Threshold dependence of bacterial growth on the protonmotive force

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The specific growth rate of phototrophic cultures of *Rhodopseudomonas capsulata* has a threshold dependence on the cytoplasmic membrane potential the major contributor to the protonmotive force. There is a strong correlation between the dependence of the membrane ionic current, the rate of ATP synthesis and the specific growth rate on the value of the cytoplasmic membrane potential.

ATP synthesis *Membrane potential* *Bacterial growth* Rhodopseudomonas capsulata *Photosynthesis*

1. INTRODUCTION

It is generally recognised that a protonmotive force (Δp) is an important energetic intermediate in the regeneration of ATP by electron transport. The chemiosmotic hypothesis [1] states explicitly that it is a central and obligate intermediate whereas in other models of energy coupling Δp is assigned to a parallel or side reaction. Free living bacteria are exposed to fluctuations in the supply of electron transport substrates and in the biosynthetic demand for ATP but it may be necessary to maintain within limits the Δp across their cytoplasmic membrane (see [2–5]). In photosynthetic bacteria, the passive ionic current across the cytoplasmic membrane (J_{dis} has a non-ohmic dependence on its driving force, the membrane potential ($\Delta\psi$) [6,7], usually the main contributor to Δp. This might be a device to confine Δp within limiting values. For chromatophore membranes from photosynthetic bacteria it was concluded that the conductance properties are dominated by proton flux through the ATP synthase [7]. The rate of ATP synthesis had a threshold dependence on $\Delta\psi$ which correlated with the flow of ionic current across the membrane. If a similar situation exists in intact bacterial cells then, since ATP synthesis is likely to be a major determinant in bacterial growth, we should expect to see a threshold dependence of specific growth rate on the magnitude of $\Delta\psi$. Evidence for such a relationship is presented below.

Abbreviations: Δp, electrochemical potential difference of H^+ across the membrane; $\Delta\psi$, electric potential difference across the membrane; J_{dis}, dissipative ionic current across the membrane; μ, specific growth rate; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone

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2. MATERIALS AND METHODS

Rhodopseudomonas capsulata was grown phototrophically on RCV medium [8]. Cells were harvested in late exponential phase, washed and resuspended in 10 mM sodium phosphate buffer (pH 7.0) and stored on ice for no more than 6 h before use. Bacteriochlorophyll concentrations were determined by extraction with acetone/methanol [9].

Electrochromic measurements of the dependence of J_{dis} on $\Delta\psi$ were performed as in [6,10]. The experiments were performed under argon in 2.5 ml fresh RCV medium in a 1×1 cm cuvette at

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30°C at a final bacteriochlorophyll concentration of either 10 or 40 μM (see figure legends). Each data point was taken from a single recording of the light-induced change in % $\Delta T_{503-487\,nm}$ with a rapidly responding dual-wavelength spectrophotometer. $\Delta\psi$ was estimated from the extent of the change in steady state. The value of $\Delta\psi$ was corrected for the potential which exists in these bacteria even under dark, anaerobic conditions as revealed by the addition of 5 μM FCCP at the end of the experiment [11]. J_{dis} was estimated from the initial rate of decay of the transmittance change on extinguishing the light [6]. In each case the illumination period was 30 s [10]. In successive recordings performed 5 min apart on the same sample, the photosynthetic light intensity was reduced with calibrated neutral density filters. Maximum light intensity was obtained from a 150 W quartz-halogen lamp collimated with an appropriate lens system and passed through 5 cm water and 2 thicknesses of Wratten 88A gelatin filter (approx. 3.5×10^5 erg $\cdot$ cm$^{-2}\cdot$ s^{-1} as measured with a silicon photodiode calibrated against a Hewlett Packard thermopile). Transmittance changes were stored digitally on a split time base to facilitate measurement on both a fast and slow scale.

Specific growth rates were determined in an identical cuvette in the same spectrophotometer housing. Sterile pre-cultures were grown anaerobically with magnetic stirring at maximum photosynthetic light intensity until absorbance measurement showed that the bacteriochlorophyll concentration reached approx. 10 μM. During this time exponential growth was established. The light intensity was then abruptly reduced and the absorbance was sampled over the next few hours. The reduced light intensities were chosen to match those employed in the electrochromic measurements.

