-11], HCO_3^- must be present in PSII preparations with deleted Mn cluster in addition

Abbreviations: LDAO, N,N-dimethyldodecylamino-N-oxide; P, primary electron donor; PSII, photosystem II; RC, reaction center; WOC, water-oxidizing complex.

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to the stoichiometric quantity of Mn^{2+} (2-4 atoms per RC), Ca^{2+}, and Cl^-. In accordance with several hypotheses, bicarbonate may be either a mediator involved in the assembly of the Mn cluster [1, 10], or an integral cofactor within PSII WOC [8], or a base necessary for proton removal during water oxidation [12].

Based on the fact that the chemical composition, pH, and temperature of the ocean in the Archaean period (more than 2.5 billion years ago) significantly differed from modern parameters and the concentration of dissolved bicarbonate could be up to 200 mM due to the high CO_2 concentration in the atmosphere, it seems possible that HCO_3^- ions played a key role in the evolutionary origin of the first oxygenic photosynthesizing organisms [13]. The data of electrochemical studies show that Mn^{2+} in aqueous solution is liganded by HCO_3^- ions, which results in a substantial shift of the Mn^{2+} oxidation potential: from 1.18 V for the aqua cation to 0.61-0.52 V depending on stoichiometry of the formed Mn-bicarbonate complex [14-16]. In the presence of high bicarbonate concentrations (over 50 mM), electrically neutral complexes $[Mn(HCO_3)_2]$ are prevalent in the solution; the value of Mn^{2+} oxidative potential for these complexes decreases to 0.52 V [14-16] and thereby becomes comparable to E_m of the primary electron donor in the RC from purple bacteria (about 0.5 V) [4-6, 17]. The hypothesis based on these data [13] suggested that such "low-potential" Mn^{2+}-bicarbonate complexes could be secondary electron donors for anoxygenic bacteria (precursors of the first O_2-evolving organisms) in the Archaean period. Later on, as a result of evolutionary changes in protein structure, the complexes of this kind could bind to the RC and form a tetramanganese-bicarbonate cluster at the donor side, a precursor of the modern Mn-containing WOC.

The possibility of Mn^{2+} oxidation in the presence of HCO_3^- by the cation radical of the primary electron donor of the purple bacteria, P_{870}^+, was shown for the first time in an isolated mutant RC from *Rhodobacter sphaeroides* R-26, where the redox potential of the P_{870}^+/P_{870} pair was raised to 0.62-0.77 V by a series of point mutations near P_{870} [18]. Later, considerable acceleration of the dark reduction of photooxidized P_{870} was shown in the presence of $MnCl_2$ and bicarbonate in the pigment–protein "core" complexes B890 from three species of wild type anoxygenic purple bacteria: *Rhodovulum iodosum*, *Rh. robiginosum*, and *Thiorhodospira sibirica* (with type II reaction centers) [19]. The data were interpreted in the framework of a model assuming the direct interaction between Mn^{2+} and P_{870}^+ at the level of electron transfer. Preliminary results showed the possibility of an analogous reaction between Mn^{2+}-bicarbonate complexes and the isolated RC from the purple bacterium *Rb. sphaeroides* R-26 [19]. The influence of (i) E_m values of the primary electron donor and (ii) steric availability of P_{870}^+ for the "low-potential" Mn^{2+}-bicarbonate complexes on the P^+ dark reduction rate in type II RCs has been studied in this work to obtain additional evidence of the redox nature of this effect. For this purpose, the objects of research in this work were the isolated RC from *Rb. sphaeroides* R-26 and the green filamentous bacterium *Chloroflexus aurantiacus*, as well as the "core" complexes B890 from the purple bacterium *Chromatium minutissimum*.

MATERIALS AND METHODS

Reaction centers from *Rb. sphaeroides* R-26 and *Cf. aurantiacus* were isolated by the standard method by treating chromatophores with LDAO detergent followed by purification on DEAE-cellulose [20, 21]. The pigment–protein ("core") complexes B890 from *Ch. minutissimum* were isolated according to the method published previously for three species of purple bacteria – *Rh. iodosum*, *Rh. robiginosum*, and *Thiorhodospira sibirica* – by incubation of the chromatophores in the presence of 2% dodecyl-β-D-maltoside followed by purification on a sucrose step gradient and DEAE-cellulose [19].

