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ON THE ROTATIONAL BROWNIAN MOTION OF A BACTERIAL IDLE MOTOR

II. THEORY OF FLUORESCENCE CORRELATION SPECTROSCOPY

Hiroshi HOSHIKAWA [a] and Hiroshi ASAI [b]

[a] *Ichimura College, Inuyama, Aichi Prefecture 484, and* [b] *Department of Physics, Waseda University, Shinjuku, Tokyo 160, Japan*

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The photon flux autocorrelation function of a fluorescent label attached to a bacterial motor shaft is calculated for the case in which the bacterial motor is considered to be actively but idly rotating. It is shown that even when the fluorescent label has a very short lifetime, fluorescence correlation spectroscopy should provide a useful tool for determining the rate of revolution of the bacterial motor under various solution conditions.

1. Introduction

It is almost certain that bacteria can swim by rotating their helical flagellar filament and that the flagellar filament is driven by a motor which is embedded in the membrane [1,2]. Measurement of the rate of revolution of the motor is important for clarifying the driving mechanism. Revolution in the loaded state, i.e., revolution with a flagellar filament, is easy to observe, since the flagellar filament can be observed by optical microscopy. However, measurement of the rate of revolution in the load-free state, i.e., revolution without a flagellar filament, is impossible using optical microscopy, since the motor shaft itself cannot be seen.

In a previous paper [3] (hereafter referred to as Part I), we developed the theory of time-dependent fluorescence depolarization for an actively rotating bacterial motor in a load-free state, and showed that this technique is one of the possible methods to measure the rate of revolution of the bacterial motor in the load-free state. However, to apply this technique to the measurement of the rate of revolution of the bacterial motor, the fluorescent label (or phosphorescent label) should have a lifetime of the order about of 10^{-2} s. From the experimental point of view, this condition is by no means desirable. Another promising method is fluorescence correlation spectroscopy [4,5]. Since this method is essentially independent of the fluorescence lifetime, one can more readily measure the rate of revolution of the bacterial motor. It is noteworthy that active rotating motion under the condition of energy flow may take place not only in a bacterial motor but also in other biological apparatus embedded in a membrane, such as the H^+-ATPase system in chloroplasts and mitochondria, the postsynaptic acetylcholine receptor system, and the calcium-pump system in sarcoplasmic reticulum [6–8]. Fluorescence correlation spectroscopy may also be applicable to investigations of these systems. In this, the second paper of the series, we apply the procedures developed in Part I to the theory of fluorescence correlation spectroscopy.

2. Rotational diffusion equation and its Green's function

In this section we summarize briefly the theory of the rotational Brownian motion of a particle

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coupled with an actively driving motor, which was developed in Part I.

An appropriate hydrodynamical model of a bacterial cell body is a spheroid. Let D_i ($i = 1, 2, 3$) be the rotational diffusion coefficient around the three principal axes of the spheroid, and put D_1 equal to D_2. We assume that the bacterial cell body has only one motor and that the motor shaft coincides with the longer axis. Then the rotational diffusion equation for a fluorescent label attached to the motor shaft is

$$\frac{\partial f}{\partial t} = -\mathrm{div}_{\zeta}\left(\vec{\Omega} f - \vec{D}\,\mathrm{grad}_{\zeta} f\right), \tag{1}$$

where $\vec{\Omega}$ is the angular velocity of the motor and ζ represents the angles around the three principal axes of the spheroid. We introduce the Euler angles (θ, ϕ, ψ) which designate the relationship between the laboratory Cartesian coordinate system and the coordinate system fixed to the cell body, and rewrite eq. 1 as follows:

$$\begin{aligned}\frac{\partial f}{\partial t} = D_1 \Bigg(& \frac{\partial^2 f}{\partial \theta^2} + \mathrm{cosec}^2\theta \frac{\partial^2 f}{\partial \phi^2} + \cot^2\theta \frac{\partial^2 f}{\partial \psi^2} \\ & - 2\,\mathrm{cosec}\theta \cot\theta \frac{\partial^2 f}{\partial \phi \partial \psi} \\ & + \cot\theta \frac{\partial f}{\partial \theta} \Bigg) + D_3 \frac{\partial^2 f}{\partial \psi^2} - \Omega \frac{\partial f}{\partial \psi}.\end{aligned} \tag{2}$$

