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Fluctuations in sliding motion generated by independent and random actions of protein motors

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Abstract

We consider theoretical fluctuations in the in vitro sliding movement of individual cytoskeletal filaments generated by an ensemble of protein motors whose actions are assumed to be statistically independent and random. We show that the mean square deviation of the sliding distances of a filament for a given period of time around their average is proportional to the inverse of the filament length. This result provides a basis for an experimental test of the general assumption on the independent and random actions of protein motors. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Protein motors generate active force to produce unidirectional sliding movements along cytoskeletal filaments; the power for the motile processes is supplied by the chemical energy of ATP hydrolysis that the motors catalyze [1,2]. Their motility assays in vitro have provided new experimental clues to the elucidation of their mechanism [3]. In one such assay, protein motors are attached on a substrate surface and the movements of individual cytoskeletal filaments driven to slide by a single protein motor or an ensemble of the motors are measured under a microscope [4,5]. When a filament is driven to slide by an ensemble of protein motors, their actions are generally assumed to be statistically independent and random. This will be referred to as the assumption of independent force generation by protein motors in this paper.

In muscle physiology, there are observations: (i) that the isometric tension in active skeletal muscle is proportional to the overlap between the thin and thick filaments, i.e. the number of myosin motor cross-bridges between the two filaments; and (ii) that the unloaded shortening velocity of skeletal muscle is independent of the overlap

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between these filaments [6]. This first observation was taken to be evidence for the independent force generation by myosin motors in isometric contraction of muscle [7]. A recent fluctuation analysis of the force generation by myosin in an in vitro assay system has provided solid evidence for the independent force generation in the isometric condition [8]. The second observation has been taken to indicate the independent force generation of the motors when there is a relative sliding motion between the thick and thin filaments under no externally-applied load [7], although this is not conclusive. A fluctuation analysis of the sliding movement of filaments in vitro provides an alternative, more adequate method for examining the assumption of independent force generation under the latter sliding condition. This is what we will discuss in this paper.

2. Premises

Here we address a statistical analysis of the fluctuation of sliding distances of cytoskeletal filaments driven to move along their longitudinal direction by protein motors. For the analysis, we define a motional diffusion coefficient, an effective diffusion coefficient, for a sliding cytoskeletal filament of a given length, as follows. The fluctuation of the sliding distance is given by the following equation:

$$\langle(\Delta X - \langle \Delta X \rangle)^2\rangle = 2D_{\mathrm{m}}\Delta t \qquad (1)$$

where ΔX is the sliding distance of a filament for a given time period Δt, $\langle \ldots \rangle$ is the average and D_{m} is an effective diffusion coefficient (= motional diffusion coefficient) of the filament. D_{m} is a measure of the fluctuation of sliding motion generated by protein motors.

The assumption of the independent force generation of protein motors leads to the inverse linear dependence of the motional diffusion coefficient D_{m} on the filament length L as will be shown below. Analyzing the length dependence of the effective diffusion coefficient experimentally, therefore, we can examine the validity of the assumption of independent force generation. The fluctuation we address here is not that in a thermal equilibrium system but that in a non-equilibrium dynamical system. To the analysis of such non-equilibrium fluctuation we can not apply the theoretical treatment which leads to the Einstein's relation: $v = D/(k_{\mathrm{B}}T) \times f$, where v is the drift velocity, D is the diffusion coefficient, k_{B} is the Boltzmann constant, T is the absolute temperature and f is the force. So it is legitimate to show first the mechanism that leads to this inverse linear dependence of D_{m} on L. The premises we will pose are:

1. each molecular motor interacts with a cytoskeletal filament independently of the other motors;
2. the characteristics of the motors are spatially homogeneous on the substrate; and
3. each motor's action has no long-term temporal correlations.

From the above three premises, we will show that the mean-square displacement of the position of the filament around its average is inversely proportional to the number of the motors involved in generating the sliding movement of the filament. This is a purely statistical consequence that is related to the central limit theorem [9].

