# Light-Induced Generation of Electric Potential Difference in Membranes of Purple and Green Sulfur Bacteria

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## Abstract

When associated with a planar phospholipid membrane, chromatophores isolated from photosynthetic sulfur bacteria *Chromatium minutissimum*, *Ectothiorhodospira shaposhnikovii*, and *Chlorobium limicola f. thiosulfatophilum* were shown to generate a light-induced transmembrane electric potential difference measured by a direct method using macroelectrodes and a voltmeter. The maximal photoelectric responses were observed upon the addition of 1,4-naphthoquinone in combination with phenazine methosulfate (or TMPD*) and ascorbate. The photoeffects were inhibited by CCCP and gramicidin. The data demonstrate that similar mechanisms of photoelectric generation function in membranes of the different bacteria studied.

## Introduction

Photosynthetic bacteria are subdivided into three families: nonsulfur purple (*Rhodospirillaceae*), purple sulfur (*Chromatiaceae*), and green sulfur (*Chlorobiaceae*) bacteria. Bioenergetic mechanisms of nonsulfur purple bacteria, a typical member of which is *Rhodospirillum rubrum*, have been studied to the greatest extent.

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* Abbreviations: CCCP = 2,4,6-trichlorocarbonyl cyanide phenylhydrazone; TMPD = $N,N,N',N'$-tetramethyl-$p$-phenylenediamine.

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By a direct method, using macroelectrodes and a voltmeter, it was shown that the energization of *R. rubrum* chromatophores by light, ATP, or inorganic pyrophosphate leads to the generation of a transmembrane electric potential difference [1], in accordance with Mitchell's chemiosmotic hypothesis [2]. The photoelectric generator function in the chromatophores is brought about by the bacteriochlorophyll reaction center complexes [3].

As to purple sulfur bacteria, the data on membrane potential generation are scant and were obtained only by indirect methods. It was shown that the chromatophores of *Chromatium minutissimum* are capable of an uptake of synthetic penetrating anions upon illumination [4]. Light-induced fluorescence changes of atebrin and 8-anilinonaphthalene-1-sulfonate, which indicate a $\Delta pH$ and electric potential difference, were observed in the suspensions of intact cells of *Ectothiorhodospira shaposhnikovii* [5]. Electrochromic shifts of bacteriochlorophyll absorption bands were found in the illuminated cells of both bacteria [6].

Up to now, there has been no work done to detect the generation of membrane potential in green sulfur bacteria. Isolated membranes of *Chlorobium limicola f. thiosulfatophilum* as well as the membranes of *E. shaposhnikovii* do not exhibit a capacity to photophosphorylation [7]. This may be explained by a disturbance in the native state of the membranes on isolation from the bacterial cells.

The purpose of the present communication was to set up a comparative study of the generation of an electric potential difference in the membranes of purple and green sulfur bacteria using the direct method of measurement described earlier [8].

### Materials and Methods

Members of the purple and green sulfur bacteria differed considerably in the structural organization of their photosynthetic apparatus. *Chr. minutissimum* forms a vesicular type of chromatophore membrane [9]. Lamellar structures consisting of stacks of short double membranes were found in *E. shaposhnikovii* [10]. Fuller et al. [11] suggest that the cytoplasmic membrane is operating as a photosynthetic apparatus in *Chl. limicola*.

Bacteria were grown anaerobically in light on modified Larsen's medium with acetate or malate for purple bacteria and with acetate and thiosulfate for green sulfur bacteria [12]. Chronatophores were isolated using a procedure similar to that described earlier [7]. Bacterial cells from 3- to 4-day-old cultures were washed in deionized water, suspended in 50 mM Tris-HCl buffer (pH 8.2) containing 250 mM sucrose and 5 mM $MgSO_4$ and sonicated (22 kc) in an ice bath for 2–3 min. Subsequent

operations were carried out at 0–2°C. The homogenates were centrifuged for 15 min at $40,000 \times g$ to separate the unruptured cells and large particles. The supernatant was centrifuged for 60–90 min at $165,000 \times g$ to obtain the membrane fraction. The pellet of *Chl. limicola* membranes was used in experiments without a washing. The membranes of purple sulfur bacteria were washed once or twice in 50 mM Tris-HCl buffer (pH 7.6) containing 250 mM sucrose and 2 mM $MgSO_4$, and suspended in the same buffer.

