Biochemistry (Moscow), Vol. 69, No. 8, 2004, pp. 890-896. Translated from Biokhimiya, Vol. 69, No. 8, 2004, pp. 1096-1103.

Temporary Stabilization of Electron on Quinone Acceptor Side of Reaction Centers from the Bacterium *Rhodobacter sphaeroides* Wild Type and Mutant *SA(L223)* Depending on Duration of Light Activation

P. P. Knox[1]*, N. I. Zakharova[1], N. H. Seifullina[1], I. Yu. Churbanova[1], M. D. Mamedov[2], and A. Yu. Semenov[2]

[1]*Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, Moscow 119899, Russia; fax: (7-095) 939-1115; E-mail: knox@biophys.msu.ru.*
[2]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119992, Russia; fax: (7-095) 939-3188; E-mail: semenov@genebee.msu.su*

Received October 20, 2003
Revision received November 19, 2003

Abstract—The dark reduction of photooxidized bacteriochlorophyll (P^+) by photoreduced secondary quinone acceptor (Q_B^-) in isolated reaction centers (RC) from the bacterium *Rhodobacter sphaeroides* wild type and mutant strain *SA(L223)* depending on the duration of light activation of RC was studied. The kinetics of the dark reduction of P^+ decreased with increasing light duration, which is probably due to conformational changes occurring under prolonged light activation in RC from the wild type bacterium. In RC from bacteria of the mutant strain in which protonatable amino acid Ser L223 near Q_B is substituted by Ala, the dependence of reduction kinetics of P^+ on duration of light was not observed. Such dependence, however, became observable after addition of cryoprotectors, namely glycerol and dimethylsulfoxide, to the RC samples from the mutant strain. It was concluded that substitution of Ser L223 with Ala disturbs the native mechanism of electrostatic stabilization of the electron in the RC quinone acceptor site. At the same time, an additional modification of RC hydrogen bonds by glycerol and dimethylsulfoxide probably includes various possibilities for more effective time delay of the electron on Q_B.

Key words: purple bacteria, photosynthetic reaction center, quinone acceptors, electron transport

It is well known that quinone acceptors play an important physiological role in photosynthetic reaction centers (RC) of purple bacteria; they couple very fast light-induced initial reactions of charge separation and electron transfer along the cofactors incorporated in RC protein structure with much slower diffusion-controlled process of further transport of reduced equivalents to the photosynthetic membrane. The secondary quinone acceptor (Q_B) under physiological conditions of functioning of the electron-transport chain sequentially accepts two electrons from the photoactive dimer of bacteriochlorophyll molecule (P). After that, the doubly reduced quinone acceptor binds two protons transported from the cell cytoplasm along special proton-conducting structures of the RC. The resulting hydroquinone diffuses to the membrane, being replaced with an electrically neutral quinone from the membrane pool.

* To whom correspondence should be addressed.

The time of stabilization of a photomobilized electron in the primary quinone acceptor (Q_A) relative to its back transfer to photooxidized P in RC from *Rhodobacter sphaeroides* at 295 K is ~100 msec [1]. In the case of the anion-semiquinone Q_B, this time increases to several hundreds of milliseconds and seconds. Kinetics of P^+ reduction mediated by Q_B^- usually deviates from a monoexponential curve. Perhaps this is due to variation in the structural dynamic state of the pigment–protein RC complex in studied samples. Various variants of Q_B binding in the interior of RC complexes occurring prior to their photoactivation have been described in the literature [2-5]. It is conceivable that these variants are associated with different states of protonation of amino acid residues closest to Q_B [3]. Cooling of RC crystals in the dark and in the light gives rise to so-called distal and proximal conformations of Q_B, respectively (with respect to Q_A) [6]. Perhaps, the distal and proximal conformations described in [6] are extreme cases. In addition, in dark conditions Q_B is in

0006-2979/04/6908-0890 ©2004 MAIK "Nauka / Interperiodica"

dynamic equilibrium with the site of its binding. This factor also contributes to kinetics of return of photomobilized electron back to P^+ after its photoactivation [7, 8].

