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Swimming speed and chemokinetic response of *Rhodobacter sphaeroides* investigated by natural manipulation of the membrane potential

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Abstract

The $\Delta\Psi$ of *R. sphaeroides*, grown under high light to reduce the levels of light-harvesting bacteriochlorophyll, was naturally manipulated using light intensity. The relationship between $\Delta\Psi$ and the swimming speed of free swimming populations of cells was investigated. After de-energisation by incubation in the dark there was an apparent threshold of about −13 mV which had to be overcome before functional motor rotation could resume and at −45 mV the motor saturated. Further increases in $\Delta\Psi$ over −45 mV did not increase the free swimming velocity. However, when a chemokinetic effector was added there was an increase in swimming speed, even though the $\Delta\Psi$ was well above saturation, indicating that the chemokinetic response is independent of normal relationship between motor rotation and $\Delta\Psi$.

Key words: Rhodobacter sphaeroides; Motility; Bacteria; Membrane potential; Flagellar motor; Chemokinesis

1. Introduction

The bacterial flagellum is rotated at its base by a rotary motor which is driven by the electrochemical ion gradient (usually protons, Δp) [1–3]. Considerable data has been gathered on the structure and function of the motor and flagellum; for recent reviews see [4–7]. The means by which electrochemical energy is converted into thrust by the motor is unknown. In order to understand this, the investigation of the relationship between the proton flux and the power output of the flagellar motor provides a means of studying the mechanism of the motor. Experiments have looked at body rotation of cells tethered to glass by a single flagellum, free swimming cells and the measurement of the rotation rate of the flagellar bundle [3,8,9]. But in most experiments cells have been artificially deenergised, by use of uncouplers and ionophores, and reenergised, by pH or ion jumps. In this study the effect on thrust generation by the flagellar motor of *Rhodobacter sphaeroides* of naturally manipulating the $\Delta\Psi$ has been investigated.

The purple non-sulphur photosynthetic bacterium *R. sphaeroides* has a single flagellar motor which stops or rotates, unlike the motor found in *Escherichia coli* which switches direction of rotation [10,11]. The motor is able to stop instantaneously, while Δp is maximal, and stopping frequency is under the control of the chemosensory system (Packer, Gauden and Armitage, unpublished). In addition, the rate of rotation of the flagellar motor is increased, i.e. shows chemokinesis, by the addition of certain chemoeffectors e.g. organic acids [12]. As each cell has only a one motor, by studying this organism the properties and performance of an individual motor can be elucidated. In this organism it is possible to manipulate the $\Delta\Psi$ using light intensity and measure the resulting $\Delta\Psi$ non-invasively by measuring the electrochromic bandshift of the carotenoids. The ability to 'naturally' manipulate and readily quantify $\Delta\Psi$ has enabled a direct study on the performance of the flagellar motor of *R. sphaeroides*.

2. Materials and methods

2.1. Growth media and conditions

R. sphaeroides WS8 was grown in 200 ml medical flat bottles anaerobically as previously described [13]. However, for this experiment the cells were grown at three different light intensities: 8, 20 and 120 $\mu M \cdot m^{-2} \cdot s^{-1}$. The bright light intensity (120 $\mu M \cdot m^{-2} \cdot s^{-1}$) was achieved by placing the culture in a water bath containing copper sulphate (to prevent overheating) between two opposing tungsten filament light sources. Cell growth was followed using a Coulter Counter and the cultures were analysed in the mid-exponential phase. The bacteriochlorophyll concentration was also measured [14]. All experiments were carried out at pH 7.2 where almost all of the Δp has been shown to be as $\Delta\Psi$.

2.2. Membrane potential measurements

The membrane potential ($\Delta\Psi$) was determined by measuring the electrochromic absorbance shift of the membrane-bound carotenoids at 523 nm with respect to 510 nm. The absorbance was measured using a DW2000 dual-wavelength spectrophotometer (SLM-Aminco, USA) as described previously [13]. $\Delta\Psi$ was calibrated by using the absorbance change after the addition of K^+ to chromatophores suspended in buffer (10 mM $MgCl_2$, 40 mM Na_2HPO_4, pH 7.4) treated with valinomycin as previously described [13].

2.3. Measurement of motility

Samples of cells in medium were withdrawn directly from the culture into optically flat microslides (0.05 mm diameter, Camlabs). The microslides were sealed at both ends with vaseline to maintain the anaerobic environment. Free swimming cells were tracked and their motile behaviour measured automatically using a computerised motion analysis system as described previously [15]. The light intensity of the microscope was adjusted using neutral density filters and the intensity was

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measured using a light meter (Skye Instruments). Under dim light (< 10 $\mu M \cdot m^{-2} \cdot s^{-1}$) the image analysis system was unable to detect motile cells, therefore, motility was analysed manually by recording the images onto a video-tape (Sony Umatic video recorder) and then tracing the tracks of the cells individually onto acetate sheets. The mean run speed (the velocity between stops) of each population of cells was determined.