Association of isolated chromatophores with an artificial planar membrane, made of a mixture of soybean phospholipids (azolectin) and decane, was achieved by adding 10–30 mM $Mg^{2+}$ or $Ca^{2+}$ as previously described [1]. Measurements of transmembrane electric potential difference in the chromatophores–planar membrane system were carried out using Ag/AgCl electrodes and a high input impedance voltmeter by the method of Drachev et al. [8]. Experiments were performed with the use of the thick (not thin bilayer) planar membrane. Azolectin, 100–120 mg, was dissolved in 1 ml of decane. Planar membrane made of a mixture of azolectin, decane, and chromatophores was applied in some experiments, as described earlier [1]. Actinic light of saturating intensity was obtained from a tungsten lamp.

## Results

Energized chromatophores of *R. rubrum* associated with planar phospholipid membrane were found to generate a transmembrane electric potential difference [1]. A photoelectric effect in the chromatophores of *R. rubrum*–planar membrane system was observed in the presence of ubiquinone (or its analogs) and cofactors of cyclic redox chain (TMPD or phenazine methosulfate). Independent of these compounds, dark electric responses arose on addition of ATP or inorganic pyrophosphate.

Generation of a membrane potential in chromatophores of *Chr. minutissimum* takes place under similar conditions. The data of such an experiment are presented in Fig. 1. Chromatophores were added to one of the two compartments separated by a Teflon partition with an 0.8-mm aperture closed by the planar phospholipid membrane. $MgSO_4$ was added then to both compartments. $Mg^{2+}$ cations neutralize the negative surface charges on the membranes and induce the association of chromatophores with the planar membrane. As a result of this the membrane becomes asymmetric, having only one of its sides associated with the chromatophores. As Fig. 1 shows, a small photoelectric response is observed subsequent to the addition of 1,4-naphthoquinone. It is enhanced by TMPD and is reversed in the dark. The addition of inorganic pyro-
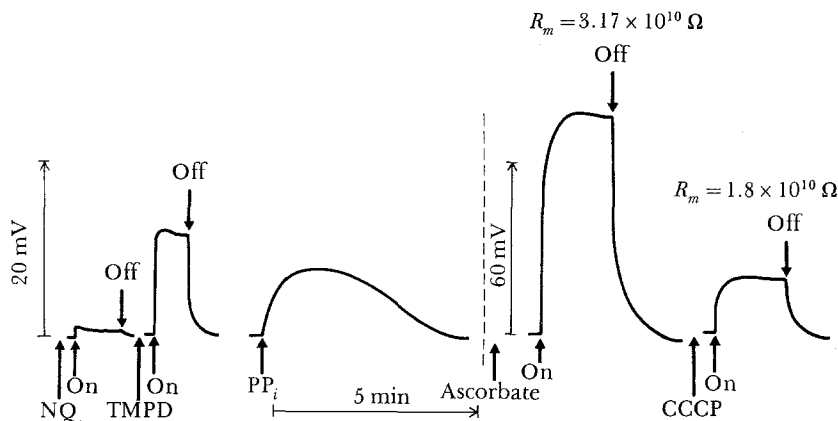
Figure 1. Energy-dependent generation of the electric potential difference by *Chr. minutissimum* chromatophores associated with the planar azolectin membrane. Incubation mixture: 50 mM Tris-HCl Buffer (pH 7.6), 10 mM $MgSO_4$, and chromatophores $(D_{860\,nm})= 1.4$; the length of the optical pathway is 1 cm). Additions (here and in all figures, to both compartments of the experimental cell): 0.1 mM 1,4-naphthoquinone (NQ), 0.5 mM TMPD, 5 mM sodium ascorbate, $1 \times 10^{-7}$ M CCCP. On and Off, switching on or off the light; $R_m$, electric resistance of the planar membrane; $R_s$, electric resistance, shunting the planar membrane, is $1 \times 10^{16}$ Ω. Positive charging of the chromatophore-free compartment is shown as an electric potential increase.

phosphate in the dark causes an electric response that disappears on exhaustion of the pyrophosphate from the medium. The photoelectric effect is greatly enhanced by ascorbate and inhibited by the protonophorous uncoupler CCCP, producing a decline in the electric resistance of the planar and the chromatophore membranes. Both light and inorganic pyrophosphate induce the formation of an electric potential difference which is positive in the chromatophore-free compartment of the cuvette. This result indicates that the generation of a membrane potential across the chromatophore membrane has a positive polarity on the inside [1].

