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Generation of Electric Potential Difference by Chromatophores from Photosynthetic Bacteria in the Presence of Trehalose under Continuous Illumination

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Abstract—Measurement of electrical potential difference (Δψ) in membrane vesicles (chromatophores) from the purple bacterium *Rhodobacter sphaeroides* associated with the surface of a nitrocellulose membrane filter (MF) impregnated with a phospholipid solution in decane or immersed into it in the presence of exogenous mediators and disaccharide trehalose demonstrated an increase in the amplitude and stabilization of the signal under continuous illumination. The mediators were the ascorbate/N,N,N'N'-tetramethyl-*p*-phenylenediamine pair and ubiquinone-0 (electron donor and acceptor, respectively). Although stabilization of photoelectric responses upon long-term continuous illumination was observed for both variants of chromatophore immobilization, only the samples immersed into the MF retained the functional activity of reaction centers (RCs) for a month when stored in the dark at room temperature, which might be due to the preservation of integrity of chromatophore proteins inside the MF pores. The stabilizing effect of the bioprotector trehalose could be related to its effect on both the RC proteins and the phospholipid bilayer membrane. The results obtained will expand current ideas on the use of semi-synthetic structures based on various intact photosynthetic systems capable of converting solar energy into its electrochemical form.

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Keywords: chromatophores, reaction center, cytochrome bc_1 complex, membrane filter, semiconductor, continuous illumination, electrical potential

INTRODUCTION

Over the past decades, multiple research efforts have been focused on the development of highly efficient artificial systems based on various dyes and semiconductors capable of converting light energy into electricity (see reviews [1, 2]). However, such systems have some disadvantages, such as high costs and the need for expensive components, technical maintenance, and disposal after a certain time. In view of this, one of the promising approaches to the development of photoelectrochemical energy converters is the use of natural photosynthetic systems (see reviews [3, 4]).

Vesicles formed by invagination of the intracytoplasmic membrane (chromatophores) of nonsulfur purple bacteria contain a photosynthetic apparatus ideally suited for such studies. Unlike the thylakoid membranes of cyanobacteria and chloroplasts, chromatophores of

Abbreviations: Δψ, transmembrane electric potential difference; Asc, ascorbate; bc_1, cytochrome bc_1 complex; cyt, cytochrome; ITO, indium-tin oxide semiconductor; MF, membrane filter; P_{870}, chlorophyll dimer; Q_A and Q_B, primary and secondary quinone acceptors, respectively; RC, reaction center; TMPD, N,N,N'N'-tetramethyl-*p*-phenylenediamine; UQ_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone.

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photosynthetic bacteria are the minimal structural and functional units capable of using the energy of absorbed photons for ATP synthesis [5]. Light-dependent cyclic electron transfer in chromatophores, for example, in *Rhodobacter sphaeroides*, occurs between the reaction center (RC) and the cytochrome bc_1 complex with the involvement of mobile membrane pool of ubiquinones (UQ_{10}) and endogenous soluble cytochrome (cyt) c_2.

After RC excitation by a light quantum, the photoactivated electron is transferred from the bacteriochlorophyll P_{870} dimer (P) to the primary (Q_A) and then to the secondary (Q_B) quinone acceptors. Reduction of the photooxidized P_{870} occurs as a result of electron transfer from the peripheral cyt c_2 (electron donor for RC). According to the modified concept of the Q cycle (see review [6]), oxidation of ubiquinol molecule formed in the RC and transferred to the ubiquinol-oxidizing center Q_o of the bc_1 complex is organized in such a way that one electron returns through the Rieske iron-sulfur protein (Fe_2S_2) and cytochromes c_1 and c_2 (high-potential chain) to the photooxidized P_{870} and the other electron is transferred through the two-heme cytochrome *b* (low-potential chain) to the ubiquinone reductase center Q_i for the reduction of ubiquinone molecule from the ubiquinone pool. Repeated initiation of the above reactions leads to the formation of "extra" ubihydroquinone in the Q_i site and increase in the number of protons exiting into chromatophore lumens per absorbed photon. It should be also noted that the light-induced vectorial transport of charges in the RCs and cyt bc_1 in the membrane vesicles is associated with the generation of the transmembrane electric potential difference ($\Delta\psi$) [7-11], which is a primary form of energy storage.

A relatively simple procedure of chromatophore preparation from bacterial cells, intact lipid environment of energy-converting enzymes in chromatophores, ability of RCs to convert light energy with a high quantum efficiency, and detailed knowledge of the mechanism of $\Delta\psi$ generation in some parts of the electron transport chain make chromatophores an attractive object for photoelectrochemical studies. When using chromatophores in hybrid systems *in vitro*, one of the important steps is their immobilization on supports (conductors, semiconductors, polymers, phospholipid membranes) and measurement of the electric potentials [12-14] and/or currents under continuous illumination [12, 15-19]. However, in the above-mentioned studies [12-14], $\Delta\psi$ generation under long-term continuous illumination has not been investigated. The current in the continuously illuminated chromatophores in [15-19] was measured mainly with the three-electrode system with the applied external electric field, so that the photocurrent amplitude depended on the field magnitude.