3. Results

The growth of *R. sphaeroides* when incubated under three different light intensities, in bright light (120 $\mu M \cdot m^{-2} \cdot s^{-1}$), in dim light (8 $\mu M \cdot m^{-2} \cdot s^{-1}$) and under normal conditions of illumination (20 $\mu M \cdot m^{-2} \cdot s^{-1}$) was found to be light-limited with a mean doubling time of 75 min under bright light whereas under dim light the mean doubling time was 600 min. The amount of bacteriochlorophyll synthesised under the two extreme light conditions was measured during growth. Cells grown in dim light contained $-11.03 \times \log[BChl]$ μM/cell, approximately four times the amount of bacteriochlorophyll found in cells grown in bright light ($-11.65 \times \log[BChl]$ μM/cell), presumably to maximise the amount of light harvested in a light-limited environment. In bright light, less bacteriochlorophyll was required to harvest the plentiful light to meet the cell's energy requirements.

The motility of cells grown under bright and dim light was measured in bright light and found to be identical, with mean swimming speeds of 17.2 ± 1.8 $\mu m \cdot s^{-1}$ and 15.2 ± 1.4 $\mu m \cdot s^{-1}$, respectively. When dim light grown cells were transferred to bright light their motile behaviour was not altered. However, when cells grown in bright light were transferred to dim light, their mean swimming speed was reduced to 9.5 ± 1.3 $\mu m \cdot s^{-1}$ and the motion of these cells appeared to be jerky, with more frequent stoops than cells incubated under high light conditions. These cells were probably swimming at sub-optimal speeds because under dim light the reduced bacteriochlorophyll concentration was unable to harvest sufficient light for maximum reaction centre turnover and the Δp was therefore reduced below that required for saturation of the flagellar motor. Therefore the relationship between swimming speed and $\Delta\Psi$ was investigated using light intensity to naturally manipulate the $\Delta\Psi$ in cells grown under bright light.

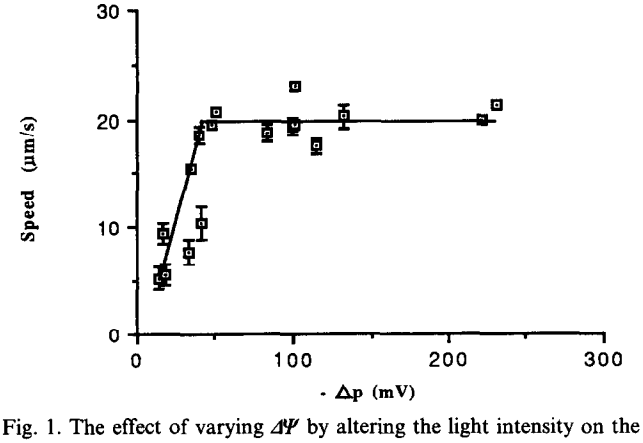

Fig. 1. The effect of varying $\Delta\Psi$ by altering the light intensity on the mean population run speed of *R. sphaeroides*. The data shown is the mean of three populations and the error bars represent one standard derivation. The measurement of mean run speed was made over the first 3 min after exposure to each light intensity and a new sample of cells was used at each intensity.

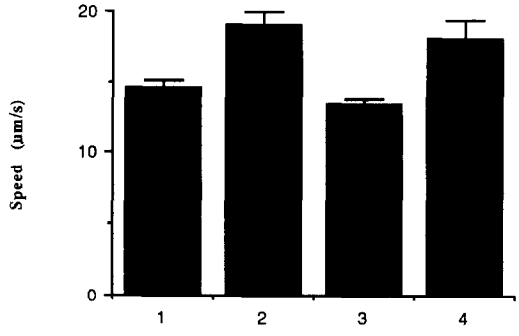

Fig. 2. Observation of chemokinesis when Δp was above saturation for motor rotation. The cells were harvested and suspended in N_2 sparred 10 mM HEXES (pH 7.2) and 50 μg/ml chloramphenicol. The cells were starved for 1 h before the measurement of the mean population run speed in the presence and absence of 1 mM pyruvate. (1) Cells grown in bright light (120 $\mu M \cdot m^{-2} \cdot s^{-1}$) measured in the absence of pyruvate. (2) Cells grown in bright light measured in the presence of 1 mM pyruvate. (3) Cells grown in dim light (8 $\mu M \cdot m^{-2} \cdot s^{-1}$) measured in the absence of pyruvate. (4) Cells grown in dim light measured in the presence of 1 mM pyruvate.