ATP synthesis was determined essentially as described [10]. The bacterial suspension in fresh RCV medium (40 μM bacteriochlorophyll) after a 45 min dark, anaerobic preincubation in a 1.2 cm diameter clear plastic syringe was positioned in the spectrophotometer housing so that it would receive the same photosynthetic light intensity as the bacteria used in the accompanying set of electrochromic measurements. Following an illumination period of either 0, 2, 3, 4, or 60s the entire contents of the syringe (2.5 ml) were injected into 1.5 ml ice-cold 14% perchloric acid. The samples were neutralised, centrifuged, frozen and assayed with a crude luciferase preparation [10]. Initial rates of ATP synthesis were calculated from the 0–4 s data points by regression to a straight line.

3. RESULTS

In intact cells of *Rps. capsulata* the dependence of ionic current on membrane potential can be followed from the electrochromic absorbance changes of endogenous carotenoid pigments [6,10]. In fig.1A, J_{dis} and $\Delta\psi$ were recorded in the steady state reached after 30 s of photosynthetic illumination. By this time transient changes in J_{dis} and $\Delta\psi$ at the onset of illumination, which can probably be attributed to activation of the ATP synthase [10], have subsided. From 30 s of illumination to at least several minutes the value of $\Delta\psi$ remained constant. Upon extinguishing the light the initial rate of relaxation of the carotenoid absorbance change gives a measure of that value of J_{dis} which is appropriate to the steady-state value of $\Delta\psi$. Fig.1A shows the dependence of J_{dis} on $\Delta\psi$, the 'current/voltage' curve of the bacterial membrane, for a series of experiments performed at progressively decreased light intensities. As explained in [7] reduction of photosynthetic light intensity leads to a heterogeneity in the sample which complicates detailed analysis. This distortion however, does not affect the qualitative significance of the results. In agreement with earlier data [6,10] the membrane ionic current had a 'diodic' dependence on $\Delta\psi$: the ionic conductance of the membrane increased with $\Delta\psi$.

Specific growth rates (μ) of the bacteria were measured in spectrophotometer cuvettes under conditions which closely resembled those employed in the current/voltage determinations. The objective was to express μ measured from the absorbance of the growing culture (fig.1B) as a function of $\Delta\psi$ measured electrochromically. Because the effective light intensity decreases due to self-shading effects in the growing culture and because these bacteria can respond to light intensity changes by altering the size of their photosynthetic antenna [12],for comparison with the short time-scale electrochromic data μ was estimated immediately following the abrupt reduction in the incident light intensity. The accuracy of the extrapolation was