In the present work, we studied generation of electric potential difference in response to continuous illumination in *Rba. sphaeroides* chromatophores immobilized on the surface of a nitrocellulose membrane filter (MF) impregnated with a solution of phospholipids using direct electrometric method [13], which involves $\Delta\psi$ registration using a pair of silver chloride (Ag/AgCl) macroelectrodes immersed in an electrolyte solution and connected to an operational amplifier. The obtained results were compared with the electrometric data for chromatophores immersed into the MF clamped on both sides by semiconductor glass plates based on indium-tin oxide (ITO) [20]. The obtained data clearly demonstrated long-term retention of the stable photoelectric activity of chromatophores in these system in the presence of disaccharide trehalose.

MATERIALS AND METHODS

Cell cultivation and preparation of chromatophores. Wild-type *Rba. sphaeroides* cells were grown under anaerobic conditions at 30°C in the Ormerod medium [21] at a light intensity of 800 $W\cdot m^{-2}$. To obtain membrane vesicles with a high content of endogenous soluble cyt c_2, we used the extraction procedure described in [22] with minor modifications. Cells suspended in 25 mM Hepes-NaOH (pH 7.5) containing several crystals of DNase and protease inhibitors were disrupted with a French press (Aminco, USA). After removing the pellet, the supernatant was applied on a sucrose density gradient (5-35%, *wt/wt*) and centrifuged in a vertical VTi-50 rotor (acquired through the Moscow State University Development Program) for 2 h at 27,000 rpm. The lower chromatophore band was collected, dialyzed twice against 25 mM Hepes-NaOH (pH 7.5) for 2 h, and concentrated. Chromatophore suspension [~2 mg of bacteriochlorophyll (bChl) per ml] was frozen in liquid nitrogen and stored at −70°C.

The concentration of bChl in chromatophores was determined as described earlier [23]. Chromatophore absorption spectra were recorded with a Hitachi 3400 spectrophotometer.

Measurement of the electric potential difference in chromatophores immobilized on the MF surface. $\Delta\psi$ was measured using direct electrometric method developed in our laboratory [13]. MF (Millipore, USA) with a pore size of 0.22 µm and thickness of 150 µm was used as a support for chromatophore immobilization. Circular MF (diameter, 2.0 cm) impregnated with a solution of azolectin (L-α-lecithin, type II-S; Sigma) in decane (80 mg/ml) was clamped between two compartments of a collapsible Teflon cuvette so that the filter blocked an opening (diameter, 0.4 cm) in the partition separating the two compartments. Both compartments were filled with 25 mM Hepes-NaOH (pH 7.5) containing 20 mM $MgCl_2$ and chromatophores were added to one of the compartments. After stirring for 1 h, both compartments were washed with a tenfold volume of the buffer without

$MgCl_2$ using a peristaltic pump to remove magnesium and unbound chromatophores.

Exogenous mediators were added before the measurements to the cell compartment containing chromatophores immobilized on the MF surface.

Photoelectric responses were recorded using a pair of silver chloride macroelectrodes (Ag/AgCl/3 M KCl) immersed in the electrolyte solution on both sides of the MF. The electrodes were connected to an operational amplifier (Burr Brown 3554BM, USA); the latter was connected to a Gage CS8012 analog-to-digital converter (ADC) and a computer. An incandescent lamp with a power of 90 W (12 V) was used as a constant light source.

Measurement of the electric potential difference in chromatophores immersed into the MF. The measurements were performed in the ITO|chromatophores–MF|ITO system. For this, 2.5 × 5 cm MF (pore diameter, 0.22 μm; thickness, 150 μm; Millipore GSTF) was placed on the surface of a glass slide coated with the ITO semiconductor. Next, 30-40 μl of suspension of chromatophores with the bChl concentration of ~2 mg/ml was applied dropwise to the MF surface (~1.0 cm^2). After adsorption for 10 min in the dark, the MF surface was washed (2 × 250 μl) with 25 mM HEPES-NaOH (pH 7.5) and then the second ITO electrode was placed on the top.

It should be noted that in the ITO|chromatophores–MF|ITO system, the mediators had been added to the chromatophore suspension before it was used for the incorporation into MF.

Registration of electrical potentials in the ITO|chromatophores–MF|ITO system was carried out using copper wires connected on one side to the glass slides coated with ITO and to the operational amplifier on the other side. The signal from the amplifier was fed to a Gage CS8012 ADC connected to a computer. Saturating continuous illumination was provided by an incandescent lamp (12 V, 90 W).

Each photoelectricity measurement was repeated at least 3 times. The range of errors for the photoresponses was ~5%. All measurements were carried out at 20 ± 1°C.

The figures were prepared with the Origin 7.5 software package (OriginLab Corporation).

