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Effect of Oxygen on Temporary Stabilization of Photoreduced Quinone Acceptors in *Rhodobacter sphaeroides* Reaction Centers

P. P. Knox[1]*, M. Heinnickel[2], and A. B. Rubin[1]

[1]Faculty of Biology, Lomonosov Moscow State University, Moscow 119899, Russia; fax: (7-095) 939-1115; E-mail: knox@biophys.msu.ru

[2]Department of Biochemistry and Molecular Biology, Pennsylvania State University, S-310 Frear Building, University Park, PA 16802, USA; fax: 814-863-7405; E-mail: mlh300@psu.edu

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Abstract—The effect of molecular oxygen on the photochemical activity of the *Rhodobacter sphaeroides* reaction centers frozen to 160 K under actinic illumination was investigated by the ESR method. About 90% of initially photochemically active bacteriochlorophyll (P) were fixed at 160 K for a long time in aerobic samples in an inactive form. In anaerobic samples, not more than 65% were fixed in an inactive form under the same conditions. In aerobic preparations, a small portion of photochemically active bacteriochlorophyll (about 10%) that retains its photochemical activity at 160 K after freezing under illumination has dark reduction kinetics similar to that of samples at room temperature after several seconds of actinic illumination. In anaerobic samples frozen under illumination, the remaining photochemically active reaction centers (35%) have the same dark reduction kinetics as samples illuminated at 295 K for 1-2 min. The conclusion is that the irreversible stabilization of bacteriochlorophyll P in the oxidized inactive state formed in the reaction centers frozen under illumination is brought about by light-induced conformational changes fixed under low temperatures.

Key words: purple bacteria, photosynthetic reaction center, electron transport, conformational changes, quinones, oxygen

It is well known that catalytic activity of proteins is closely associated with their conformational mobility. It was also demonstrated that in light-transforming complexes of photosynthetic reaction centers (RC) of purple bacteria that conformational dynamics of proteins played an important role at all stages of the light-induced electron transport in the chain of the electron transfer cofactors integrated in RC structure: photoactive dimer of bacteriochlorophyll molecules (P), bacteriopheophytin, and primary (Q_A) and secondary (Q_B) quinone acceptors of the electron. The protein dynamics during the fast stage of the electron transfer from P to Q_A (τ ~ 150-200 psec at room temperature) is primarily responsible for high-efficiency relaxation processes that prevent dark recombination between photooxidized P and photoreduced primary quinone [1, 2]. As a result of these processes the time of dark reduction of P^+ from Q_A^- in RC from *Rhodobacter sphaeroides* at 295 K is ~0.1 sec. Further electron transfer from Q_A^- to Q_B (in *Rb. sphaeroides* the two acceptors are ubiquinone-10 molecules) and its stabilization in the secondary quinone is accompanied by more significant changes in RC structure [3]. According to X-ray diffraction analysis of *Rb. sphaeroides* RC crystals, fixation of light-induced conformational changes by rapid immersion of illuminated crystals into liquid nitrogen is accompanied by a 5 Å displacement and reorientation of the photoreduced secondary quinone in the RC protein structure [4]. The time of the dark reduction of P^+ from Q_B^- increases to 1 sec. However, this time increases upon increasing the intensity and duration of light exposure of RC. In addition to 1-sec kinetic component, increase in the intensity and duration of actinic light brings about appearance of a significantly slower (τ ~ tens of seconds and more) component of the dark reduction of P^+. The contribution of the slower component increases upon increasing the duration of the light exposure of RC. The increase in the efficiency of temporary stabilization of the electron on Q_B after prolonged light activation is due to "deepening" of conformational changes in RC structure caused by multiple repetitions of the elementary act of photoinduced electron transfer between P and Q_B during exposure of RC to continuous light [5, 6].

However, it is well known that reduced secondary quinone acceptor in RC of purple bacteria is able to interact with molecular oxygen [7]. Therefore, it is rather con-

* To whom correspondence should be addressed.

ceivable that deepening of temporary stabilization of the electron on Q_B after prolonged light activation can also be regarded as a result of interaction of the anion-semiquinone Q_B with Q_2. To test this suggestion, we studied earlier [8] the effect of molecular oxygen on dark stabilization of electron on Q_B in *Rb. sphaeroides* RC. It was found that removal of oxygen brought about a significant decrease in the time of electron transfer from Q_B^- to P^+ in the dark after photoactivation with continuous light (exposure time, from 10 sec to 5 min). This decrease was mainly due to a decrease in the characteristic time of the second (slow) component of dark reduction of P^+. The characteristic time of the component under anaerobic conditions does not exceed 10 sec. Although kinetics of dark electron transfer from Q_B^- to P^+ depended on the duration of the preceding photoactivation both in aerobic and in anaerobic samples, this dependence in aerobic preparations is more pronounced than in anaerobic ones. These findings can be regarded as evidence that duration of light exposure does induce conformational rearrangement of RC structure, which manifests itself as an increase in the time of dark recombination between Q_B^- and P^+ under these conditions.

