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The antimycin-sensitive electrogenesis in *Rhodopseudomonas sphaeroides* chromatophores

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Electrogenesis linked to cyclic electron transfer through the ubiquinol-cytochrome c_2 oxidoreductase complex in *Rhodopseudomonas sphaeroides* chromatophores was investigated by a direct electrometric method. Rapid kinetic measurements of laser flash-induced $\Delta\psi$ generated in chromatophores associated with a phospholipid-impregnated collodion film revealed an exponential rise of $\Delta\psi$ with a τ of $\sim$ 700 μs. This exponential electrogenic phase contributes about 11% of the total photoelectric response and is sensitive to antimycin.

Membrane potential; Kinetics; Ubiquinol-cytochrome c_2 oxidoreductase; *(Rhodopseudomonas sphaeroides)*

1. INTRODUCTION

The ubiquinol-cytochrome c_2 oxidoreductase complex of *Rhodopseudomonas sphaeroides* catalyses cyclic electron transport, accepting electrons from the photochemical reaction center through ubiquinol, and delivering them through cytochrome c_2.

The electrogenic stages linked to electron transfer in reaction centers (RCs) were obtained previously with chromatophores in studies of electrochromic absorbance changes of carotenoids [1,2] and then by a direct electrometric method, developed in our group [3]. Three main electrogenic stages, i.e. (i) the formation of the primary dipole $P870^+ \cdot Q_A^-$; (ii) the reduction of photooxidized $P870^+$ by cytochrome c_2; (iii) protonation of the Q_B in response to even laser flashes were revealed in the kinetics of flash-induced responses of *Rhodospirillum rubrum* [4] and *Rps. sphaeroides* [5] chromatophores associated with the phospholipid-impregnated collodion film.

Three main stages were also observed in the flash-induced carotenoid changes [2], two of which were identical to stages (i) and (ii) registered by a direct electrometric method. The antimycin-sensitive stage has not been observed in our system. We explain this as the result of ubiquinone extraction from chromatophore membranes into the hydrophobic volume of the artificial membrane [6].

In this study, we attempted to reconstitute the cyclic electron transport of the photosynthetic redox chain and to study electrogenesis linked with the ubiquinol-cytochrome c_2 oxidoreductase complex in *Rps. sphaeroides* chromatophores associated with an artificial membrane.

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Abbreviations: RC, reaction center; P870, reaction center bacteriochlorophyll dimer; $\Delta\psi$, transmembrane electric potential difference; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine

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2. MATERIALS AND METHODS

Asolectin (phosphatidylcholine type II S), TMPD and ubiquinone-10 were from Sigma, Hepes and antimycin from Serva, and myxothiazol was a kind gift from Professor Trowitzsch.

Isolation of chromatophores from *Rps. sphaeroides*, their association with the phospholipid-impregnated collodion film and rapid kinetics measurements of $\Delta\psi$ by the direct electrometric method were as described [7]. In the case of continuous illumination the illuminator OU-28 was used, supplied with light-filter KC-11. Saturating light pulses were delivered from a LOMO OGM-40 ruby laser ($\lambda \sim 694$ nm; pulse half-width, 20 ns; 50 mJ output), linked to a home-built programmed pulse generator. The data storage and processing system consisted of a transient recorder DL-1070 (Datalab) interfaced to a NOVA-3D minicomputer (Data General).

Reconstitution of the Q_B function in the collodion film-associated chromatophores was achieved by adding 20 mg/ml Q-10 to the solution of asolectin in decane, used to impregnate the collodion film directly to the measuring cell as described [8].

3. RESULTS AND DISCUSSION

As previously shown, upon chromatophore association with the phospholipid-impregnated collodion film, extraction of ubiquinone from the chromatophore membrane and oxidation of cytochrome c_2 and partially of P870 take place [5].

Light-induced electron transfer under these conditions is confined to primary dipole $P870^+ \cdot Q_A^-$ formation within the RC. The generation of stable $\Delta\psi$ upon continuous illumination was only observed in the presence of high concentration of redox mediators as a result of noncyclic electron transfer from exogenic donors to acceptors [6].

