Statistical kinetics of the bacterial flagellar motor

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The statistical behavior of the bacterial flagellar motor matches that of a Poisson stepper that takes at least 400 steps per revolution. Using this fact, we study the effect of motor stochastics on experiments in which fluorescent motors, initially synchronized by polarization photobleaching, become uncorrelated. [S1063-651X(97)06106-0]

I. INTRODUCTION

A bacterial flagellum is driven at its base by a rotary motor [1]. Numerous studies have classified the subunits of this motor into functional and structural groups. The stator includes a discrete set of torque-generating units comprising the proteins MotA and MotB [2,3]. Each unit steps independently along a ring of lattice sites attached to the rotor [4]. The likely location of these sites is in the C-terminal domain of the protein FliG, a component of the MS and C rings [5]. For a recent review of motor structure see Macnab [6].

Motor function is usually studied by tethering a single flagellar filament to glass; the relative motion of the stator and rotor is inferred from the motion of the tethered cell body [7]. However, the relative motion of subunits within the motor might be directly measurable using polarization anisotropy. Which of these subunits rotate and which do not? The rotation of the γ subunit relative to the α and β subunits of ATP synthase was recently demonstrated using this technique [8].

Polarization photobleaching labels the orientation of an individual motor by destroying the ability of fluorophores attached to that motor to absorb light of a particular polarization. The ability of a motor population to absorb light at different polarizations, i.e., the polarization anisotropy, is a measure of the numbers of photobleached motors at different orientations. Thus polarization photobleaching is a means of ''synchronizing'' the orientation of a population of motors, and polarization anisotropy is a means of tracking their subsequent orientations. To understand the dynamics of the anisotropy, one needs to know how the distribution of motor orientations relaxes from the initial synchronized and highly anisotropic state to equilibrium, where orientations are uniformly distributed.

The stochastic dynamics of flagellar rotation contribute to this relaxation. The statistical behavior of the flagellar motor matches that of a Poisson stepper—a Poisson stepper takes discrete steps that are separated by exponentially distributed waiting times—that takes at least 400 steps per revolution [9]. Therefore an understanding of the relaxation of polarization anisotropy following polarization photobleaching requires considerations of the stochastic dynamics of a Poisson stepper walking along a cyclic lattice. These considerations are the subject of this paper.

II. EVOLUTION OF PROBABILITY DENSITY OF A POISSON STEPPER ON A CYCLIC LATTICE

Given a stepper initially at the zeroth site on a cyclic lattice of N sites, the probability of the stepper being at the ith site at time t is the probability that the stepper has taken $jN+i$ steps where j is a positive integer. So the probability that the stepper is at the zeroth site at time t is the sum of the probabilities that the stepper has made $0,N,2N,\ldots$ steps. If the stepper is Poisson, each of these probabilities is given by the Poisson formula (e.g., [10]) yielding the sum

$$x_0(t) = e^{-\mu} \sum_{j=0}^{\infty} \frac{\mu^{jN}}{(jN)!}. \quad (1)$$

Here μ is the mean number of steps taken in time t. The generalization to the ith site is

$$x_i(t) = e^{-\mu} \sum_{j=0}^{\infty} \frac{\mu^{jN+i}}{(jN+i)!}. \quad (2)$$

Although exact the infinite sums are not so useful. However, they can be collapsed into useful finite sums. Before proceeding to the general case, consider the case $N=2$. For $N=2$, Eqs. (1) and (2) are

$$x_0(t) = e^{-\mu} \left(1 + \frac{\mu^2}{2!} + \frac{\mu^4}{4!} + \cdots \right) = \frac{1 + e^{-2\mu}}{2} \quad (3)$$

and

$$x_1(t) = e^{-\mu} \left(\mu + \frac{\mu^3}{3!} + \frac{\mu^5}{5!} + \cdots \right) = \frac{1 - e^{-2\mu}}{2}. \quad (4)$$

Here the Taylor expansions of $\cosh(\mu)$ and $\sinh(\mu)$ have been substituted with their identities. These results were obtained by [11].