Optical spectroscopy. Photoinduced changes in the absorbance at 603 nm, which reflect the redox properties of the primary electron donor P_{870}, were recorded with a single-beam differential spectrophotometer constructed in our laboratory. The measuring light from a KGM-98 lamp passed through an HL-1 Jobin Ivon monochromator (France), cuvette with a sample, glass light filter, and second UM-2 monochromator, and then hit a photomultiplier (PMT). The signal from the PMT was fed through the operational amplifier to the ADC (Gage CS8012) connected to the computer.

RESULTS

The aim of this work was to study Δψ generation in intact bacterial membrane vesicles under continuous illumination. Previously, it was shown [24] that chromatophores isolated under certain conditions (cell disruption with a French press at low ionic strength) from the nonsulfur purple bacterium *Rba. sphaeroides* contain ~70% cyt c_2 inside the vesicles (see “Materials and Methods” section). In these chromatophores, reduction of photooxidized P_{870} in the RCs can occur as a result of direct electron transfer from endogenous cyt c_2 [24, 25]. In other words, functionally active chromatophores should contain cyt c_2, which along with a pool of ubiquinones, acts as a redox carrier between the RCs and cyt bc_1. The content of cyt c_2 was determined by recording changes in the absorbance at 603 nm (which reflects the redox properties of P_{870}) in a suspension of chromatophores in response to single light flashes in the absence and presence of 2% Triton X-100 (not shown) (see [26]).

Figure 1 shows Δψ generation under constant illumination in *Rba. sphaeroides* chromatophores associated with the surface of MF impregnated with a phospholipid solution. No photoelectric response was observed in the absence of additives (mediators) (Fig. 1a, curve *1*). It should be noted that as in the case of a collodion film [9, 10], association of chromatophores with MF impregnated with a solution of phospholipids in decane results in the extraction of UQ both from the site of the secondary quinone acceptor Q_B in the RC protein and the membrane pool. In addition, cyt c_2 and the primary electron donor bChl P_{870} dimer are oxidized in the RC. Under these conditions, light-induced electron transfer in chromatophores is limited by the formation of the $P^{+}_{870}Q^{-}_{A}$ radical ion pair inside the RC protein. However, charge transfer outside the RC in the described system can be reconstructed by the saturation of immobilized chromatophores with exogenous quinone and reduction of endogenous cyt c_2 (see [14]).

No photoelectric response was observed in chromatophores associated with the MF surface in the presence of ascorbate (Asc) and N,N,N'N'-tetramethyl-p-phenylenediamine (TMPD) only in the assay medium (Fig. 1a, curve *2*). The use of 2.5 mM Asc/150 μM TMPD pair as an electron donor was needed to reduce soluble cyt c_2 localized inside chromatophores, because of poor Asc penetration through the membranes [9].

Figure 1a, curve *3* shows Δψ generation under continuous illumination in the presence of only 150 μM 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ_0, electron acceptor). The photoresponse amplitude under these conditions was ~1.8 mV. UQ_0 is an efficient exogenous electron acceptor from quinones in the RCs [27, 28] that is widely used in photoelectrochemical