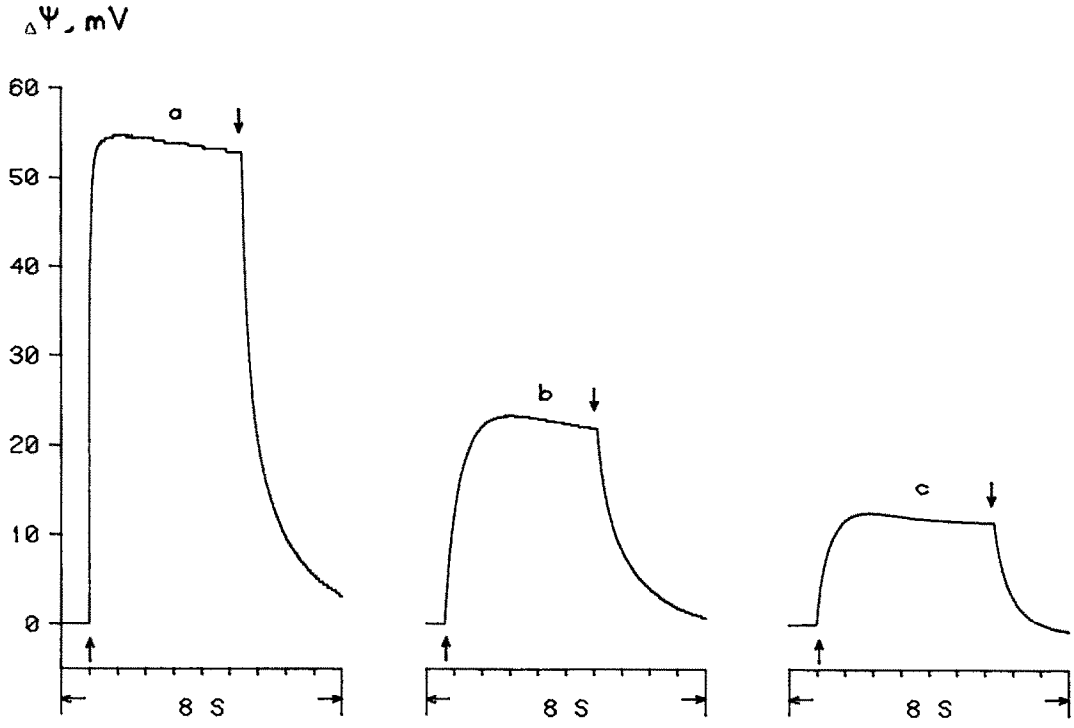

Fig.1. Typical electric responses of *Rps. sphaeroides* chromatophores associated with a phospholipid-impregnated collodion film under continuous illumination. The reaction medium contained 20 mM Hepes-KOH, pH 7.5, 2 mM ascorbate, 3 μM TMPD. The total bacteriochlorophyll concentration was ~25 μM. 20 mg/ml of CoQ_{10} was added to the decane solution of phospholipids used to impregnate the collodion film. (a) Saturated light; (b) half-saturated light; (c) as (b) but 6 μM antimycin was added. The arrows indicate switching on and turning off of continuous illumination.

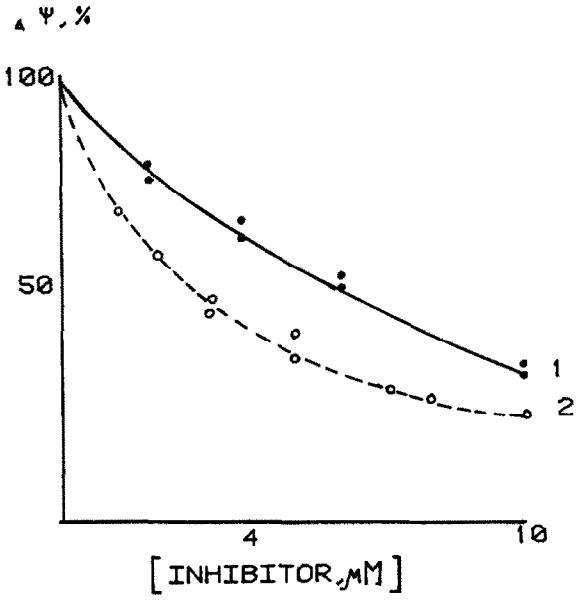

Fig.2. Dependence of the amplitude of photoelectric responses on the concentration of inhibitors upon continuous illumination. Conditions as in fig.1b. (1) Antimycin; (2) myxothiazol.