Now consider the case of arbitrary N. Call the summation in Eq. (1) f_0. By inspection

$$\frac{d^N f_0}{d\mu^N} = f_0. \quad (5)$$

The general solution of Eq. (5) is

$$f_0 = \sum_{k=0}^{N-1} c_k e^{\omega_k \mu}, \qquad (6)$$

where c_k is a constant and $\omega_k = \exp(i2\pi k/N)$. At $\mu=0$, f_0 satisfies the conditions

$$\frac{d^n f_0}{d\mu^n}(\mu=0) = \begin{cases} 1 & \text{if } n=0 \\ 0 & \text{if } n\neq 0 \end{cases} \qquad (7)$$

in which $0 \leq n < N$. Substituting Eq. (6) into Eq. (7) produces N linearly independent equations:

$$\sum_{k=1}^{N} c_k \omega_k^n = d_n, \qquad (8)$$

where $d_n=1$ if $n=0$ and $d_n=0$ if $n=0$. Note that

$$\omega_k^n = \exp(i2\pi kn/N) = W_{kn}. \qquad (9)$$

But W_{kn} are Fourier modes and d_n is a discrete Fourier transform of the coefficients c_k. Therefore the inverse transform of d_n yields the unknown coefficients [12]:

$$c_k = \frac{1}{N}\sum_{n=0}^{N-1} d_n W_{nk}. \qquad (10)$$

Since $d_n=1$ only if $n=0$,

$$c_k = \frac{1}{N} W_{0k} = \frac{1}{N}. \qquad (11)$$

In summary, the occupancy probability of the zeroth lattice site given initial conditions (7) is

$$x_0(t) = e^{-\mu} f_0 = \frac{1}{N}\sum_{k=0}^{N-1} e^{(\omega_k-1)\mu}, \qquad (12)$$

where $\omega_k = \exp(i2\pi k/N)$.

Call the summation in Eq. (2) f_i. Adjacent lattice points are related by a single differentiation:

$$f_i = \frac{df_{i+1}}{d\mu}. \qquad (13)$$

Using Eqs. (12) and (13), the occupancy probabilities of all lattice points are easily calculated.

III. AN ALTERNATE DERIVATION

Although the approach in Sec. II is physically appealing, another approach exploiting the mathematical structure of the problem provides a different perspective. In Sec. II, features of the problem, e.g., Eqs. (5) and (7), emerged which facilitated its solution. Here the inevitability of these features is shown.

Consider a Poisson stepper walking unidirectionally along a cyclic lattice of N sites. Each step occurs with a constant probability per unit time λ. Therefore the probability that a stepper steps in an interval of time δt is $\lambda \delta t$. Let x_i be the occupancy probability of site i. How do the N occupancy probabilities evolve with time?

Consider the change in occupancy probability of site $i+1$, δx_{i+1}, in a small interval of time δt. The positive probability flux to site $i+1$ is due to the stepping of motors at site i. If the occupancy probability of site i is x_i, the positive flux is $x_i \lambda \delta t$. Also the negative flux due to steppers leaving site $i+1$ for site $i+2$ is $x_{i+1}\lambda \delta t$. Therefore

$$\delta x_{i+1} = x_i \lambda \delta t - x_{i+1}\lambda \delta t. \qquad (14)$$

With the substitution $\delta\mu = \lambda \delta t$ and rearrangement, Eq. (14) becomes a first-order inhomogeneous differential equation:

$$\frac{dx_{i+1}}{d\mu} + x_{i+1} = x_i. \qquad (15)$$

The occupancy probabilities of sites $i+1$ and i are related to the homogeneous solution of Eq. (15), $\exp(-\mu)$, and an integrating factor f by

$$x_i = e^{-\mu}\frac{df}{d\mu} \qquad (16)$$

and

$$x_{i+1} = e^{-\mu}f. \qquad (17)$$

Therefore x_i and x_{i+1} are related by

$$x_i = \hat{T}x_{i+1}, \qquad (18)$$

where

$$\hat{T} = e^{-\mu}\frac{d}{d\mu}e^{\mu}. \qquad (19)$$

Successive operations by $\hat{T}$ relate the occupancy probability of any lattice site to any other lattice site. Due to lattice cyclicity

$$x_0 = \hat{T}^N x_0. \qquad (20)$$

Calling $g = e^{\mu}x_0$, Eqs. (18) and (20) produce

$$\frac{d^N g}{d\mu^N} = g. \qquad (21)$$

Let the initial condition for the probability density over the lattice be

$$x_i(0) = \begin{cases} 1 & \text{if } i=0 \\ 0 & \text{if } i\neq 0 \end{cases}. \qquad (22)$$

The initial condition translates into the condition

$$\frac{d^n g}{d\mu^n}(\mu=0) = \begin{cases} 1 & \text{if } n=0 \\ 0 & \text{if } n\neq 0 \end{cases} \qquad (23)$$

in which $0 \leq n < N$. From this point, the derivation of x_0 follows as in Sec. II. The evolution of the occupancy probability of other lattice points from x_0 is calculated using $\hat{T}$.