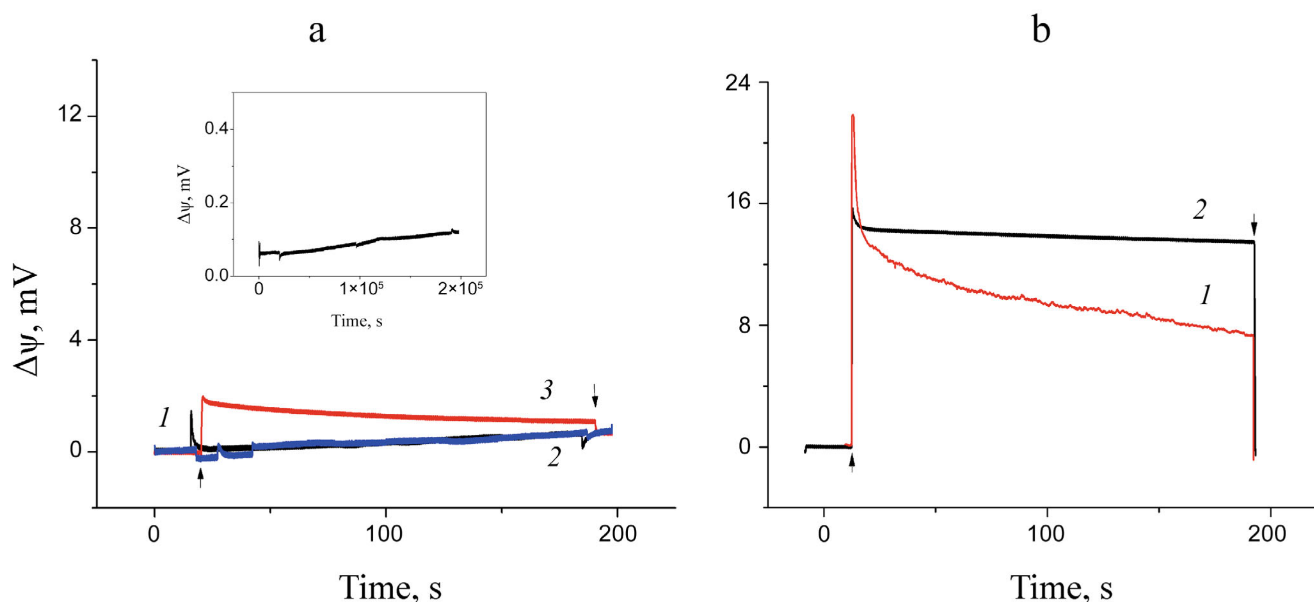

Fig. 1. a) Generation of electric potential difference under continuous illumination by chromatophores associated with the surface of MF (pore size, 0.22 µm; filter thickness, 150 µm) impregnated with azolectin solution in decane (80 mg/ml) without additives (curve *1*), in the presence of 2.5 mM ascorbate (Asc)/150 µM N,N,N'N'-tetramethyl-*p*-phenylenediamine (TMPD) pair (curve *2*), and in the presence of 150 µM UQ_0 only (curve *3*). Incubation medium: 25 mM HEPES-NaOH (pH 7.5). Inset: photoresponse in the presence of 5 mM Asc/150 µM TMPD and 150 µM UQ_0 in the absence of chromatophores. Arrows (↑) and (↓) indicate light on and off, respectively. b) Generation of electric potential difference in the medium containing 2.5 mM Asc/150 µM TMPD and 150 µM UQ_0 in the absence (curve *1*) and presence (curve *2*) of 0.75 M trehalose.

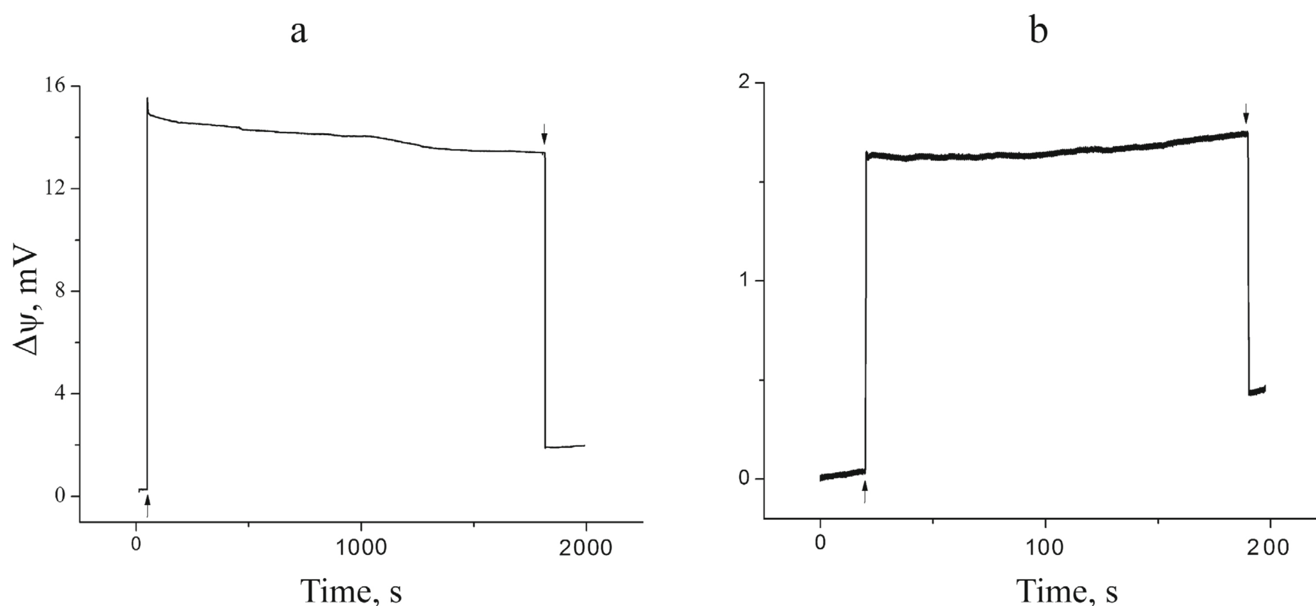

Fig. 2. Generation of electric potential difference under stationary illumination in the presence of 0.75 M trehalose by associated with the MF surface: a) chromatophores isolated by a standard method; b) chromatophores deficient in cyt c_2. Experimental conditions are as in Fig. 1b, curve *2.*

systems [28]. No photoresponse was observed in the control samples containing Asc/TMPD and UQ_0, but no chromatophores (inset in Fig. 1a).

Figure 1b, curve *1* shows the photoelectric response of chromatophores under continuous illumination in the presence of both electron donor (Asc/TMPD) and acceptor (UQ_0). It can be seen that only in the presence of both mediators, a significant increase in the photoelectric response was observed, followed by a two-component decrease in Δψ with the fast (~3 s) and slow (~180 s) phases.