This effect was not sensitive to inhibitors of the ubiquinol-cytochrome c_2 oxidoreductase complex. However the charge transfer beyond the primary dipole in the described system may be reconstituted by saturating the artificial membrane with ubiquinone-10 and by reducing cytochrome c_2 in the presence of ascorbate and 3 μM TMPD [5]. Under appropriate conditions the cyclic charge transfer in *Rps. sphaeroides* chromatophores associated with a collodion film takes place. The results of a typical experiment are given in fig.1. One can see (fig.1a) that the continuous illumination causes generation of a stable electric potential difference of 55–60 mV.

Antimycin A at micromolar concentrations does not inhibit completely the cyclic electron transfer [9]. Thus, one could expect that the addition of antimycin will not cause a decrease in the amplitude of the photoelectric response, but rather a slowing down of its increase, since the experiment was performed at saturating light intensity. At the same time when the intensity of the actinic light was lowered so that the photoresponse decreased by

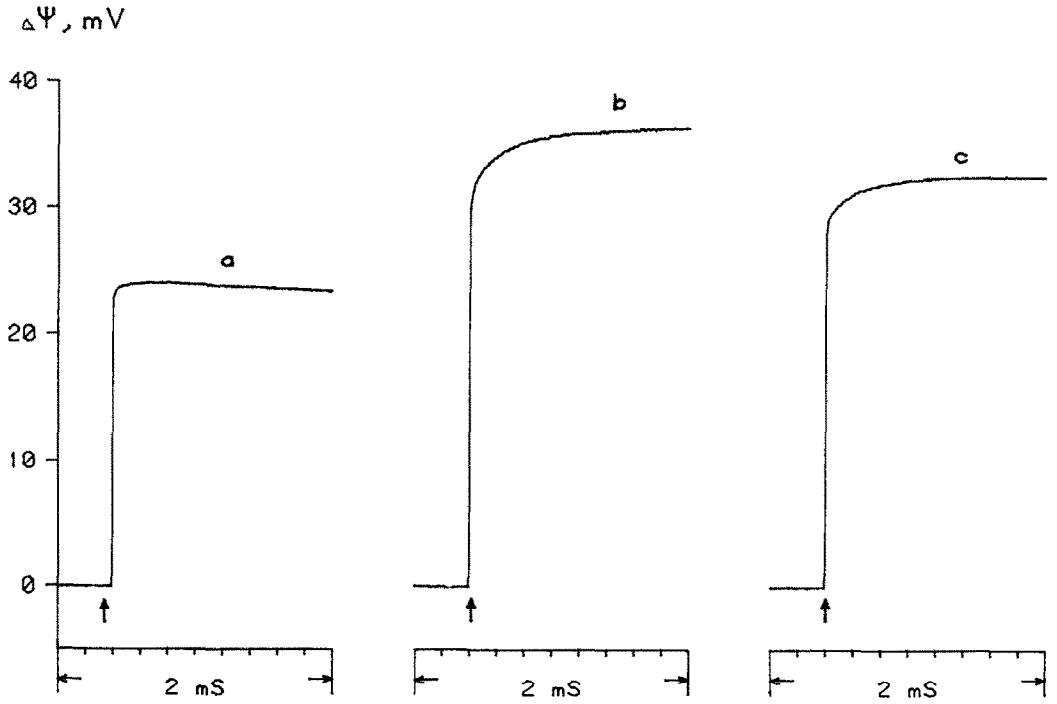

Fig.3. Typical laser flash-induced electric responses of *Rps. sphaeroides* chromatophores. The reaction medium contained 20 mM Hepes-KOH, pH 7.5. (a) No other additions; (b) 2 mM ascorbate and 3 μM TMPD were added; (c) as (b) but 6 μM antimycin was added.

~50% (of its maximal value) the addition of ~6 μM antimycin resulted in an approx. 2-fold decrease of $\Delta\psi$.