Analogous findings were obtained in membranes isolated from *E. shaposhnikovii* (Fig. 2). Maximal photoelectric response (positive in the chromatophore-free compartment) is observed on addition of phenazine methosulfate, 1,4-naphthoquinone, and ascorbate. Substitution of phenazine methosulfate for TMPD leads to a decrease in the value of photoeffect (not shown). Shunting of the planar membrane by an external resistance $(R_s)$ causes an inhibition and differentiation of the photoelectric response. The uncoupler CCCP strongly lowered the photoeffect.

Figure 3 shows the data of an experiment performed with the use of the thick planar membrane made of a mixture of azolectin, decane, and *E.*
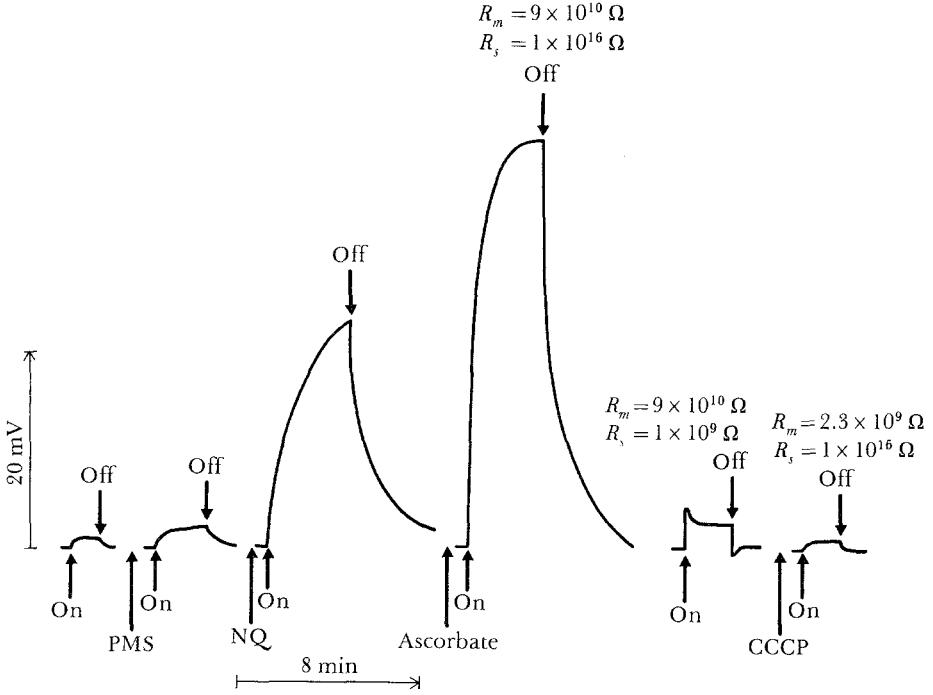
Figure 2. Light-induced generation of the electric potential difference by *E. shaposhnikovii* chromatophores associated with the planar azolectin membrane. Incubation mixture: 50 mM Tris-HCl buffer (pH 7.6), 30 mM $CaCl_2$, and chromatophores $(D_{860nm} = 1.3$; the length of the optical pathway is 1 cm). Additions: 0.1 mM phenazine methosulfate (PMS), 0.1 mM 1,4-naphthoquinone (NQ), 5 mM sodium ascorbate, $2 \times 10^{-6}$ M CCCP.