It is important to note that longevity of stabilization of photomobilized electron on Q_B under conditions of one-electron reduction substantially depends on the duration of RC photoactivation. Perhaps, this is due to an increase in the conformational changes in RC structure associated with multiple repetition of the elementary act of light-induced charge separation between P and Q_B and causing a decrease in the rate of the process of dark recombination between P^+ and Q_B^- [9, 10]. Effective electrostatic stabilization of photomobilized electron in the quinone acceptors Q_A and Q_B is due to proton displacement in the protein interior of the quinone acceptors. Not only the closest vicinity of the quinone cofactors is involved in these processes. It was found that modification of the charge state of the quinone acceptors could be accompanied by changes in the values of p*K* of protonatable amino acids at distances up to 15-17 Å [11-13].

Site-directed mutagenesis proved to be a very effective tool for studying processes of electron transfer in RC. For example, in the RC of *Rb. sphaeroides* this approach was effectively used to study possible pathways of proton transfer from cytoplasm to photoreduced Q_B [14]. These studies revealed that if mutation inhibited one pathway, alternative variants could be activated. It is fairly conceivable that these pathways interact with one another. This interaction can be mediated by various mechanisms, including a network of hydrogen bonds of water molecules localized in the RC structure [15, 16]. Ser L223 is a key terminal amino acid residue of RC protein capable of interacting with a cluster of carbonyl groups of some amino acids located near Q_B. The state of protonation of the amino acids significantly depends on the process of generation of the semiquinone form of Q_B [17]. In addition, theoretical calculations demonstrated that splitting of the hydrogen bond between Ser L223 and Asp L213 during restoration of Q_B and formation of hydrogen bond between serine and Q_B^- facilitated stabilization of anion semiquinone of Q_B [18]. It is of interest to study the influence of Ser L223 on kinetics of recombination between quinone acceptors and photooxidized P. In particular, it is interesting to study the dependence of the process on duration of photoactivation. The pigment–protein RC complex isolated from the photosynthetic bacterium *Rb. sphaeroides* wild type and mutant strain *SA(L223)*, in which Ser L223 capable of forming hydrogen bonds with Q_B^- through its hydroxyl group was replaced with alanine incapable of forming such bond.

MATERIALS AND METHODS

Cells of wild-type non-sulfur purple bacteria *Rhodobacter sphaeroides* were grown in liquid Ormerod culture medium [19] under anaerobic conditions in a luminostat at a temperature of about 30°C for 3 days. Ammonium malate or sodium malate was used as a source of carbon. The pH of the Ormerod medium was adjusted to 6.8 using 10% solution of NaOH and sterilized at 0.5 atm for 40-60 min. Yeast autolysate (0.5 ml of concentrated solution per 10 ml medium) was added to culture medium before inoculation.

Cells of the *Rb. sphaeroides* mutant strain *SA(L223)* were grown in liquid Sistrom culture medium [20]. When the culture medium was prepared, its pH was adjusted to 6.8-7.0. Culture medium was sterilized at 0.5 atm for 40-60 min. Solution of vitamins was prepared separately. It contained (per 20 ml of water): 20 mg of nicotinic acid, 10 mg of thiamin, 2 mg of *p*-aminobenzoic acid, and 0.2 mg of biotin. The following antibiotics were also added to the culture medium before inoculation: tetracycline (10 mg per 1 ml water) and kanamycin (100 mg per 1 ml water). For inoculation, bacterial colonies were washed off from a Petri dish and diluted in 100-150 ml of culture medium filled in a 250- to 300-ml flask. The bacteria were grown in the dark in a rotary flask shaker (110-120 rpm) at 30°C for 3-4 days.

To prepare chromatophores, cells of wild-type *Rb. sphaeroides* were separated from the culture medium by centrifugation (6000*g*, 20 min). The resulting pellet was suspended in 0.01 M sodium phosphate buffer (pH 7.2) and disrupted using a UZDN-1 ultrasonic disintegrator (frequency, 22 kHz; 3 min; 4°C). Undisrupted cells and large particles were separated by centrifugation (10,000*g*, 15 min). Fraction of chromatophore membranes was sedimented from the supernatant by centrifugation (144,000*g*, 90 min, 4°C).

