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The influence of structural-dynamic organization of RC from purple bacterium *Rhodobacter sphaeroides* on picosecond stages of photoinduced reactions

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Abstract

Effects of the hydrogen bond network on the rate constants of energy migration (k_m), charge separation (k_e), electron transfer to Q_A (k_Q) and P^+I^- recombination in RC of *Rhodobacter sphaeroides* were analysed in control and modified RC preparations at different temperatures. Modification of RC were made by the addition of 40% v/v DMSO. The rate constants k_m, k_e, k_Q were evaluated from pump-and-probe measurements of the absorption difference kinetics at 665 nm corresponding to BPh_L^- formation and subsequent electron transfer to Q_A. For the investigation of P^+I^- recombination a primary quinone acceptor was pre-reduced in the dark by adding of 1 mg/ml of dithionite and 1 mM sodium ascorbate. Recombination kinetics were measured at 665 and 870 nm. The numerical analysis of the temperature dependence of k_e and k_Q was performed on the basis of the model proposed by Kakitani and Kakitani (T. Kakitani and H. Kakitani (1981), Biochim. Biophys. Acta, 635, 498–514). It was found that: (a) in control samples the molecular rate constants k_m, k_e and k_Q were about $(3.4\ ps)^{-1}$, $(4.5\ ps)^{-1}$ and $(200\ ps)^{-1}$, respectively; (b) under modification by DMSO these rates decrease up to $(5.3\ ps)^{-1}$, $(10.3\ ps)^{-1}$ and $(500\ ps)^{-1}$, respectively; (c) as the temperature drops from 300 K to 77 K the rate constant k_m decreases by 1.8 times in control and by 3.2 times in modified samples. In contrast to the observed k_m changes the increase in k_e and k_Q values by 2 and more times under cooling was found in control and modified RC; (d) in control preparations with Q_A acceptor pre-reduced in the dark the lowering of the temperature caused the increase in the time of P^+I^- recombination from 10 to 20 ns. After DMSO modification the kinetics of charge recombination in RC was biexponential at room temperature with $\tau = 10$ ns and $\tau_1 = 0.8$ ns, and at 77 K with $\tau = 20$ ns and $\tau_1 = 0.6$ ns, correspondingly. The results obtained reveal that in RC isolated from *Rb. sphaeroides* the processes of energy migration, charge separation, electron transfer to Q_A and ion-radical pair P^+I^- recombination depend on the state of hydrogen bonds of water–protein structure. Fast relaxation processes in RC structure including polarization of H-containing molecules in the

Abbreviations: BChl, bacteriochlorophyll; Bph, bacteriopheophytin; LHC, light harvesting complex; RC, reaction center; P, primary electron donor; I, primary electron acceptor BPh_L; Q_A, primary quinone; PSII, photosystem II; DMSO, dimethylsulphoxide; FWHM, full width at half maximum

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surrounding of electron carriers can accept electron energy dissipated at the initial steps of energy and electron transfer.

Keywords: Reaction center; Hydrogen bond; Cryoprotectant; Energy migration; Electron transfer; Temperature dependence

1. Introduction

The key steps of primary processes of photosynthesis include the efficient excitation energy migration within the LHC, excitation trapping by photoactive pigments in RC, charge separation and primary stabilization in the form of an ion-radical pair. Numerous investigations of these processes performed for the last years by means of femtosecond and picosecond time resolution technique revealed multicomponent character of energy migration and charge separation kinetics. According to data [1] the bleaching of the absorption band at 847 nm of *Rhodobacter sphaeroides* reaction centers occurs after 90 fs excitation followed by the final wavelength shift of the band to 860 nm in 250 fs. The finding is discussed in terms of the time behaviour of the vibronic wave packets at the ground state as a result of the interaction with phonons at 293 K. Three components in the kinetics of the light-induced absorption changes in membranes of *H. mobilis* have been recorded [2]. The fastest component (1–2 ps) was ascribed to the process of exciton equilibration at the thermally relaxed excited state. In Ref. [3] a femtosecond spectral evolution of the excited state of bacterial RC was studied. Initial spectral evolution is related to the fast relaxation processes at the excited state. According to Ref. [3] these relaxation processes involve: (i) vibrational relaxation of higher frequency modes, (ii) protein rearrangement coupled to the reorganization of small side dipolar groups, (iii) electronic relaxation. In other measurements [4] energy transfer in light-harvesting pigment-protein complexes of purple bacteria *Rb. sphaeroides* and *Rps. palustris* was studied by pump-probe absorption spectroscopy method with ~ 100 fs time resolution. It was concluded that fast transient processes observed in the experiments were mainly related to solvation processes at the room temperature.

Among factors that are responsible for the multicomponent kinetics of the photoinduced optical changes in RC an important role belongs to protein and bound water as possible active participants in the primary steps of photosynthesis. The role of water in photosynthetic primary processes has been analysed in the work [5]. Polarization of water molecules located in the close vicinity of the RC photoactive pigments gives rise to the new state in tens (hundreds) of femtoseconds after light flash and is accompanied by the heat dissipation of 0.03–0.12 eV of the electron excitation energy. Kinetics studies of absorbance changes of *Rb. sphaeroides* RC at 938 nm revealed [6] low frequency (~ 145 cm^{-1}) vibrations in the time domain of 0–2 ps after femtosecond excitation. Earlier it was shown [7] that such frequencies are characteristic for vibrations of water–protein component of RC structure.

