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歩行運動と筋蛋白の確率過程

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Appendix

Stochastic relations between cellular and molecular events in muscle mechanics

Abstract

If a myosin head in a sarcomere could walk, it would step towards the M-line or towards the Z-disc. The probable result of innumerable units would be expressed by the wave form of the contraction. The probability density function of a Poisson process fits a twitch wave of skeletal muscle very well. Results of experiment reveal that (1) Poisson's parameter λ is load-sensitive and specifies the velocity of sliding; (2) when a nerve impulse triggers the SR, Ca concentration increases throughout the muscle. The uniformization of independent events takes place and the condition for a Poisson process is fulfilled. Though it looks dramatic, twitch is an "unusual" event. A 1-dimensional Brownian process with random configuration would be the "usual" process. Brownian motion of innumerable contractile proteins never stops in life. The internal energy produced by ATP is not only for the contraction but also for stabilizing structures. Each cycle of shortening and elongation is a phenomenon that consumes energy. The result of experiment related to the energy distribution is summarized in the diagram (Fig.2.C). The energy of a single myosin-actin unit is proportional to $\beta^* k_B T$ and is found to be $9.52 \cdot 10^{-21}$ J, at 20℃.

Key words: Muscle mechanics; force-velocity relation; Poisson process.

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Introduction

When ATP is applied to the glycerinated muscle its stiffness begins to decrease. At the slight touching an unstrained muscle specimen shortens, then immediately returns to the original length. It looks as if the muscle actively determines the resting length itself. Skeletal muscle has a structure composed of vast numbers of molecules which are assembled to respond with sufficient stress against a 1-dimensional strain.

The beautiful striation pattern of fresh skeletal muscle lies in quiet when seen under the microscope. However if it were possible to observe with an accuracy of 1 nanometer, innumerable fidgety molecules would be seen. Water molecules are violently shaking the high polymers. Myosin heads surrounded by the actin cylinder are swinging independently, and whenever myosin contacts actin ATP is hydrolyzed. These molecular transient events within an actual cell are very likely the real case. However, in practice there are technological limits to our space-time resolution capacity.

In spite of the random molecular motion, muscle works tidily. Not only chemical but also mechanical interaction between myosin and actin is fundamental to muscle mechanics. If myosin in a sarcomere could walk, it would step towards the M-line or towards the Z-disc so the probable result would be expressed by the waveform of muscle contraction.

To find a model for the contraction, the formula for the binomial distribution is tested. It was able to converge on a best fit curve, but the fit is not unique. However, an unexpectedly good result is obtained by selecting the formula for a Poisson process for simulating an old record of a twitch wave (frog's m.sartorius). The fit is unique, and the goodness of fit (R2) is 0.9981, (Fig. 1).

Assuming that the time course of a single twitch is the unit interval of a Poisson process, (0, T], an experimental curve is classified into very small intervals. The χ-square test shows that its significant level is 0.05.

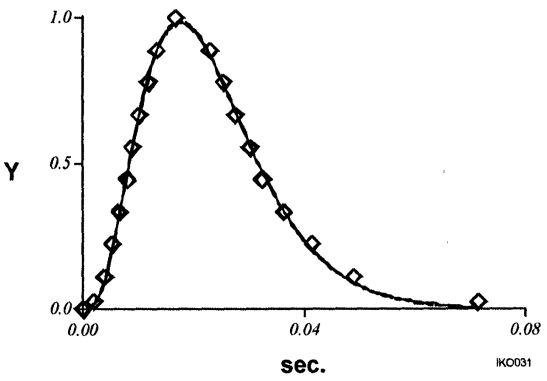

Fig. 1 Digitalized reading of twitch wave (open diamonds frog's m.sartorius). The solution curve of Eq [1] is superimposed where k and λ ; 3 [−]; 179.3 [s^{-1}] respectively. A is constant [mN].

Results and parameters

By making following formula into a practical tool, a new series of experiments has been attempted.

$$Y(t) = A\lambda \exp(-\lambda t)^* (\lambda t)^k / k! \qquad [1]$$

When a sample is extended at every 1 mm step, elastic tension (open circles) and peaks of isotonic twitch (black diamonds) above the elastic tension are recorded. All data from muscles (m.semitendinosus, Rana nigromacrata) are plotted in Fig. 2B.