A milder buffer solution was used to prepare chromatophores from cells of the mutant strain of *Rb. sphaeroides*: 0.02 M Tris, 0.02 M Hepes, 0.1 M KCl, and 0.15 M sucrose (pH 7). Further stages of the isolation procedure were as described above.

To isolate RC preparations from the wild-type photosynthetic membranes of *Rb. sphaeroides*, 0.5% solution of zwitterion detergent lauryl dimethylamine oxide (LDAO) was added to suspension of chromatophores (optical density at 850 nm in 1-cm cuvette, 40 units). After 30 min of incubation at 20°C, the suspension was centrifuged (144,000*g*, 60 min, 4°C). The supernatant was treated with 22% solution of ammonium sulfate. Floating precipitate containing RC was dissolved in 0.01 M sodium phosphate buffer (pH 7.2) containing 0.05% LDAO and dialyzed against the same buffer for 24 h at 4°C to remove residual ammonium sulfate. Final purification of this preparation was performed using chromatography on a column with hydroxyapatite. These procedures were described in more detail in [21].

To isolate RC preparations from the mutant strain of the photosynthetic bacterium *Rb. sphaeroides*, the supernatant obtained after centrifugation at 144,000*g* omitting

preliminary precipitation with ammonium sulfate was applied to a column with hydroxyapatite (concentration of LDAO was preliminarily adjusted to 1%). Conditions of chromatography were the same as described above in case of preparations of wild-type *Rb. sphaeroides*. The RC-enriched fraction eluted from the column was concentrated by precipitation with ammonium sulfate, dissolved in buffer solution containing 0.05% LDAO, and dialyzed for one day. Additional purification of the preparation was performed using chromatography on a column with hydroxyapatite. The preparation was chromatographed after the concentration of LDAO in the dialyzate was adjusted to 3%.

Photochemical activity of RC preparations was studied spectrophotometrically using a computer-assisted single-beam differential spectrophotometer described in [22]. Detected signal was digitized using a Datalab DL 912 transient recorder (UK) or recorded with a chart recorder. This spectrophotometer allows the photoreactions in samples to be activated with both pulse and continuous light. Intensity of continuous actinic light was up to 10^2 J·m^{-2}·sec^{-1}. Actinic and monitoring light beams were separated in time using a phosphoroscope. Pulse light photoactivation was performed using an ISSh-100 3M stroboscopic lamp (pulse duration, 10 μsec; spectral range, 400-600 nm; pulse energy, 9 mJ). Actinic and monitoring light beams were separated using complementary glass filters. Kinetics of light-induced absorption changes of bacteriochlorophyll P in RC samples was measured in the Q_y absorption band of the pigment (at 870 nm). The processes of one-electron interaction between photoactive bacteriochlorophyll and quinone acceptors were studied, because the RC preparations studied contained neither physiological electron donors for photooxidized P (cytochrome *c*) nor exogenous donors. Usually, four to seven flash-induced signals were averaged. The resulting kinetic curves of dark reduction of P^+ were decomposed into exponential components using the Marquardt method of nonlinear regression analysis [23].