Protein and bound water play their role in the primary steps of photosynthesis affecting dynamic properties of RC structure. Thus, as shown in [8] the isotope replacement of H by ^{2}H in *R. rubrum* chromatophores affects charge recombination in the primary ion-radical pair P^+I^-. After such substitution the increase in the life time of nanosecond luminescence decay component related to the stabilization of separated charges was recorded. It has been shown before that ^{2}H-bonds in water–protein bridges in RC are more rigid than H-bonds [9]. Proceeding from the literature data [9] and our experimental results [8] we suggested that the effect of H_2O/D_2O exchange is related to the changes of the state of hydrogen bonds. It may be due to the corresponding decrease in the mobility and increase in the rigidity of the RC protein structure. As a result, both the energy barrier between P^* and P^+I^- states and the life time of P^+I^- state decrease, whereas the quantum efficiency of recombinant luminescence increases. Similar effects were observed with solvents which affect protein hydrogen bonds state and thereby affects protein dynamics. In our previous experiments conducted with the use of the picosecond absorbance and fluorescence spectroscopy methods [10–15] it was shown that isotopic substitution of H by ^{2}H in RC leads to the slowing down of the electron transfer rate from I^- to the

quinone acceptor Q_A (k_Q) and to the increase in the efficiency of recombinant luminescence by ~2 times. Incorporation of multiatomic alcohols and dimethylsulphoxide (DMSO) in the reaction center structure gave similar results. It was found that the degree of the effect was correlated with proton-accepting capacity and the ability of the reagents to penetrate into the hydrophobic part of the RC structure.

Up to now there are no generally accepted explanation of the effects of DMSO on the RC structure. However it was demonstrated in Refs. [16,17] that the addition of DMSO to the H-bonded liquids causes the increase in the rigidity of their hydrogen bonds. In Refs. [10–15] we observed similar effects both of ^2H for H substitution and DMSO addition on the kinetics of the primary events in RC of purple bacteria. In accordance with the results of the model experiments [16,17] we suggested that the effect of DMSO is due to the alteration of the H-bond state in the RC structure. Of course, detailed investigation of the mechanism of DMSO action on the RC structure is needed.

In the literature, data were reported on the effect of H-bonds modification in the vicinity of electron transfer cofactors on the electron transfer in mutants, having different number of H-bonds between P and RC protein. It was shown that the addition of new H-bonds between P and protein after site-directed mutagenesis led to the increase in the midpoint redox potential of P [18]. According to the author's opinion this results in the decrease in the 'driving force' and the rate of the initial charge separation [18]. On the other hand from the paper [19], it follows that there is no direct correlation between the changes of P redox potential and the observed changes of the initial charge separation rate as a result of such mutagenesis. It was also shown that the shift of the pH values of RC suspension to the acid (pH 5.5–5.8) or alkaline regions (pH 10–10.5) led to the corresponding change of the rates of the primary electron-transport processes [11]. We believe the observed and somewhat controversial effects of H-bonds modification on the electron transfer processes should be rather related to their contribution to the dynamic properties of RC water–protein structure.

The study of the temperature dependence of the rate constant k_Q ($I \rightarrow Q_A$ step) in *Rb. sphaeroides* reaction centers modified by DMSO has shown that the lowering of the temperature brings about the k_Q increase both in the control and modified preparations [13]. Recently [14,15], we have studied the effect of hydrogen bonds modification on the charge separation rate constant k_e after addition of DMSO to *Rb. sphaeroides* RC. After such modification the k_e value drops by two times. This result correlates with the corresponding increase in the intensity of the nanosecond recombinant luminescence component [10–12]. The effect was explained by polarization of hydrogen bonds in the primary reactant surrounding. It was suggested that small H-containing molecular groups in the RC structure could play the immediate role in the electron transfer steps within the time domain of 10^{-9}–10^{-12} s. In the recently published paper [20] the effect of the substitution of H by ^2H and the addition of multiatomic alcohols and DMSO on the primary processes in RC of Photosystem II (PSII) in higher plants was studied. It was shown that isotope of $H/^2H$ exchange had little effect on the charge separation rate, whereas incorporation of multiatomic alcohols and DMSO into the RC structure slows down the rate of this reaction by 1.5–2 times. According to [20] such modifying effect on the rate of the electron transfer to Q_A and charge recombination processes in the primary ion-radical pair $P680^+Pheo^-$ in higher plants RC were less pronounced than in bacterial RC. The differences in the degree of the modifying effects on the kinetics of primary steps in reaction centers of PSII and purple bacteria the authors [20] explain by structural differences of the corresponding RC preparations.

In the present work we continue our investigations started in Refs. [10,11,13–15] on the modifying effects of the protein matrix on the processes of energy migration, ion-radical pair P^+I^- formation and recombination and electron transfer to the quinone acceptor Q_A in the reaction centers of purple bacteria *Rb. sphaeroides*.

2. Materials and methods

Reaction center preparations from *Rb. sphaeroides* were isolated as described in Ref. [21]. During experiments the samples were kept in 10^{-2} M sodium phosphate buffer, pH 7.0. Concentration of P870 was

about $0.5 \cdot 10^{-3}$ M. The reaction centers were modified by the addition of 40% v/v DMSO. When it was necessary to accelerate the cycle reactions to reduce P^+ N,N,N',N'-tetramethyl-*p*-phenylenediamine (10^{-4} M) and sodium ascorbate (10^{-3} M) were added before measurements to the particular samples. For the investigation of recombination processes in the RC the primary quinone acceptor Q_A was pre-reduced in the dark by adding of 1 mg/ml of dithionite and 1 mM sodium ascorbate. Pump-and-probe measurements of the absorption changes were performed with the absorption picosecond spectrometer as described in Ref. [22]. Samples were excited by 5 ps (FWHM) laser pulses with the wavelength of 532 nm (main frequency doubled output) or 590 nm (frequency doubled stimulated Raman scattering output) derived from Nd^{3+}-doped [$KGd(WO_4)$] laser system operating at the repetition frequency of 2 Hz. It's known that RC of purple bacteria contains two symmetrical branches of cofactors designated as M and L. The latter is an active branch in relation to the electron transport. Q_x absorption bands of Bph_M and Bph_L are shifted with respect to each other: their absorption maxima are localized at 530 and 545 nm, correspondingly [23]. In our experimental conditions excitation of RC can be realized at $\lambda_{ex} = 532$ nm (Q_x absorption band of BPh_M) or 590 nm (Q_x absorption band of RC's bacteriochlorophylls). It is clear that under excitation at $\lambda_{ex} = 590$ nm and registration of flash-induced absorption difference kinetics at 665 nm (BPh_L^- formation) charge separation process between P^* and Bph_L will be tested. If $\lambda_{ex} = 532$ nm is used for the excitation BPh_M will be excited. In this situation the following sequence of photosynthetic primary steps is triggered: (i) energy migration $Bph_M \xrightarrow{k_m} P870$; (ii) charge separation in the state $|P870^* Bph_L>$ and ion-radical pair $|P870^+ Bph_L^->$ formation with the rate constant k_e; (iii) the electron transfer reaction from BPh_L^- to Q_A with the rate constant k_Q. Evidently by comparing two types of kinetics of Bph_L^- formation generated either after P870 or BPh_M excitation the rate constant k_m of energy migration from Bph_M^* to P can be evaluated.

