The *Rhodobacter sphaeroides* flagellar motor is a variable-speed rotor

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Abstract The rotation rate of the unidirectional stop/start motor of *Rhodobacter sphaeroides* was investigated using computerised motion analysis of tethered cells. The *R. sphaeroides* motor was found to have a variable rotation rate compared to the virtually constant-speed motor of wild-type and CheR mutant (smooth swimming) *Escherichia coli*. In addition, the dynamics of the *R. sphaeroides* motor during stopping was analysed with no consistent correlation behaviour. The motor could go from full rotation to stop, or stop to full rotation within one video frame, i.e. 0.02 s, but it could also slow down into a stop or restart slowly, taking up to 0.25 s. The *R. sphaeroides* motor under chemokinetic stimulation was also analysed and was found to show increased torque generation and reduced variation in rotation rate.

Key words: *Rhodobacter sphaeroides*; Chemokinesis; Flagellar motor; Bacterial motility

1. Introduction

The bacterial flagellum is powered by a rotary motor energised by a transmembrane electrochemical ion gradient, usually a proton motive force. Investigation of the dynamics of the motor has been centred on the flagellar motors of *Escherichia coli*, *Salmonella typhimurium* and *Streptococcus* spp. These motors rotate at a virtually constant rate and are able to switch direction of rotation, an event which happens within a video frame (0.02 s). The structure and dynamics of the motor are reviewed in Blair [1] and Berg [2]. Recently, environmentally important organisms have been shown to swim at varying speeds and evidence for speed itself being involved in motile responses has been found for several organisms [3–5]. Yet the dynamics of these motors has not been addressed.

The motor in *Rhodobacter sphaeroides* rotates in one direction propelling the cell forwards, and periodically stops to allow a change in swimming direction [6]. *R. sphaeroides* has been shown to modulate stopping frequency in response to temporal changes in chemoeffector concentration [7]. Its motor shows a sustained increase in the rate of rotation and a decrease in the stopping frequency in response to the presence of weak organic acids (chemokinesis) [4,8]. The mechanism controlling the stopping and starting is unknown but stops occur even when the cell is fully energised [9] and in cells in which all chemosensory response regulators have been deleted [P.A. Hamblin and J.P. Armitage, unpublished]. The speed and stopping frequency of both free swimming and tethered cells are very variable. In this study the dynamics of the *R. sphaeroides* flagellar motor have been measured to analyse the speed fluctuations, changes in fluctuation during chemokinesis and investigate the stop/start mechanism.

2. Methods

2.1. Growth, media and conditions

R. sphaeroides WS8 was grown photoheterotropically as described previously [4]. The cells were harvested in late log phase, washed and resuspended in 10 mM Na-HEPES (pH 7.2) with 50 μg/ml chloramphenicol. The cells were starved under light for 40 min and then tethered using antiflagellar antibody, as previously described, before loading into the flow chamber [4]. High light on the microscope ensured that the proton motive force was maintained well above saturation for motor rotation [10].

E. coli RP437 (wild type) and RP2154 (a *che*R mutant with a smooth-swimming phenotype, supplied by J.S. Parkinson) were grown aerobically in M9 minimal medium by shaking at 30°C. Cells were harvested in mid-exponential phase and tethered as previously described [11].

2.2. Motion analysis

The tethered cells were observed using phase contrast microscopy (100× lens and a 5× lens before the camera) with a CCD camera (Sony HYPERHAD model SPT-M108CE). The tracks of tethered cells were recorded onto video tape (Panasonic video recorder NV-SD400) and a computerised motion analysis system from HTS Ltd. (Sheffield, UK) was used for the analysis of the rotation rates with time of individual cells [12]. Data points were taken at 50 Hz frame rate (0.02 s) using the interlacing images.

The raw ASCII data files were processed to eliminate system noise, each point in the curve was replaced by the weighted average of its nearest nine neighbours by the method of Savitsky and Golay [13].