RESULTS AND DISCUSSION

Kinetic curves of dark reduction of bacteriochlorophyll P photooxidized by single light pulses in isolated reaction centers from the bacteria *Rb. sphaeroides* wild type and mutant strain *SA(L223)* are shown in Fig. 1. These kinetic curves were decomposed into two exponential components, three exponential components, or two exponential components plus a constant background component. The constant background component corresponds to a very long time of electron return to oxidized bacteriochlorophyll from Q_B^- in a fraction of reaction centers of the sample. Taking into account experimental error in determination of time and amplitude parameters, biexponential approximation was found to provide the best fit of experimental curves. In the wild-type RC, characteristic time (τ) and relative amplitude (A) of kinetic components were: $\tau_1 = 353 \pm 30$ msec, $A_1 = 54 \pm 5\%$; $\tau_2 = 2.0 \pm 0.09$ sec, $A_2 = 46 \pm 5\%$. In the mutant strain RC they were: $\tau_1 = 163 \pm 20$ msec, $A_1 = 50 \pm 5\%$; $\tau_2 = 1.8 \pm 0.09$ sec, $A_2 = 50 \pm 3\%$. The characteristic time of the second component is typical of recombination between P^+ and Q_B^-. Perhaps lower value of τ of the first component is due to the fact that gross kinetics of dark reduction of P^+ contains a component attributed to the process of recombination between P^+ and Q_A^- ($\tau < 0.1$ sec) in the fraction of RC complexes in which Q_B is not bound to the binding site at the moment of photoactivation. It is also possible that the presence of the faster kinetic component represents structural dynamic variability of the RC state in the preparations. As a result, the time of electron stabilization in Q_B may differ in different RC complexes. It follows from these data that the time of stabilization in the anion-semiquinone Q_B in mutant RC is in general shorter than in the wild-type RC. This result seems to be fairly natural, because it was noted above that Ser L223 played the role of a proton donor in the process of stabilization of the anion-semiquinone Q_B generated during RC photoactivation. An increase in the rate of the process of dark reduction of P^+ caused by electron transfer from Q_B^- was earlier observed in studies of membrane preparations (chromatophores) of the *Rb. sphaeroides* mutant strain *SA(L223)* [24].

However, it interesting to note that substitution of Ser L223 by Ala also decreases the time of stabilization of the anion-semiquinone Q_A. The rate of this process was

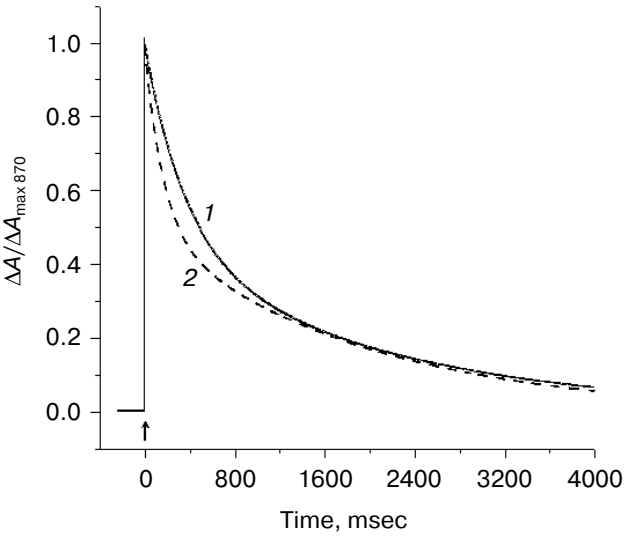

Fig. 1. Kinetic curves of absorption changes of photoactive bacteriochlorophyll (wavelength, 870 nm) induced by single short light pulses in RC from bacteria *Rb. sphaeroides* wild type (*1*) and mutant strain *SA(L223)* (*2*).

measured in the presence of 10^{-2} M *o*-phenanthroline. It is well known that *o*-phenanthroline is an inhibitor of direct electron transfer from Q_A to Q_B, which competitively displaces the secondary quinone from its binding site in the RC structure. The time of the process of dark recombination between P^+ and Q_A^- measured in the wild type and mutant RC was found to be 76 ± 6 and 59 ± 6 msec, respectively. The trend toward an increase in the rate of dark recombination between P^+ and Q_A^- in the *SA(L223)* mutant RC preparations relative to the wild-type RC preparations of *Rb. sphaeroides* was also reported in [25]. The decrease in the time of dark recombination between P^+ and Q_A^- in the mutant RC preparations can be explained by involvement of various protonatable amino acids in the process of stabilization of semiquinone. Quinone acceptors in bacterial RC are actually surrounded by charge clusters, in which the state of ionization of amino acid residues gives rise to a large energy of electrostatic interaction between them. About 20 amino acid residues are arranged in two such clusters near Q_A and Q_B [26]. These clusters share a number of amino acid residues. Therefore, electrostatic influence of the protein on electron and proton transport in such a cooperative system may include the effect of amino acid residues at distances larger than 15 Å. Redox state of one quinone acceptor is able to modify the value of p*K* of ionizable groups near the other quinone acceptor [11-13]. Electrostatic response induced by changes in the charge state of quinones is mediated by networks of hydrogen bonds formed by water molecules and polar groups in the RC structure between the Q_A and Q_B loci. Substitution of a protonatable amino acid residue in this system by non-protonatable amino acid residue modifies the state of the whole system [27].