Eq [1] fits to all experiment records well. The mean value of R_2 is 0.997 ± 0.002, and the constant k is 4.889 ± 0.7803 (n=15). Values of λ are plotted in Fig. 3. Normalized λ/x relation is as follows;

$$\lambda(x) = 0.57 \exp(-\beta x) + 0.43 \qquad [2]$$
$$0 \leq x \leq 1$$

The value of β is 2.352, goodness of fit (R2)=0.942. λ is the load sensitive parameter and β determines the curvature of F/V relation.
As a result, Eq.1 becomes;

$$Y(x,t) = A\lambda(x) \exp(-\lambda(x)t)^* (\lambda(x)t)^k / k! \quad [3]$$

When $t = (k - \sqrt{k})/\lambda(x)$, the velocity is maximum. The force-velocity curve can be expressed;

$$[F/V] = (V_{mx} - V_{m1}) / (V_{m0} - V_{m1}) \qquad [4]$$

Where V_{m0} V_{m1} V_{mx} correspond to λ(x=0) λ(x=1) λ(x=x) respectively.

Y(x, t) reaches its peak at $t = k/\lambda(x)$. Muscle develops the force proportional to load, while it maintains elastic tension.
If the rate of available units to work is considered, the relation between the work and load is expressed as follows;

$$W(x) = x^* (1-x)^* (a + b \exp(\beta x))^* \exp(-k - \beta x)^* k^k / k! \qquad [5]$$
$$0 \leq x \leq 1$$

Where a=0.57, b=0.43, β=2.352, k=5.
Eq [5] fits the data of Fig. 2, B well, R2 is 0.853.

Correlation between results and Poisson process

Originally Eq [1] is the probability density function of a Poisson process, which is an limiting case of the binomial distribution based on specific mathematical conditions.

In general a Poisson process is supposed to treat the phenomenon which rarely happens and the most important feature of it is the uniform configuration.

The question is whether there is any correspondence between the strict conditions of Eq [1] and physiological phenomena.

The distinctive characteristics of Poisson processes are that the sum of an independent Poisson variable is a Poisson variable too and that parameters are additive.

Skeletal muscle works voluntarily. When the nerve impulse triggers the SR, a simultaneous increase of Ca concentration throughout the cell occurs. This could satisfy the condition of uniform configuration. Therefore, what the mathematics implies is that the twitch wave looks so dramatic but is an "unusual" event. Innumerable molecules in a cell should be playing some other important "usual" roles continuously.

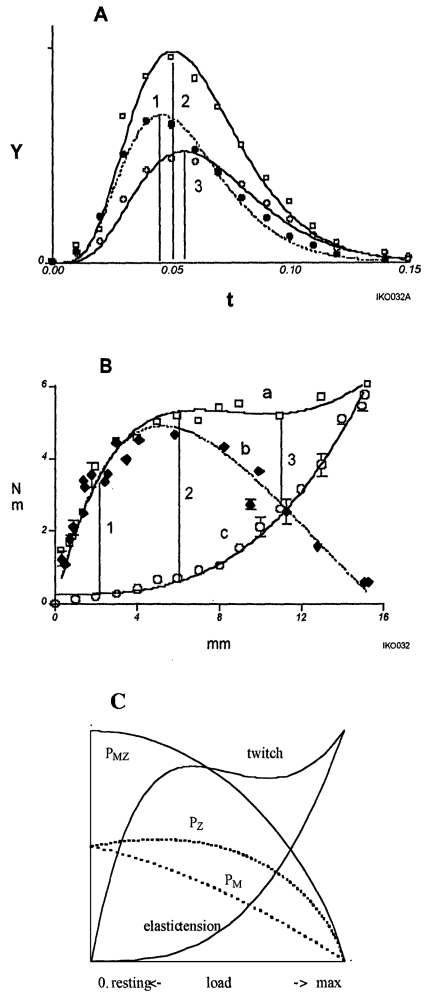

Fig. 2 A; three twitch waves and fitting curves. B; elastic tension (open circles); peak of twitch wave (black diamond); total tension (open squares); and fitting curves; (a) total force, (b) peak of twitch, (c) elastic tension. C; Diagram by Eq [10] (see text).

Resonance vibration of myosin-actin complex (MAC)

When myosin chemically binds to actin, it becomes like a molecular joint. As a composition ingredient of the body molecules in the living body are restrained somewhat spatially. The Lagrangean function L will be useful to express spatially restricted 1-dimensional motion of the system.