3. Results and discussion

3.1. Comparison of the behaviour of the unstimulated *R. sphaeroides* flagellar motor and the *E. coli* motor

The rotation rates of each of 30 fully energised, unstimulated, *R. sphaeroides* cells were analysed for 80 s. The population mean rotation rates ranged from 2.58 ± 4.74 to 12.13 ± 6.8 Hz. A typical tethered cell is shown in Fig. 1A. A frequency distribution of rotation rate is shown for 20 s of unsmoothed data collected from 10 cells (Fig. 2A). The mean rotation rate for the cell population was 6.9 ± 6.3 Hz. The distribution is bimodal with data for rotating cells greater than 3 Hz and the data below this corresponding to stops. Stopped cells are buffeted by vibrational and Brownian forces resulting in a scattering of the values around zero.

The data show that the unidirectional motor of *R. sphaeroides* is a variable-speed motor and that the speed variation can be seen both in individual motors and between cells in a population. The standard deviation for each cell was between 25 and 200% of the mean rotation rate. Similar variations have been found in populations of free swimming cells with individuals showing a broad distribution of speeds. For comparison the well-characterised switching motors of fully energised *E. coli* RP437 and a smooth-swimming RP2154 were also analysed. Each time the graph crosses the x-axis a switch

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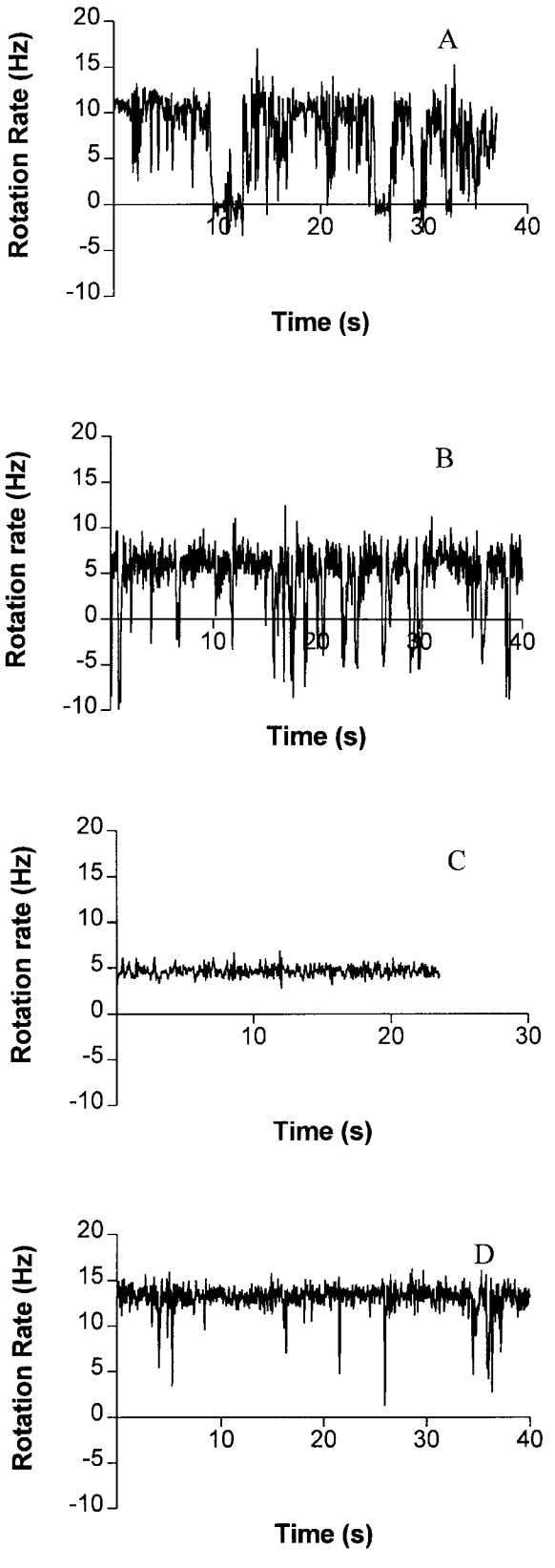

Fig. 1. The rotation rates of typical tethered cells of *R. sphaeroides* and *E. coli* are shown. A: Rotation rate of unstimulated *R. sphaeroides* WS8 over a 40 s period. (The rotation rate of stopped cells was below 2.5 Hz, not zero, due to vibrational forces acting on the cells) The cells were in Na-HEPES buffer (pH 7.2). B: Rotation rate of *E. coli* RP437 (wild type). C: Rotation rate of *E. coli* RP2154 (the smooth swimming CheR mutant). D: A tethered cell of *R. sphaeroides* during chemokinetic stimulation in the presence of 1 mM Na-acetate in the Na-HEPES buffer (pH 7.2).
←

in the direction of rotation of the flagellar motor had occurred. Ten wild-type cells were analysed and their mean rotation rates were found to vary between 3.1 ± 1.7 and 6.33 ± 3.2 Hz in the CW direction. In the CCW direction the rotation rate varied between 5.2 ± 3.0 and 0.5 ± 2.7 Hz.

