EVIDENCE THAT THE IONIC CONDUCTIVITY OF THE CYTOPLASMIC MEMBRANE OF *RHODOPSEUDOMONAS CAPSULATA* IS DEPENDENT UPON MEMBRANE POTENTIAL

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1. Introduction

In mitochondria, chloroplasts and bacteria, a protonmotive force [1] ($\Delta\bar{\mu}_{H^+}$) across the coupling membrane is believed to be an energetic intermediate in electron-transport phosphorylation. The dependence of the magnitude of $\Delta\bar{\mu}_{H^+}$ upon the rate of electron transport is usually non-linear [2–6]. Here, I show that in intact cells of photosynthetic bacteria a voltage-dependent ionic conductance [2] probably accounts for this.

In steady-state, the rate at which $\Delta\bar{\mu}_{H^+}$ is generated by electron transport (J_{gen}) is equal to the rate at which it is dissipated by electrophoretic ion fluxes (J_{dis}). So that:

$$J_{gen} = J_{dis}$$

or

$$J_e \times n = G \times \Delta\bar{\mu}_{H^+} \quad (1)$$

where J_e is the rate of electron transport, n is the stoichiometry of H^+ translocated per electron transferred and G is the ionic conductivity of the membrane. If n is a constant and if G is independent of $\Delta\bar{\mu}_{H^+}$ then inhibition of the electron-transport rate should lead to a proportionate lowering of $\Delta\bar{\mu}_{H^+}$. However in several kinds of mitochondrial preparation [2–4], in thylakoids [5] and in membrane vesicles from *Paracoccus denitrificans* [6] the rate of coupled respiration can be substantially inhibited without significantly lowering $\Delta\bar{\mu}_{H^+}$. This could be because G is not constant but decreases with decreasing $\Delta\bar{\mu}_{H^+}$ (i.e., the 'current/voltage' relation of the membrane is non-Ohmic). This view was concurred with in [2,6] and several factors suggested to affect membrane conductivity. In [5] relation between the rate of photosynthetic electron transport and ΔpH in thylakoid membranes was found to be non-linear. It was concluded that the ionic conductance of the membrane was dependent on ΔpH [5]. In [4] data was obtained with mitochondria that were not compatible with this general explanation. Slip in the electron-transport proton pumps (variable n in eq. (1)) was proposed to provide a better explanation [4].

The ion distribution methods normally used to measure $\Delta\bar{\mu}_{H^+}$ are not fast enough to follow membrane ion currents. The electrochromic pigment shifts in photosynthetic membranes have an adequate time response [7]. Following single turnover flash excitation of either chloroplasts [8,9] or chromatophores [10,11] the decay of the electrochromic signal does not follow a single exponential, indicating that the conductivity of the membranes is non-Ohmic. The aim of these experiments was to use the electrochromic shift of photosynthetic bacteria to measure the J_{dis} *vs* $\Delta\bar{\mu}_{H^+}$ relation under conditions analogous to those commonly used in mitochondria and chemosynthetic bacteria, i.e., during the steady-state, in which eq. (1) applies.

Anaerobic suspensions of intact cells of *Rps. capsulata* have been used here under conditions in which $\Delta\bar{\mu}_{H^+}$ is almost entirely membrane potential, $\Delta\Psi$ [12]. It was found that the cytoplasmic membrane of the cells was non-Ohmic but that the conductance was steeply dependent on $\Delta\Psi$. In contrast, the current/voltage relation in FCCP-treated cells was approximately linear. These observations explain why a reduction in the rate of photosynthetic electron flow (by decreasing the actinic light intensity) led to a much less extensive fall in $\Delta\Psi$ in untreated cells than in partially uncoupled bacteria.

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2. Methods

Rps. capsulata strain N22 was grown to late exponential phase under anaerobic, photosynthetic conditions as in [13]. Cells were harvested and washed in a medium containing 10 mM Na_2HPO_4 (pH 7.0) with H_3PO_4 and stored on ice. Bacteriochlorophyll was determined in 7:2 acetone:methanol extracts [14].