Additional evidence in favor of this suggestion was obtained in experiments with cooling of RC from purple bacteria (*Rb. sphaeroides*, *Rhodospirillum rubrum*) to cryogenic temperatures under aerobic conditions in the light. Cooling under these conditions brings about fixation of two RC fractions, which differ significantly from one another in photochemical activity. The majority of RC complexes are frozen in the state with oxidized P (for an indefinitely long interval of time). In the fraction of RC that retain photochemical activity under these conditions, the kinetics of the dark reduction of P^+ after illumination at 160 K is close to the kinetics of P^+ reduction at room temperature in RC samples subjected to short-term (up to ~10 sec) photoactivation [9-13]. It is safe to suggest that the fraction of RC with bacteriochlorophyll P fixed in oxidized state in samples frozen in the light may correspond to the fraction of reaction centers in which increase in the duration of illumination at room temperature causes a substantial decrease in the characteristic time (to tens of seconds and more) of further dark electron transfer from Q_B^- to P^+. As noted above, such a decrease in the reaction rate at room temperature is thought to be associated with conformational changes in RC protein. Under these conditions, molecular oxygen facilitates electron hold-up in the Q_B locus [8].

However, experimental studies with cooling of illuminated anaerobic RC preparations had not yet been performed. Therefore, it was of considerable interest to study the effect of molecular oxygen on the relative contribution of the fraction with irreversibly photooxidized P and on the kinetics of pigment–acceptor interaction in reaction centers with retained photochemical activity in frozen in the light RC preparations of purple bacteria.

MATERIALS AND METHODS

Cells of the non-sulfur purple bacterium *Rhodobacter sphaeroides* were disrupted using an ultrasonic disintegrator. Chromatophore membranes were separated by centrifugation and incubated for 30 min at 4°C in 10 mM sodium phosphate buffer (pH 7.0) containing 0.5% solution of zwitterion detergent lauryl dimethylamine oxide (LDAO). The resulting preparation was centrifuged at 144,000*g* for 90 min at 4°C. The fraction of RC contained in the supernatant was isolated using chromatography on a column with hydroxyapatite as described in more detail in [14]. Detergent LDAO, which caused solubilization of hydrophobic membrane RC complexes, was substituted by dialysis with sodium cholate, an anion-active detergent. The resulting preparations of RC were more resistant to long-term storage than initial RC preparations. In experiments, we used preparations of RC solubilized in 10 mM sodium phosphate buffer containing 0.1% sodium cholate (pH 8.1). The concentration of RC in these preparations was ~100 μM. A fraction of photochemically active bacteriochlorophyll of RC during storage might be auto-oxidized in the dark. In this case, 10^{-5} M sodium ascorbate was added to experimental samples before measurements.

Photoinduced reactions in RC samples were investigated by the ESR method in the laboratory of Prof. J. Golbeck (Pennsylvania State University, USA) using a Bruker ECS-106 spectrometer of 3-cm microwave range (signal of cation-radical of P with *g*-factor 2.0026 was measured). Samples were activated with continuous light (wavelength range, 400-800 nm; intensity, ~1 kW/m^2) through a special window in the spectrometer cavity. To obtain samples frozen in a dark state, an ESR capillary (diameter, ~3 mm) with RC preparation was placed in the dark in the spectrometer cavity precooled with liquid nitrogen to 160 K. To measure signals of samples frozen in a light state, RC preparation was cooled for 2-3 min with liquid nitrogen to 77 K in a transparent Dewar flask under exposure to actinic light and then placed in the light in the spectrometer cavity precooled with liquid nitrogen to 160 K.

To provide more complete removal of oxygen from samples in certain experiments, RC preparations suspended in water-buffer medium containing no cryoprotectors were used. The same samples were used in low-temperature measurements. To remove oxygen from samples, suspension of RC was kept for several hours in a gas-tight chamber in a nitrogen atmosphere.

RESULTS AND DISCUSSION

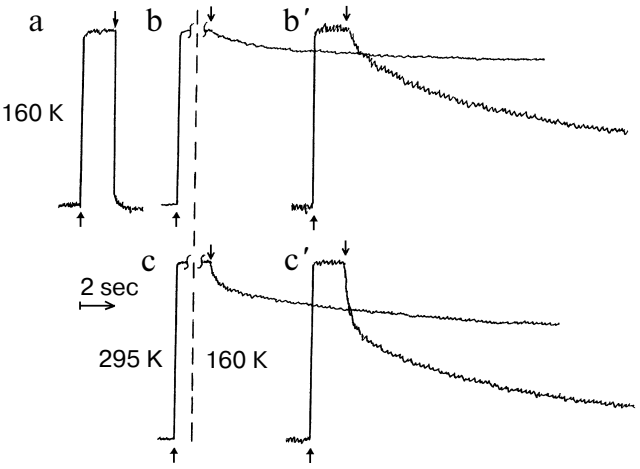
We found in our experiments that dark signal of bacteriochlorophyll cation-radical (P^+) was observed in nei-

ther aerobic nor anaerobic RC preparations of *Rb. sphaeroides* cooled in the dark to 160 K. Further exposure to actinic light was accompanied by generation of signal P^+, the rise-time and decay time of the signal (after actinic light on and off, respectively) was shorter (<1 sec) than the time resolution of the ESR spectrometer used in our experiments (figure, (a)). These results are consistent with the well-known facts that light activation of RC from *Rb. sphaeroides* cooled in the dark induces transfer of an electron to the primary quinone within the time interval ~100 psec. Further electron transfer to the secondary quinone under these conditions is inhibited. As a result, the electron from the primary quinone returns to P^+ with characteristic time 30-40 msec [2, 3, 11, 15].