In other words, protein relaxation under conditions of changing charge state of protein cofactors is thought to affect protein structure at rather large distances. This conclusion is confirmed by the results of our experimental study of the dependence of the efficiency of stabilization of photomobilized electron in the quinone acceptor site on duration of photoactivation of the RC. In this case, multiple repetitions of electron trapping on the acceptor is accompanied by proton displacement in its vicinity and induces conformational changes in RC structure, which cause an additional decrease in the rate of dark reduction of P^+. Perhaps electrostatic interaction of the photomobilized electron with deprotonated amino acid groups is the driving force of the conformational changes.

The results of the study of the dependence of the kinetics of dark reduction of P^+ on duration of photoactivation of RC with continuous light are given in Table 1. Kinetic curves of RC bacteriochlorophyll dimer absorption changes during photoactivation of preparations for 10 and 100 sec are shown in Fig. 2, curves (a) and (b), respectively. It follows from Fig. 2 that in RC from wild-type bacteria the rate of P^+ reduction progressively decreases upon increasing the photoactivation duration. This result is fully consistent with our earlier findings and can be adequately explained within the framework of the concept of conformational changes of RC progressively increasing during continuous photoactivation [9, 10]. On the other hand, kinetics of dark reduction of P^+ in mutant RC was independent on the photoactivation duration (see Table 1). This result may represent the fact that the proto-

Table 1. Dependence of characteristic times and relative amplitudes of kinetic components of dark reduction of photooxidized bacteriochlorophyll on duration of photoactivation of RC from bacteria *Rb. sphaeroides* wild type and mutant strain *SA(L223)*

Duration of photoactivation, sec	Wild-type		Mutant strain	
	amplitude of fast ($t_{1/2}$ < 1 sec) kinetic component of P^+ reduction	time ($t_{1/2}$, sec) and amplitude of slow kinetic component of P^+ reduction	amplitude of fast ($t_{1/2}$ < 1 sec) kinetic component of P^+ reduction	time ($t_{1/2}$, sec) and amplitude of slow kinetic component of P^+ reduction
10	40%	2.5 (18%) 45-50 (42%)	80%	3-5 (20%)
20	40%	3-5 (12%) 100 (48%)	80%	3-5 (20%)
60	30%	3 (10%) 140 (60%)	80%	3-5 (20%)
100	22%	3-5 (8%) 150 (70%)	80%	3-5 (20%)
200	17%	150 (83%)	—	—