3. Langevin dynamics model

In the following, we consider the movement of a cytoskeletal filament driven to slide by an ensemble of protein motors fixed on a substrate surface, which satisfy the above three premises. Our major concern is the fluctuation in the sliding distance of a filament owing to the fluctuation of the active driving force by protein motors. We assume that the filament is a linear elastic rod and we assign the number from $i = 1$ to $i = N$ to the sites on the filament with which the protein motors can make the force generating interaction [10]. Hereafter, these sites will be referred to as 'binding sites'. The phenomenological equation of motion of the filament is introduced as follows:

$$-\gamma(\mathrm{d}x_i/\mathrm{d}t) + f + \xi_i(t) - (\partial U/\partial x_i) = 0 \qquad (2)$$

where x_i is the position of the i-th binding site, γ is a friction coefficient, f and $\xi_i(t)$ are the mean part and the fluctuating part of the sliding force acting at the i-th binding site, and U is the elastic energy along the filament, which depends on the deviation of the length between the neighboring binding sites from the rest length.

Eq. (2) represents the balance of force acting at the i-th binding site. The first term on the left hand side (l.h.s.), $-\gamma(\mathrm{d}x_i/\mathrm{d}t)$, represents the friction force acting at the i-th binding site. It should be pointed out that the friction force in Eq. (2) is not due to the solvent because the solvent friction is too small to limit the sliding velocity of the filament movement generated by protein motors [11,12]. The friction force in Eq. (2) is generated by protein motors and its entity has been discussed before [12]. We should stress that the main purpose of the present model is not to be very realistic but to show as simple as possible the essential mechanism that leads to the formula $D_\mathrm{m} \propto 1/L$, which actually sometimes disaccords with experimental findings [13,14].

The sliding force acting at the i-th binding site consists of the mean part f and the fluctuating part around the former, $\xi_i(t)$. We require that the average of the latter vanishes; $\langle \xi_i(t) \rangle = 0$. We also require that the time correlation is of short memory time and independent of the force fluctuations at the other binding sites;

$$\langle \xi_i(t)\xi_i(t') \rangle = \mu(|t-t'|),$$
$$\langle \xi_i(t)\ \ \xi_j(t') \rangle = 0 \text{ for } i \neq j \qquad (3)$$

Typically we may assume $\mu(s) = \mu_0 \mathrm{e}^{(-s/\tau)}$ with $\mu_0 > 0$ and $\tau > 0$. Here we have neglected the thermally-driven fluctuation of the friction coefficient, since our major concern is the fluctuation in the sliding distance due to the fluctuation of the active driving force by protein motors. The negative sliding force due to 'overshot' myosin cross-bridges after their 'power stroke' proposed in Huxley 57 model [15], however, should be included in the fluctuation force $\xi_i(t)$. The last term on l.h.s. of Eq. (2) is the elastic force along the filament which depends on the relative displacement of the i-th binding site with respect to its neighbors. We may thus assume the form $U(x_1,\ldots,x_N) = \sum_{i=1}^{N-1} \Phi(x_{i+1} - x_i)$ with $\Phi(l)$ having the minimum at the rest length l_0 between the neighboring binding sites.

Our interest is in the motion of the 'center of mass' of the filament, $X = (x_1 + \ldots + x_N)$, for the time interval much longer than the memory time τ of the individual fluctuating force. Noting that the elastic forces acting at the binding sites are cancelled out upon the summation, we obtain the equation of motion for the center of mass as follows:

$$-\gamma(\mathrm{d}X/\mathrm{d}t) + f + \sum_{i=1}^{N} \xi_i(t)/N = 0 \qquad (4)$$

First it is evident from Eq. (4) that the mean velocity of the center of mass is independent of N; $\langle \mathrm{d}X/\mathrm{d}t \rangle = \gamma^{-1} f$: since N is proportional to the length of the filament L, the mean velocity is independent of L.

The motional diffusion coefficient D_m is written as follows:

$$D_\mathrm{m} = \lim_{t\to\infty} \langle (X(t) - [X(0) + \gamma^{-1} f t])^2 \rangle / (2t) \qquad (5)$$

Integrating Eq. (4) from the time 0 through t and using Eq. (3), we find that

$$\begin{aligned} D_\mathrm{m} &= \lim_{t\to\infty} \left(\int_0^t \mathrm{d}t_1 \int_0^t \mathrm{d}t_2 \mu(|t_1 - t_2|) \right) / (2N\gamma^2 t) \\ &= \lim_{t\to\infty} \left(\int_0^t \mathrm{d}t_1 \int_0^{t_1} \mathrm{d}t_2 \mu(|t_1 - t_2|) \right. \\ &\quad \left. + \int_0^t \mathrm{d}t_1 \int_{t_1}^t \mathrm{d}t'_2 \mu(|t_1 - t'_2|) \right) / (2N\gamma^2 t) \\ &= \lim_{t\to\infty} \left(\int_0^t ds \int_0^{t-s} dt_1 \mu(s) \right. \\ &\quad \left. + \int_0^t ds' \int_{s'}^t dt_1 \mu(s') \right) / (2N\gamma^2 t) \\ &= \lim_{t\to\infty} \left(t \int_0^t \mu(s)\mathrm{d}s - \int_0^t s\mu(s)\mathrm{d}s \right) / (\gamma^2 N t) \\ &= M_0/(\gamma^2 N) \end{aligned} \qquad (6)$$