IV. AUTOCORRELATION ANALYSIS

Let ν be a stochastic variable that switches between values 0 and 1 over time. Its autocorrelation function $\langle \nu(t)\nu(0) \rangle$ is the mean probability that ν is 1 at both a randomly chosen initial time and at a time t later. Let $\Psi_0(t)$ have the value 1 if the Poisson stepper is at the zeroth lattice site and 0 if it is elsewhere. So the autocorrelation function of $\Psi_0(t)$ is the mean probability that the stepper is at the zeroth site at an initial time and at a time t later. However, $x_0(t)$ is the probability that a stepper is at the zeroth lattice site at time t given occupancy there at $t=0$. Let p be the probability of being at the origin at $t=0$. For a lattice with N sites, $p=1/N$. Hence

$$\langle \Psi_0(t)\Psi_0(0) \rangle = p x_0(t) = \frac{x_0(t)}{N}. \tag{24}$$

We have the stochastic variable $N^{1/2}\psi_0$ of which $x_0(t)$ is the autocorrelation. Let Ψ_i be the stochastic variable that has the value 1 if the stepper is at the ith lattice site and zero if elsewhere. By similar arguments

$$\langle \Psi_i(t)\Psi_0(0) \rangle = \frac{x_i(t)}{N}. \tag{25}$$

Hence the solutions for intermediate lattice sites are cross-correlations of well-defined stochastic variables. This identification may prove useful in numerical studies or computation.

V. CALCULATION OF PROBABILITY DISTRIBUTION RELAXATION TIMES

The probability amplitude at the zeroth site should oscillate with a frequency equal to the motor rotation rate. Also the oscillations should dampen as the probability distribution broadens over the lattice points. Substituting $\omega_k = \exp(i2\pi k/N)$ into Eq. (12) gives

$$x_0(t) = \frac{1}{N} + \frac{2}{N} \sum_{k=1}^{E[(N-1)/2]} \exp\left(\frac{-t}{\tau_k}\right) \cos\left(\frac{2\pi t}{T_k}\right), \tag{26}$$

where τ_k is the time constant for decay of the kth Fourier mode which has oscillatory period T_k. $E(m)$ is the integral part of m. The constants are

$$\tau_k = \frac{1}{\lambda \ \mathrm{Re}(1-\omega_k)} \tag{27}$$

and

$$T_k = \frac{2\pi}{\lambda \ \mathrm{Im}(\omega_k)}. \tag{28}$$

The time constant of a mode is related to its rotation frequency $f_k = 1/T_k$ by

$$\tau = \frac{1}{\lambda - [\lambda^2 - (2\pi f)^2]^{1/2}}. \tag{29}$$

In Eq. (26), the dominant contribution is that which decays slowest, i.e., has the largest time constant. This mode corresponds to the root of unity with the largest real component: ω_1. For arbitrary N, Eq. (29) exactly relates τ_1 and T_1. However, for the bacterial flagellar motor, $N \gg 1$ and a reasonable estimate can be calculated in this limit. The Taylor expansion of ω_1 keeping the first three terms is

$$\omega_1 \approx 1 + i\frac{2\pi}{N} - \frac{1}{2}\left(\frac{2\pi}{N}\right)^2. \tag{30}$$

Combining Eqs. (27), (28), and (30)

$$T_1 \approx \frac{N}{\lambda} \tag{31}$$

and

$$\tau_1 \approx \frac{NT_1}{2\pi^2}. \tag{32}$$

T_1, the oscillatory period for the slowest decaying mode, predictably corresponds to the mean rotation period of the motor. The oscillations decay, more slowly with larger N; as the number of steps increases, fluctuations in rotation rate of the flagellar motor are reduced [4,9].

VI. ATP SYNTHASE AND THE BACTERIAL FLAGELLAR MOTOR

For $N=3$, the summation in Eq. (26) has one term:

$$x_0(t) = \frac{1}{3} + \frac{2}{3} \exp\left(\frac{-t}{\tau_1}\right) \cos\left(\frac{2\pi t}{T_1}\right). \tag{33}$$

Here, $\omega_1 = \exp(i2\pi/3)$. Using Eqs. (27) and (28), $\tau_1 = 2/3\lambda$ and $T_1 = 4\pi/\lambda\sqrt{3}$. Using $\hat{T}$, x_1 and x_2 are readily calculated. These results were obtained by Sabbert and Junge [11] using matrix methods.