Flash-induced absorbance difference kinetics of Bph_L^- formation was tested at 665 nm. Kinetics of absorbance changes related to the photooxidation of photoactive pigment BChl RC was tested at 870 nm. Experimental data were fitted using the set of kinetic equations which describes the time evolution of the population of different states of RC. The theoretical curve for the absorption changes at the wavelength of a probing pulse was convoluted with the function describing the form of a probing pulse. The rate constants of transitions between different states of RC were evaluated using nonlinear least-squares fitting algorithm based on the Marquardt method.

3. Experimental results

Kinetics of photoinduced absorption changes at 665 nm after excitation at the Q_x absorption band of BChl RC ($\lambda_{ex} = 590$ nm) in the control and modified samples are presented in Fig. 1. It shows that the modification of the reaction centers by DMSO results in the slowing down of the charge separation process between P and I. To interpret this effect the following simplified kinetic scheme (Eq. (1)) is presented below. It suggests that the electronically excited state $|P^* I>_i$ initially generated is further transformed into the intermediate vibronically relaxed equilibrated state

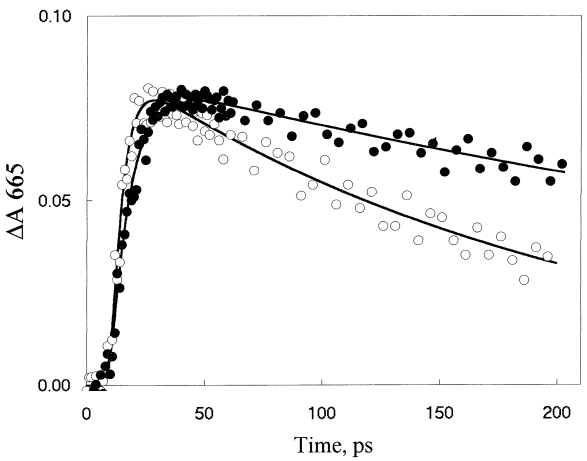

Fig. 1. Kinetics of the absorption changes in the Bph anion-radical band (665 nm) in the RC of *Rb. sphaeroides*. $\lambda_{ex} = 590$ nm (BChl Q_x absorption band); ○: control; ●: modified by DMSO. Calculated parameters, in control samples: $\tau_e = 1/k_e = 4.4 \pm 0.5$ ps; $\tau_Q = 1/k_Q = 205 \pm 10$ ps; in modified samples: $\tau_e = 1/k_e = 10.8 \pm 0.7$ ps; $\tau_Q = 1/k_Q = 510 \pm 20$ ps.

$|P^*I>_r$ which precedes the state with separated charges $|P^+I^->$:

$$|P^*I>_i \xrightarrow{k_r} |P^*I>_r \xrightarrow{k_e} |P^+I^-> \xrightarrow{k_Q} |P^+IQ_A^-> \quad (1)$$

Here k_r, k_e, k_Q are the rate constants of vibrational relaxation, charge separation processes and the electron transfer to the quinone acceptor, respectively. Approximation of the experimental kinetic curves according to the scheme (Eq. (1)) for the control samples (open circles, Fig. 1) and modified preparations (closed circles) gave the values $\tau_e = 1/k_e = 4.4 \pm 0.5$ ps and 10.8 ± 0.7 ps, respectively. From Fig. 1 it is also evident that the characteristic time $\tau_Q = 1/k_Q$ of the electron transfer from I^- to Q_A in control samples is equal to 205 ± 10 ps, whereas after modification of RC samples this time increased up to 510 ± 20 ps. This result suggests the similar role of relaxation processes in the $I \rightarrow Q_A$ electron transfer step.

Kinetics of photoinduced absorption changes in RC samples after excitation at the Q_x absorption band of Bph_M in the nonactive branch of electron carriers ($\lambda_{ex} = 532$ nm) are shown in Fig. 2. Under these conditions an additional step of the excitation energy migration from Bph_M^* to P with the rate constant k_m is introduced (see Section 2). The sequence of events in the case of light excitation at $\lambda_{ex} = 532$ nm can be described by the following scheme:

$$|Bph_M^*PI> \xrightarrow{k_m} |P^*I>_i \xrightarrow{k_r} |P^*I>_r \xrightarrow{k_e} |P^+I^-> \xrightarrow{k_Q} |P^+IQ_A^-> \quad (2)$$