Optical absorption spectra of the preparations were measured in a Shimadzu UV-1601 PC spectrophotometer (Japan) in a 1-cm cuvette. For accumulation of RC in the long-lived oxidation state, the preparations were illuminated for 30 sec with red light ($\lambda > 600$ nm, 1900 μmol photon/($sec \cdot m^2$)). Difference light-minus-dark absorption spectra were obtained by subtracting the absorption spectra measured in different time intervals after switching off the actinic light from the absorption spectra measured before the illumination of the preparations. The kinetics of photoinduced changes in absorption (ΔA) associated with reversible photooxidation of the primary electron donor was measured in a 1-cm cuvette with an Agilent 8453 spectrophotometer (USA). All measurements were made at room temperature. Before the study the samples were diluted 10-fold in buffer with 50 mM Hepes (pH 8.2). For removal of CO_2/HCO_3^- from the buffer, the buffer was aerated with air that had passed through a 20-cm layer of Ascarite and a 50% NaOH solution [7].

RESULTS

The RC isolated from *Rb. sphaeroides* R-26 and *Cf. aurantiacus*, as well as the pigment–protein complexes B890 from *Ch. minutissimum*, were in full agreement with the previously published data as concerned their spectral characteristics [22, 23]. The absorption spectra in the near-infrared region, as is shown in Fig. 1a, are characterized by maxima at 865, 800, and 760 nm for the RC from *Rb. sphaeroides* R-26 and at 865, 813, and 756 nm and a 792-nm shoulder for the RC from *Cf. aurantiacus*. The absorption spectrum of the "core" complexes B890

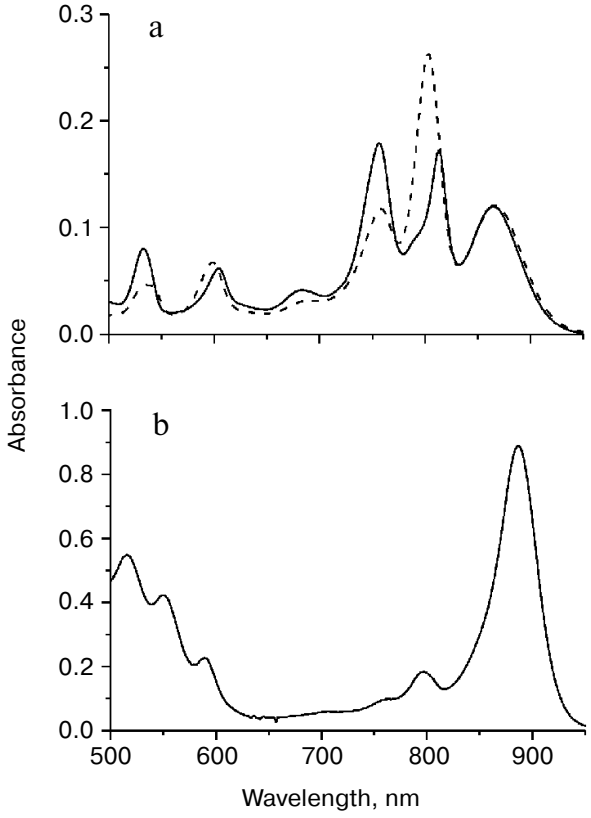

Fig. 1. Optical absorption spectra: a) RC from *Rb. sphaeroides* R-26 (dashed line) and *Cf. aurantiacus* (solid line). The spectra are normalized by the absorption band of the primary electron donor at 865 nm; b) pigment–protein complexes B890 from *Ch. minutissimum*. Measurements were made in medium containing 50 mM Hepes (pH 8.2).

from *Ch. minutissimum* (Fig. 1b) in the near-infrared region was characterized by the predominant absorption band of the antenna with maximum at 890 nm, as well as by maxima at 800 and 760 nm.

Illumination of RC initiates the process of charge separation accompanied by formation of a $P^+Q_A^-$ redox pair with recombination time ~100 msec under usual conditions for the purple bacteria [3, 24]. However, as has been shown previously, under long-term and intensive illumination some part of RC may pass into the so-called long-lived oxidation state characterized (in the absence of exogenous electron donors) by slower kinetics of dark reduction of P^+. In this case, the time of complete relaxation of the primary electron donor in purple bacteria is several minutes [18, 19, 25, 26] to several hours for *Cf. aurantiacus* [23, 27] depending on the duration and intensity of illumination.