The following experiments were carried out to study the effect of the disaccharide trehalose (bioprotector) on the light-dependent formation of Δψ by chromatophores immobilized on the MF surface. In the presence of Asc/TMPD and UQ_0, addition of 0.75 M trehalose

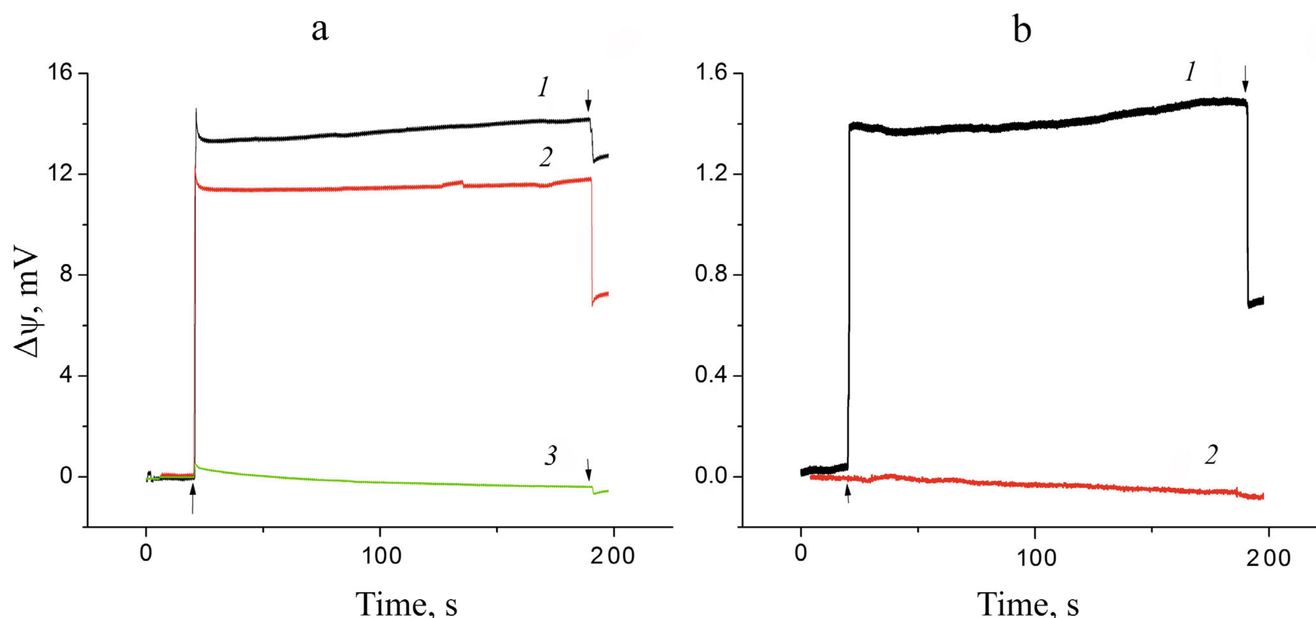

Fig. 3. a) Generation of electric potential difference in chromatophores associated with the surface of MF impregnated with a solution of phospholipids. Conditions are as in Fig. 1b (curve *2*) in the absence (curve *1*) and presence (curve *2*) of 5 μM AntA; curve *3*, conditions are as in Fig. 3a (curve *1*) in the presence of 10 μM atrazine. b) Generation of electric potential difference by chromatophores after 18 h (curve *1*) and 40 h (curve *2*) of storage at room temperature. Experimental conditions are as in Fig. 3a, curve *1*.

under continuous illumination stabilized Δψ for ~190 s (Fig. 1b, curve *2*). Under these conditions, stabilization of Δψ signal was also observed under much longer illumination (~1800 s) (Fig. 2a).

Figure 2b shows Δψ generation in the presence of Asc/TMPD, UQ_0, and trehalose by *Rba. sphaeroides* chromatophores deficient in cyt c_2. Illumination of these chromatophores led to the generation of electric potential difference with an amplitude of ~1.8 mV, indicating that the reduced 150 μM TMPD penetrating through the membrane was unable to efficiently reduce photooxidized P_{870} under stationary conditions [11].

Charge transfer in the RC and bc_1 protein complexes in chromatophores is associated with the generation of Δψ that can be measured by the electrochromic shift of carotenoid absorption bands and direct electrometric method [10]. Figure 3a (curve *1*) shows Δψ generation under continuous illumination in the presence of Asc/TMPD, UQ_0, and trehalose. To determine the contribution of the bc_1 complex to the total photoresponse, antimycin A (AntA, inhibitor of the ubiquinone reductase site Q_i of the bc_1 complex) was added to the medium, which reduced the photoresponse amplitude by ~12% (Fig. 3a curve *2*). The observed effect could be due to the fact that UQ_0 binds to the Q_B site in the RC [28] and receives an electron from Q_A^-; its reduced form (UQ_0H_2) acts as a "substrate" only for a small fraction of the bc_1 complexes.