Another pattern was observed in case of cooling of RC preparations in the light. When aerobic RC preparations had been cooled in the light to 160 K, switching actinic light off (at 160 K) brought about up to 90% RC complexes for an indefinitely long interval of time to a stabilized state with oxidized P (figure, (b)). The kinetics of the dark reduction of P^+ in the fraction of RC, which retained photochemical activity under these conditions, contained two exponential components with $t_{1/2}(1)$ ~ 1-2 sec (contribution, 20-30%) and $t_{1/2}(2)$ ~ 7-8 sec (contribution, 70-80%). Repeated photoactivation of these samples with continuous light 5-10 min after they had been kept in the dark at 160 K was characterized by the same kinetics. The kinetic pattern in this case was also independent of duration of photoactivation with continuous light and was close to the kinetic pattern recorded under aerobic conditions at room temperature after short-term (up to several seconds) photoactivation of RC preparations [8]. The kinetic pattern of the redox reactions of P in the photochemically active fraction of RC as measured after 5-min dark adaptation of the sample at 160 K is illustrated in the figure, (b′).

The fraction of photoactive pigment P fixed in an oxidized state in anaerobic RC preparations cooled under exposure to continuous light to 160 K was smaller (~65%) than in aerobic samples cooled under identical conditions to the same temperature. The kinetics of the dark reduction of P^+ in the fraction of RC (~35%) that retained photochemical activity of bacteriochlorophyll P under these conditions (figure, (c)) contained two exponential components with $t_{1/2}(1)$ < 1 sec (contribution, 30-40%) and $t_{1/2}(2)$ ~ 5-6 sec (contribution, 60-70%). This kinetics was close to the kinetic pattern recorded in anaerobic RC at room temperature after 1-2-min photoactivation [8]. Kinetics of redox transformations of P in the photochemically active fraction of RC as measured after dark adaptation of the sample for 5 min at 160 K is shown in the figure, (c′).

It follows from the results described above that there was no drastic difference between patterns of photoreactions of P in aerobic and anaerobic RC preparations of *Rb. sphaeroides* cooled in the light. Neither the contribution of the fraction of RC with irreversibly oxidized bacteriochlorophyll P nor the kinetics of the dark reduction of P^+ in the RC fraction with retained photochemical activity differed substantially from each other. It is safe to conclude from these findings that stabilization of a fraction of photochemically active bacteriochlorophyll P in the oxidized state during cooling of RC preparations to 160 K under exposure to continuous actinic light is due predominantly to the light-induced conformational changes in RC structure, which are fixed at low temperature. The role of molecular oxygen as a trap of the photomobilized electron in this phenomenon is thought to be significantly smaller than the role of the conformational changes. This conclusion is supported by the following data. As mentioned above, it was the effect of oxygen that was believed to be responsible for a substantial increase in the characteristic time of the second kinetic component of reduction of P^+ after prolonged photoactivation of RC at room temperature. It would be fairly natural to bring the RC fraction with the slow kinetics of dark reduction of P^+ at 295 K into predominant correlation with the RC fraction in samples cooled in the light, in which photochemically active bacteriochlorophyll is fixed in an oxidized state. However, the contribution of slower component of dark reduction of P^+ at room temperature did not exceed 50% even after 5-min-long light activation. On the other hand, it follows from the results obtained in this work that up to 90% of bacteriochlorophyll P was irre-



a) Kinetic curve of light-induced changes in the ESR signal of the bacteriochlorophyll P cation-radical in RC preparations of *Rb. sphaeroides* cooled in the dark to 160 K under aerobic and anaerobic conditions. b, c) Kinetic curve of dark reduction of P^+ in RC cooled in the light to 160 K under (b) aerobic and (c) anaerobic conditions; b′) kinetics of redox transformations of P in RC cooled in the light to 160 K under aerobic conditions as measured after 5-min dark adaptation (scale of the amplitude of the light-induced changes in the ESR signal is ten times larger than in the figure, (b)); c′) similar kinetics in anaerobic sample (scale of amplitude is three times larger than in the figure, (c)). Upward and downward arrows indicate moments of actinic light on and off, respectively

versibly fixed in the oxidized state during cooling of aerobic RC samples to 160 K in the light.

Thus, the new results obtained in this work support the viewpoint of the leading role of the light-induced conformational changes of the RC of purple bacteria in long-term temporary stabilization of photoreduced quinone acceptors in RC of purple bacteria. Effective stabilization of the electron in this site of the photosynthetic electron-transport chain is of considerable physiological importance for coupling of extremely fast reactions of primary charge separation in RC with much slower diffusion-controlled processes of further transfer of reduced equivalents in the photosynthetic membrane.

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