In our experiments, intensive illumination (λ > 600 nm, 1600 μmol photons/(sec·m^2), 30 sec) of the RC from *Rb. sphaeroides* R-26 converted about 30% of photoreactive RC into the long-lived oxidation state. It is well seen on the kinetics of photooxidation and the dark reduction of P^+_{870} obtained during the measurement of photoinduced ΔA at 865 nm under illumination followed by the dark incubation of the preparations (Fig. 2, curve *1*). The change in intensity of the bleaching band at 865 nm on the difference light-minus-dark absorption spectrum (Fig. 3) was also used to measure the kinetics of the dark reduction of long-lived P^+_{870} (Fig. 4). These results show that the relaxation of long-lived P^+_{870} in the absence of exogenous additives is much slower, and the presence of oxidized RC (about 7-10%) is observed even after 20-min dark incubation of the samples. The time of 50% reduction of P^+_{870} in the absence of exogenous additives was about 445 sec. In the presence of 0.1 mM $K_4Fe(CN)_6$, a known artificial electron donor for P^+_{870} of purple bacteria, there was quick and complete reduction of the photooxidized RC, so that the photoinduced ΔA completely disappeared in 10 sec after switching off the light (Fig. 2, curve *3*). Acceleration of the dark relaxation of P^+_{870} was also observed upon the addition of 0.5 mM $MnCl_2$ together with 50 mM $NaHCO_3$ (Figs. 2 and 4). In this case, the time of 50% reduction of oxidized RC decreased more than 4-fold compared to the control and was approximately 100 sec. At the same time, the addition to the RC of 0.5 mM $MnCl_2$ or 50 mM $NaHCO_3$ separately had no substantial effect on the dark reduction rate of oxidized P^+_{870} (Fig. 4a). Acceleration of the dark reduction of P^+_{870} upon the addition of Mn^{2+} depended on bicarbonate concentration: in the presence of 0.5 mM Mn^{2+}, the effect was not observed when HCO_3^- concentration in the medium was 10 and 15 mM; it was clearly manifested beginning from 30 mM concentration of HCO_3^- and

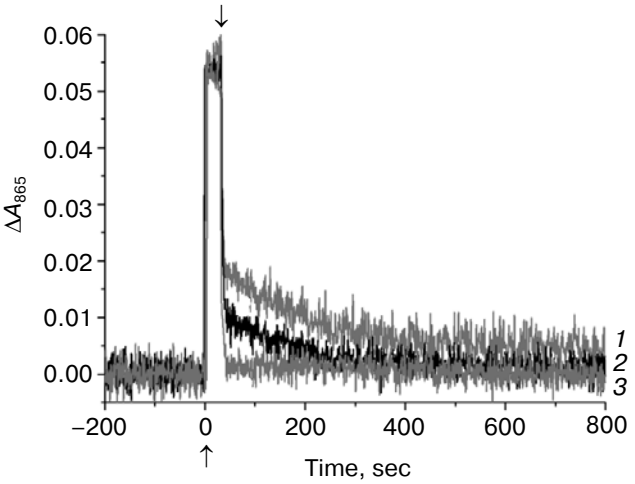

Fig. 2. Kinetics of photooxidation and dark reduction of P_{870} from *Rb. sphaeroides* R-26 measured by the photoinduced change in absorption at 865 nm during illumination followed by the dark incubation of preparations: *1*) in the absence of additives (control); *2*) in the presence of 0.5 mM $MnCl_2$ and 50 mM $NaHCO_3$; *3*) in the presence of 0.1 mM $K_4Fe(CN)_6$; ↑↓, switching actinic light on and off. Measurements were made in CO_2-depleted medium containing 50 mM Hepes (pH 8.2).