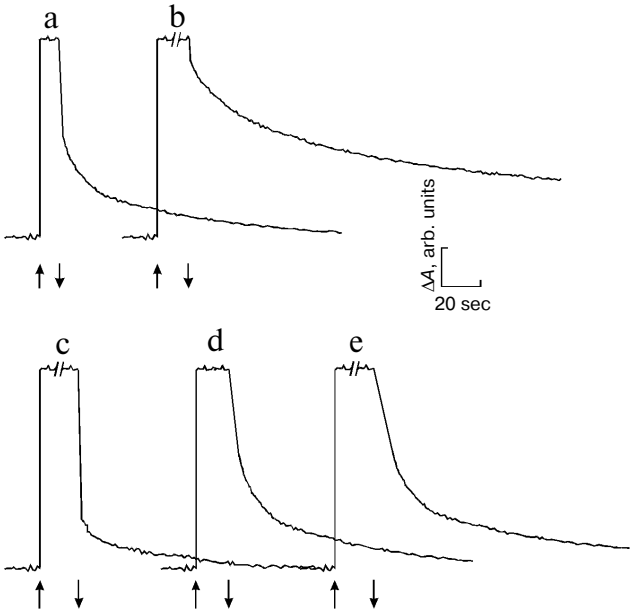

Fig. 2. Kinetic curves of light-induced absorption changes of photoactive bacteriochlorophyll (wavelength, 870 nm) in RC from bacteria *Rb. sphaeroides* wild type (a, b) and mutant strain *SA(L223)* (c-e) and dependence on duration of photoactivation. Duration of photoactivation: a) 10 sec; b) 100 sec; c) 15 sec; d) 15 sec; e) 120 sec; a-c) RC in sodium phosphate buffer without additions; d, e) samples with 87% glycerol (by volume). Upward and downward arrows indicate moments of actinic light on and off, respectively.

natable amino acid is absent in position L223 of the mutant RC, and another variant of electrostatic stabilization of anion-semiquinone Q_B is implemented. Perhaps the variants of proton displacement in the vicinity of Q_B less effective for stabilization of Q_B^- are also possible in this case (this is evidenced by more rapid reduction of P^+ in experiments with pulse photoactivation of RC). However, these processes do not lead to structural changes of RC developing with time, which can be regarded as evidence of lower degree of cooperation between local changes in the closest vicinity of Q_B and more extended structural changes of RC. It is safe to suggest that the structural changes of RC developing with time are substantially determined by the state of the network of intraprotein hydrogen bonds involving Ser L223 and directly connected to Q_B^-.

However, it is interesting to note that stabilization of an electron on Q_B, which progressively increases upon increasing the time of RC exposure to continuous actinic light, in RC preparations isolated from mutant bacteria can be observed after modification of RC hydrogen bonds with cryosolvents. It should be noted that the addition of cryosolvents itself causes an increase in the time of dark reduction of P^+ from Q_B^- (Fig. 2, curves c, d, e). Obviously, this fact is due to the cryosolvent-induced modification of the network of hydrogen bonds of the RC and can be regarded as additional evidence in favor of the suggestion that hydrogen bonds play an important role in the temporary stabilization of an electron on the quinone acceptors of RC. It follows from the X-ray diffraction data that there are deep invaginations on the cytoplasmic side of the RC complex. These invaginations contain ordered water molecules and allow solvent molecules to permeate deep into the protein structure. Two large cavities filled with water were revealed in the RC structure near Q_B and close to the cytoplasm side of the RC protein. The shortest distance between water molecules in these cavities is 3.9 Å [28]. The intraprotein water molecules together

Table 2. Effect of cryosolvents and partial dehydration on kinetics of dark reduction of photooxidized bacteriochlorophyll from Q_B^- in RC preparations from bacteria *Rb. sphaeroides* wild type and mutant strain *SA(L223)* and dependence on duration of photoactivation

Sample	15 sec of illumination		2 min of illumination		Ratio of $(t_{1/2}(1)\cdot A_1 + t_{1/2}(2)\cdot A_2)$ values at 2 min and 15 sec of illumination
	time ($t_{1/2}(1)$, sec) and amplitude (A_1, %) of the first kinetic component	time ($t_{1/2}(2)$, sec) and amplitude (A_2, %) of the second kinetic component	time ($t_{1/2}(1)$, sec) and amplitude (A_1, %) of the first kinetic component	time ($t_{1/2}(2)$, sec) and amplitude (A_2, %) of the second kinetic component	
Addition of glycerol:					
70%	4 (45%)	30 (55%)	3 (30%)	27 (70%)	1.1
87%	3 (40%)	35 (60%)	5 (45%)	55 (55%)	1.5
Addition of DMSO:					
30%	1.5 (50%)	10 (50%)	1.5 (50%)	12 (50%)	1.2
40%	1.5 (60%)	12 (40%)	1.5 (45%)	15 (55%)	1.56
RC film	1 (70%)	23 (30%)	1.5 (70%)	23 (30%)	1.05