The Green's function $G(\theta, \phi, \psi; t | \theta', \phi', \psi'; t')$ of eq. 2 is obtained as

$$\begin{aligned} & G(\theta, \phi, \psi; t | \theta', \phi', \psi'; t') \\ & = \frac{1}{(2\pi)^2} \sum_l \sum_m \sum_n \exp\left[-\left\{ D_1 (\lambda_{lm}^n - m^2) \right.\right. \\ & \qquad \left.\left. + m^2 D_3 + im\Omega \right\} t \right] \\ & \qquad \times \phi_{lm}^n(\theta) \phi_{lm}^n(\theta') \exp\{ il(\phi - \phi') \\ & \qquad + im(\psi - \psi') \}, \end{aligned} \tag{3}$$

where $\phi_{lm}^n(\theta)$ is the eigenfunction of the eigenvalue equation [9] (see Part I),

$$\begin{aligned}\phi_{lm}^n(\theta) = N_{lm}^n & (1 + \cos\theta)^{(m+l)/2} (1 - \cos\theta)^{(m-l)/2} \\ & \times F\Bigg(m + n + 1,\ m - n,\ m - l \\ & + 1;\ \frac{1 - \cos\theta}{2} \Bigg) \quad (m \geqq l),\end{aligned} \tag{4}$$

and N_{lm}^n is the normalization constant,

$$\begin{aligned} N_{lm}^n = & \left(\frac{2n + 1}{2^{2m+1}} \right)^{1/2} \frac{1}{(m - l)!} \\ & \times \left\{ \frac{(n - l)!(n + m)!}{(n + l)!(n - m)!} \right\}^{1/2} \quad (m \geqq l), \end{aligned} \tag{5}$$

λ_{lm}^n is the eigenvalue:

$$\begin{aligned} & \lambda_{lm}^n = n(n + 1),\ n = 0, 1, 2, \ldots \\ & l = -n, -n + 1, \ldots, 0, 1, 2, \ldots, n \\ & m = -n, -n + 1, \ldots, 0, 1, 2, \ldots, n \end{aligned} \tag{6}$$

As the exact Green's function is thus obtained, we can calculate the photon flux autocorrelation function.

3. Fluorescence correlation spectroscopy

Aragon and Pecora [5] developed the theory of fluorescence correlation spectroscopy for a spherical rotor. Their expression for the photon flux autocorrelation function $\Gamma(\tau)$ is

$$\begin{aligned}\Gamma(\tau) = I_0^2 \epsilon^2 Q_f^2 \Bigg[& \int_{-\infty}^{0} \frac{\mathrm{d}t'}{\tau_f} \int_{-\infty}^{\tau} \frac{\mathrm{d}t''}{\tau_f} \\ & \times \exp\{ (t' + t'' - \tau)/\tau_f \} \\ & \times \langle E(\Xi_0)^2 A(\Xi_{t'})^2 E(\Xi_\tau)^2 A(\Xi_{t''})^2 \rangle \\ & - \left\{ \int_{-\infty}^{0} \frac{\mathrm{d}t'}{\tau_f} \exp(t'/\tau_f) \langle E(\Xi_0)^2 A(\Xi_{t'})^2 \rangle \right\}^2 \Bigg],\end{aligned} \tag{7}$$

where I_0 is the incident radiation flux, ϵ the extinction coefficient of the fluorescent molecule, Q_f the quantum efficiency, and τ_f the fluorescence lifetime. $A(\Xi_t)^2$ and $E(\Xi_t)^2$ are the absorption and emission probabilities, respectively, and are equal to the square of the scalar product of the transition moment and the polarization vector. Ξ_t represents the angle of the fluorescent label at time t with respect to the laboratory coordinate system. Let $\vec{\mu}_a(\Xi_t)$ and $\vec{\mu}_e(\Xi_t)$ be the respective absorption and emission transition moments of the fluorescent label. Then $A(\Xi_t)^2$ and $E(\Xi_t)^2$ can be written as