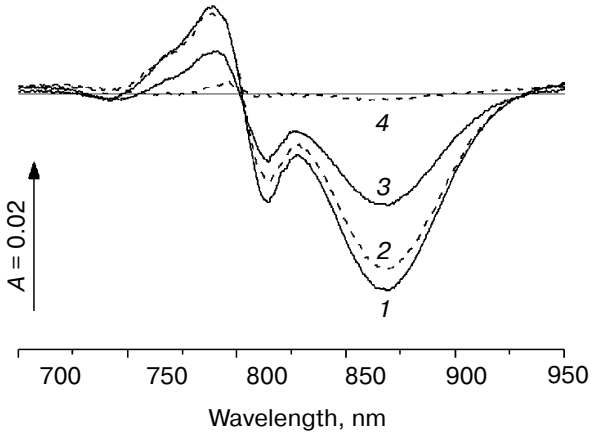

Fig. 3. Difference light-minus-dark absorption spectra of the RC from *Rb. sphaeroides* R-26 obtained 10 sec (*1*, *2*) and 420 sec (*3*, *4*) after switching the actinic light off in the absence of additives (*1*, *3* – solid lines) and in the presence of 0.5 mM $MnCl_2$ and 50 mM $NaHCO_3$ (*2*, *4* – dashed lines). Measurements were made in the CO_2-depleted medium containing 50 mM Hepes (pH 8.2).

reached saturation at 50-75 mM HCO_3^-, i.e. under the conditions required, as has been shown previously [14-16], for the formation of "low-potential" Mn^{2+}-bicarbonate complexes. The study of dependence of the effect on Mn^{2+} concentration showed that acceleration of the dark reduction of P_{870}^+ in the presence of 50 mM $NaHCO_3$ is observed already at 10 μM Mn^{2+}, and the effect reaches its maximum value at 0.3-0.5 mM $MnCl_2$. The Mg^{2+} or Ca^{2+} cations (used for comparison with Mn^{2+}) added together with bicarbonate (Fig. 4b) did not increase the rate of dark reduction of P_{870}^+. Similarly, the substitution of formate (HCO_2^-) or acetate for bicarbonate without changing the Mn^{2+} concentration also resulted in the loss of the effect demonstrated in the presence of 0.5 mM $MnCl_2$ added together with 50 mM $NaHCO_3$, which was evidence of the specificity of Mn^{2+}-bicarbonate complexes in electron donation to P_{870}^+.

The kinetics of photooxidation and dark reduction of P_{870}^+ of the second purple bacterium under study, *Ch. minutissimum*, the RC of which (in contrast to *Rb.*