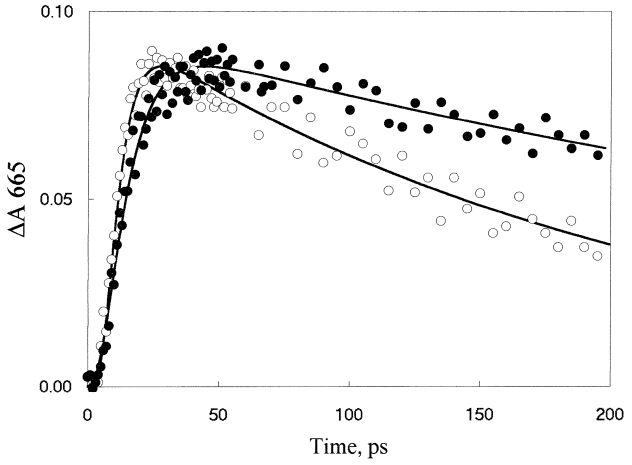

Fig. 2. Kinetics of the absorption changes in the Bph anion-radical band (665 nm) in the RC of *Rb. sphaeroides*. $\lambda_{ex} = 532$ nm (Bph_M Q_x absorption band), ○: control, ●: modified by DMSO. Calculated parameters, in control samples: $\tau_m = 1/k_m = 3.4 \pm 0.5$ ps; $\tau_e = 1/k_e = 4.7 \pm 0.5$ ps; $\tau_Q = 1/k_Q = 195 \pm 10$ ps; in modified samples $\tau_m = 1/k_m = 5.3 \pm 0.5$ ps; $\tau_e = 1/k_e = 9.8 \pm 0.7$ ps; $\tau_Q = 1/k_Q = 490 \pm 20$ ps.

For the control samples the following values of parameters indicated on scheme 2 were obtained: $\tau_m = 1/k_m = 3.4 \pm 0.5$ ps, $\tau_e = 1/k_e = 4.7 \pm 0.5$ ps and $\tau_Q = 1/k_Q = 195 \pm 10$ ps. For the preparations modified by DMSO these values are: $\tau_m = 5.3 \pm 0.5$ ps, $\tau_e = 9.8 \pm 0.7$ ps and $\tau_Q = 490 \pm 20$ ps. One can see that values τ_e and τ_Q for control and modified samples are similar to those obtained both with the direct excitation of P870 (Fig. 1) or BPh_M (Fig. 2). Decrease in k_m after addition of DMSO may be the evidence that the modification of intraprotein hydrogen bonds in RC leads also to the retardation of the excitation energy migration from Bph_M to the photoactive pigment preceding charge separation in PI pair.

To study in more details relaxation processes at the $I \rightarrow Q_A$ step quinone acceptor molecules Q_A were reduced in the dark before light excitation. It is well known that in RC where quinone acceptor Q_A is pre-reduced in the dark the direct electron transport from I^- is blocked ($k_Q = 0$), so that life-time values of the primary ion-radical pair $|P^+I^->$ state rise from 0.2 to ~ 10 ns [24]. Since relaxation processes contribute to the kinetics of a short-lived (~ 3 ps) $|P^*I>$ state we expected to demonstrate also their role in the deactivation of a long-lived state $|P^+I^->$ (~ 10 ns).

Absorption difference kinetics $\Delta A870$ and $\Delta A665$ in control and modified RC at 300 K with quinone acceptor Q_A pre-reduced in the dark are shown in Fig. 3. One can see that under these conditions the decay of P^+I^- proceeds mainly through the charge recombination process which is accompanied by the transition of $|P^+I^->$ to the ground state: $|P^+I^-> \rightarrow |PI>$.

It was found that in control preparations at the room temperature the kinetics of charge recombination is monoexponential with τ equal to 10 ns. The residual bleaching of $\Delta A870$ remained in the time

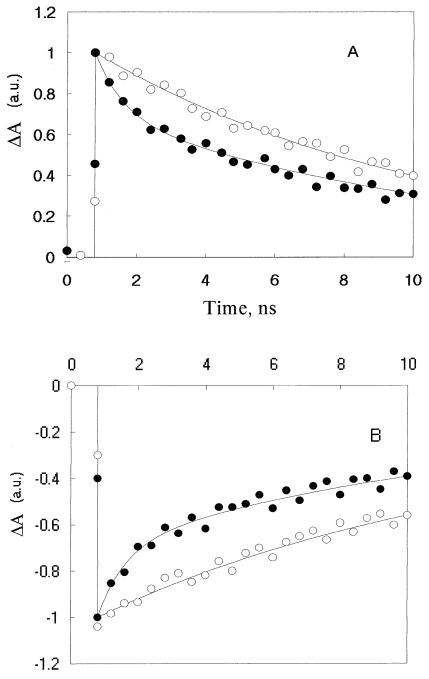

Fig. 3. Kinetics of laser-induced absorption changes in the closed RC's at 665 (A) and 870 nm (B) at room temperature. For pre-reduction of Q_A in the dark, 1 mg/ml of dithionite and 1 mM sodium ascorbate were added. ○: control samples; ●: modified by adding of 40% v/v DMSO.

domain >10 ns corresponds to the portion of $|P^+I^->$ pairs which underwent the transition to the triplet state.

However, in RC modified by DMSO, the kinetics of $\Delta A870$ and $\Delta A665$ absorption changes are no more monoexponential (Fig. 3, dark circles). Analytically the experimental kinetics of the photoinduced changes of $\Delta A870$ and $\Delta A665$ on Fig. 3 were fitted with expression (3) convoluted with the apparatus function of the spectrometer.

$$\Delta A = A\exp(-t/\tau) + A_1\exp(-t/\tau_1) + A_2 \qquad (3)$$

In this equation A, τ are the amplitude and duration of a slow recombination component, A_1, τ_1 are the amplitude and life time of the fast component, A_2 reflects the efficiency of the triplet state formation.

We found that in modified samples a new fast component with $\tau_1 = 0.8$ ns appears additionally to the component with $\tau = 10$ ns, registered for the control preparations. Magnetic field is known to alter the life time of ion-radical pair $^1|P^+I^->$ and the yield of the triplet state $^3|P^+I^->$ formation [24]. In our experiments magnetic field had no effect on τ_1 and A_1 values of the fast component (data not shown). The latter result testified that a new path for the decay of $|P^+I^->$ states is generated. This result can be explained if we assume that there exist two states of ion-radical pair—initial $|P^+I^->_i$ and relaxed $|P^+I^->_r$, differing from each other in their energy levels, life times and decay pathways. We suggest that after charge separation the initial state of ion-radical pair $|P^+I^->_i$ is generated. The latter relaxes with a rate constant k'_r to the vibronically equilibrated state $|P^+I^->_r$. As a result the energy level of the nonrelaxed state $|P^+I^->_i$ lowers and only after such relaxation the subsequent electron transfer to Q_A (k_Q) takes place. Thus, the relaxation processes, coupled to the movements of small H-containing groups of the protein surrounding, can precede both primary charge separation ($P \rightarrow I$) and electron transfer from I^- to Q_A.