In the system where two vibrating particles, (p_1, p_2) are connected by a spring, (k_2), and both ends are fixed to side walls also by springs, (k_1, k_3), resonance will take place. The Lagrangian equation is able to express resonance vibration of this system. The mass of the particles is set to m_1 and m_2. When m_1 is fixed to 1.0 and value of m_2 is varied from 0.1 to 1.0, assuming that the mass of actin increases in proportion to external load, the resonance frequency of the system changes. The natural frequency ω_n of p_1 and p_2 are;

$$\omega_{np1} = ((k_1+k_2+k_3)/m_1)^{1/2} \quad [6]$$

$$\omega_{np2} = (k_2/m_2)^{1/2} \quad [7]$$

Change of L only depends on the value of m_2. Then it is examined how much the formula which made m_2 in ω_{np2} variable x would fit experimental values;

$$\lambda(x) = (0.03368/x)^{1/2} + 0.3526 \quad [8]$$
$$0.1 \leq x \leq 1.0$$

The curve (a) in Fig. 3 A is drawn by Eq [8], which fits to the data, λ (open circles); R_2: 0.9263. Elasticity of the spring between molecules is 0.034; [N/m]. The curve (b, dotted line) is based on Eq [2] which is the ordinary one phase exponential decay; R_2: 0.9422.

Fig. 3 B is to show the behavior of L.
When m_2 is increased from 0.2 (dotted line) to 0.6, resonance vibration slows down ($m_1=1.0$, $k_1=k_3=0.3$). It means that single MAC responds proportionally to the load following the Hooke's law.

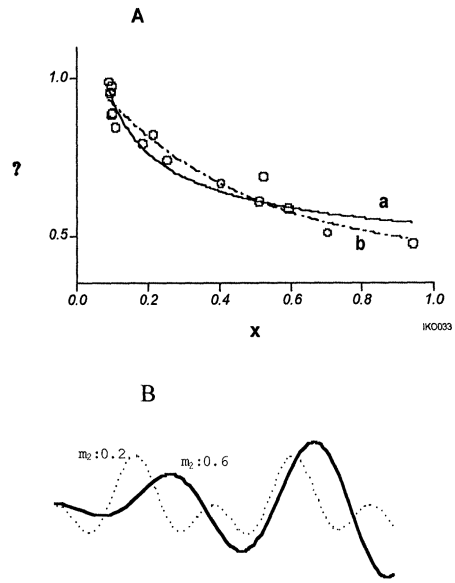

Fig. 3 A; λ (open circle); the curves of Eq [6] (a); and Eq [2] (b). B; resonance vibration of Lagrangean L; mass 0.2 (dotted line); 0.6 (black line).

The elastic tension increases with stretching a muscle (Fig. 2 B). There is a lattice structure of all MAC units. Resonance vibration will be far more complicated. Since single MAC behaves as Hookian, the following formula is used. The curve (c) along open circles in Fig. 2 B fits well, R_2: 0.9869. Formula is;

$$Y(x) = 0.2645 + (0.1177\gamma)^3 \qquad [9]$$
$$1 \leq \gamma \leq 15.3 \quad \gamma:[\mathrm{mm}].$$

Right hand side of Eq [9] implies that the reaction of each unit to load follows the Hooke's law. In the 3-dimensionsional lattice structure, elasticity can be approximated assuming that each unit works like 3 units. The normalized formula becomes;

$$Y(x) = 0.036 + x^3, \ (x = 1.0\gamma, 0 \leq \gamma \leq 1)$$

This is a simple but useful model to treat the non-Hookian elasticity of muscle.

In the experiment twitch tension is recorded above the elastic tension and the net height is plotted in Fig. 2 B (black diamonds). It suggests that work will be possible if the excess energy is stored.

Since λ [s^{-1}] is related to velocity, (Eq.2), the amount of stored energy can be estimated by using the relative V(x) as follows;

$$V(x) \approx (\lambda(x) - \lambda_{x=1}) / (\lambda_{x=0} - \lambda_{x=1}) \qquad [10]$$

V(x) [m/s] times x, [N] gives the energy for work, (W(x), [J/s=W]). Formula describing the relation between twitch height and external load becomes as follows;

$$W(x) = 2.66 \cdot x \cdot (\exp(-0.154x) - 0.09) \qquad [11]$$
$$0 \leq x \leq 15.3$$

Dotted curve (b) in Fig. 2 B is drawn by Eq [11]; R_2: 0.8476.

Open square mark in Fig. 2B shows the sum of elastic and twitch tensions at each level of stretching. The number of Fig. 2B is to show the point of each twitch wave in Fig. 2A. The curve along total tension is drawn by the sum of two formulas; R_2: 0.9675.