*shaposhnikovii* chromatophores. The photoelectric responses in this system are a combination of the electrogenic effects of the chromatophores associated with the right and left sides of the planar membrane. The direction and the form of the photoeffect in such a system are variable and are determined by the quantitative ratio of the chromatophores associated with the two sides of the planar membrane. As is shown in Fig. 3, the electric responses to switching the light on and off are biphasic. The ionophorous antibiotic gramicidin added to one of the compartments of the cuvette (thus only increasing the ion permeability of the chromatophore membranes in this compartment) results in the formation of an asymmetric system, and a clear increase in the electric responses is now observed due only to the chromatophores associated with the opposite side of the planar membrane. The electric resistance of the planar (thick)
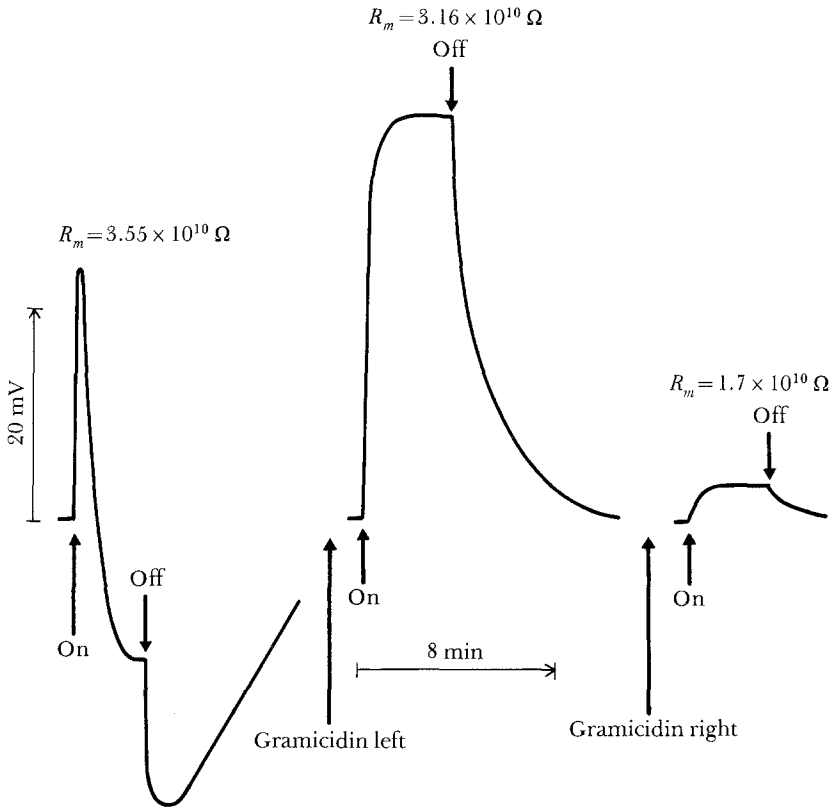
Figure 3.   Effect of gramicidin on the light-induced electric responses of the planar membrane made of a mixture of *E. shaposhnikovii* chromatophores, azolectin, and decane. Incubation mixture: 50 mM Tris-HCl buffer (pH 7.6), 0.1 mM 1,4-naphthoquinone, 0.1 mM phenazine methosulfate, 5 mM sodium ascorbate. Gramicidin additions: $5 \times 10^{-7}$ M in the left and $1 \times 10^{-6}$ M in the right compartment of the experimental cell. $R_s = 1 \times 10^{16}$ Ω.

membrane $(R_m)$ decreases only slightly by the action of gramicidin. The addition of gramicidin to the second compartment of the cuvette also, leads to an inhibition of the photoeffect.