Temperature dependencies of the rate constants of the energy migration k_m (Fig. 4), charge separation k_e (Fig. 5) and electron transfer to the quinone acceptor k_Q (Fig. 6) were studied in control and modified preparations. For this purpose the kinetics of the absorbance changes at 665 nm and 870 nm was measured at different temperatures and rate constants of energy migration, charge separation, electron transfer to Q_A and charge recombination were evalu-

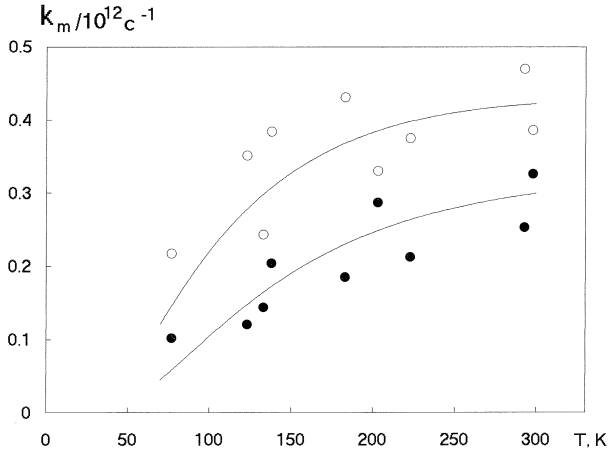

Fig. 4. The temperature dependence of the rate constant of energy migration from Bph_M to P in the RC of *Rb. sphaeroides*. ○: control, ●: modified by DMSO samples. Solid lines were fitted according to the formula (4). Calculated parameters, in control samples: $E_a = 0.015$ eV; in modified samples: $E_a = 0.02$ eV.

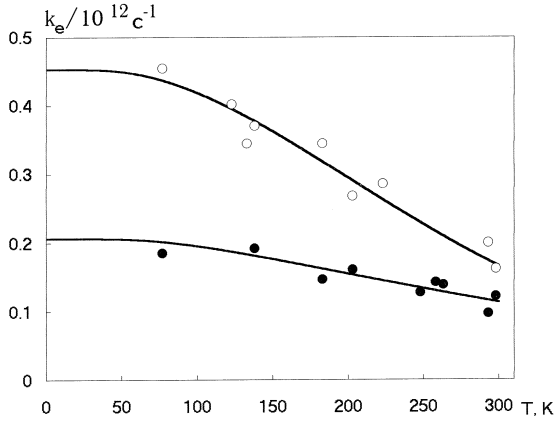

Fig. 5. The temperature dependence of the rate constant of charge separation in the RC of *Rb. sphaeroides*. ○: control, ●: modified by DMSO samples. Solid lines were fitted according to the formula (6). Calculated parameters, in control samples: $S = 0.9$; $\Delta G(295) = 3500$ cm^{-1}; $\Delta E_1 = 2400$ cm^{-1}; $\Delta E = 80$ cm^{-1}; $\nu_q = 1700$ cm^{-1}; $\nu_c = 150$ cm^{-1}; $V_{if} = 40$ cm^{-1}; in modified samples: $S = 0.9$; $\Delta G(295) = 4200$ cm^{-1}; $\Delta E_1 = 1500$ cm^{-1}; $\Delta E = 2540$ cm^{-1} $\nu_q = 1700$ cm^{-1}; $\nu_c = 200$ cm^{-1}; $V_{if} = 40$ cm^{-1}.

ated from these data. It was found that as the temperature drops from 300 to 77 K k_m decreases by ~ 1.8 times in control and by ~ 3.2 times in modified

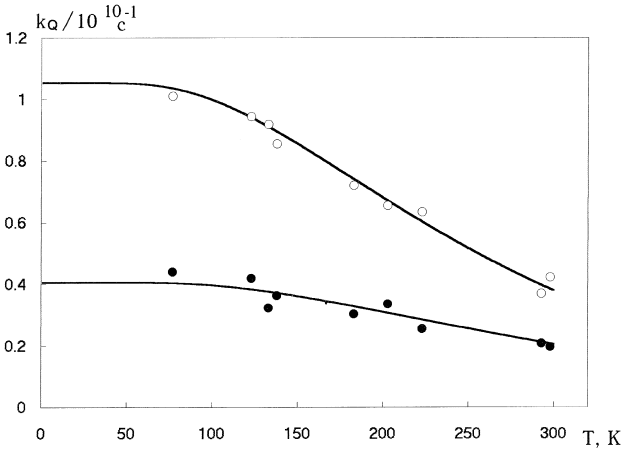

Fig. 6. The temperature dependence of the rate constant of electron transfer from I^- to Q in the RC of *Rb. sphaeroides*. ○: control, ●: modified by DMSO samples. Solid lines were fitted according to the formula (6). Calculated parameters, in control samples: $S = 0.6$, $\Delta G(295) = 3800$ cm^{-1}; $\Delta E_1 = 2400$ cm^{-1}; $\Delta E = 1720$ cm^{-1}; $\nu_q = 1400$ cm^{-1}; $\nu_c = 270$ cm^{-1}; $V_{if} = 12$ cm^{-1}; in modified samples: $S = 0.6$; $\Delta G(295) = 4300$ cm^{-1}; $\Delta E_1 = 2000$ cm^{-1}; $\Delta E = 2770$ cm^{-1}; $\nu_q = 1400$ cm^{-1}; $\nu_c = 320$ cm^{-1}; $V_{if} = 12$ cm^{-1}.

samples. In contrast to the observed k_m changes the abnormal increase by 2 and more times in k_e and k_Q values under cooling was found. For instance, in the control samples k_e increased from $\sim 0.22 \cdot 10^{12}$ to $0.45 \cdot 10^{12}$ s^{-1}, whereas in modified RC the k_e rise was from $\sim 10^{11}$ s^{-1} to $2 \cdot 10^{11}$ s^{-1}. Electron transfer rate constant k_Q changes under these conditions from $\sim 5 \cdot 10^9$ s^{-1} (at 300 K) to $1.2 \cdot 10^{10}$ s^{-1} (at 77 K) in the control samples and from $\sim 2.2 \cdot 10^9$ s^{-1} to $4.3 \cdot 10^9$ s^{-1} in the modified ones.