For the bacterial flagellar motor, $N \approx 400$ [9]. Keeping the dominant mode in Eq. (26) we have

$$x_0(t) \approx \frac{1}{400} + \frac{2}{400} \exp\left(\frac{-ft}{20}\right) \cos(2\pi ft), \tag{34}$$

where f is rotation frequency. Figure 1 depicts a stochastic simulation for $N=400$ and the theoretical prediction for its dominant mode.

VII. CONCLUSIONS

Polarization anisotropy should allow us to identify components of the flagellar motor that rotate, provided that they can be fluorescently labeled and bleached. Since the number of sites in the lattice is expected to be much larger than that of ATP synthase ($N=3$, [8]), the signal should ring at the rotation frequency equation (34). In addition, the conclusions reached earlier [4,9], namely, that the motor is not a fluid drive and that its statistical behavior matches that of a Poisson stepper that takes at least 400 steps per revolution, can be corroborated in anisotropy studies by comparing oscillatory period to decay time constant, Eq. (32). By using free-spinning fla-
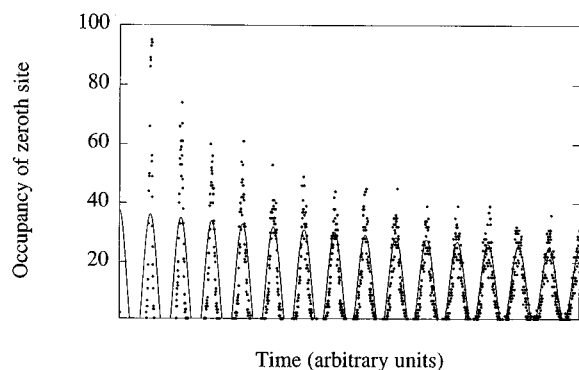
FIG. 1. The number of motors at the zeroth site as a function of time resulting from a simulation of 10 000 Poisson motors each with 400 lattice sites. Points indicate the results of the stochastic simulation. The solid line is the theoretical prediction for the slowest decaying mode, Eq. (34).

gella with proximal hooks but no filaments, anisotropy studies can explore a regime of motor function—low-torque, high speed—otherwise difficult to access. These experiments are in preparation.

As noted above, this paper complements an earlier study aimed at understanding the dynamics of polarization anisotropy following polarization photobleaching of ATP synthase, a stochastic rotary motor in which $N=3$ [11]. However, our calculations produce straight-forward analytic results without requiring matrix manipulations. Novel features of this work are (a) an operator interrelates the solutions for the occupancy probabilities of all sites (19); (b) the time constant for decay of each Fourier mode of occupancy probability is directly related to its oscillatory frequency (29); (c) an approximation valid for large N provides a simple formula to describe the behavior of the bacterial flagellar motor (34).

[1] H. C. Berg and R. A. Anderson, Nature (London) **245**, 380 (1973).

[2] S. M. Block and H. C. Berg, Nature (London) **309**, 470 (1984).

[3] D. F. Blair and H. C. Berg, Science **242**, 1678 (1988).

[4] A. D. T. Samuel and H. C. Berg, Biophys. J. **71**, 918 (1996).

[5] S. A. Lloyd and D. F. Blair, J. Mol. Biol. **266**, 733 (1997).

[6] R. M. Macnab, *Escherichia coli and Salmonella* (ASM Press, Washington, DC, 1996), pp. 125–145.

[7] M. Silverman and M. Simon, Nature (London) **249**, 73 (1974).

[8] D. Sabbert, S. Engelbrecht, and W. Junge, Nature (London) **381**, 623 (1996).

[9] A. D. T. Samuel and H. C. Berg, Proc. Natl. Acad. Sci. USA **92**, 3502 (1995).

[10] H. C. Berg, *Random Walks in Biology* (Princeton, Princeton, NJ, 1993), Eq. A.32.

[11] D. Sabbert and W. Junge, Proc. Natl. Acad. Sci. USA **94**, 2312 (1997).

[12] L. Sirovich, *Introduction to Applied Mathematics* (Springer-Verlag, Berlin, 1988).