A typical *E. coli* RP437 cell is shown in Fig. 1B with a mean rotation rate of 4.9 ± 4.7 Hz and RP2154 in Fig. 1C which had a mean rotation rate of 4.9 ± 1.9 Hz. Distributions of the rotation rate for 10 cells are also shown in Fig. 2B,C. The wild-type *E. coli* flagellar motor switched direction of rotation resulting in a skewed distribution of the data (median 7.22 Hz) whereas the smooth-swimming mutant (median 4.9 Hz) did not switch during the measurement period and has a very tight distribution of data contrasting sharply to the distribution for *R. sphaeroides*. In *E. coli* most of the changes in rotation rate occurred during switching, as has been found previously [14], with rotation rate being much smoother between periods of switching.

The fluctuations in rotation rate of *R. sphaeroides* cells occur even though they were under conditions where they are known to be fully energised and occur independently of stops. The cause of variation in rotation rate is not understood and has not been considered specifically in current models of motor rotation. The data from the enteric motor studies suggest several possible mechanisms that might produce variable torque generation in a tightly coupled motor [15–17]. The 8 or so MotA/B complexes known to be the site of torque generation have been shown during resurrection experiments in *E. coli* to produce torque independently [18,19]. The motor in *R. sphaeroides* could produce variable speed by altering the number of functioning Mot complexes interacting with the motor. Another possibility would be changing the proton flow through gating the proton transducing channels in the Mot or switch complexes. It cannot be ruled out that, instead of a tightly coupled motor, the motor in *R. sphaeroides* is loosely coupled, with the work produced per proton being variable at constant pmf. All of these possibilities would reduce torque and produce an inefficiency in the motor. Recently evidence has suggested that the speed of motor rotation may be controlled by two homologues of Che Y (a response regulator in the Che pathway) in *Rhizobium meliloti*, interacting independently with the motor and under the control of the phosphorelay system [5]. *R. sphaeroides* also has more than one Che Y homologue and these may have a role in speed control [20]. *E. coli* only has one Che Y.

3.2. Stop mechanism

The flagellar motor of *R. sphaeroides* periodically stops rotation, and direction changing occurs [6]. The stops are an intrinsic feature of the motor and occur even though the cell is fully energised and in the absence of Che Y [P.A. Hamblin and J.P. Armitage, unpublished]. A smooth-swimming mutant phenotype has not been found. Deletion of the Che pathway has no obvious effect on unstimulated motor stopping, suggesting an intrinsic control. The stopping frequency can be altered by the Che pathway as *R. sphaeroides* responds to temporal changes in chemoeffector concentration by altering