In control preparations with pre-reduced Q_A acceptor but without the addition of DMSO the lowering of the temperature caused the increase in the life time of $|P^+I^->_r$ relaxed state from 10 to 20 ns. The yield of the triplet state $^3|P^+I^-Q_A>$ formation rose from 0.12 to 0.60 (data not shown). In samples modified by DMSO with pre-reduced quinone acceptor the kinetics of absorbance changes at 870 and 665 nm were biexponential (see formula (3) and Fig. 3). The temperature dependences of A, A_1, A_2 and τ, τ_1 parameters describing the kinetics of photoinduced ΔA870 changes in the modified RC with pre-reduced quinone acceptor Q_A are summarized in Table 1.

It is seen that under cooling from 300 K to 77 K the duration of the fast component τ_1 in the kinetics of the absorbance changes at 870 nm decreases insignificantly (from 0.8 to 0.6 ns)—whereas the amplitude A_1 of the same component diminishes essentially. That means that the life time of the initial state $|P^+I^-Q_A>_i$ $\tau_1 = 1/(k_1 + k'_r)$ does not depend on temperature. As seen from Table 1 after cooling to

Table 1
Effects of temperature on the kinetic parameters in the relationship (3) describing the absorbance changes at 870 nm in RC when electron transfer to Q_A is blocked

T, K	A	τ, ns	A_1	τ_1, ns	A_2
77	0.20	20.0	< 0.05	0.6	0.76
105	0.25	20.0	0.05	0.5	0.70
140	0.36	15.2	0.15	0.66	0.50
170	0.40	14.2	0.18	0.59	0.42
200	0.40	12.0	0.22	0.6	0.38
295	0.55	11.2	0.30	0.8	0.15

A, τ: amplitude and life time of a slow component of charge recombination in the relaxed state of the primary ion-radical pair. A_1, τ_1: amplitude and life time of a fast component, A_2 reflects the efficiency of the triplet state formation.

the liquid nitrogen temperature, $k_1 \sim A_1$ decreases by 6 times. Obviously, since the sum $(k_1 + k'_r)$ remains almost constant, the value of k'_r should increase as the temperature drops from 300 K to 77 K. At the same time the amplitude of the slow component A decreases twice whereas its life time τ increases by ~ 2 times. It corresponds to the increase in the yield of the triplet state formation (A_2) from 0.15 to 0.76 under cooling. This result is in a good agreement with data of Schenck et al. [25] where the triplet state formation yield was found to increase from 0.10 at 300 K to almost 1.0 at 10 K.

4. Discussion

The temperature dependence of the rate constant k_m of the energy migration from Bph_M to P can be interpreted within the framework of Förster theory as caused by the decrease in the widths of acceptor absorption and donor luminescence bands under cooling. To approximate the temperature dependence of the rate constant k_m, Förster formula was used where the spectra of the acceptor absorption and donor emission are assumed to have Gaussian form. This dependence is true for temperatures higher than Debye temperature for the phonons of the environment [26]:

$$k_m = A\exp(-E_a/kT) \tag{4}$$

Here E_a is the activation energy of the $|Bph^*_M P> \rightarrow |Bph_M P^*>$ transition; A is a factor taking into account the resonance interaction of donor and acceptor molecules. The calculations show that for the control samples $E_a = 0.015$ eV and for the DMSO modified samples $E_a = 0.02$ eV. Thus, the modification of the reaction centers results in the decrease in the rate constant of the energy migration from Bph_M to P which is due to the increase in the activation energy of the transition. It is known, that E_a is related to the frequencies of vibrations in the nearest surrounding of chromophores. As a result of our earlier studies [8,10–15] we have concluded that DMSO modification affects the rigidity of hydrogen bonds so that the corresponding change in the vibration frequencies of the nearest BPh_M and P surrounding in RC may lead to the change of E_a.

There are at least two possibilities to explain the temperature dependence of the electron transfer rate constants k_e and k_Q in the RC of purple bacteria: (i) temperature affects directly the reaction rates as a consequence of the changes of the Frank–Condon factors (the overlapping of the wave functions of vibrational modes of donor and acceptor molecules), (ii) the observed temperature dependence is due to the changes in conformational dynamics of the RC protein. The first version was analysed in the works of Jortner [27] and Jortner and Bixon [28]. According to their theory in the temperature dependent region. the rate constant of the electron transfer is expressed by the 'Marcus' form:

$$k_{et} = \frac{K_{e1}}{(4\pi E_r k_B T)^{\frac{1}{2}}} \exp\left(-\frac{E_a}{k_B T}\right) \tag{5}$$

where k_{el} is a preexponential factor, describing the electron coupling between initial and final states, E_r is the reorganization energy of the transition, k_B is Boltzmann constant, E_a is the activation energy:

$$E_a = \frac{(\Delta E - E_r)^2}{4E_r} \tag{5a}$$

Here ΔE is the thermal effect of the reaction. In the case of the activationless electron transfer $\Delta E = E_r$ and therefore $E_a = 0$. Thus, the rate constant for the activationless electron transfer is proportional to $1/\sqrt{T}$ and increases as the temperature lowers. Such abnormal temperature dependence of the rate constant is due to the changes of the population of vibrational levels of reactant molecules with temperature and corresponding changes of the overlapping factor between initial and final vibrational states. At low temperatures only the zero-point vibrational levels will be populated where the overlapping factor is maximum. The transfer rate will decrease as the temperature is raised, since higher vibrational levels of the reactants will be thermally populated where the overlapping factor is smaller. Due to this mechanism of the activationless processes it is only possible to gain the maximum increase of 1.7-fold in the rate constant for the investigated temperature range (300–100 K). In the work [29] based on Jortner theory, different types of the temperature dependence of