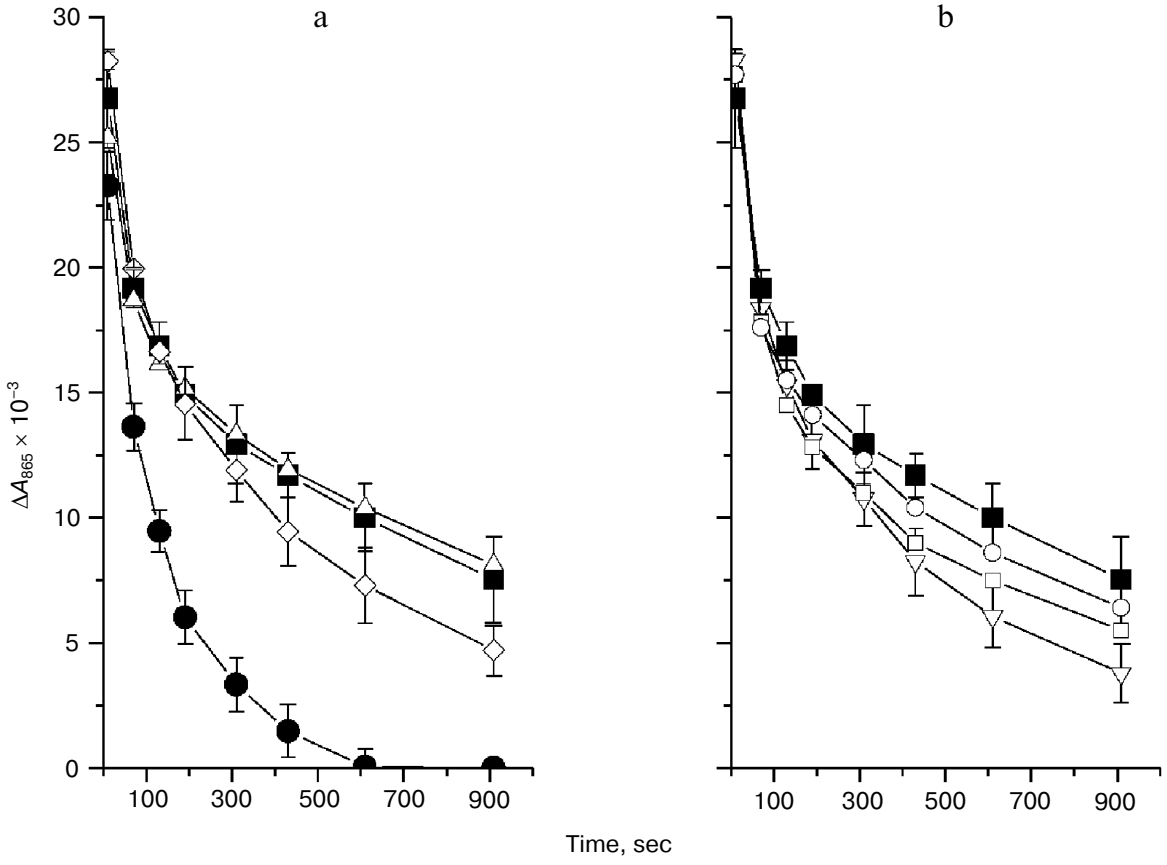

Fig. 4. Kinetics of dark reduction of P_{870}^+ from *Rb. sphaeroides* in the absence of additives (closed squares) and in the presence of: a) 0.5 mM $MnCl_2$ (open rhombs), 50 mM $NaHCO_3$ (open triangles), 0.5 mM $MnCl_2$ plus 50 mM $NaHCO_3$ (closed circles); b) 0.5 mM $MgCl_2$ plus 50 mM $NaHCO_3$ (open circles), 0.5 mM $CaCl_2$ plus 50 mM $NaHCO_3$ (open squares), 0.5 mM $MnCl_2$ plus 50 mM $NaHCO_2$ (open inverted triangles). The kinetics were obtained on the basis of ΔA values in the bleaching band of the special pair of BChl at 865 nm calculated from difference oxidized-minus-reduced absorption spectra. The measurements were made in the CO_2-depleted medium containing 50 mM Hepes (pH 8.2).

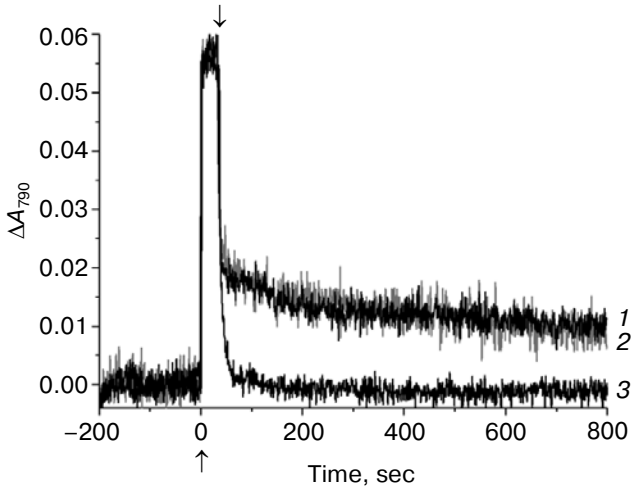

Fig. 5. Kinetics of photooxidation and dark reduction of the primary electron donor of *Ch. minutissimum*, P^+_{870}, measured by photoinduced ΔA at 790 nm under illumination followed by the dark incubation of preparations: *1*) in the absence of additives (control); *2*) in the presence of 0.5 mM $MnCl_2$ plus 50 mM $NaHCO_3$; *3*) in the presence of 0.1 mM $K_4Fe(CN)_6$; ↑↓, switching actinic light on and off. The measurements were made in the CO_2-depleted medium containing 50 mM Hepes (pH 8.2).

sphaeroides R-26) contains a cytochrome subunit [28], was obtained by measuring the photoinduced ΔA at 790 nm (Fig. 5), also associated with the reversible photooxidation of P_{870}. This wavelength was chosen because the major absorption band of the primary electron donor in the "core" complexes B890 is overlapped with and the intensive absorption band of the antenna having the maximum at 890 nm, which may complicate interpretation of the kinetics of photoinduced ΔA. Intensive illumination of the preparations ($\lambda > 600$ nm, 1600 μmol photons/(sec·m^2), 30 sec) converted into the long-lived oxidation state about 30% of the RC, as was shown for the RC from *Rb. sphaeroides* R-26, while the addition of 0.1 mM $K_4Fe(CN)_6$ caused a rapid and complete reduction of P^+_{870}. However, the presence of "low-potential" Mn^{2+}-bicarbonate complexes had no stimulating effect on the dark reduction rate of the long-lived oxidized RC from *Ch. minutissimum* (in contrast to the effects described for the RC from *Rb. sphaeroides* R-26). As one can see from Fig. 5, both in the absence of additives (control) and in the presence of 0.5 mM $MnCl_2$ added together with 50 mM $NaHCO_3$ in the solution, the kinetics of the dark reduction of P^+_{870} are indistinguishable.