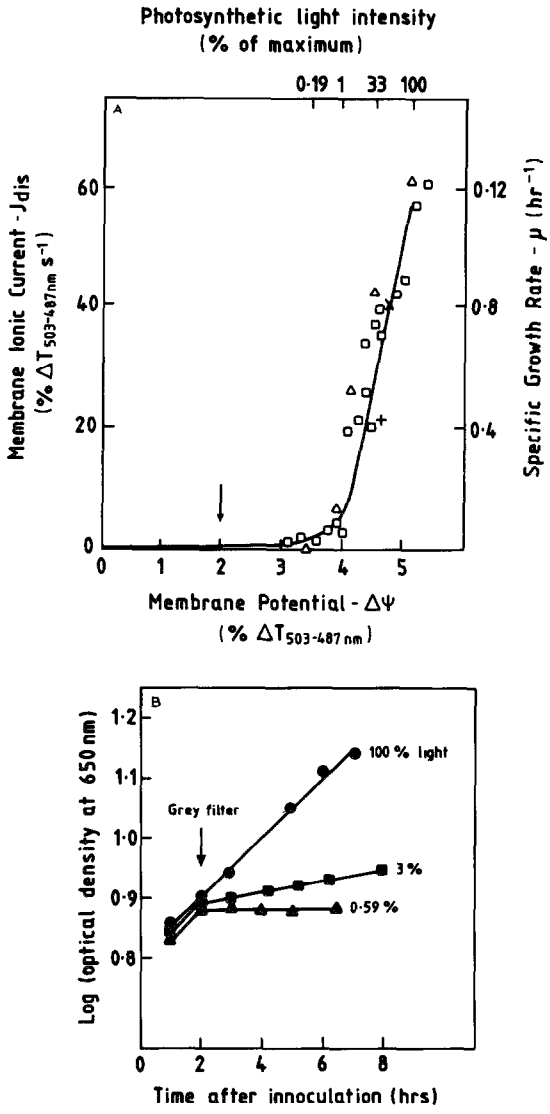

Fig.1. Dependence of membrane ionic current (J_{dis} and specific growth rate (μ) on membrane potential ($\Delta\psi$). The dependence of J_{dis} on $\Delta\psi$ (□, A) was recorded from carotenoid transmittance changes at 503–487 nm. The arrow (A) shows the level of $\Delta\psi$ which exists in dark, anaerobic cells (see section 2). The bacteriochlorophyll concentration was 10 μM. The dependence of μ on $\Delta\psi$ (Δ, A) was determined in an identical cuvette with the same maximum intensity of illumination (see section 2, B), When the bacteriochlorophyll concentration reached 10 μM (arrow in B) the light intensity was reduced to the same levels as had been employed in the electrochromic measurements (shown as a percentage of the maximum light intensity in B). Specific growth rates from data similar to that from B (see text) were plotted against the electrochromically determined $\Delta\psi$ (Δ, A). Points x and + (A) show the effect of myxothiazol (0.018 μM) and FCCP (1.0 μM), respectively, on the relationship between μ on $\Delta\psi$. Their effect on μ was determined in experiments similar to those shown in B: the reagents were added as methanolic solutions (final methanol concentration < 1%) instead of the light intensity reduction. In separate experiments the effects of the reagents on $\Delta\psi$ were determined from the steady-state extent of the carotenoid band shift.

justified by the simple logarithmic dependence shown in the figure. The dependence of μ on $\Delta\psi$ is plotted in fig.1A. The specific growth rate achieved at the highest $\Delta\psi$ recorded in these experiments was equivalent to a biomass doubling time of 5.7 h. Even a slight reduction of $\Delta\psi$ led to a pronounced decrease in growth rate. Similar results were obtained whether $\Delta\psi$ was lowered by reducing the photosynthetic light intensity or by treating the cells either with a low concentration of myxothiazol partially to inhibit the cyclic electron transport chain or with the protonophore FCCP to increase the passive membrane H^+ conductance (fig.1A). At $\Delta\psi < 0.67$ of the maximum, growth was extremely slow. This represents a threshold region of $\Delta\psi$ for growth. A correlation between the specific growth rate and the membrane ionic current is evident from fig.1A. The normalised values of μ and J_{dis} expressed as a function of $\Delta\psi$ coincide.

In chromatophore membranes isolated from these bacteria there is a major contribution to J_{dis} from proton translocation through the ATP synthase during photophosphorylation [7]. ATP synthesis in intact bacteria is difficult to measure because of simultaneous consumption during metabolism and biosynthesis. As an indication of the in vivo rate of ATP synthesis and its dependence on $\Delta\psi$, the initial rate of change at the onset of illumination and the level of ATP after 60 s illumination were measured as a function of light intensity. The experiments were performed under similar conditions to a set of electrochromic measurements of J_{dis} and $\Delta\psi$. A higher density of bacterial cells than in fig.1 was used to increase the sensitivity of the ATP assays. The results are shown in fig.2. Both the initial rate of change of