Carotenoid absorption changes were measured in a cross beam spectrophotometer operating with a response time of 100 μs. The amplified signal from the photomultipliers was stored digitally. The 10 X 10 mm cuvette was completely filled with a medium containing 10 mM Na_2HPO_4, 30 mM Na-malate, 7 mM $(NH_4)_2SO_4$ (pH 7.0), pregassed with argon. The bacterial cells were added (with or without uncoupler or inhibitor) and the cuvette was stoppered with a tight-fitting teflon plug. The suspension (final vol. 3.7 ml) was incubated in the dark at room temperature for ~30 min to ensure thorough anaerobiosis. The actinic light was provided by a 150 W quartz halogen lamp. About 20% of the emitted light was collected, collimated and passed through 4 cm of water, a spring-loaded shutter and 2 layers of Wratten 88A gelatin filter before being directed with a mirror through the bottom of the cuvette. The intensity of the actinic light was decreased with neutral density filters whose transmission was measured at 850 nm.

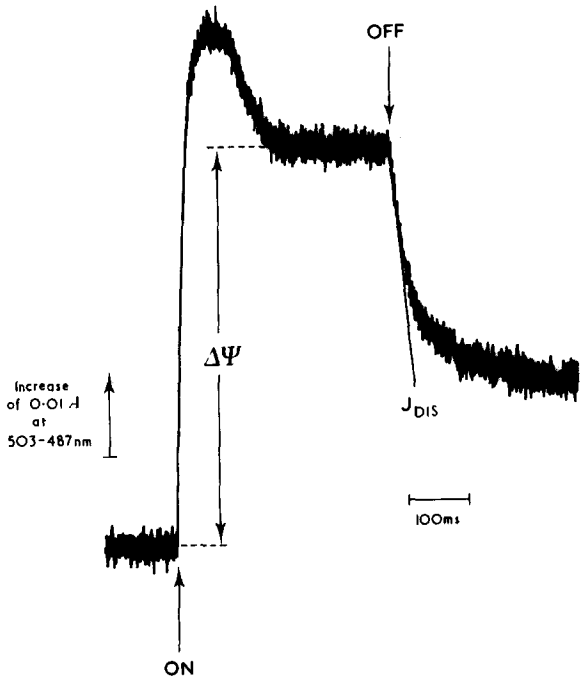

Fig.1. Carotenoid absorption changes induced by a short period of illumination in intact cells of *Rps. capsulata*. The anaerobic cell suspension containing 11.9 μM bacteriochlorophyll was prepared as in section 2. The suspension was illuminated and darkened where indicated by manual opening and closing of the shutter. The initial rate of decay upon darkening gives the membrane current (J_{dis}) for the steady-state value of $\Delta\Psi$.

3. Results

3.1. *The current/voltage curves for the cytoplasmic membrane of intact cells of* Rps. capsulata

The carotenoid absorption bands of many species of photosynthetic bacteria are shifted to slightly longer wavelengths during illumination with photosynthetic light [7]. Fig.1 shows characteristic data for intact anaerobic cells of *Rps. capsulata*. When the actinic light was switched on, the carotenoid absorption change reached a maximum value in ~0.06 s. Following a small transient decline the carotenoid absorption reached a steady-state value in ~0.25 s. When the actinic light was extinguished the carotenoid absorption decayed at first rapidly and then more slowly to its original dark level. These data were accurately reproducible on the same suspension of cells for several hours provided that the time between the illumination periods was $\geqslant$2 min.

There is adequate evidence to show that the carotenoid band shifts are a rapid and linear response to membrane potential [7]. It was decided to determine the current/voltage curve in the steady-state of illumination when the carotenoid shift (and therefore $\Delta\Psi$) had become constant (eq. (1)). The membrane potential (in units of carotenoid absorption change) can be read off directly from fig.1. The corresponding value of the dissipative ionic current, J_{dis} is given by the initial rate of decay of the carotenoid absorption change at the instant at which the shutter is closed.