The RC of the green filamentous bacterium *Cf. aurantiacus* is characterized by lower value of redox potential of the primary electron donor compared to the purple bacteria in spite of the fact that the RC of *Cf. aurantiacus* is also of type II in structural and functional organization. In accordance with the literature data, the redox potential value of the P^+_{865}/P_{865} pair is $E_{m\ 8.2}$ = 0.362 V [29] or $E_{m\ 8.0}$ = 0.420 V [30] for membrane complexes and $E_{m\ 8.0}$ = 0.386 V for isolated RC [20]. In addition, the dark reduction of P^+ in the RC from *Cf. aurantiacus* proceeds much more slowly than in *Rb. sphaeroides* R-26, and complete relaxation of P^+_{865} after intensive illumination may take several hours [23, 27]. Figure 6a shows that about 60% of RC passes into the long-lived state with the oxidized primary electron donor, P^+_{865}, with a lifetime of several tens of minutes, under illumination of the

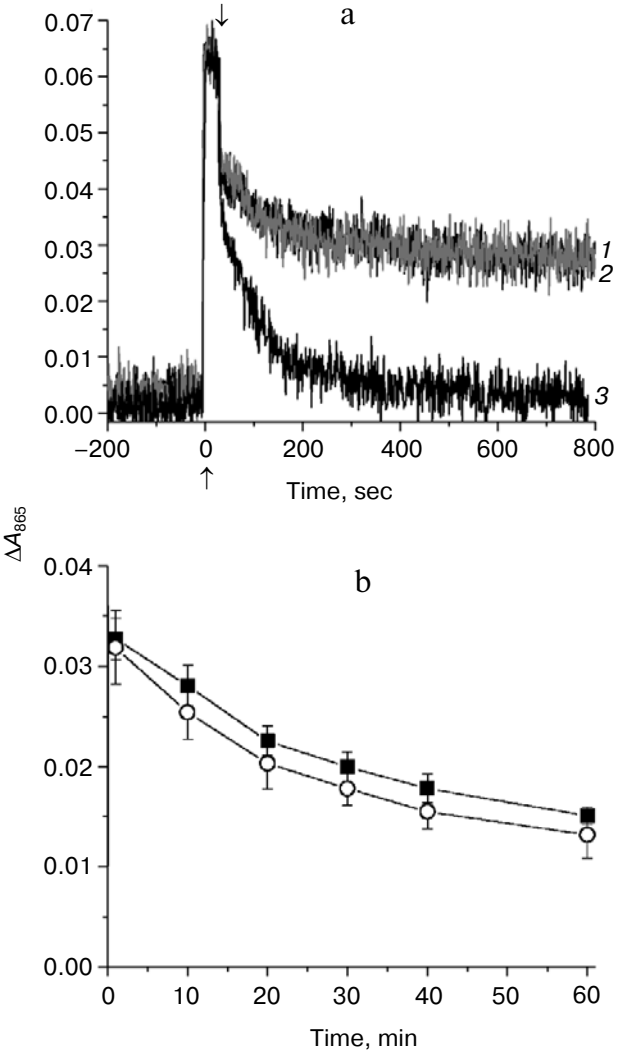

Fig. 6. a) Kinetics of photooxidation and dark reduction of the primary electron donor from *Cf. aurantiacus*, P^+_{865}, measured by photoinduced ΔA at 865 nm under illumination followed by the dark incubation of preparations: *1*) in the absence of additives (control); *2*) in the presence of 0.5 mM $MnCl_2$ plus 50 mM $NaHCO_3$; *3*) in the presence of 0.1 mM $K_4Fe(CN)_6$; ↑↓, switching actinic light on and off. b) The kinetics of dark reduction of P^+_{865} from *Cf. aurantiacus* obtained on the basis of ΔA values in the bleaching band of the special pair of BChl at 865 nm calculated by difference oxidized-minus-reduced absorption spectra in the absence of additives (closed squares) and in the presence of 0.5 mM $MnCl_2$ plus 50 mM $NaHCO_3$ (open circles). The measurements were made in CO_2-depleted medium containing 50 mM Hepes (pH 8.2).