Probability process with random configuration

Muscle works by twitch. However the twitch wave occurs only if the configuration is uniformized. Twitch is like a big sudden wave on a lake where tiny ripples never cease. Even if twitch does not happen, other process should be proceeding because Brownian motion never stops.

The Gaussian distribution in which stochastic configuration differs from Poisson process is to be the "usual" process. It is likely that the mean square variance of Gaussian distribution is proportional to load.

Myosin is swinging back and forth and the internal energy is produced constantly regardless to the direction. However, if ATP is depleted, the rigor will take place.

As for the swinging direction in the sarcomere, myosin head should step towards M-line or Z-disc. When muscle is relaxed, probabilities of the step direction are equal. If one end is bearing load, difference of probability will be more pronounced.

In the binomial distribution it is set to p as probability of the swing toward M-line and to q (=1-p) as probability of the swing toward Z-disc. Energy produced by M-ward step is set to P_M, and energy produced by Z-ward step is set to P_Z. Then sum of two energies is set to P_{MZ} which means the amount of stored energy available for work. Assuming that x is proportional to load, the amount of stored energy can be expressed as follows;

$$P_{MZ} = p \log_2(1/p) + q \log_2(1/q) \qquad [12]$$
$$-1 \leq x \leq +1$$

Where p=(1-x)/2; x is the relative load.

Fig. 2 C is the diagram based on Eq [12]. The abscissa is the displacement and the ordinate is for the energy. The left end of the abscissa is the resting point (x=0). Since P_M and P_Z are related to p and q. P_{MZ} reaches its maximum value at the resting point where p=q=0.5. The right end of abscissa is the point of maximum load, where all random swing of myosin stops.

The probability that the micro system in a macro system is in the state of energy $\varepsilon^{(j)}$ is proportional to $\exp(-\varepsilon^{(j)}/k_BT)$. Therefore in the present system $\exp(-\beta x)$ is expressing the state of stored energy at different conditions. The energy barrier is highest at x=1, which is proportional to $\exp(-\beta)$. So it seems that βk_BT is the energy for single MAC. ($9.52 \cdot 10^{-21}$ J , 20℃).

If the muscle length is fixed tightly, muscle does not work and can utilize maximum stored energy of P_M. This is the isometric contraction.

In Fig. 2 C curves of the elastic tension and the twitch height are superimposed. A normalized formula for the latter is as follows; R_2: 0.8786;

$$W(x) = 6.164 \times V(x) \qquad [13]$$
$$0 \leq x \leq 1$$

Although the characteristic between energy and load is expressed by $\{x^*V(x)\}$, constant of 6.164 is necessary to fit. It may be related to the short range neighbor interaction in the hexagonal pipe of actin and myosin heads spreading in six directions.

What the diagram indicates is that the elastic tension is developed with the expense of stored energy which will shorten the muscle as soon as load is disappeared. (Entropy elasticity)

The internal energy produced by ATP hydration is not only for work but also for the elastic tension and relaxation. The length of muscle is dependent on the balance of the intrinsic mechanism and each of shortening and elongation is to be the phenomenon consuming energy.

It is tempting to assume that this stress-strain mechanism is related to the periodicity of various muscles, if the external strain is periodic.

Actuator and slider

A boy has to bend and stretch twice for every cycle in order to continue to and fro on rocking a swing. ATP hydrolysis occur twice for every cycle of myosin vibration. Energy should be supplied twice into one cycle which makes the self-generating motion smooth.

A pair of thick and thin filament of half sarcomere length represents the bio-motor unit in a cell. Thick filament is the actuator and thin filament is the slider corresponding to those units of the ultrasonic driven motor (USDM)[1]. Actuator of USDM which put piezo-electric element of the shape of a tooth of a comb in order is tightly forced on the slider. Then a traveling wave is started by periodic minute vibration of element, and the slider is slid. If power is off, two parts stick to each other just like rigor in muscle.

Swing of myosin is calculated with the equation of pendulum motion. Pendulum with 20 nanometer length swings in a cycle of $1/\lambda$ second and the width of a swing is less than ±6 nm. And then it is estimated as the USDM type motor. The tip of myosin head carries out small ellipse movement with inclination to actin. The major axis of ellipse is 0.2 nm or less. Inclination of major axis of an ellipse is determined by the stepping direction of myosin.

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References

1) Kenzyo, N., Sashida, T., (1993) Choonpa-Nyumon, Sogodensi-shuppan, Tokyo., 119-158.