with protonatable amino acids from the quinone vicinity produce a joint network of hydrogen bonds, which is obviously involved both in stabilization of the semiquinone form of Q_B and in proton transport from cytoplasm after two-electron reduction of the secondary quinone acceptor. The results of one series of experimental study of the effect of glycerol and dimethylsulfoxide (DMSO) on kinetics of dark recombination between P^+ and Q_B^- in RC preparations isolated from the mutant strain *SA(L223)* are given in Table 2. In samples containing these agents, the kinetics of P^+ reduction was adequately approximated by a sum of two exponential components. Perhaps, like in case of pulse photoactivation of RC considered above, approximation of kinetics of P^+ reduction by a sum of two exponential components corresponds to the presence of two RC fractions with different times of electron stabilization in Q_B. As noted in introduction, this is seemingly due to different variants of Q_B binding to RC complexes and dynamic equilibrium between Q_B and its binding site, which is attained in the dark before application of a light pulse. To provide quantitative evaluation of the dependence of the kinetics of dark reduction of P^+ on duration of photoactivation of RC, characteristic times of two kinetic components were multiplied by their relative amplitudes and resulting products were added to each other: $t_{1/2}(1)\cdot A_1 + t_{1/2}(2)\cdot A_2$. The right column in Table 2 gives the values of ratio of such integral parameters of kinetics of dark reduction of P^+ as decimated after 2-min-long and 15-sec-long exposure of RC to actinic light. It follows from these data that the addition of glycerol and DMSO to mutant RC results in the appearance of the dependence of the kinetics of dark reduction of P^+ on duration of photoactivation of RC. It should be noted in this context that DMSO, which has substantially larger values of dipole moment and proton-acceptor strength [29, 30], exerts a more pronounced effect in significantly lower concentrations than glycerol. It is obvious that changes in sample viscosity caused by addition of cryosolvents do not modify significantly the dependence of the time of electron stabilization in Q_B under conditions of prolonged photoactivation of RC, because the viscosity of glycerol is significantly greater than the viscosity of DMSO. This conclusion is also supported by the results of studies of air-dried (equilibrated to atmospheric humidity) films of mutant RC preparations. It follows from Table 2 that kinetics of dark reduction of P^+ in such partially dehydrated samples is independent of duration of photoactivation of the RC (like in aqueous solution of RC in the absence of cryosolvents).

Thus, the results of this study support the suggestion discussed above, that the network of hydrogen bonds of RC protein is involved in structural changes of the RC that develop upon increasing the photoactivation duration. Substitution of protonatable amino acid residue Ser L223 in the vicinity of Q_B by non-protonatable amino acid residue alanine breaks the native mechanism of development of these changes induced by electron donation to Q_B. However, additional modification of the network of hydrogen bonds of RC by cryosolvents is thought to include other possible structural pathways of semiquinone stabilization in the Q_B site.

We are grateful to Dr. S. I. Bibikov and Prof. D. Oesterhelt (Institute of Biochemistry, Max Planck Society, Germany) for the mutant strain of the bacterium.

This study was supported by the Russian Foundation for Basic Research (project Nos. 01-04-49268, 03-04-48983, 03-04-49219) and ISTC (project No. 2296).