preparations isolated from *Cf. aurantiacus*. The time of 50% dark reduction of P^+_{865} in the absence of exogenous additives (control) was about 50 min (Fig. 6). The presence of 0.1 mM $K_4Fe(CN)_6$ considerably accelerated the kinetics of P^+_{865} relaxation, but in this case complete reduction occurred during 300-400 sec, while the complete reduction of P^+_{870} upon the addition of 0.1 mM potassium ferrocyanide in similar experiments with RC from the purple bacteria was observed in a few seconds (Figs. 2 and 5). Note that the addition of Mn^{2+} together with bicarbonate under the conditions typical of the formation of "low-potential" Mn^{2+}-bicarbonate complexes had no effect on the kinetics of dark reduction of the long-lived oxidized RC from *Cf. aurantiacus* (Fig. 6).

DISCUSSION

The RC from anoxygenic purple bacteria, like the RC of PSII, are of type II or pheophytin–quinone type of RC [3, 31], in spite of the structural and functional differences on the donor side. It is probable that the type II RC from anoxygenic photosynthesizing bacteria is an evolutionary precursor of the RC of PSII, though the pathways of evolutionary transition from anoxygenic to oxygenic photosynthesis are still unclear. The previously formulated hypothesis of evolutionary origin of the inorganic core of PSII WOC [13] suggested that bacterial precursors (probably purple bacteria) of the first O_2-evolving organisms (cyanobacteria) could use the "low-potential" Mn^{2+}-bicarbonate complexes as an exogenous electron donor, and later on these complexes could form a primitive tetra-Mn cluster on the donor side of the RC (a precursor of the modern inorganic core of WOC). This assumption was based on research results demonstrating the necessity of HCO_3^- for maximum reactivation of the donor side of PSII [7-11] and on results of the study of electrochemical properties of Mn^{2+} under its liganding to HCO_3^- ions [14, 15] showing a considerable decrease in the redox potential of Mn^{2+} in these complexes compared to the aqua cation Mn^{2+} liganded by water molecules (for which E_m of the Mn^{2+}/Mn^{3+} pair is 1.18 V). At the same time, it was shown that positively charged Mn^{2+}-bicarbonate complexes with 1 : 1 stoichiometry ($[Mn(HCO_3)]^+$) and Mn^{2+} oxidation potential about 0.61 V dominate in solution at pH 8.2 and a HCO_3^- concentration of 10-50 mM, while electrically neutral complexes with 1 : 2 stoichiometry ($[Mn(HCO_3)_2]$) and Mn^{2+} oxidation potential of 0.52 V prevail at HCO_3^- concentrations above 50 mM [15, 16]. Thus, formation of 1 : 2 Mn^{2+}-bicarbonate complexes thermodynamically enables the redox interaction between Mn^{2+} and the oxidized primary electron donor of anoxygenic bacteria with redox potential of the P^+/P pair about 0.5 V [4-6].

