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Effects of Extraction of the H-Subunit from *Rhodobacter sphaeroides* Reaction Centers on Relaxation Processes Associated with Charge Separation

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Abstract—Effects of extraction of the H-subunit from *Rhodobacter sphaeroides* photosynthetic reaction centers (RC) on the characteristics of the photoinduced conformational transition associated with electron transfer between photoactive bacteriochlorophyll and primary quinone acceptor were studied. Extraction of the H-subunit (i.e., the subunit that is not directly bound to electron transfer cofactors) was found to have a significant effect on the dynamic properties of the protein–pigment complex of the RC, the effect being mediated by modification of parameters of the relaxation processes associated with charge separation.

Key words: photosynthetic reaction center, electron transport, conformational relaxation, *Rhodobacter sphaeroides*

The relaxation mechanisms of the initial stages of charge separation in photosynthetic reaction centers (RC) are closely associated with the conformational dynamics of these protein–pigment complexes. A network of hydrogen bonds in the RC is obviously involved in these processes [1]. In addition to the primary charge separation, conformational mobility also provides stabilization of photomobilized electrons in the quinone acceptors Q_A and Q_B [2]. Stabilization of an electron on the secondary quinone Q_B is accompanied by significant conformational changes that they are detected even by X-ray diffraction in a RC crystal frozen to 77 K upon exposure to light [3]. Both external and intramembrane factors affecting structural and dynamic properties of RC (e.g., temperature, humidity, composition of membrane environment) were found to modify the electron transfer activity of the complexes [1, 2, 4]. For example, the electron transfer processes mediated by quinone acceptors were found to depend on the phospholipid composition, lipid phase polarity and mobility, and type and concentration of detergents forming the amphiphilic environment of isolated RC complexes [5, 6].

Reaction centers of purple bacteria consist of three hydrophobic protein subunits (L, M, and H). Subunits L and M each have five transmembrane α-helical strands. All electron transport cofactors of the RC are bound to the strands. Subunit H contains only one transmembrane α-helical strand. This subunit is less hydrophobic than the L- or M-subunits, and the main part of the H-subunit covers the cytoplasmic side of the binding site of quinones Q_A and Q_B in the RC complex [7, 8]. The functional role of this subunit is still obscure. It was shown in [9] that removal of the H-subunit from *Rhodobacter sphaeroides* RC complexes causes a decrease in the rate of transfer of a photomobilized electron from Q_A to Q_B, a simultaneous decrease in the stability of the anion-semiquinones Q_A^- and Q_B^-, and an increase in the sensitivity of the complexes to detergent-induced denaturation. According to [10], extraction of the H-subunit has virtually no effect on the rate of electron transfer from the intermediate acceptor (bacteriopheophytin) to Q_A unless an atom of nonheme iron located in the RC between Q_A and Q_B is also extracted. Because transmembrane potential is generated in proteoliposomes containing only the *Rhodospirillum rubrum* L-subunit and M-subunit, it was concluded that the H-subunit is

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not directly associated with electrogenic activity [11]. According to [12], the *Rhodopseudomonas viridis* RC complexes with the H-subunit removed are characterized by electron transport activity similar to that in native RC. A shift of the midpoint redox potential of photoactive bacteriochlorophyll from 376 to 462 mV (pH 8.2), observed in these preparations upon H-subunit extraction was mainly due to the effects of the detergent coating of the hydrophobic RC complexes in solution.