To prove that the observed photoelectric activity of chromatophores was due to the functioning of RCs, atrazine (inhibitor of electron transfer between Q_A^- and Q_B) was added to the medium. Indeed, addition of 10 μM atrazine almost completely inhibited generation of the photoelectric response (Fig. 3a, curve *3*), indicating that in the studied system, electron transfer in the acceptor site proceeded from the primary quinone Q_A to the exogenous acceptor Q_0.

To study the stability of photoresponses, chromatophores associated with the MF surface were stored in the dark at room temperature and illuminated at regular intervals. The amplitude of Δψ, which was initially ~14 mV (Fig. 3a, curve *1*), decreased to ~1.4 mV after 18 h of storage (Fig. 3b, curve *1*). No photoresponse was observed after 40 h of storage (Fig. 3b, curve *2*).

Figure 4 shows Δψ generation in chromatophores immersed into MF and clamped on both sides by semiconductor indium-tin oxide glass plates (ITO|chromatophores–MF|ITO) in the presence of mediators (Asc/TMPD, UQ_0), antimycin A, and trehalose. It was assumed that the redox mediators moved freely inside the MF and could interact with the RCs and ITO electrodes. When the system was illuminated for ~4000 s, a stable nondecreasing photoelectric response with an amplitude of ~30 mV was generated [20].

It should be noted that the 18-h dark adaptation of the ITO|chromatophores–MF|ITO system at room temperature resulted in a significant decrease in the Δψ signal upon illumination for 180 s (not shown). The inset in Fig. 4 shows Δψ generation upon addition of ~40 μl of fresh 25 mM HEPES-NaOH (pH 7.5) containing redox mediators (Ask/TMPD and UQ_0) directly to the ITO|chromatophores–MF|ITO system after its storage

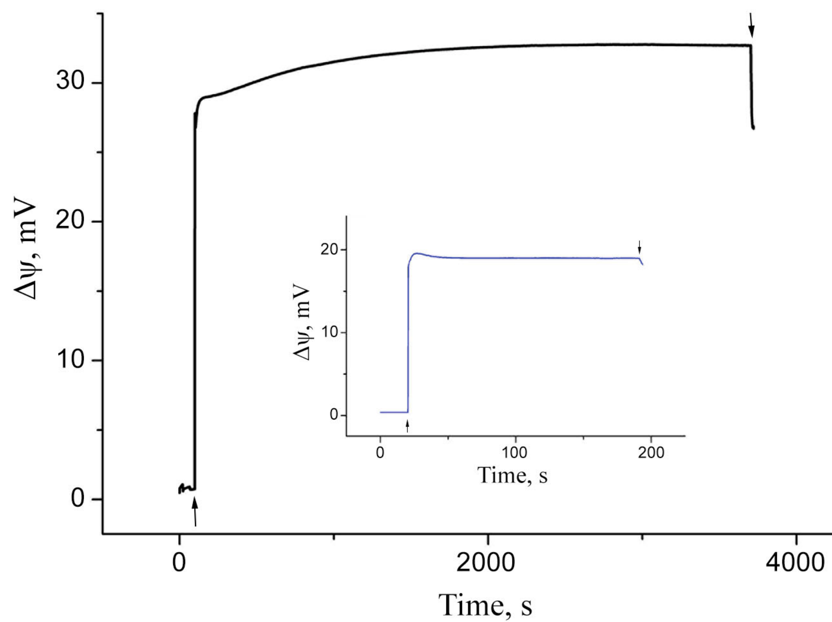

Fig. 4. Generation of Δψ in chromatophores immersed into MF (ITO|chromatophores–MF|ITO system) in the presence of 2.5 mM Asc, 150 μM TMPD, 150 μM UQ_0, 0.75 M trehalose, and 5 μM AntA and illuminated for 4000 s (control). Inset: Δψ generation after addition of fresh 25 mM HEPES-NaOH (pH 7.5) containing Asc/TMPD and UQ_0 directly to the ITO|chromatophores–MF|ITO system on the day 30 of storage at room temperature.