The experiment shown in fig.1 was repeated on the same sample of bacteria at a series of lower actinic light intensities. In each case the cells were illuminated until the carotenoid shift reached a steady-state and then the suspension was darkened and the initial rate of decay was measured. The dependence of the initial rate of decay of the carotenoid shift upon the steady-state magnitude of the shift before darkening is shown in fig.2. This is equivalent to the current/voltage relation for the cytoplasmic membrane in units of carot-

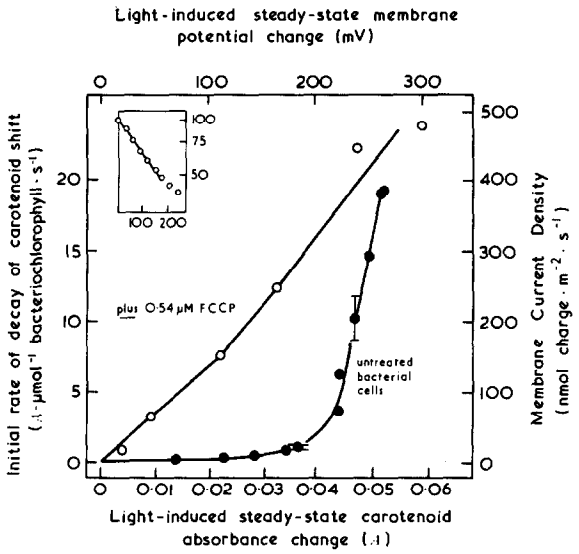

Fig.2. Current/voltage relationship for the cytoplasmic membrane of *Rps. capsulata*. Taken from a series of data similar to that in fig.1 in which the actinic light intensity was varied with neutral density filters. The left and lower axes are expressed in units of carotenoid absorption change. The right and upper axes were calculated as described in the text. The error bars shown on 2 of the points represent standard deviations in the visual estimation of the initial rate of decay from 5 meas. The inset shows a log plot of the decay of the carotenoid shift (100 units is equivalent to 10.8 A . μmol bacteriochlorophyll^{-1} . s^{-1}) as a function of time (ms) after closing the shutter. The first part of the decay can be approximated by a single exponent.

enoid absorption (an estimate of more conventional units is described below). The profile is not linear but reveals a steep dependence of the ionic conductance upon membrane potential ($G = f(\Delta\bar{\mu}_{H^+})$ in eq. (1)).

When the experiment was repeated in the presence of a low concentration of the uncoupler FCCP (fig.2) the current/voltage relation became almost linear (G = constant in eq. (1)). The low concentration of FCCP used in this experiment had an insignificant effect upon the membrane conductance at high values of $\Delta\Psi$ but led to large increases in net G at low $\Delta\Psi$.

3.2. *Quantitative evaluation of the current/voltage relation*

The axes of fig.2 were converted from carotenoid absorption change units into conventional units by the following procedures.

3.2.1. Membrane current density

The bacteria were treated with 2.7 μM antimycin A and the carotenoid band shift generated by a single, saturating 20 μs flash was measured and found to be 0.23 A/μmol bacteriochlorophyll. Under these conditions almost all of the photosynthetic reaction centres in the suspension catalyse the separation of 1 charge across the membrane [15]. The reaction is so rapid that it is not obscured by the much slower discharging processes. In the photosynthetic membranes of *Rps. capsulata* there is av. 1 reaction centre/370 nm^2 [16]. Therefore the carotenoid absorbance change of 0.23 A/μmol bacteriochlorophyll is generated by the translocation of 1/370 charges/nm^2. The rate of discharge (J_{dis}) in charges . nm^{-2} . s^{-1} follows directly (see [8] for a similar calculation based on similar assumptions in thylakoid membranes).

3.2.2. Membrane potential

The light-induced carotenoid shift in intact cells can be calibrated by applying a series of K^+-diffusion potentials to chromatophores subsequently prepared from the cells [17]. The membrane potential axis of fig.2 was estimated from the data of [17] in which the cells were grown and harvested by similar procedures. The carotenoid shift elicited by a 20 μs flash in antimycin-treated cells was used as the normalising standard.

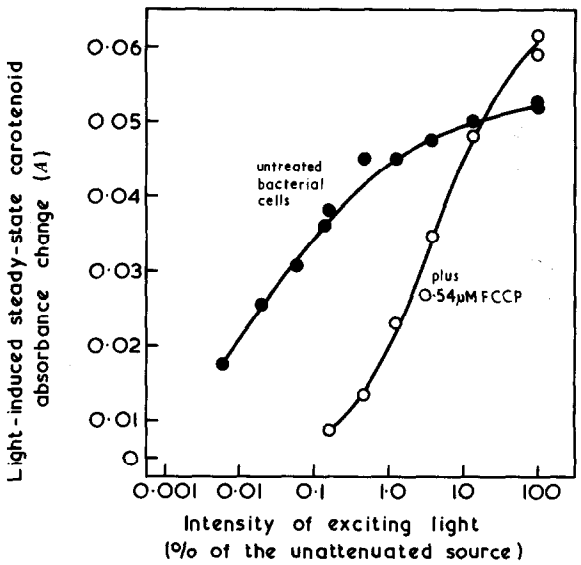

Fig.3. Light intensity dependence of the light-induced carotenoid shift in intact cells of *Rps. capsulata*. Taken from the series of experiments used in fig.1 and 2.