$$A(\Xi_t)^2 = (\vec{\mu}_a(\Xi_t) \cdot \vec{\epsilon}_i)^2, \tag{8a}$$

$$E(\Xi_t)^2 = (\vec{\mu}_e(\Xi_t) \cdot \vec{\epsilon}_f)^2, \tag{8b}$$

where $\vec{\epsilon}_i$ and $\vec{\epsilon}_f$ are the polarization vectors of the incident and emitted light, respectively. Although, in general, the directions of $\vec{\mu}_a$ and $\vec{\mu}_e$ are different, we assume for simplicity that $\vec{\mu}_a$ and $\vec{\mu}_e$ are parallel.

Now, let our apparatus be such that the incident light which has polarization parallel to the Z axis is radiated along the X axis and the observation of the fluorescence which has polarization parallel to the Z axis is made on the Y axis. Then, $A(\Xi_t)$ is equal to $E(\Xi_t)$ and can be expressed in terms of the Euler angles as

$$A(\Xi_t) = E(\Xi_t) = \cos p \sin\theta \sin\psi + \sin p \cos\theta, \tag{9}$$

where $(\frac{\pi}{2} - p)$ is the angle between the transition moment and the motor shaft.

Returning to eq. 7, let us consider the four-time correlation function appearing in the first term of the right-hand side. To evaluate the four-time correlation function, the joint probability $P_4(\Xi_t, \Xi_{t'}, \Xi_{t''}, \Xi_{t'''})$ must be known. However, as the process described by eq. 1 has Markovian nature, P_4 can be expressed by using the Green's function of eq. 3 as

$$\begin{aligned} P_4(\Xi_t, \Xi_{t'}, \Xi_{t''}, \Xi_{t'''}) &= G(\Xi_{t'''}; t'''|\Xi_{t''}; t'')G(\Xi_{t''}; t''|\Xi_{t'}; t') \\ &\times G(\Xi_{t'}; t'|\Xi_t; t)f(\Xi_t)\ (t''' > t'' > t' > t), \end{aligned} \tag{10}$$

where $f(\Xi_t)$ is the initial distribution. Following the procedure of Aragon and Pecora [5], we split the integral including the four-time correlation function into two parts:

$$\begin{aligned} Q_1 = &\int_{-\infty}^0 \frac{dt'}{\tau_f} \int_{-\infty}^0 \frac{dt''}{\tau_f} \exp\{(t' + t'' - \tau)/\tau_f\} \\ &\times \langle A(\Xi_{t'})^2 A(\Xi_{t''})^2 E(\Xi_0)^2 E(\Xi_\tau)^2 \rangle \\ &(\tau > 0 > t'' > t'), \end{aligned} \tag{11a}$$

$$\begin{aligned} Q_2 = &\int_{-\infty}^0 \frac{dt'}{\tau_f} \int_0^\tau \frac{dt''}{\tau_f} \exp\{(t' + t'' - \tau)/\tau_f\} \\ &\times \langle A(\Xi_{t'})^2 E(\Xi_0)^2 A(\Xi_{t''})^2 E(\Xi_\tau)^2 \rangle \\ &(\tau > t'' > 0 > t'). \end{aligned} \tag{11b}$$

The ensemble averages appearing in eqs. 11a and 11b are evaluated by using the joint probabilities $P_4(\Xi_{t'}, \Xi_{t''}, \Xi_0, \Xi_\tau)$ and $P_4(\Xi_{t'}, \Xi_0, \Xi_{t''}, \Xi_\tau)$, respectively. Substituting eq. 3 into eq. 10 and carrying out the integrations (eqs. 11a and 11b) with respect to t' and t'', we obtain