The use of the planar membrane technique also provides a means for measuring the photoelectric responses in membrane structures of green sulfur bacteria. Illumination causes the generation of an electric potential difference across the planar membrane made of a mixture of azolectin, decane, and the membrane particles of *Chl. limicola* (Fig. 4). Shunting of the planar membrane by an external electric resistance leads to inhibition of
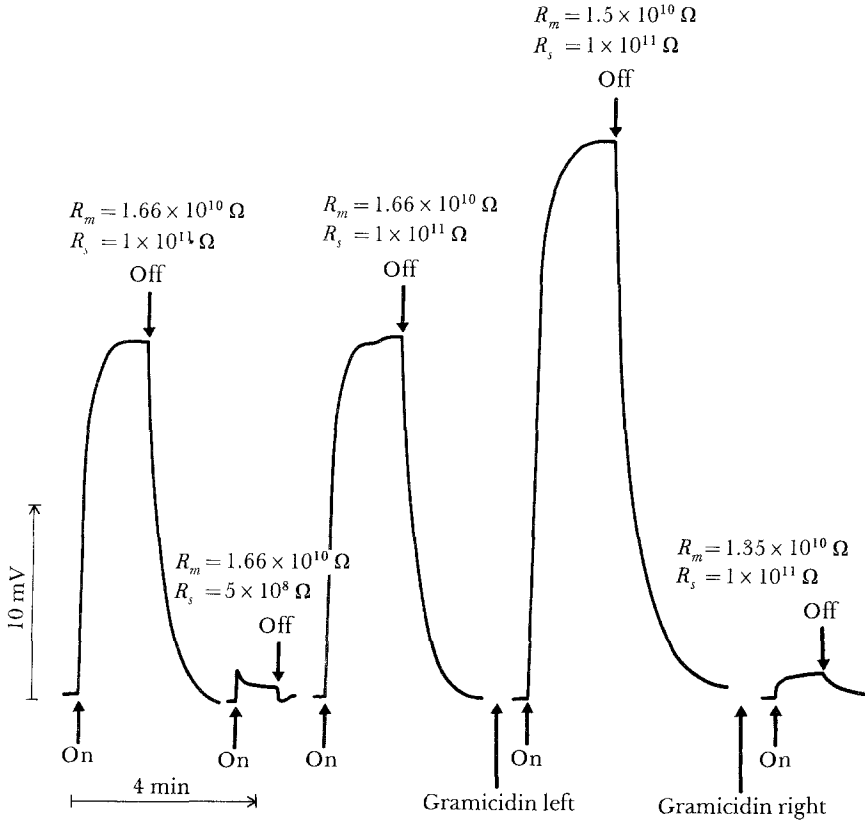
Figure 4. Effect of gramicidin on the light-induced electric responses of the planar membrane made of a mixture of *Chl. limicola* chromatophores, azolectin, and decane. Incubation mixture: 50 mM Tris-HCl buffer (pH 7.8), 0.1 mM 1,4-naphthoquinone, 0.1 mM phenazine methosulfate, 5 mM sodium ascorbate. Additions: $5 \times 10^{-7}$ M gramicidin.

the photoelectric response and its differentiation. The value and the form of the response are restored as the shunting electric resistance $(R_s)$ increases to the original level. Gramicidin added to one of the cuvette compartments causes an increase in the asymmetry of the system and in the electric response of the chromatophores associated with the opposite side of the planar membrane. The transmembrane electric potential difference is generated with a "plus" in the gramicidin-free compartment of the cuvette. The photoelectric response is inhibited on addition of gramicidin to both compartments.

*Discussion*

The data obtained demonstrate that membranes isolated from the photo-synthetic sulfur bacteria generate an electric potential difference upon the illumination. The membrane potential generation is inhibited by the ionophorous antibiotic gramicidin, by the protonophorous uncoupler CCCP, and also by an inhibitor of nonheme iron proteins, *o*-phen-anthroline, as was shown earlier for *R. rubrum* [1, 3].

Among the different methods for membrane potential measurement, the use of the planar phospholipid membrane technique is of particular interest. This system possesses the advantage of providing a means for measuring the electric potential difference in the native chromatophores, as well as measuring the electrogenic activity of the isolated sub-chromatophore pigment–protein complexes incorporated into the planar membrane. In this respect disruption of the membrane structure upon isolation does not seem to impair its electrogenic activity, which can be demonstrated when the isolates are incorporated in a lipid membrane. That is, the planar membrane, which may be far in excess by weight of the associated chromatophores, seems to provide a "curative" effect, causing a restoration of the electroinsulating properties of the damaged chromato-phore membranes. A disturbance of the electroinsulating properties during preparation seems to lead to a loss of photophosphorylation activity and the ability to generate a membrane potential (measured by the shifts of bacteriochlorophyll absorption bands, by the transport of penetrating ions, and by the fluorescence change of atebrin and anilinonaphthalene-sulfonate) in the isolated *E. shaposhnikovii* and *Chl. limicola* membrane particles, which show *in vivo* a distinctive type of construction [10, 11] as compared to the vesicular chromatophores of *R. rubrum* and *Chr. minutissimum* [9].