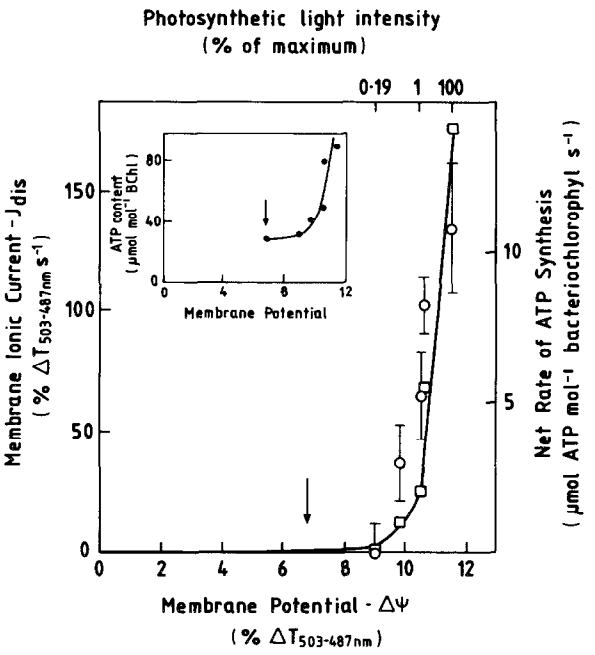

Fig.2. The dependence of membrane ionic current and changes in the cellular ATP content on membrane potential. The dependence of J_{dis} on $\Delta\psi$ (□) was followed as in fig.1A except that the bacteriochlorophyll concentration was 40 μM. The cytoplasmic ATP levels were estimated under similar conditions of light intensity (see section 2). The apparent rate of ATP synthesis was estimated from the level of ATP after 0, 2, 3 and 4 s of illumination by regression to a straight line and plotted against the electrochromically determined $\Delta\psi$ (○). The error bars show 90% confidence limits. The inset shows the dependence of the ATP the level after 60 s illumination on the steady-state value of $\Delta\psi$.

the ATP level upon illuminating and the level after 60 s in the light (inset) had a threshold dependence on $\Delta\psi$ which was related to the dependence of J_{dis} on $\Delta\psi$.

4. DISCUSSION

Cyclic electron transport in chromatophores of *Rps. capsulata* leads to the translocation of protons with a stoichiometry which is independent of the magnitude of $\Delta\psi$ [13]: there is no significant slip in the electron transport-driven proton pump (cf. predictions from the application of the linear equations of non-equilibrium thermodynamics [14]. It can therefore be assumed that the steady-state value of J_{dis} is directly proportional to the electron transport rate. On this basis the decrease in specific growth rate following a reduction of photosynthetic light intensity in the manner described by fig.1 can be explained. Fig.1A shows that a large decrease in the photosynthetic light intensity (e.g. from 100 to 1% of the maximum) leads to a considerable fall in the steady-state electron transport rate (90% from the maximum value). Because of the diodic properties of the membrane, this fall in the rate of electron transport and associated proton translocation produces only a small drop in the value of $\Delta\psi$ (a reduction of only 26% of $\Delta\psi$ for a 100-fold decrease in light intensity from the maximum): the protonmotive force (equal to $\Delta\psi$ under the conditions of our experiments [6,15] is conserved. However, the gated properties of the ATP synthase ensure that even the small drop in $\Delta\psi$ results in a large decrease in the rate of ATP synthesis (e.g. (fig.2) and as a result the growth rate, which depends crucially on ATP synthesis, is strongly inhibited. The control point in the sequence of events leading to the threshold dependence of growth rate on $\Delta\psi$ is the ATP synthase. Experiments with the ATP synthase inhibitor, venturicidin, have shown that in intact bacterial cells more than 60% off the total membrane ionic current is a proton flux through this enzyme [7]. Thus, the ionic gating of the ATP synthase dominates the conductance properties of the membrane and is responsible for the homeostatic control of $\Delta\psi$. The survival value of this device may be 2-fold: (i) wasteful hydrolysis of ATP is prevented by severe reduction in enzyme activity when $\Delta\psi$ falls below the value which is necessary thermodynamically to drive net ATP synthesis; (ii) $\Delta\psi$ is reserved under non-growing conditions for the maintenance of basal cellular functions not directly involving ATP, including for example, osmotic regulation and motility.

At extremely low photon fluence rates the intrinsic proton permeability (leakage) of photosynthetic membranes might represent a significant loss factor in energy conversion [16]. It is possible that such losses are confined to those values of $\Delta\psi$ at which μ, J_{dis} and the rate of ATP synthesis become vanishingly small (e.g. $<70\%$ of the maximum $\Delta\psi$ in our experiments). Thus at low photon fluence rates, although significant values of $\Delta\psi$ can be attained, the electron transport-driven proton flux

across the membrane is only sufficient to overcome the intrinsic proton leak: the magnitude of $\Delta\psi$ might partly determine whether the bacteria begin to grow or whether only maintenance functions are carried out.

The correlation between J_{dis} and μ and the threshold $\Delta\psi$ for growth can be uniquely demonstrated in phototrophic bacteria owing to the convenience of the electrochromic determinations and because of the ease with which the energy supply can be adjusted through alterations in light intensity. It seems likely that bacteria which rely on oxidative phosphorylation for the regeneration of ATP will display similar characteristics although the proof of this might be difficult to establish. Fermenting organisms in which Δp is generated by ATP hydrolysis might be expected to behave differently. Indeed it was found that in *Streptococcus cremoris* Δp was independent of growth rate [17] or varied inversely with growth rate [18] depending on the culture conditions.

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