Thus, further studies of the role of the H-subunit in the initial stages of energy conversion are of considerable interest. The goal of this work was to compare the temperature dependence of dark recombination between photooxidized bacteriochlorophyll dimer (P) and photoreduced Q_A in native RC complexes and RC complexes with extracted H-subunit (LM-complex). This reaction is characterized by an atypical temperature dependence (the recombination rate increases as temperature decreases) and nonexponential kinetics. The deviation of the recombination kinetics from a exponential curve is more pronounced at low temperatures [13-16]. In the preceding work [15], we interpreted such a deviation in terms of different conformational states of the RC, the characteristic times of the recombination between P^+ and Q_A^- in individual conformational states being temperature-independent. As temperature decreases, the “high-temperature” conformational states (states with longer recombination time) are converted in the dark to states with shorter recombination time. If an RC sample is frozen in the light, the “high-temperature” conformational state is trapped, and the characteristic time of recombination between P^+ and Q_A^- at 77 K in the sample is close to that at room temperature [13, 15]. It was suggested in [17] that nonexponential recombination kinetics may also be due to photoinduced conformational transitions in the RC. According to this concept, photoinduced charge separation triggers relaxation to a new conformational state. If the rate of the relaxation is significantly higher than the rate of charge recombination, the relaxation kinetics do not contribute to the recombination kinetics. Otherwise (i.e., if the rates of the processes are comparable), the photoinduced conformational transition dynamics is superimposed on the recombination dynamics. The term “momentary recombination rate constant” $k_r(t)$ was introduced to characterize the rate of the process at current time instances and given values of conformational coordinate $x(t)$ in RC exposed to short-term photoactivation. An automodel solution of the set of simultaneous differential equations describing the photoinduced conformational transition was obtained [18]. The values of $k_r(t)$ can be calculated from the experimental decay curves of the photoinduced absorption signal of dark recombination between P^+ and Q_A^- as:

$$k_r(t) = -\partial \ln A_Q^-(t) / \partial t,$$

where A_Q^- is the amplitude of the detected signal. Mathematical processing of recombination kinetics revealed that the characteristic time of conformational relaxation at room temperature is significantly shorter than the characteristic time of charge recombination in the initial conformational state (~80 msec). However, at temperatures below 200 K, the relaxation rate decreases, and kinetics of photoinduced conformational transition is superimposed on the recombination kinetics. The momentary recombination rate constant in this case decreases with time. However, it takes no more than ~120 msec to complete the relaxation process throughout the whole temperature range.

In the present work we used this approach to compare the relaxation characteristics of the photoinduced conformational transition in native RC complexes (LMH-complex) and RC complexes with extracted H-subunit (LM-complex) at cryogenic temperatures in samples frozen both in the light and in the dark.

MATERIALS AND METHODS

Reaction center preparations were isolated from chromatophores of *Rb. sphaeroides* using detergent lauryl dimethylamine oxide (LDAO) as described in [19]. A chaotropic agent, $LiClO_4$ (0.75 M), was used for extracting the H-subunit [9]. Isolated LMH- and LM-complexes were suspended in 10 mM sodium phosphate buffer (pH 8.0) containing 0.1% sodium cholate.

Photoinduced reactions were measured in the Q_Y absorption band (870 nm) using a computer-assisted single-beam spectrophotometer with pulse excitation (pulse energy, 9 mJ; half-maximum pulse duration, 10 µsec; spectral range, 400-600 nm). To analyze the process of recombination between P^+ and Q_A^-, RC were treated with *o*-phenanthroline (10^{-2} M), an inhibitor of electron transfer from Q_A to Q_B. In low-temperature measurements, 60-70 vol. % glycerol was added to the RC preparations. Samples were frozen during exposure to light with a spectral composition 400-600 nm and intensity of $10^2\ J \cdot m^{-2} \cdot sec^{-1}$.

RESULTS AND DISCUSSION

Kinetic curves of dark reduction of photooxidized P in LM- and LMH-complexes frozen to 100 K in the dark or during exposure to actinic light are shown in Fig. 1. The temperature dependences of the characteristic time of recombination between P^+ and Q_A^- (monoexponential approximation) in these preparations are shown in Fig.

2. The main difference between these curves is a longer recombination time in LM-complexes. Perhaps such an increase in the recombination time is due to a slight increase in the distance between P and Q_A in LM-complexes. Because recombination between P^+ and Q_A^- is a process of electron tunneling, its rate significantly depends on the donor–acceptor distance. It can be easily shown that in case of direct tunneling in a uniform medium through a 2.8-nm barrier (according to X-ray diffraction [20], this is the distance between P and Q_A in RC), a twofold increase in the recombination time is observed if the barrier width increases by less than 0.1 nm. It is even more probable that in the case of structural tunneling along specific pathways in the RC protein molecule [21], removal of a protein domain (the H-subunit) modifies the RC interior, thereby affecting the efficiency of electron tunneling.