3.3. *Light-intensity dependence of the carotenoid band shift*

During the collection of data for fig.2 it became clear that the steady-state magnitude of the membrane potential in untreated bacterial cells was rather insensitive to the intensity of the actinic light source: to reduce the membrane potential by 50% it was necessary to attenuate the actinic source to <0.1% of the original intensity. The complete set of data is plotted in fig.3. The situation changed dramatically in the presence of low concentrations of FCCP. The steady-state membrane potential became much more sensitive to actinic light-intensity (fig.3). Reduction of 50% of $\Delta\Psi$ was achieved by attenuation of the source to ~3% of the original intensity.

4. Discussion

The current/voltage relation for untreated, intact cells of *Rps. capsulata* is non-linear. The conductance is strongly dependent upon membrane potential, varying from ~700 mS/m at $\Delta\Psi$ = 250 mV to <5 mS/m^2 at $\Delta\Psi$ = 50 mV (calculated from fig.2). This pronounced non-Ohmic behaviour occurs across the normal range of membrane potential developed by photosynthesising cells at 'physiological' light intensities. Note that electrochromic measurements yield values of membrane potential in intact cells ~1.7-times higher than those determined by the redistribution of phosphonium salts [17]. The results support the postulation of non-Ohmic behaviour of energy-coupling membranes [2,3,5]. The alternative proposal of variable thermodynamic efficiency in the proton pumps [4] is perhaps unnecessary.

The finding that extensive inhibition of electron transport leads to only a small drop in $\Delta\bar{\mu}_{H^+}$ in a variety of organelles and bacteria [2–6] testifies to the generality of the response. It is not easy to demonstrate directly in purple bacteria because the photosynthetic electron-transport chain is cyclic. Nevertheless the data of fig.3 are probably an expression of this effect – when photosynthetic electron flow is restricted by lowering the intensity of the actinic light, the membrane potential is not strongly depressed. The drop in membrane potential (0.052–0.044 carotenoid absorption units) is minimal in the region of high membrane conductance (compare fig.2), in accordance with the above thesis. In the enforced absence of data for the rate of cyclic electron transport, the weak dependence of membrane potential on light intensity (fig.3) could otherwise be attributed to insensitivity of the electron flow rate to light intensity but this possibility can be excluded by the effect of FCCP on the profile. The steeper dependence in FCCP-treated cells (fig.3) can be explained by the finding that this protonophore has a voltage-independent conductance in the cytoplasmic membrane of the bacteria (fig.2): from eq. (1) a decrease in the rate of photosynthetic electron transport (J_e) at constant G (and n) should lead to an equivalent fall in $\Delta\bar{\mu}_{H^+}$.

The non-Ohmic current/voltage relation of fig.2 is probably another aspect of an apparent $\Delta\Psi$ dependence for a fast component in the decay of the carotenoid shift seen in pre-steady-state conditions after short actinic light flashes [18]. From the sensitivity of the fast decay component to venturicidin it was concluded that H^+ flux through the F_0F_1-ATPase and consequent ATP synthesis only takes place when a threshold membrane potential is exceeded [18]. Extending this conclusion to these data, the increasing conductivity at high values of $\Delta\Psi$ may be due to a disproportionate increase in the proton flux through the ATPase. A similar increase in the phosphorylating proton flux at high $\Delta\bar{\mu}_{H^+}$ was proposed [20]. From experiments on the rate of H^+ transport in response to artificially imposed K^+-diffusion potentials [19] it was argued that the ATPase in the cytoplasmic membranes of *Streptococcus lactis* also has a gated response. These experiments demonstrate the changing membrane conductivity directly. We are currently pursuing experiments with ATPase inhibitors with the view to assessing the contribution of proton flux through the ATPase to the total ionic current across the membrane.

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