REFERENCES

1. Woodbury, N. W., and Allen, J. P. (1995) in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., eds.) Kluwer Academic Publishers, The Netherlands, pp. 527-557.
2. Lancaster, C. R. D., and Michel, H. (1997) *Structure*, **5**, 1339-1359.
3. Grafton, A. K., and Wheeler, R. A. (1999) *J. Phys. Chem.*, **103**, 5380-5387.
4. Rabenstein, B., Ullmann, G. M., and Knapp, E.-W. (2000) *Biochemistry*, **39**, 10487-10496.
5. Zacharie, U., and Lancaster, C. R. D. (2001) *Biochim. Biophys. Acta*, **1505**, 280-290.
6. Stowell, M. H. B., McPhillips, T. M., Rees, D. C., Soltis, S. M., Abresch, E., and Feher, G. (1997) *Science*, **276**, 812-816.
7. Shinkarev, V. P., and Wraight, C. A. (1997) *Biophys. J.*, **72**, 2304-2319.
8. Ambrosone, L., Mallardi, A., Palazzo, G., and Venturoli, G. (2002) *Phys. Chem. Chem. Phys.*, **4**, 3071-3077.
9. Abgaryan, G. A., Goushcha, A. O., Kapustina, M. T., Kononenko, A. A., Knox, P. P., and Kharkyanen, V. N. (1997) *Biofizika*, **42**, 1088-1093.
10. Barabash, Yu. M., Berezetskaya, N. M., Christophorov, L. N., Goushcha, A. O., and Kharkyanen, V. N. (2002) *J. Chem. Phys.*, **116**, 4339-4352.
11. Okamura, M. Y., and Feher, G. (1992) *Annu. Rev. Biochem.*, **61**, 861-896.
12. Lancaster, C. R. D., Michel, H., Honig, B., and Gunner, M. R. (1996) *Biophys. J.*, **70**, 2469-2492.
13. Miksovska, J., Maroti, P., Tandori, J., Schiffer, M., Hanson, D. K., and Sebban, P. (1996) *Biochemistry*, **35**, 15411-15417.
14. Okamura, M. Y., Paddock, M. L., Graige, M. S., and Feher, G. (2000) *Biochim. Biophys. Acta*, **1458**, 148-163.
15. Abresch, E. C., Paddock, M. L., Stowell, M. H. B., McPhillips, T. M., Axelrod, H. L., Soltis, S. M., Rees, D. C., Okamura, M. Y., and Feher, G. (1998) *Photosynth. Res.*, **55**, 119-125.
16. Beroza, P., Fredkin, D. R., Okamura, M. Y., and Feher, G. (1992) in *The Photosynthetic Bacterial Reaction Center II* (Breton, J., and Vermeglio, A., eds.) Plenum Press, New York, pp. 363-374.
17. Mezzetti, A., Nabedryk, E., Breton, J., Okamura, M. Y., Paddock, M. L., Giacometti, G., and Leibl, W. (2002) *Biochim. Biophys. Acta*, **1553**, 320-330.

18. Alexov, E. G., and Gunner, M. R. (1999) *Biochemistry*, **38**, 8253-8270.
19. Pimenova, M. N., Grechushkina, N. N., Azova, L. G., Semenova, E. V., and Myl'nikova, S. I. (1983) *Practical Handbook of Microbiology* [in Russian], MGU Publishers, Moscow.
20. Sistrom, W. R. (1960) *J. Gen. Microbiol.*, **22**, 778-785.
21. Zakharova, N. I., and Churbanova, I. Yu. (2000) *Biochemistry (Moscow)*, **65**, 149-159.
22. Timofeev, K. N., and Shaitan, K. V. (1988) in *Modern Methods of Biophysical Research* (Rubin, A. B., ed.) [in Russian], Vysshaya Shkola, Moscow, pp. 226-282.
23. Himmelblau, D. (1973) *Analysis of Processes by Statistical Methods* [Russian translation], Mir, Moscow.
24. Bibikov, S. I., Bloch, D. A., Cherepanov, D. A., Oesterhelt, D., and Semenov, A. Yu. (1994) *FEBS Lett.*, **341**, 10-14.
25. Paddock, M. L., McPherson, P. H., Feher, G., and Okamura, M. Y. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 6803-6807.
26. Roy, C., Lancaster, D., Gunner, M. R., and Michel, H. (1995) in *Photosynthesis: From Light to Biosphere* (Mathis, P., ed.), Vol. 1, Kluwer Academic Publishers, The Netherlands, pp. 903-906.
27. Tandori, J., Baciou, L., Alexov, E., Maroti, P., Schiffer, M., Hanson, D. K., and Sebban, P. (2001) *J. Biol. Chem.*, **276**, 45513-45515.
28. Beroza, P., Fredkin, D. R., Okamura, M. Y., and Feher, G. (1992) in *The Photosynthetic Bacterial Reaction Center II* (Breton, J., and Vermeglio, A., eds.) Plenum Press, New York, pp. 363-374.
29. Gordon, A., and Ford, R. (1976) *Chemist's Textbook* [Russian translation], Mir, Moscow.
30. Kleeberg, H., Heinje, G., and Luck, W. A. P. (1986) *J. Phys. Chem.*, **90**, 4427-4430.