The obtained results show that the light-induced $\Delta\psi$ generation is observed as a result of electron transfer through the ubiquinol-cytochrome c_2 oxidoreductase complex.

Fig.2 shows the dependence of the $\Delta\psi$ amplitude on the concentration of antimycin and myxothiazol. It can be seen that the inhibition is incomplete, but a 2-fold decrease in the amplitude of $\Delta\psi$ is reached at an antimycin concentration of ~6 μM or at ~3 μM myxothiazol.

Fig.3 demonstrates the typical laser flash-induced electric responses in *Rps. sphaeroides* chromatophores. It can be seen (fig.3b) that in the presence of excess ascorbate and 3 μM TMPD, a slow phase is observed in the course of the $\Delta\psi$ rise. As shown in [5] this effect is partially due to reduction of $P870^+$ by cytochrome c_2. However the kinetics of this phase is markedly slower than that reported for $P870^+$ reduction by cytochrome c_2. Addition of antimycin leads to some acceleration in the rise and to a decrease of its amplitude (fig.3c). The total amplitude is decreased by ~10% (see fig.4).

A computer analysis of the kinetics of $\Delta\psi$ generation shows that the sum of the slow phases contributes about 23% of the total photoelectric response and is reasonably well approximated by two exponents with τ_1 ~200 μs and τ_2 ~700 μs (the contribution of these phases being about equal) (see table 1). After addition of antimycin the phase with τ_2 disappears but the amplitude of phase with τ_1 does not change. The rest of the 200 μs phase is mainly due to reduction of $P870^+$ by cytochrome

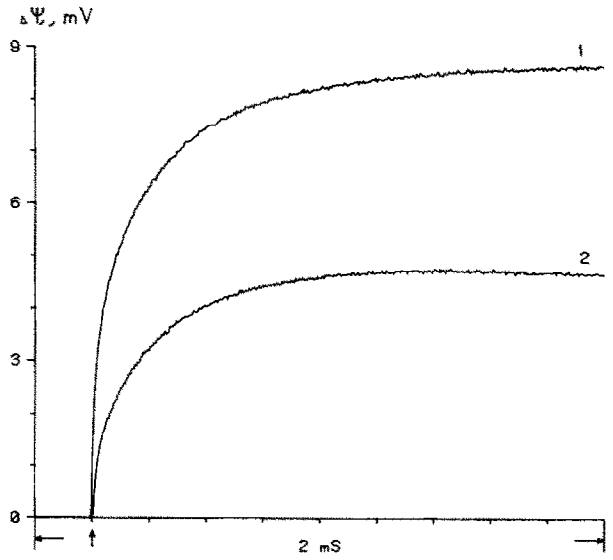

Fig.4. The upper parts of the curves (fig.3b and c) normalized by the amplitude of the fast unresolvable phase of $\Delta\psi$ generation. 1, conditions as in fig.3b; 2, conditions as in fig.3c.

c_2 and slightly to vectorial protonation of Q_B^{2-} [5].

Both amplitude and τ of the antimycin-sensitive electrogenic stage recorded by the electrochromic absorbance changes of carotenoids are essentially dependent on the ambient redox potential [10,11]. Unfortunately, the redox condition in our case is ambiguous. It is also obvious that the quantitative comparison of the amplitude of discrete phases of $\Delta\psi$ generation is impossible without knowledge on the level of system reconstruction. In the case of our chromatophore preparations about 30% of cytochrome c_2 present is capable of reducing

Table 1

The quantitative characteristics of the kinetics of $\Delta\psi$ generation in *Rps. sphaeroides* chromatophores

Additions	Total amplitude of photoresponse (mV)	τ_1 (μs)	τ_2 (μs)	Amplitude (mV)	
				Phase τ_1	Phase τ_2
2 mM ascorbate + 3 μM TMPD	37	190	700	4.7	4
+ 6 μM antimycin	33	210	–	4.5	–.

$P870^+$. Furthermore, the reconstruction level of Q_B function amounted to 70% [8].

Thus, we cannot estimate the real amplitude of the assayed electrogenic stage. The results of this study allow us to conclude that there exists an electrogenic charge transfer through the ubiquinol-cytochrome c_2 oxidoreductase complex in *Rps. sphaeroides* chromatophores.

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