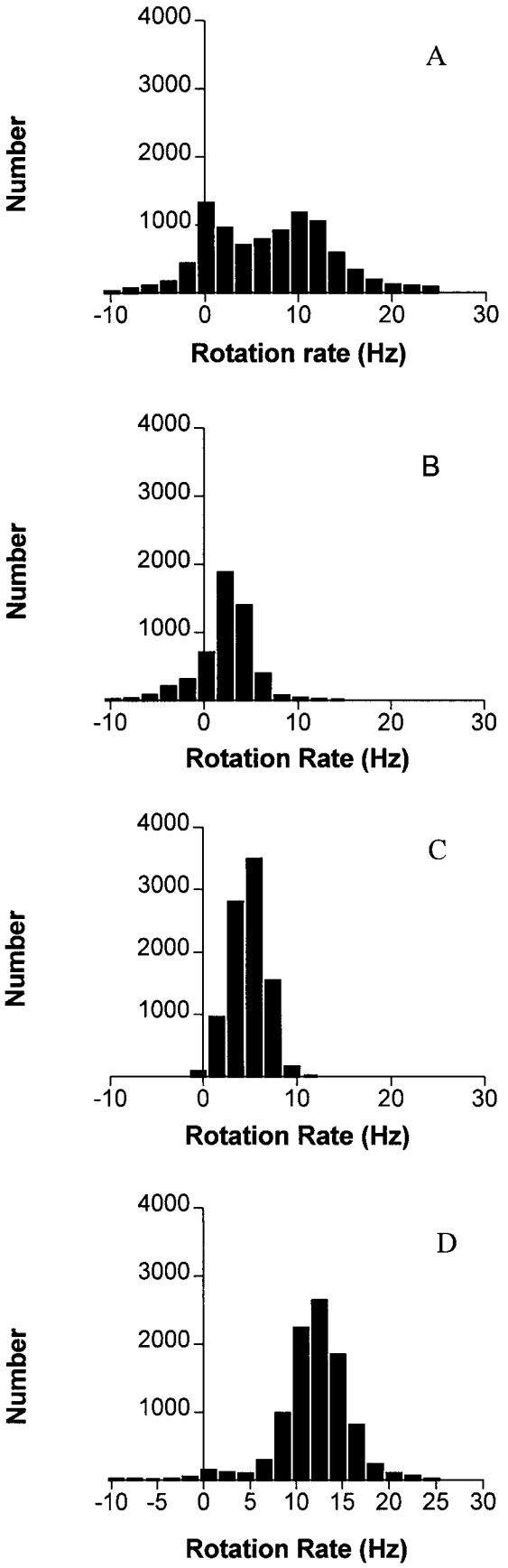

Fig. 2. The distributions of the rotation rates for each population of 10 cells over 20 s. A: Unstimulated *R. sphaeroides*. B: *E. coli* RP437 (wild type). C: *E. coli* RP2154 (the smooth swimming CheR mutant). D: *R. sphaeroides* during chemokinetic stimulation after the addition of 1 mM Na acetate to Na-HEPES buffer (pH 7.2). ←

stopping frequency [7]. Additional as yet unidentified proteins may be involved in stop control. The mechanism of stopping a fully energised rotating motor is not understood. It is not known whether on stopping the motor is disengaged from the driving force, rather like a clutch, or whether a brake of some form is applied. One hundred stops from 30 cells of *R. sphaeroides* were analysed and the mean population stop duration was found to be 0.54 ± 0.45 s (minimum 0.04 s and maximum 2.9 s). The rotation rate was analysed immediately before and after a stop. Many of the stops were abrupt changes, with the rotating motor stopping within a video frame (0.02 s), while other stops were preceded by the cell slowing down. When restarting this was again found to be either within a video frame, or gradual. No correlation was found between rotation rate and stopping frequency and no consistent pattern was found within individual cells or the population. The motor of *E. coli* switches within one video frame and does not slow into a switch. Fig. 3 shows two stops occurring in one cell. The motor very rapidly changed from fast rotation to zero within 0.04 s (two video frames) but could stop slowly over 0.245, the stop duration ranged from 0.1 to 0.6 s and the time for resumption of prestimulus rotation from zero varied from 0.04 to 0.24 s. No evidence was found for quantal decreases in speed on stopping which would have indicated that the number of torque generating units were individually deactivated as the cells slowed and stopped although it is possible that this event could be too rapid for detection using video tracking or be damped by the compliance of the hook.

3.3. Chemokinesis

As well as the unusual feature of a stop/start motor, *R. sphaeroides* has been shown to increase the rate of rotation of the flagellar motor independently of proton motive force on the addition of certain chemoeffectors, such as the weak organic acids [4,9]. The chemokinetic behaviour of *R. sphaeroides* again demonstrates that its motor can change speed under fully energised conditions. In the presence of a chemokinetic effector the motor rotates at an apparent maximum rate for a sustained period, independently of the proton motive force [9,10]. Rotation rate analysis show that chemokinetically stimulated cells have reduced variation compared to cells in the absence of effector. Although the motor may still stop the probability is decreased [7]. Fig. 1D shows the rotation rate of a cell chemokinetically stimulated with 1 mM Na-acetate and Fig. 2D the distribution of the data for a cell population of 10 cells. The rotation rates of chemokinetically stimulated cells were faster and less variable than that of unstimulated cells. In the example shown the mean rotation rate of the individual cell was 13.1 ± 2.8 Hz while the mean rotation rate of the population was 12.0 ± 3.7 Hz. Under chemokinetic stimulation the rotation rates fell within a tight distribution and this distribution was shifted to faster rates compared to the broad rather flatter distribution of unstimulated cells, i.e. cells showed a comparatively constant rotation