The relaxation characteristics of the photoconformational transition were estimated in these samples at ~100 K using experimental data averaged over five experiments. In accordance with [17], in the LMH-complexes frozen in the dark the values of recombination rate constant k_r at specific moments of time (momentary recombination rate constant) were measured 1, 40, 80, and 120 msec after photoactivation with a light flash. These values of the momentary rate constant are given in the first row of the top part of the table. The values of k_r were determined accurate to ~7-8%. The second row of the top part of the table contains similar results obtained for LMH-complexes frozen during exposure to actinic light. The bottom part of the table contains corresponding data obtained for LM-complexes. The values of k_r normalized to the minimum value of k_r, as measured 120 msec after the light flash in LM-complexes frozen during exposure to actinic light ($k_r = 0.0037$), are given in parentheses. This method of normalization was chosen because the highest degree of relaxation of the charge-transfer state was observed in samples frozen during exposure to actinic light.

It follows from the table that the values of k_r in LM-complexes are generally less than in LMH-complexes. Therefore, the former preparations are subjected to faster and deeper initial relaxation ($t < 1$ msec) than the latter. Further stages in relaxation in LM-complexes are slower than in LMH-complexes. This can also be regarded as evidence that the initial stages of the conformational relaxation induced by charge separation in LM-complexes are faster and more complete than in LMH-complexes. This finding is thought to be due to either loose protein structure or low effective microviscosity of complexes with removed H-subunit. This effect is almost equally pronounced in samples frozen in the dark or in the light. The energy profiles of the conformational relaxation (recombination rate constant dependence on conformational coordinate) in LM- and LMH-complexes are shown in Fig. 3. It follows from Fig. 3 that the amplitude of the conformational changes induced by charge separation in samples frozen upon exposure to actinic light (i.e., samples with lower values of k_r) is significantly larger than the amplitude of the changes induced by a single flash in samples frozen in the dark. This may indicate

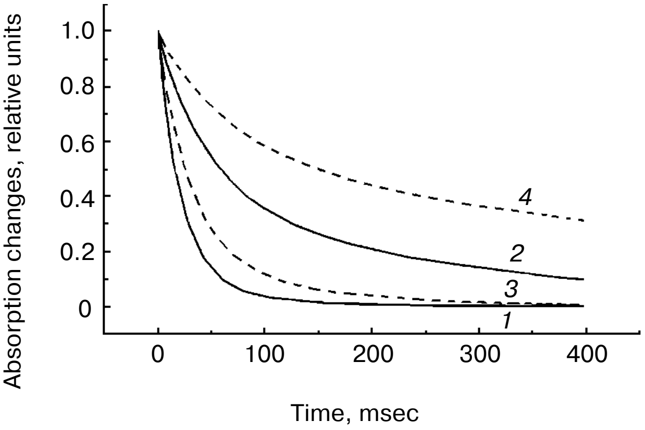

Fig. 1. Kinetic curves of dark reduction of photooxidized bacteriochlorophyll in LMH- (*1*, *2*) and LM-complexes (*3*, *4*) of *Rb. sphaeroides* RC frozen to 100 K in the dark (*1*, *3*) or during exposure to actinic light (*2*, *4*).

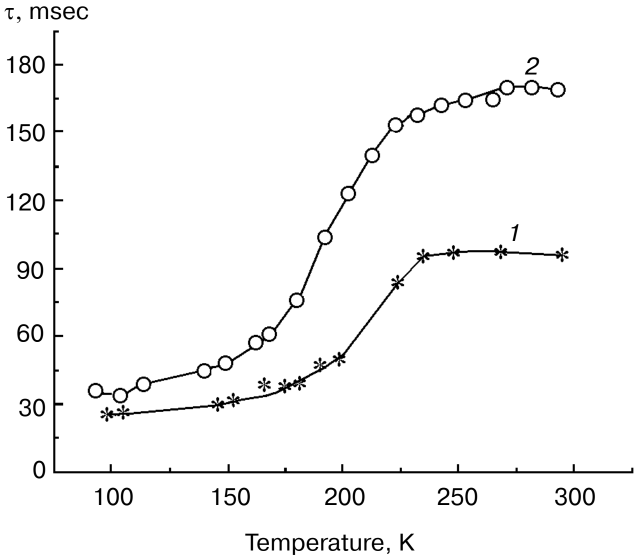

Fig. 2. Temperature dependences of the characteristic time (τ, monoexponential approximation) of dark recombination between photooxidized bacteriochlorophyll and photoreduced primary quinone acceptor in LMH- (*1*) and LM-complexes (*2*) of *Rb. sphaeroides*.