The effect of varying light intensity on the speed and on $\Delta\Psi$ is shown in Fig. 1. The results showed a linear relationship between $\Delta\Psi$ and swimming speed up to approximately −45 mV. Above −45 mV, the swimming speed was independent of $\Delta\Psi$ and remained constant when $\Delta\Psi$ was increased. −45 mV therefore represented a saturation point above which the cells swam at optimal speeds and below which the speed was dependent upon the value of $\Delta\Psi$. At a potential of −13 mV motion was approximately equal to Brownian motion. This may represent a value below which the cells were unable to swim. *R. sphaeroides* grown in dim light was able to swim at optimal speed over the full range of light intensities used in this experiment.

In a previous study *R. sphaeroides* has been shown to increase the rate of flagellar rotation in response to the presence of certain chemoeffectors, such as the organic acids [16] and previous work has suggested that this chemokinetic response is independent of Δp [17]. Cells grown in bright light were incubated under high light where the $\Delta\Psi$ was maximal and the flagellar motor was above its saturation point, and 1 mM of the chemokinetic effector pyruvate was added to cell suspension and the effects of this were examined (Fig. 2). The mean swimming speed of the cells increased by over 30% confirming that the increase in swimming speed, shown to be a direct result of an increase in flagellar rotation, was

Table 1
The measured thresholds and saturation values of Δp for the flagellar motors of different species of bacteria

Species	Motility state	Threshold Δp (mV)	Saturation Δp (mV)	Method of energisation
R. sphaeroides	free-swimming	−13	− 45	light
B. subtilis [8]	free-swimming	−30	− 60	DNP
B. subtilis [9]	free-swimming	−30	−100	K^+ diffusion
B. subtilis [9]	tethered	−30	− 80	K^+ diffusion
Streptococcus [19]	tethered	−10/none	not found (above 100 mV)	K^+ diffusion

DNP = dinitrophenol.

independent of $\Delta\Psi$, and, as the speed increase was maintained for several hours, it was not subject to adaptation.

4. Discussion

A natural manipulation of $\Delta\Psi$ was conducted on cells of *R. sphaeroides* with reduced quantities of bacteriochlorophyll by varying the light intensity. During all measurements the external pH was maintained at 7.2 and it has been shown that at an external pH of 7.2 the intracellular pH of cells of *R. sphaeroides* is also 7.2; therefore under these conditions Δp is equivalent to $\Delta\Psi$, and thus a natural manipulation of Δp was achieved. The relationship between $\Delta\Psi$ and the swimming speed of the population was studied. A threshold of −13 mV was determined for flagellar motor rotation in free swimming cells and a saturation $\Delta\Psi$ of −45 mV. Further increases in $\Delta\Psi$ above this did not increase the rate of rotation of the flagellar. Values for the saturation and threshold Δp for motor rotation have also been determined in *Bacillus subtilis* and *Streptococcus sp.* and are shown for comparison in Table 1.

The saturation of Δp of *R. sphaeroides* (−45 mV) was lower than those found for other organisms and below this a linear relationship was found between swimming speed and Δp. The technique used to measure $\Delta\Psi$ the carotenoid bandshift is known to give higher values than the methods used for the other organisms in Table 1, therefore the low values obtained for *R. sphaeroides* are, if anything, an overestimate. The non-invasive technique used to measure $\Delta\Psi$ gives more reliable data at low values and this combined with the method used to naturally manipulate $\Delta\Psi$ probably means the data are reliable. The differences in the values may reflect species differences as all previous measurements have been conducted on Gram-positive cells whereas *R. sphaeroides* is Gram-negative. In addition, free swimming *B. subtilis* use a bundle of flagellar filaments and the higher values may reflect the problems of coordination of the different filaments at low Δp. The behaviour of tethered cells is expected to be different as the load on the motor caused by rotating a cell body rather than the flagellar bundle is much greater. The motors of tethered cells of *Streptococcus* do not appear to saturate which is probably due to the load on the motor and may reflect these differences.

Free swimming cells of *R. sphaeroides*, once deenergised by incubation in the dark, appear to require a threshold Δp before flagellar rotation can resume. A similar delay to motility has been found previously in *R. sphaeroides* [18] with a lag between light-induced energisation and motility after anaerobic incubation in the dark and also after the addition of the uncoupler CCCP. In tethered cells a threshold for motor rotation was identified when cells were energised but not when they were deenergised [19]. It has been postulated that the barrier could be a structural change in the motor apparatus.

Most results conclude that there is a linear relationship between Δp and flagellar rotation, as a result of the tight coupling of the motor. The ability of *R. sphaeroides* to swim faster under certain conditions even though the motor is theoretically saturated is therefore interesting. We confirmed that chemikinesis occurs when the driving force for the motors is well over that required to apparently saturate the motor and is therefore probably independent of the normal relationship between $\Delta\Psi$ and motor rotation. It is possible that transport of the effectors result in physical changes in the structure of the motor perhaps increasing the number of Mot proteins interacting with the motor.

This study has used the natural manipulation of $\Delta\Psi$ and thus of Δp to characterise the relationship between the driving force and flagellar rotation in *R. sphaeroides* under natural and chemokinetically stimulated conditions.

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