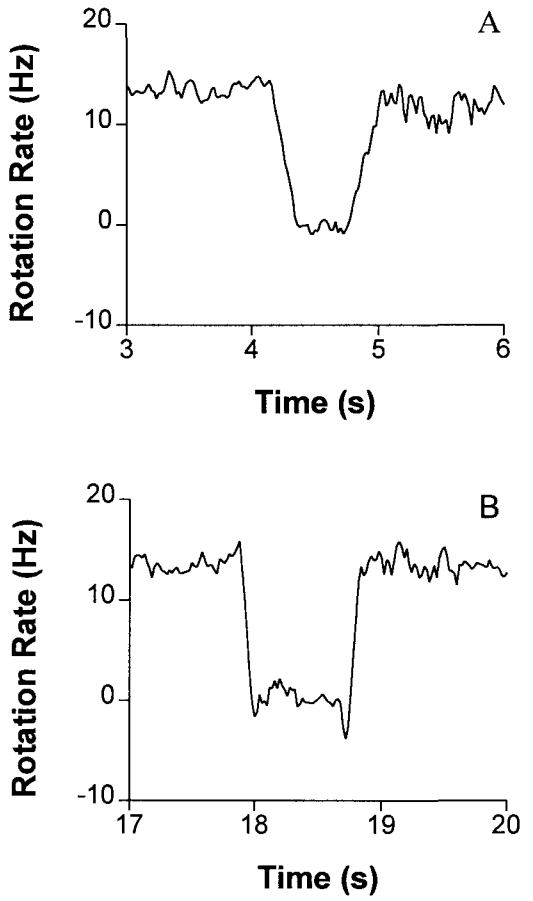

Fig. 3. Two examples of the rotation rate during typical stops in rotation of the flagellar motor of *R. sphaeroides* and during resumption of rotation.

rate. Very few stops occurred and thus chemokinetically stimulated cells of *R. sphaeroides* may be comparable with the smooth-swimming mutant of *E. coli* which also showed less variability in rotation rates when compared to the wild type.

The mechanism of chemokinesis and the differences between a stimulated and unstimulated motor are unknown. It has been postulated that the presence of chemokinetic effectors such as the organic acids or potassium ions may cause a physical change in the motor or the membrane, increasing the effectiveness of the interaction of the torque generating proteins with the rotor. In chemokinetically stimulated cells and smooth-swimming mutants the motors are either no longer switching direction or rarely stopping. The variation in rotation rate may in part be the result of factors controlling switching or stopping. The study by Eisenbach et al. [14] also found that non-switching motors of *E. coli* had a steadier rotation rate than switching motors. The variation in rotation rate in *R. sphaeroides* may therefore be linked in some way to direction changing, perhaps involving speed changes rather than complete stops. Under chemokinetic stimulation the increase in torque generation suggests that all the Mot complexes are fully engaged, all proton transducing channels in the motor may be open or perhaps an as yet unidentified protein/s may be controlling the speed increase. *R. sphaeroides* mutants in which Che Y1 and Che Y2 are deleted still show chemokinesis (unpublished observation).

Current models of the mechanism of the motor have focused on the constant speed, switching, enteric motor and have not been extended to consider variable speed motors. The *R. sphaeroides* motor behaviour provides a model organism to study torque generation in a different species which shows the chemokinetic response as well as a stop/start motility. It is monoflagellate so there is only one motor and thus one thrust generator per cell, its bioenergetics are well characterised and therefore are more easily manipulated.

Recently MotA and MotB have been identified in *R. sphaeroides* [21,22] and therefore repetition of the resurrection experiments in this organism may elucidate the role of the Mot complexes in variable torque generation.

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