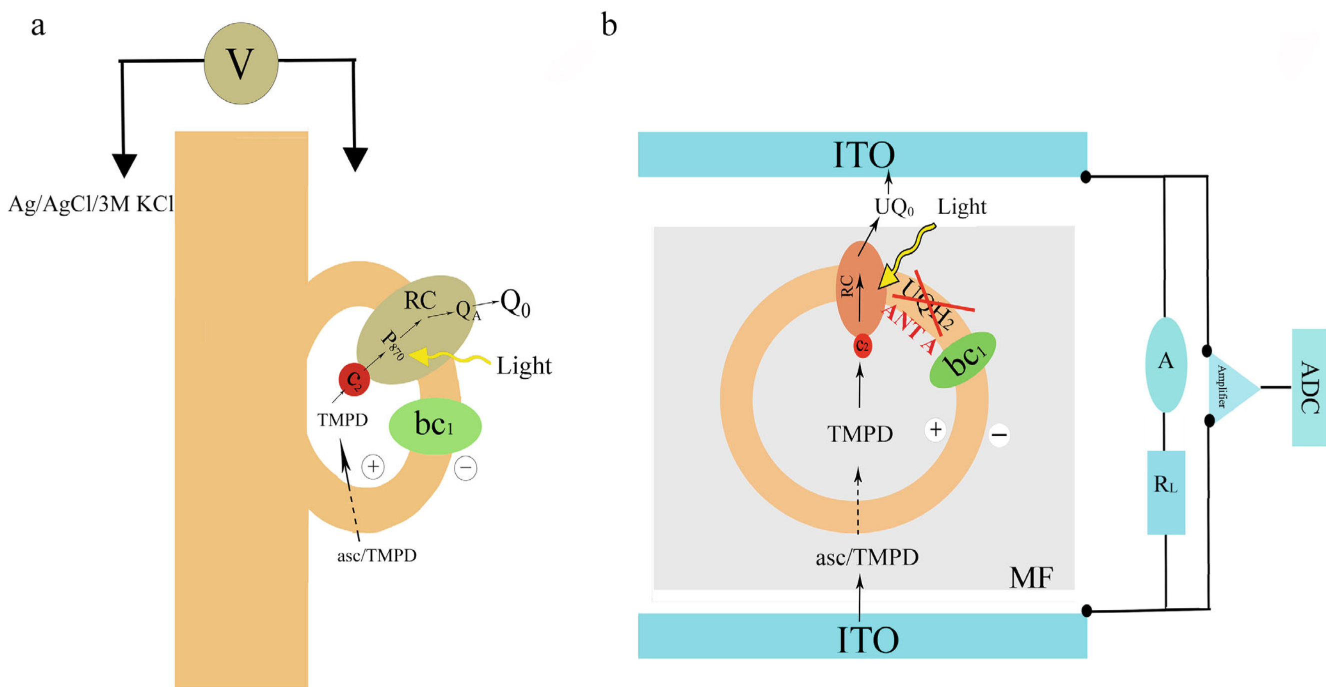

Fig. 5. Electron transfer pathways induced by stationary illumination in *Rba. sphaeroides* chromatophores associated with the MF surface (a) and immersed into MF (b).

for 30 days in the dark at room temperature. Under these conditions, the photoresponse amplitude was ~70% of the control.

Figure 5 shows presumable electron transfer pathways in chromatophores immobilized by two different methods.

DISCUSSION

The studies of the mechanism of Δψ generation in chromatophores associated with the surface of a collodion film impregnated with a lipid solution in response to single light flashes using direct electrometric method

demonstrated the presence of the following electrogenic reactions: 1) charge separation between P_{870} and Q_A; 2) re-reduction of photooxidized P_{870} by endogenous cyt c_2; 3) protonation of the doubly reduced secondary quinone acceptor Q_B (see review [11]); 4) electron and proton transfer in the cyt bc_1 complex [10].

The purpose of this work was to demonstrate $\Delta\psi$ generation under stationary illumination of chromatophores immobilized on the surface of MF and immersed into it and to study its mechanism.

It should be noted that adsorption of biomaterials on a solid surface (Au, TiO_2, ITO electrodes) can lead to structural changes and emergence of electrostatic repulsion between the studied sample and the support surface ([29] and references therein). Taking into account the biocompatibility, biodegradability, nontoxicity, and low cost of porous nitrocellulose membrane filter [30, 31], we used it as a matrix for chromatophore immobilization. Interaction of chromatophores with the MF surface was achieved by neutralization of negative charges of the polar heads of chromatophore membrane phospholipids and MF by addition of Mg^{2+} or Ca^{2+} cations [13] (see "Materials and Methods" section).

To generate stable electric signals, we used functionally active chromatophores with a high content of endogenous cyt c_2. Since the redox cofactors in the RC and bc_1 transmembrane complexes are immersed deeply in the protein matrix, the recording of $\Delta\psi$ under continuous illumination *in vitro* (in a semi-synthetic system) was performed in the presence of exogenous mediators. The Asc/TMPD pair was used because of the chromatophore membrane permeability for the reduced TMPD and capability of this compound to reduce endogenous cyt c_2 [9]. Soluble UQ_0 maintained the functional activity of RCs by accepting electrons from the primary quinone Q_A^- in chromatophores associated with the MF surface and from the secondary quinone Q_B^- in the case of chromatophores immersed into the MF [20, 28]. UQ_0 was an efficient mediator between the quinone acceptor site of the RC and electrodes [28, 32].

In *Rba. sphaeroides* chromatophores associated with the MF surface, loosely bound Q_B and UQ molecules was washed out with n-decane used as a hydrophobic solvent for phospholipids during MF impregnation, which resulted in the blockade of cyclic charge transfer [13]. Under these conditions, ubihydroquinone (UQ_0H_2) generated under stationary illumination could not act as a substrate for the bc_1 complex, probably, because of its high hydrophilicity (Fig. 5a).