$$\begin{aligned} Q_1 = &\frac{1}{2^9\pi^8} \sum_{lmn} \sum_{l'm'n'} \sum_{l''m''n''} \exp\left\{-\left(M_{lmn} + \frac{1}{\tau_f}\right)\tau\right\} S_1 \\ &\times \frac{1}{\tau_f^2} \cdot \frac{1}{M_{l''m''n''} + (1/\tau_f)} \\ &\cdot \frac{1}{M_{l'm'n'} - M_{l''m''n''} + (1/\tau_f)}, \end{aligned} \tag{12a}$$

$$\begin{aligned} Q_2 = &\frac{1}{2^9\pi^8} \sum_{lmn} \sum_{l'm'n'} \sum_{l''m''n''} \exp\left\{-\left(M_{lmn} + \frac{1}{\tau_f}\right)\tau\right\} S_2 \\ &\times \frac{1}{\tau_f^2} \cdot \frac{1}{M_{l''m''n''} + (1/\tau_f)} \\ &\cdot \frac{1}{M_{lmn} - M_{l'm'n'} + (1/\tau_f)} \\ &\times \left[\exp\left\{\left(M_{lmn} - M_{l'm'n'} + \frac{1}{\tau_f}\right)\tau\right\} - 1\right], \end{aligned} \tag{12b}$$

where S_1 and S_2 are defined by

$$\begin{aligned} S_1 = &\int d\Xi_\tau \int d\Xi_0 \int d\Xi_{t''} \int d\Xi_{t'} A(\Xi_{t'})^2 A(\Xi_{t''})^2 E(\Xi_0)^2 \\ &\times E(\Xi_\tau)^2 L_{lmn}(\Xi_\tau|\Xi_0) L_{l'm'n'}(\Xi_0|\Xi_{t''}) \\ &\times L_{l''m''n''}(\Xi_{t''}|\Xi_{t'}), \end{aligned} \tag{13a}$$

$$\begin{aligned} S_2 = &\int d\Xi_\tau \int d\Xi_{t''} \int d\Xi_0 \int d\Xi_{t'} A(\Xi_{t'})^2 E(\Xi_0)^2 A(\Xi_{t'})^2 \\ &\times E(\Xi_\tau)^2 L_{lmn}(\Xi_\tau|\Xi_{t''}) L_{l'm'n'}(\Xi_{t''}|\Xi_0) \\ &\times L_{l''m''n''}(\Xi_0|\Xi_{t'}). \end{aligned} \tag{13b}$$

M_{lmn} and $L_{lmn}(\Xi_t|\Xi_{t'})$ are abbreviated forms:

$$M_{lmn} = D_1(\lambda_{lm}^n - m^2) + m^2 D_3 + im\Omega, \tag{14}$$

$$\begin{aligned} L_{lmn}(\Xi_t|\Xi_{t'}) = &\phi_{lm}^n(\theta)\phi_{lm}^n(\theta')\exp\{il(\phi - \phi') \\ &+ im(\psi - \psi')\}. \end{aligned} \tag{15}$$

We can calculate the photon flux autocorrelation function exactly by evaluating the integrals in eqs. 13a and 13b and performing the summations in eqs. 12a and 12b. As mentioned in section 1, however, fluorescence correlation spectroscopy is a method which is essentially independent of the fluorescence lifetime. Therefore, we confine ourselves to the case where our fluorescent labels have lifetimes much shorter than the time scale of the revolution of the bacterial motor. If this is not done, the exact expression of $\Gamma(\tau)$ becomes extremely complex and virtually useless for experimental purposes.

If we take the limit $\tau_f \to 0$ in eqs. 12a and 12b, we obtain

$$\lim_{\tau_f \to 0} Q_1 = 0, \tag{16a}$$

$$\lim_{\tau_f \to 0} Q_2 = \frac{1}{2^9 \pi^8} \sum_{lmn} \sum_{l'm'n'} \sum_{l''m''n''} \exp(-M_{l'm'n'}\tau) S_2. \tag{16b}$$