where we have assumed that both $M_0 = \int_0^\infty \mu(s)\mathrm{d}s$ and $M_1 = \int_0^\infty s\mu(s)\mathrm{d}s$ are positive and finite, which is valid as far as the correlation function $\mu(s)$ decays to zero faster than s^{-2} as $s \to \infty$. (In moving from the second line to the third line on the right hand side, we have introduced new variables of integration, s and s', which are related to t_2 and t'_2 via the relations $t_2 = t_1 - s$ and $t'_2 = t_1 + s'$ in the first and second integrals on the second line, respectively. Since the number of binding sites N is proportional to the length of the filament L, the result then shows the relationship, $D_\mathrm{m} \propto 1/L$.

4. Discussion

The basic picture of the present model is that the independently acting N molecular motors yield the net fluctuating force of only the $\sqrt{N}$ order, while the friction is of the N order. As a result, the fluctuation of the filament's displacement becomes $1/\sqrt{N}$ of that we expect from a single motor. Since D_m is related to the square of the fluctuation of the displacement, we obtain $D_\mathrm{m} \propto 1/N$, i.e. $D_\mathrm{m} \propto 1/L$. This is a direct consequence of the assumption of independent force generation by protein motors when there is a relative sliding movement between a cytoskeletal filament and the motors.

The same argument can be applied to the motion of microtubules driven to slide by an ensemble of two-headed kinesin, between the paired heads of which is cooperativity [2]. If the actions of individual kinesin molecules are statistically independent and random, the above argument thus again leads to $D_\mathrm{m} \propto 1/L$.

Below we discuss situations where this $D_\mathrm{m} \propto 1/L$ relationship breaks. First, if the characteristics of motors' function are spatially heterogeneous on the substrate surface [the violation of the present premise 2], we can theoretically show that the D_m has a component that is independent of L, in addition to a $1/L$-dependent component [16]. The essential mechanism of the former is that the duration time of the interaction between a specific molecular motor and the sliding filament is proportional to the length of the filament, L. The motion of a filament will, therefore, have the time correlation of the order of L. This invalidates the condition required for the central limit theorem, and thus breaks the $D_\mathrm{m} \propto 1/L$ relationship. The theory [16] reproduces quantitatively the L-independent part of D_m obtained in the experiments cited therein [13,14] without adjustable parameters.

Second is the presence of long-term temporal correlations in the activity of the individual motors [the violation of the present premise 3]. In this case, the $D_\mathrm{m} \propto 1/L$ relationship breaks again. In fact, if the correlation time of motor's activity is much larger than the duration time of the interaction between a specific motor molecule and a sliding filament, the problem can be reduced to the previous case of the spatial heterogeneity. In the second case, however, D_m that deviates from the $1/L$ relationship will eventually disappear in the limit of long filament, $L \to \infty$.

The third is that the action of a molecular motor in driving a filament to slide by splitting ATP is influenced by the actions of the other motor molecules involved in the sliding of the same filament. [This is the condition where the present premise 1 is violated.] If so, the dependence on L of the coefficient D_m may be different from $1/L$, perhaps with a slower decaying exponent $L^{-\alpha}$ $(0 \le \alpha < 1)$. For this to be the case, we require a certain feed-back (or cooperative) mechanism in the motor's function. One possibility could be a feed-back mechanism brought about through the displacement of a sliding filament.

From the above discussion it is clear that use of an ensemble of homogeneously oriented protein motors is crucial in the application of the present method; one possibility for this purpose is to use long native thick filaments isolated from molluscan smooth muscle [17,18]. The present method would then provide more detailed clues concerning the assumption of the independent force generation by protein motors when there is a relative sliding motion between the motors and a filament. Such an experiment with native thick fila-

ments from molluscan smooth muscle has been in progress.

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