On the other hand, when chromatophores were immersed into MF, the functioning of the cyclic electron transport chain with the participation of the RC and bc_1 complexes, UQ pool, and cyt c_2, was preserved. This was confirmed by the fact that addition of AntA (bc_1 complex inhibitor) led to a significant (almost twofold) increase in the amplitude of the steady-state $\Delta\psi$ [20]. The increase in the amplitude of the photoelectric response upon inhibition of the bc_1 complex was due to the redirection of cyclic electron flow (which does not contribute to the membrane potential in the studied system) toward linear electron transfer between the ITO electrodes and RCs (Fig. 5b). This type of chromatophore immobilization allowed to record stable non-decreasing $\Delta\psi$ signal during prolonged illumination (~4000 s) [20].

Therefore, the recorded maximal photoelectric response was probably due to the light-induced direct electron transfer along the cyt $c_2 \rightarrow RC \rightarrow Q_A \rightarrow Q_0 \rightarrow O_2$ path in the case of chromatophores associated with the MF surface (Fig. 5a) or ITO → cyt $c_2 \rightarrow RC \rightarrow Q_A \rightarrow Q_B \rightarrow$ $\rightarrow UQ_0 \rightarrow$ ITO path for chromatophores immersed into MF pores (Fig. 5b). In other words, in both cases, generation of stationary $\Delta\psi$ was due to the operation of RCs themselves. This was evidenced by the suppression of electrical response generation in the presence of atrazine, an inhibitor of electron transfer between quinone acceptors in RCs (Fig. 3a, curve *3*).

In studies on the conversion of light energy, it is important not only to obtain a significant amplitude of electric current under continuous illumination, but also to identify conditions for maintaining the functional activity of samples during their long-term storage at room temperature. In chromatophores associated with the MF surface, an almost complete drop in the $\Delta\psi$ amplitude was observed after 40 h of storage (Fig. 3b, curve *2*). The decrease in the photoresponse amplitude could be associated either with a decrease in the resistance of MF impregnated with a lipid solution [13] or with the 'degradation' of added mediators, in particular, Asc. However, the recovery of the MF resistance (2×10^8 Om) and addition of a fresh portion of the buffer containing mediators (Asc/TMPD, UQ_0) to the assay medium does not lead to recovery of $\Delta\psi$ (not shown). Therefore, the decrease in the $\Delta\psi$ amplitude with time in the case of chromatophores associated with the MF surface was most likely associated with the conformational and structural changes in the RCs.

As for the stability of photoelectric responses of chromatophores immersed into the MF, addition of a fresh buffer with the electron donor and acceptor to the ITO|chromatophores–MF|ITO system stored at room temperature under aerobic conditions for 30 days, caused a recovery of the $\Delta\psi$ amplitude to ~70% (inset in Fig. 4) relative to the control sample (Fig. 4), indicating that the majority of chromatophores immobilized on the MF remained functionally active for at least 30 days.

Hence, in both systems, stable maximal photoresponses were observed only in the presence of trehalose, a disaccharide with unique physicochemical properties. Previously, similar stable photoelectric responses were detected in the presence of this osmolyte in isolated pigment–protein complexes of PSI and PSII immersed into MF [33, 34]. Note that trehalose also affects the nature

of the electric response (Fig. 1b, curve *2*), namely, suppresses the rapid burst and decay of $\Delta\psi$ at the start of illumination (Fig. 1b, curve *1*), which can be due to a decrease in the ionic permeability of the chromatophore membrane [35, 36]. A similar effect of trehalose on the photoresponse rapid burst and decay was observed in chromatophores immersed into MF [20]. Stabilization of photoresponses could be associated with an improvement in the efficiency of interactions between the mediator(s) and proteins of the photosynthetic electron transport chain. Preservation of a thin hydration shell of transmembrane proteins in the presence of this disaccharide can change their conformation into a conformation more optimal for their efficient functioning [37-39]. Trehalose might also stabilize lipid bilayers by replacing water during formation of hydrogen bonds between its hydroxyl groups and polar lipid heads [40]. In this case, interactions of trehalose with the transmembrane proteins and phospholipids in chromatophores can occur only near the outer side of the membrane because of the impermeability of chromatophore membrane for trehalose.

Therefore, trehalose stabilized photoelectric signals generated by both types of immobilized chromatophores; however, the most stable photoresponses were observed when chromatophores were immersed into the MF. This extremely simple method of sample immobilization might help to preserve intact photosynthetic proteins of chromatophore inside the MF pores at room temperature for a long period of time.

The obtained results will expand modern ideas on the use of semisynthetic structures based on various intact photosynthetic systems (cyanobacteria and purple bacteria cells [41], plant thylakoid membranes) capable of converting solar energy into its electrochemical form.

Contributions. M.D.M. developed the concept and supervised the study, analyzed electrical measurements, and wrote the text of the article; L.A.V. and A.A.Z. isolated bacterial membrane vesicles (chromatophores) and performed electrical measurements.

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Ethics declarations. The authors declare no conflict of interest in financial or any other sphere. This article does not contain any studies with human participants or animals performed by any of the authors.

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