electron transfer rate constants upon electron-vibrational coupling were analysed. It was shown that only in the case of strong electron-vibratory coupling ($S \sim 20$) and large reaction heat effects the rate constant of the electron transfer can increase by 2.5 times within the temperature range of 300–100 K. However the estimations [30] of the electron transfer rate from the excited dimer P^* to Bph_L based on π-electron approximation showed that for this reaction the dimensionless S values are small and correspond to the weak coupling. In another paper [31] proceeding from Marcus theory an attempt was made to estimate the effect of the dielectric relaxation in the vicinity of interacting molecules on the electron transfer rate. However, since in the case of activationless electron transfer the rate constant dependence on temperature is proportional to $1/\sqrt{T}$ it could have only 1.7-fold increase after cooling down to 100 K.

As follows from experimental data on Figs. 5 and 6, the actual increase in the rate constants exceeds values predicted by both Jortner and Marcus models. To interpret our results we suggested that the observed temperature dependence of the electron transfer rate constants is mainly related to the conformational and vibrational states of the protein matrix. The corresponding formalism to treat this suggestion was firstly derived in the model presented by Kakitani and Kakitani [32]. In accordance with this model the vibrational states of the reaction centers include high frequency skeleton vibrations of porphyrine molecules with the average frequency $<\nu_q>$ and low frequency vibrations (soft modes) of water–protein matrix with the average frequency $<\nu_c>$. According to Kakitani and Kakitani [32] high-frequency skeleton vibrations do not change significantly their population above zero-point level with temperature and do not contribute to the temperature dependence of the electron transfer rate constant. It is low frequency vibrations of the water–protein matrix (soft modes) which are mainly responsible for the observed temperature effects. These modes change their frequency during transition and thereby contribute to the change in entropy. Kakitani and Kakitani derived the expression for the electron transfer rate k_{et} identical to the corresponding expression suggested by Jortner [27], except that ΔE was replaced by the free energy change ΔG, which depends on the temperature. In the limit when for the high-frequency skeletal modes $h<\nu_q> >> kT$ the expression for k_{et} simplifies to the following [32]:

$$k_{et} = \frac{4\pi^2}{\hbar^2} \frac{V_{if}^2}{\langle \nu_q \rangle} \frac{e^{-S} S^P}{\Gamma(p+1)} \tag{6}$$

In this equation V_{if} is the electronic matrix element of the donor–acceptor interaction between the primary and final states; S is a parameter of the electron-vibration coupling strength for the high-frequency modes; Γ is gamma function; $p = \Delta G/h<\nu_q>$; ΔG is the change of the free energy in the course of the reaction, $\hbar$ is Plank constant; $\Delta G = \Delta E + \Delta E_1(1/2 + n_c)$; ΔE is the energy gap between the primary and final states; $n_c = (\exp(h\nu_c/kT - 1)^{-1}$ is a mean number of low-frequency vibrational quanta of protein–water matrix vibrations (soft mode) at the temperature T; $\Delta E_1 = Nh\Delta\nu_c$ is a total change of energy of fundamental vibrations of water–protein surrounding during transition, N is a total number of low-frequency vibration modes; $\Delta\nu_c$ is the change of protein vibration frequency during electron transfer. Kirmaier et al. also used formula (6) for fitting the temperature dependence of the rate constant of the electron transfer from I^- to Q_A in nonmodified RC [33].

Theoretical curves for the temperature dependence of the rate constants were calculated according to Eq. (6). Corresponding values of their parameters are presented in the legends to Figs. 5 and 6, where the value ΔG is given for $T = 295$ K. Temperature dependence of ΔG was also calculated and corresponding curves are presented on Fig. 7. Since $h<\nu_q> >> kT$ vibrations with frequency $<\nu_q>$ will not contribute to the temperature dependence of the electron transfer rate. On the other hand, since $h\nu_c \sim kT$ the 'freezing' of soft vibration modes of the water–protein surrounding with the decrease in temperature will take place so that $n_c \to 0$. The decrease in ΔG with the temperature drop (Fig. 7) leads to the increase in k_{et} up to the limit value where $\Delta G = \Delta E + 0.5\Delta E_1$. As a result of the RC modification by DMSO the value ν_c increases (legends to Figs. 5 and 6). That means that after DMSO treatment protein bonds become more rigid. The increase in the rigidity of protein bonds leads to the decrease in the number of low-frequency modes which change their frequency

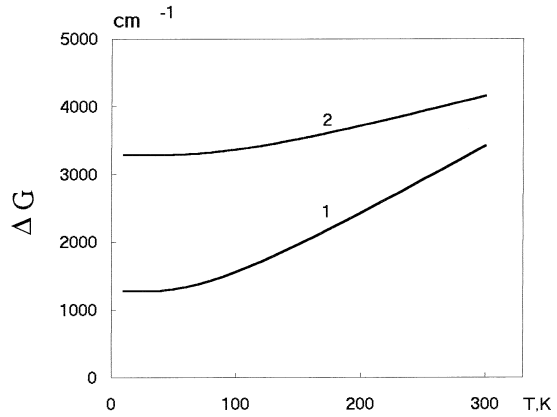

Fig. 7. Temperature dependence of free energy gap for control (1) and modified by DMSO (2) samples.

during transition. This results in the rise of the 'critical' temperature point where the temperature dependence curve of electron transfer rates (Figs. 5 and 6) reaches its steady state level. The same effect also leads to the decrease in the slope of the temperature dependence curve for the modified RC (black circles on Figs. 5 and 6). The modification of RC leads to the increase in the energy gap ΔE between the initial and final states. This gives rise to the ΔG increase (Fig. 7) and as a consequence to the drop of the electron transfer rate for the modified reaction centers (Figs. 5 and 6). It is interesting that at normal temperatures ($T \sim 300$ K) the difference between the rate constant values in normal and DMSO modified RC samples for the charge separation (Fig. 5) and electron transfer to Q_A (Fig. 6) is far less pronounced that at low temperatures ($T < 100$ K). In other words at higher temperatures the effect of DMSO treatment is somehow 'diminished'. This may be due to the lower frequency of protein vibrations in the nonmodified control samples. As a result, in control RC with temperature growth more rapid populating of higher vibrational states of water–protein matrix and thus more rapid growth of ΔG take place as compared to modified RC. Formal calculations show that the rate constants k_e and k_Q for modified and control RC would be theoretically equal at the temperature ~ 400 K. Of course the above calculation based on the model [32] are not final. These results rather illustrates the role the soft protein modes play in the electron transfer.

