GENERATION OF ELECTRIC POTENTIAL BY REACTION CENTER COMPLEXES FROM *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Several lines of indirect evidence indicate that the electrochemical H^+ potential is produced by the light-dependent cyclic electron transfer in chromatophores and intact bacterial cells [1–4], as was originally postulated by Mitchell [5].

In this paper, data on the direct measurement of electric current generation by bacteriochlorophyll reaction center complexes, isolated from *R. rubrum* chromatophores, is reported. A method of reconstitution of the reaction center complex-containing proteoliposomes and their association with planar phospholipid membranes was elaborated. Formation of light-induced electric potential difference by the proteoliposomes was demonstrated by the conventional voltmeter techniques as well as by a phenyldicarbaundecaborane (PCB^-) probe. The photoelectric effect was shown to increase on addition of TMPD or cytochrome *c* in combination with CoQ or vitamin K_3, and to decrease on addition of ferricyanide, *o*-phenanthroline and a protonophorous uncoupler.

2. Materials and methods

The bacteriochlorophyll-containing lipoprotein complexes of the photosynthetic reaction centers were isolated from chromatophores of wild type of *R. rubrum* by the method of Noël et al. [6]. The method included solubilization of chromatophores with LDAO, centrifugation to remove heavy membrane fragments, and fractionation of the supernatant with ammonium sulphate to purify the reaction center complexes. The complexes were then dialyzed and kept at 0°C in the dark in 50 mM potassium phosphate buffer, pH 7.8.

Proteoliposome reconstitution was carried out by the following method. 100 mg of azolectin were suspended in 1 ml of the mixture containing 50 mM potassium phosphate, pH 7.5, 5 mM $MgSO_4$ and 5% sodium cholate, and sonicated for 30 sec. Then the suspension was supplemented with 1.5 ml of a solution containing 50 mM potassium phosphate, pH 7.5, 0.03% LDAO and bacteriochlorophyll reaction center complexes (3 mg protein/ml). In some experiments 0.2 mM CoQ_6 was added into the same mixture. The final mixture was sonicated 10 times for 30 sec with 30 sec intervals. At the next step, the detergents were removed by 16 hr dialysis at +2°C in the dark against 50 mM potassium phosphate, pH 7.5 and 0.5 mM dithiothreitol. Proteoliposomes were sedimented at 165 000 *g* for 50 min and suspended in 0.25 M sucrose, 10 mM Tris–HCl, pH 7.5 and 2 mM $MgSO_4$.

Association of proteoliposomes with planar azolectin membrane and measurement of transmembrane electric potential differences were carried out as described by Drachev et al. [7]. The PCB^- concentration changes in the proteoliposome suspensions were monitored with the phospholipid membrane techniques [8]. The concentration of P870 in *R. rubrum* reaction

Abbreviations: CCCP, trichlorocarbonylcyanidephenylhydrazone; LDAO, laurildimethylamine oxide; PCB^-, phenyldicarbaundecaborane anion; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine;

center preparations was determined using a millimolar extinction coefficient of 112 $mM^{-1}cm^{-1}$ [9]. Actinic light of saturating intensity ($\lambda > 700$ nm) was obtained from a Tungsten lamp.

3. Results

Fig. 1 shows a result of a typical experiment with the reaction center proteoliposomes-planar membrane system. The proteoliposomes were added into one of the two compartments, separated by a Teflon partition with an 0.8 mm aperture closed by a planar azolectin membrane. To induce association of the planar and proteoliposomal membranes, the negative surface charges of phospholipids were neutralised by Ca^{2+} ions. As is seen in fig.1A, the illumination of the proteoliposome-treated planar membrane results in a transmembrane electric potential difference being generated (negative on the proteoliposome-free side of the membrane). Light-induced electric potential difference greatly increases on addition of TMPD and CoQ_6. An effect, similar to that of CoQ_6, could be

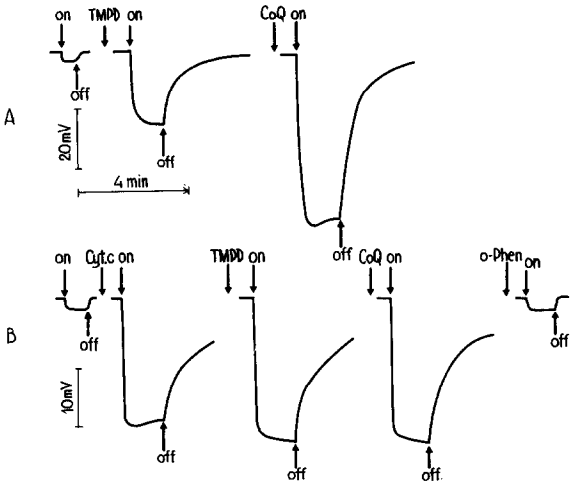

Fig. 1. Electric generation by bacteriochlorophyll reaction center proteoliposomes associated with planar azolectin membrane. Incubation mixture: 0.2 M sucrose, 0.05 M Tris–HCl, pH 7.4, 5 mM $MgSO_4$, 30 mM $CaCl_2$ and proteoliposomes. A. Proteoliposomes reconstituted without CoQ_6 (0.1×10^{-6} M P870). B. Proteoliposomes reconstituted with CoQ_6 (0.2×10^{-6} M P870). Additions: 0.5 mM TMPD, 0.2 mM CoQ_6, 10^{-5} M cytochrome *c*, 2 mM *o*-phenanthroline.