Consequently, we are left with the calculation of Q_2. The procedure for this calculation is as follows: firstly, we evaluate the integrals in eq. 13b with respect to ϕ and ψ,

$$\int_0^{2\pi} \exp\{i(l-l')\phi\}\mathrm{d}\phi = \begin{cases} 2\pi & (l-l'=0), \\ 0 & (l-l' \neq 0), \end{cases} \tag{17a}$$

$$\int_0^{2\pi} \sin^2\psi \exp\{i(m-m')\psi\}\mathrm{d}\psi = \begin{cases} \frac{\pi}{2}(3\delta_{m-m'} - 1) & (m-m' = 0, \pm 2), \\ 0 & (m-m' \neq 0, \pm 2), \end{cases} \tag{17b}$$

$$\int_0^{2\pi} \sin\psi \exp\{i(m-m')\psi\}\mathrm{d}\psi = \begin{cases} \pm i\pi & (m-m' = \pm 1), \\ 0 & (m-m' \neq \pm 1). \end{cases} \tag{17c}$$

Secondly, we evaluate the integrals with respect to θ. The integrals needed for our calculation are

$$J_s = \int_0^{\pi} \sin^2\theta \phi_{0m}^n(\theta) \sin\theta \mathrm{d}\theta, \tag{18a}$$

$$J_c = \int_0^{\pi} \cos^2\theta \phi_{0m}^n(\theta) \sin\theta \mathrm{d}\theta, \tag{18b}$$

$$J_{sc} = \int_0^{\pi} \sin\theta \cos\theta \phi_{0m}^n(\theta) \sin\theta \mathrm{d}\theta, \tag{18c}$$

$$K_s = \int_0^{\pi} \sin^2\theta \phi_{0m}^n(\theta) \phi_{0m'}^{n'}(\theta) \sin\theta \mathrm{d}\theta, \tag{18d}$$

$$K_c = \int_0^{\pi} \cos^2\theta \phi_{0m}^n(\theta) \phi_{0m'}^{n'}(\theta) \sin\theta \mathrm{d}\theta, \tag{18e}$$

$$K_{sc} = \int_0^{\pi} \sin\theta \cos\theta \phi_{0m}^n(\theta) \phi_{0m'}^{n'}(\theta) \sin\theta \mathrm{d}\theta, \tag{18f}$$

and are listed in tables 1–3. Finally, the summa-

Table 1

Values of J_s, J_c and J_{sc} for necessary n and m

n	m	J_s	J_c	J_{sc}
0	0	$\frac{4}{3}N_{00}^0$	$\frac{2}{3}N_{00}^0$	
2	0	$-\frac{4}{15}N_{00}^2$	$\frac{4}{15}N_{00}^2$	
2	1			$\frac{4}{15}N_{01}^2$
2	2	$\frac{16}{15}N_{02}^2$		

Table 2

Values of K_s and K_c for necessary n, m, n' and m'

n	m	n'	m'	K_s	K_c
0	0	0	0	$\frac{4}{3}(N_{00}^0)^2$	$\frac{2}{3}(N_{00}^0)^2$
0	0	2	0	$-\frac{4}{15}N_{00}^0 N_{00}^2$	$\frac{4}{15}N_{00}^0 N_{00}^2$
0	0	2	2	$\frac{16}{15}N_{00}^0 N_{02}^2$	
2	0	2	0	$\frac{4}{21}(N_{00}^2)^2$	$\frac{22}{105}(N_{00}^2)^2$
2	0	4	0	$-\frac{8}{105}N_{00}^2 N_{00}^4$	$\frac{8}{105}N_{00}^2 N_{00}^4$
2	0	2	2	$-\frac{32}{105}N_{00}^2 N_{02}^2$	
2	0	4	2	$\frac{16}{315}N_{00}^2 N_{02}^4$	
2	1	2	1	$\frac{16}{105}(N_{01}^2)^2$	$\frac{4}{35}(N_{01}^2)^2$
2	1	4	1	$-\frac{8}{315}N_{01}^2 N_{01}^4$	$\frac{8}{315}N_{01}^2 N_{01}^4$
2	1	4	3	$\frac{32}{315}N_{01}^2 N_{03}^4$	
2	2	4	0	$\frac{16}{315}N_{02}^2 N_{00}^4$	
2	2	2	2	$\frac{32}{35}(N_{02}^2)^2$	$\frac{16}{105}(N_{02}^2)^2$
2	2	4	2	$-\frac{32}{945}N_{02}^2 N_{02}^4$	$\frac{32}{945}N_{02}^2 N_{02}^4$
2	2	4	4	$\frac{256}{315}N_{02}^2 N_{04}^4$	