Based on our experimental results we suggested the following scheme on Fig. 8 to summarize the role of relaxation processes in the primary steps of photosynthesis. After light excitation the relaxation process in the excited state P^* with the rate constant k_r takes place. Charge separation occurs from the relaxed $|P^*IQ_A>_r$ state with the rate constant k_e. As a result of the electron transfer to I the initial nonrelaxed state of ion-radical pair $|P^+I^-Q_A>_i$ forms. This state can be deactivated via both the backward charge recombination process (k_1) with the formation of the $|P^*IQ_A>_r$ state or through the transition to the relaxed singlet state $^1|P^+I^-Q_A>_r$ with the rate constant k'_r. In control RC the transition (k'_r) from $|P^+I^-Q_A>_i$ to $^1|P^+I^-Q_A>_r$ is supposed to be very fast ($k'_r >> k_Q$) so the initial nonrelaxed state of the ion-radical pair is not detected on picosecond time scale. Modification of RC by means of isotope replacement or DMSO incorporation leads to the retardation of relaxation (solvation) processes ($k'_r < k_Q$). Therefore both the life time of $|P^+I^-Q_A>_i$ state and quantum yield of the recombination (k_1) into $|P^*IQ_A>_r$ state increase. It is because of this reason that in modified preparations with the quinone acceptor being pre-reduced in the dark the new recombinant luminescence component of ~ 0.8 ns appears (data not shown).

After relaxation of the initial state $|P^+I^-Q_A>_i$ with the rate constant k'_r the final $|P^+I^-Q_A>_r$ state

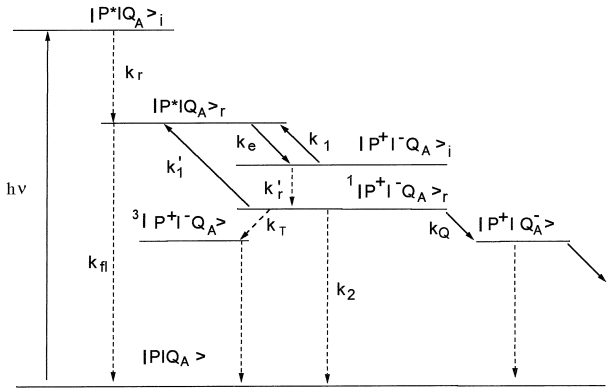

Fig. 8. Scheme of primary steps of photosynthesis in the RC of purple bacteria. k_r, k'_r: rate constants of relaxation of $|P*IQ_A>_i$ and $|P^+I^-Q_A>_i$ states; k_e: rate constant of charge separation, k_1, k'_1, k_2: rate constants of charge recombination; k_{fl}: fluorescence rate constant; k_T: rate constant of triplet state of ion-radical pair formation; k_Q: rate constant of electron transfer from I^- to Q_A.

forms. At least four paths for the deactivation of the latter are possible: (i) recombination (k'_1) into the relaxed excited state $|P^* IQ_A>_r$; (ii) recombination into the ground state $|PIQ_A>$ with the rate constant k_2; (iii) transition into the triplet state $^3|P^+I^-Q_A>$ with quantum yield A_2 and the rate constant k_T; (iv) electron transfer to the quinone acceptor Q_A with the rate constant k_Q, which is the most probable under normal conditions ($k_Q >> k'_1, k_2, k_T$).

In the RC with pre-reduced quinone acceptor the electron transfer to Q_A is blocked ($k_Q = 0$) so that processes of the triplet formation (k_T) and charge recombination prevail. At the room temperature, the recombination process is more effective ($k_2 + k'_1 > k_T$), but at the liquid nitrogen temperature the yield of the triplet formation is close to 80% [25].

5. Conclusion

High efficiency of the primary charge separation in photosynthetic RC depends on a number of factors including relaxation processes coupled to the polarization of the water–protein surrounding in the close vicinity of donor and acceptor molecules. Possible participants in such processes are water molecules, small polar protein groups, responsive to the effects of agents modifying protein structure. Small molecular groups in the RC structure containing hydrogen atoms can accept part of the dissipated electron energy both at the stages of energy migration to the photoactive pigment and in the course of the charge separation and electron transfer to Q_A.

Isotope replacement of H by ^2H and/or incorporation of specific solvents into the protein matrix influence the reaction rate of the direct and backward electron transfer. At the same time the effects of such modification on other properties of RC are unknown. Meanwhile it was shown recently [34] that after addition of 50% (v/v) of DMSO to the light-harvesting complex 2 in vitro isolated from PSII membrane fragments the samples exhibited a high degree of aggregation of pigment molecules. Our results on redox midpoint potential changes in RC after $^2H_2O \rightarrow H_2O$ substitution and DMSO addition will be published in the nearest future. Of course, high time resolution (femtosecond) is needed to measure directly the rate constants of relaxation (solvation) processes k_r and k'_r which reflect spectral evolution of $|P^* IQ_A>_i$ and $|P^+I^-Q_A>_i$ states as a result of their interaction with the water–protein surrounding. Finally, we plan to perform vibration frequencies measurements of $<\nu_c>$ and n_c by means of Raman spectroscopy in control and modified samples at different temperatures.

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