Values of momentary recombination rate constant k_r (msec^{-1}) measured at different time intervals after application of an actinic light flash at T ≈ 100 K in LMH- and LM-complexes from *Rb. sphaeroides* frozen in the dark or during exposure to actinic light

Parameter	Time interval after application of actinic light flash, msec			
	1	40	80	120
	LMH-complex			
Cooling				
in the dark	0.045 (12.2)	0.031 (8.4)	0.022 (5.9)	0.015 (4.1)
in the light	0.011 (3)	0.01 (2.7)	0.0096 (2.6)	0.0069 (1.9)
	LM-complex			
Cooling				
in the dark	0.028 (7.6)	0.023 (6.2)	0.016 (4.3)	0.013 (3.5)
in the light	0.0073 (2)	0.0056 (1.5)	0.0044 (1.2)	0.0037 (1)

Note: The values of the rate constant k_r normalized to the limiting value of k_r as measured 120 msec after the actinic light flash in LM-complexes frozen in the light are given in parentheses.

the existence of a local state corresponding to complete conformational relaxation following photoinduced charge separation. This local state is separated by an energy barrier from many other states corresponding to the relaxation time of about 100 msec (left part of Fig. 3). This state can be populated at relatively high temperatures during sample adaptation to light. It should be noted that the range of variation of momentary recombination rate in samples frozen during exposure to actinic light is reduced (table). Perhaps this is due to a decrease in the amplitude of the photoconformational changes in these samples caused by a specific energy surface relief in the vicinity of the state corresponding to complete conformational relaxation (right part of Fig. 3).

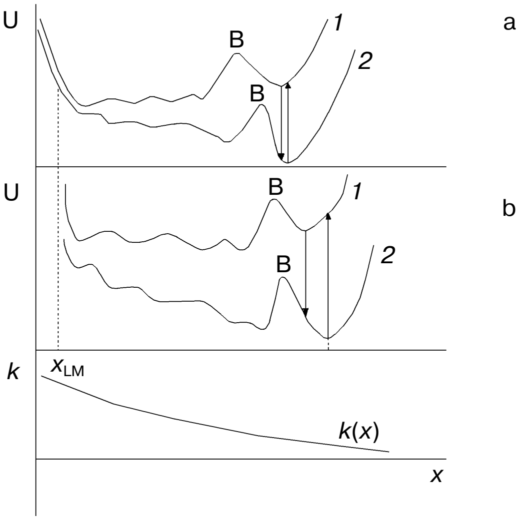

Fig. 3. Profiles of the system energy surfaces (U) in states PQ (*1*) and P^+Q^- (*2*) and dependence of the rate constant of dark recombination *k* on the conformational coordinate *x*: a) LMH-complex; b) looser LM-complex. The state fixed during freezing in the light is separated by barrier B from an array of conformational states with close energy levels. Arrows show the electron–conformational transitions accompanying charge separation and charge recombination in preparations frozen in the light (barrier B causes a noticeable decrease in the range of variation of the recombination rate in these samples). Removal of the H-subunit results in conformational barrier deformation along axis *x* and an x_{LM}, decreasing the limiting value of the recombination rate.

Thus, the results of this work show that extraction of the H-subunit from RC complexes affects both the rate and magnitude of the light-induced conformational transition of the pigment–protein complex of RC. It should be noted that the H-subunit is not directly bound to electron transfer cofactors, is the most polar of three RC subunits, and the boundary of the H-subunit with the L- and M-subunits contains dozens of water molecules [7]. Perhaps extraction of the H-subunit modifies the microenvironment of electron transfer cofactors and structure of hydrogen bond network in RC. The role of the network of RC hydrogen bonds in relaxation processes providing effective photoinduced charge separation has been experimentally demonstrated in the preceding work [1]. Other researchers also discussed possible contributions of the RC hydrogen bond network and polarizable side chains of RC proteins to charge separation and stabilization [22, 23]. Ultimately, the temperature- and light-induced effects on charge recombination between the photoreduced primary quinone acceptor and photooxidized bacteriochlorophyll observed in our experiments can be explained by modification of the structural and dynamic states of the RC.

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