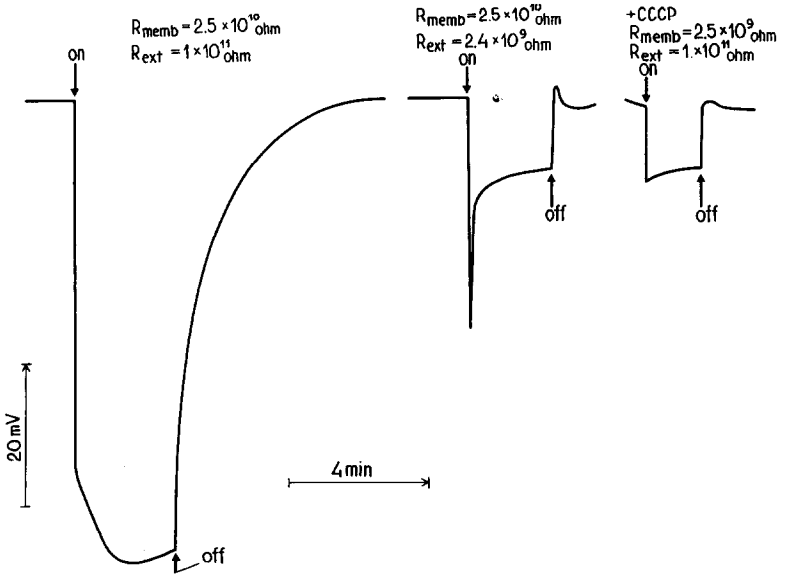

Fig. 2. The effects of shunting by an external electric resistance (R_{ext}) and uncoupler CCCP on electric generation by bacteriochlorophyll reaction center proteoliposomes associated with planar azolectin membrane. Incubation mixture: 0.2 M sucrose, 0.05 M Tris–HCl, pH 7.5, 5 mM $MgSO_4$, 30 mM $CaCl_2$, 0.5 mM TMPD, and proteoliposomes reconstituted with CoQ_6 (0.12×10^{-6} M P870).

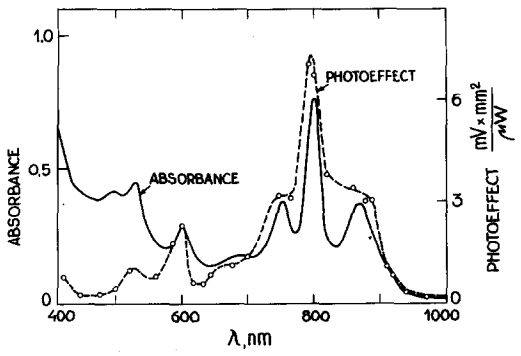

Fig. 3. Absorption spectrum of the bacteriochlorophyll reaction center complexes (solid curve) and the action spectrum of the photoelectric response in the system 'bacteriochlorophyll reaction center proteoliposomes – planar membrane' (dashed curve). Incubation mixture: 0.2 M sucrose, 0.05 M Tris–HCl, pH 7.5, 5 mM $MgSO_4$, 30 mM $CaCl_2$, 0.5 mM TMPD and proteoliposomes reconstituted with CoQ_6 (0.12×10^{-6} M P870).

produced by vitamin K_3, and to that of TMPD – by phenazinemethosulfate (not shown).

In the experiment illustrated in fig.1B, proteoliposomes reconstituted with CoQ_6 were used. It is seen that the photoelectric response of proteoliposomes increases on addition of cytochrome *c*. Subsequent additions of TMPD and CoQ_6 stimulate the photoeffect only slightly. The photoeffect is strongly inhibited by *o*-phenanthroline (fig.1B) and ferricyanide (not shown).

Shunting the planar membrane by an external electric resistance (fig.2) was found to cause a decrease in the light-induced response and a characteristic change in its form (differentiation). No pronounced differentiation was observed when electric conductance was increased by CCCP, although a strong decrease in the electric potential took place.

The action spectrum of the photoeffect is shown in fig.3. One can see that the maxima in the action spectrum coincide with those in the reaction center complexes absorption spectrum.

The last series of experiments showed that illumination induces an extrusion of PCB^- anions from the proteoliposomes, indicating the light-dependent negative charging of the proteoliposome interior. The process is activated by combination of TMPD and vitamin K_3, and inhibited by CCCP.

Antimycin A was without any influence on the photoeffect either in the planar membrane or PCB^- experiments.

4. Discussion

The above data demonstrate electric generation by bacteriochlorophyll reaction center complexes incorporated into planar and spherical (liposomal) phospholipid membranes. Maximal values of the photoeffect are observed in the presence of TMPD or cytochrome *c* in the combination with CoQ_6 or vitamin K_3.

Apparently, the role of added carriers consists in the return of reducing equivalents from the primary electron acceptor to oxidized bacteriochlorophyll P870. Such an effect should result in cyclization of the light-induced electron transfer whose first step is oxidoreduction between P870 and the primary electron acceptor presumably localized on the opposite sides of the native and reconstituted membranes. The small photoelectric response observed in the samples without added redox carriers may be mediated by endogenous CoQ which is present in the reaction center complexes.

Oxidation of P870 by ferricyanide or inhibition of the electron transfer from reduced primary acceptor to ubiquinone by *o*-phenanthroline cause a strong decrease in the photoelectric response.

In conclusion, bacteriochlorophyll reaction center complexes are competent in generating a transmembranous photocurrent.

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