Table 3

Values of K_{sc} for necessary n, m, n' and m'

n	m	n'	m'	K_{sc}
2	1	0	0	$\frac{4}{15}N_{01}^2 N_{00}^0$
2	1	2	0	$\frac{4}{105}N_{01}^2 N_{00}^2$
2	1	4	0	$-\frac{16}{315}N_{01}^2 N_{00}^4$
2	1	2	2	$\frac{16}{105}N_{01}^2 N_{02}^2$
2	1	4	2	$\frac{32}{945}N_{01}^2 N_{02}^4$
4	1	2	0	$\frac{4}{105}N_{01}^4 N_{00}^2$
4	1	2	2	$-\frac{8}{315}N_{01}^4 N_{02}^2$
4	3	2	2	$\frac{32}{315}N_{03}^4 N_{02}^2$

tions in eq. 16b are performed. These summations are tedious but straightfoward. Calculation of the second term on the right-hand side of eq. 7 is rather simple and in the limit $\tau_f \to 0$, we have

$$\int_{-\infty}^{0} \frac{dt'}{\tau_f} \exp(t'/\tau_f)\langle E(\Xi_0)^2 A(\Xi_{t'})^2 \rangle = \tfrac{1}{5}. \quad (19)$$

Thus, we obtain the final expression for the photon flux autocorrelation function $\Gamma(\tau)$ as

$$\begin{aligned}\Gamma(\tau) = I_0^2\epsilon^2 Q_f^2 \tfrac{1}{35}\Big[&\tfrac{4}{7}\exp(-6D_1\tau)(\cos^2 p - 2\sin^2 p)^2 \\ &+ \tfrac{48}{7}\exp\{-(5D_1 + D_3)\tau\}\cos \Omega\tau \sin^2 p \cos^2 p \\ &+ \tfrac{12}{7}\exp\{-(2D_1 + 4D_3)\tau\}\cos 2\Omega\tau \cos^4 p \\ &+ \tfrac{1}{315}\exp(-20D_1\tau)(9\cos^8 p \\ &- 144\sin^2 p \cos^6 p + 624 \sin^4 p \cos^4 p \\ &- 384 \sin^6 p \cos^2 p + 64 \sin^8 p) \\ &+ \tfrac{8}{63}\exp\{-(19D_1 + D_3)\tau\}\cos \Omega\tau \sin^2 p \\ &\times \cos^2 p (3\cos^2 p - 4\sin^2 p)^2 \\ &+ \tfrac{4}{63}\exp\{-(16D_1 + 4D_3)\tau\}\cos 2\Omega\tau \\ &\times \cos^4 p (\cos^2 p - 6\sin^2 p)^2 \\ &+ \tfrac{8}{9}\exp\{-(11D_1 + 9D_3)\tau\} \\ &\times \cos 3\Omega\tau \sin^2 p \cos^6 p \\ &+ \tfrac{1}{9}\exp\{-(4D_1 + 16D_3)\tau\}\cos 4\Omega\tau \cos^8 p. \end{aligned} \quad (20)$$

When $\Omega = 0$ and $D_1 = D_3$, the above equation simplifies as follows:

$$\Gamma(\tau) = I_0^2\epsilon^2 Q_f^2 \frac{1}{(35)^2}\{80 \exp(-6D\tau) + \tfrac{64}{9}\exp(-20D\tau)\}. \quad (21)$$

Eq. 21 coincides with the photon flux autocorrelation function of a spherical particle without a motor [5]. Thus our result, eq. 20, is a generalization of the result obtained by Aragon and Pecora for a spheroidal particle with a motor.