Our previous work on the pigment–protein complexes B890 isolated from the three species of wild type purple bacteria (*Rh. iodosum*, *Rh. robiginosum*, and *Th. sibirica*) showed [19] that in the presence of high bicarbonate concentrations (>30 mM) Mn^{2+} was capable of redox interaction with P^+_{870}, which manifested itself in acceleration of the dark reduction of oxidized RC, as is observed in the presence of 0.1 mM $K_4Fe(CN)_6$, a known donor for RC from the purple bacteria. The research results presented in this paper allow the comparison of redox interaction between "low-potential" Mn^{2+}-bicarbonate complexes and the type II RCs from the three species of anoxygenic bacteria – *Rb. sphaeroides* R-26, *Ch. minutissimum*, and *Cf. aurantiacus* – different in accessibility of P and in the value of redox potential of the P^+/P pair. Like $K_4Fe(CN)_6$, the "low-potential" (1 : 2) Mn^{2+}-bicarbonate complexes are also capable of electron donation to RC of the purple bacterium *Rb. sphaeroides* R-26, which manifests itself in considerable acceleration of the dark reduction of P^+_{870} in their presence. However, it should be noted that complete relaxation of P^+_{870} in this case takes about 420 sec, while in the case with potassium ferrocyanide the photoinduced ΔA belonging to P^+_{870} disappear during 10 sec after switching off the actinic light. According to the literature, the redox potential of the primary electron donor of *Rb. sphaeroides* is about 0.50 V [4-6], while the Mn^{2+} oxidation potential in the 1 : 2 Mn^{2+}-bicarbonate complexes exceeds (though only slightly) this value and is 0.52 V [14, 15]. This is probably a factor that limits the effective electron donation from Mn^{2+} to P^+_{870}. This assumption is favored by the absence of acceleration of the dark reduction of P^+_{870} with predominance of 1 : 1 Mn^{2+}-bicarbonate complexes in the solution (at bicarbonate concentration below 30 mM), within which the Mn^{2+} oxidation potential is 0.61 V, as well as the absence of redox interaction between Mn^{2+} and P^+_{870} during the formation of Mn^{2+} complexes with formate, oxalate, and acetate ions, where the Mn^{2+} oxidation potential is 0.78, 0.77, and 0.69 V, respectively [14, 15]. In addition, the work with RC from *Rb. sphaeroides* R-26, where the redox potential of the primary electron donor was raised to 0.77 V using a series of point mutations near P_{870} [18], revealed effective electron donation from Mn^{2+} to P^+_{870} at a maximum bicarbonate concentration in the solution of 15 mM, i.e. under conditions of prevalence of 1 : 1 Mn^{2+}-bicarbonate complexes in the solution.

In contrast to *Rb. sphaeroides*, according to recent data the RC from *Ch. minutissimum* contains an additional cytochrome subunit forming a stable complex with RC and carrying six heme groups. With regard to the redox potential value, the hemes are conventionally divided into high-potential ($E_{m1} = 0.39$ V and $E_{m2} = 0.32$ V), medium-potential ($E_{m3} = 0.21$ V and $E_{m4} = 0.10$ V), and low-potential ($E_{m5} = 0.02$ V and $E_{m6} = -0.05$ V) [28]. The low-potential hemes provide for interaction with the terminal electron donors for RC (oxidizable substrates). The medium-potential hemes seem to be mediators in cyclic electron transport from photoreduced quinone acceptor to the high-potential hemes directly interacting with the primary

electron donor of RC. Our studies of the preparations from *Ch. minutissimum* show the complete absence of the acceleration of the dark reduction of P^+_{870} under the formation of "low-potential" 1 : 2 Mn^{2+}-bicarbonate complexes (0.5 mM $MnCl_2$ plus 50 mM $NaHCO_3$), in spite of the fact that the redox potential of the P^+_{870}/P_{870} pair of *Ch. minutissimum* is also 0.5 V. It may be supposed that the cytochrome tightly bound to RC on the donor side prevents the Mn^{2+}-bicarbonate complexes from directly interacting with P^+_{870}, like it was in the RC from *Rb. sphaeroides*. At the same time, even the high-potential heme of the cytochrome oxidized directly by the cation radical P^+_{870} cannot oxidize the Mn^{2+}-bicarbonate complexes, because its E_m is 0.39 V [28], i.e. not high enough for oxidation of Mn^{2+} in the 1 : 2 complex with bicarbonate. Similarly, the redox potential of the primary electron donor of the green filamentous bacterium *Cf. aurantiacus* ($E_m \approx 0.4$ V [20, 29, 30]), which is insufficiently high compared to the potential of Mn^{2+}-bicarbonate complexes, seems to make the redox interaction between Mn^{2+} and oxidized RC thermodynamically unfavorable.

Thus, the data show that the primary electron donor in the type II RCs from anoxygenic bacteria, characterized by the redox potential of 0.5 V, is capable of redox interaction with electrically neutral "low-potential" Mn^{2+}-bicarbonate complexes [$Mn(HCO_3)_2$] (which may be considered as an experimental confirmation of the previously suggested hypothesis of the evolutionary origin of WOC in the Archaean period [13]). At the same time, such redox interaction between Mn^{2+}-bicarbonate complexes and P^+ becomes practically impossible in the presence of RC-bound cytochrome (in *Ch. minutissimum*) or at an insufficiently high redox potential of the primary electron donor of RC (in *Cf. aurantiacus*).

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