The planar membrane, consisting of a mixture of phospholipids and decane, extracts ubiquinone and its derivative from the associated chromatophores [1]. This generates a need for the addition of 1,4-naphthoquinone in the incubation mixture to compensate for the loss of the quinone pool in the chromatophores.

The maximal photoelectric responses in the chromatophores–planar membrane system are observed in the presence of 1,4-naphthoquinone, phenazine methosulfate (or TMPD), and ascorbate. Apparently, the role of phenazine methosulfate and TMPD is to promote the cyclic redox chain of the chromatophores (see, e.g., Trebst [13]).

The protonation of ubiquinone (or 1,4-naphthoquinone) involving its reduction by an endogenous electron acceptor on the external side of the chromatophore membrane, its subsequent transmembrane movement
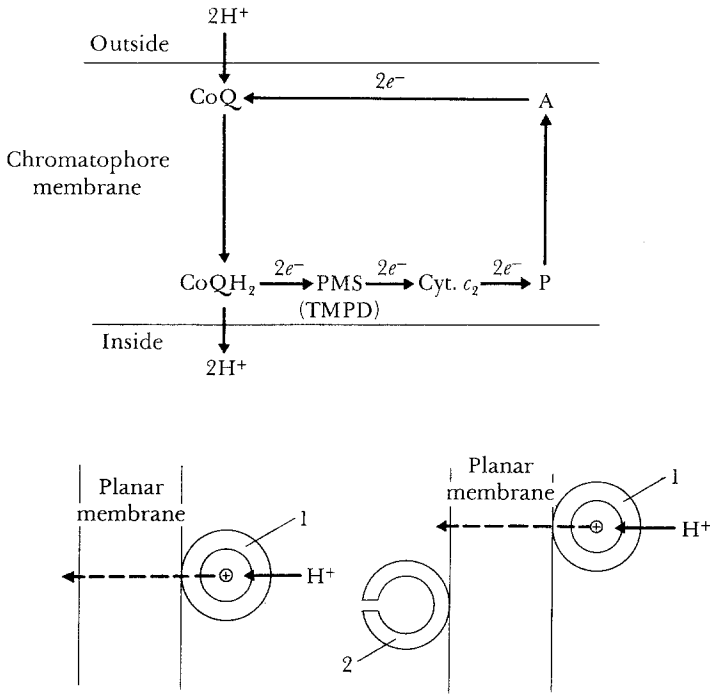
Figure 5. Schemes proposed for the light-induced generation of the electric potential difference in the chromatophores (a) and in the chromatophores–planar membrane systems (b, c). A, Endogenous electron acceptor; P, bacteriochlorophyll photoreaction center; Cyt. $c_2$, cytochrome $c_2$; 1, chromatophore; 2, chromatophore treated by gramicidin.

according to the concentration gradient, and its reoxidation by the bacteriochlorophyll reaction center on the internal side of the membrane with the help of phenazine methosulfate (or TMPD) (Fig. 5a), are likely to be the cause of the generation of the transmembrane proton gradient (positive inside the chromatophores). The movement of positive charges from the inside of the chromatophores to the chromatophore-free compartment of the experimental cuvette across the planar membrane (Fig. 5) (or to the compartment containing gramicidin in the case of chromatophores on both sides—Fig. 5) leads to the generation of the electric potential difference across the planar membrane separating the two compartments of the cuvette. Movement of negative charges in this system should be in the opposite direction. The positive pole on the planar membrane side which is not associated with chromatophores, or associated with chromatophores treated with gramacidin, indicates that

the inside of the chromatophores which is associated with the opposite side of the planar membrane also has a positive electric polarity.

Of particular interest are the data on the similarity of the conditions for light-induced generation of the electric potential difference in membranes of purple and green sulfur bacteria, in membranes of nonsulfur purple bacteria, and in the proteoliposomes containing the subchromatophore pigment–protein complexes of *R. rubrum* [1, 3]. This indicates that the same mechanism of photoelectric generation functions in the membranes of the different bacteria studied.

## Acknowledgments

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