In fig. 1, the photon flux autocorrelation function $\Gamma(\tau)$ divided by $\Gamma(0)$ is plotted vs. $D\tau$ for several values of Ω/D, where D_1 and D_3 are kept equal to D and p is taken as 0 for simplicity. If $\Omega = 0$, the first two terms of $\Gamma(\tau)$ remain and these terms give a simple decay curve as shown by the broken line. If Ω/D takes a large value, a sinusoidal function is superimposed on the decay function as shown by the solid lines a and b. The value $\Omega/D = 10^3$ is thought to be an appropriate one for the bacterial motor [10].

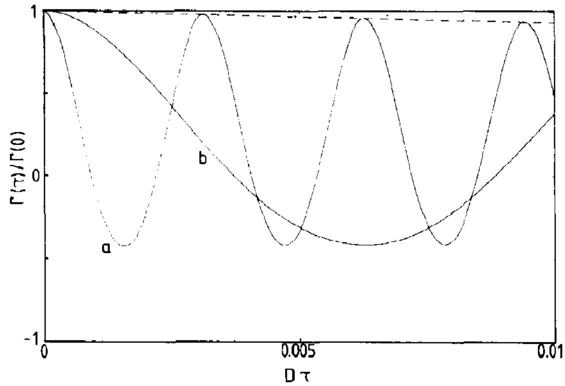

Fig. 1. The function of photon flux autocorrelation function $\Gamma(\tau)/\Gamma(0)$ plotted vs. $D\tau$, where D_1 and D_3 are kept equal to D. The transition moment of the fluorescent molecule is assumed to be perpendicular to the motor shaft. Solid line a represents the case of $\Omega/D = 1 \times 10^3$, and solid line b, $\Omega/D = 2.5 \times 10^2$.

4. Discussion

We have obtained the photon flux autocorrelation function $\Gamma(\tau)$, eq. 20, for the fluorescence correlation spectroscopy of a fluorescent label attached to the bacterial motor shaft. The solution is limited to the case where the fluorescent label has a very short lifetime. The exact solution without this restriction could be obtained by the same procedure as described in section 3, but the exact expression of $\Gamma(\tau)$ would be too complex to be of any practical value.

Eq. 20 contains up to the fourth order of the Fourier series with respect to the angular velocity Ω. In contrast, the fluorescence polarization anisotropy $r(t)$ contains up to the second order [1].

Eight relaxation times appear in eq. 20, whereas three relaxation times appear in $r(t)$ [1]. The reason for these results is that $\Gamma(\tau)$ is essentially the four-time correlation of the direction of the fluorescent label, whereas $r(t)$ is the two-time correlation.

The features of the function $\Gamma(\tau)/\Gamma(0)$ are almost the same as those of $r(t)$. *

A comparison of eq. 23 in Part I and the first three terms of eq. 20 shows that the ratio of these three terms of eq. 20 is the same as in the case of eq. 23. Furthermore, the coefficients of the remaining terms of eq. 20 have comparatively small values. This explains why $\Gamma(\tau)$ is quantitatively similar to $r(t)$.

In this series of papers, we have developed the theoretical basis for determining the rate of revolution of the bacterial motor in the load-free state. We have found that both time-dependent fluorescence depolarization and fluorescence correlation spectroscopy are possible methods for this purpose.

In addition to the bacterial motor, other kinds of biological apparatus in cells may rotate under the condition of energy flow. Our methods proposed here might be applied to determine whether H^+-ATPase systems in mitochondria or in chloroplasts, the acetylcholine receptor system in postsynaptic membranes and other biological apparatus embedded in membrane can rotate actively.

* In the caption of Part I, we stated that we had plotted $r(t)$ for $\Omega/D = 10^3$ and 2.5×10^2. However, this is erroneous; the Ω/D values are actually 17.5 and 4.4, respectively. As an appropriate value of Ω/D for the bacterial motor is 10^2 or 10^3, we should have plotted $r(t)$ for $\Omega/D = 10^2$ or 10^3.

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