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Slow and macroscopic modulation of thermal fluctuations in myofibrils

Hajime Honda, Shinji Nagao, Kuniyuki Hatori, Koichiro Matsuno *

Department of Bioengineering, Nagaoka University of Technology, Nagaoka 940-21, Japan

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Abstract

A comparison was made between fluctuations in a length of sarcomere from a myofibril during relaxed and rigor conditions. The fluctuations of the length associated with rigor states was due entirely to thermal fluctuations in the ambient. In contrast, the fluctuations associated with relaxed states were accompanied by extremely slow and macroscopically modulated thermal fluctuations. A slow, macroscopic modulation of the thermal fluctuations of the length of sarcomere was found to be unique to myofibrils in their relaxed conditions.

Keywords: Actomyosin; ATP; Fluctuations; Myofibril; Sarcomere; Thermal noise

1. Introduction

Cell motility driven by ATP hydrolysis raises at least two possibilities for materializing mechanical movements which are both asymmetric in space and time. One is to utilize the energy released from ATP to directly drive the molecular machine [1] in an asymmetric manner in space. Another possibility is to let the energy from ATP regulate thermal energy in the ambient spatially, in an asymmetric manner [2]. Of these two possibilities, however, the more serious attention should be directed toward the latter emphasizing the role of ATP hydrolysis as a regulator. The amount of energy released from a single ATP molecule is of the order of at most 10 kT (k: Boltzmann's constant, T: temperature) and does not significantly distinguish itself from that from thermal fluctuations [3,4]. In particular, if both a spatial asymmetry and fluctuations or noises with a finite time duration for their temporal correlation exist as in the case of a thermal ratchet [5,6], then a macroscopic and asymmetric movement in space would be expected.

As a matter of fact, myofibrils as a complicated assembly of actomyosin complex [7] seem to satisfy both the conditions of asymmetric structure in space and temporal correlation of a finite duration in time. Actin filament, a major constituent of myofibrils, certainly does have an asymmetric spatial structure distinguished by possessing an arrow-like polarity. Furthermore, the characteristic time for utilizing one molecule of ATP through its hydrolysis at each actomyosin complex within a myofibril is roughly of the order of or greater than 10 ms as estimated from

* Corresponding author.

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a motility assay [8]. These two aspects of asymmetric structure in space and temporal correlation with a finite duration in time may provide us with a condition that states that the energy released from ATP could serve as a factor regulating thermal fluctuations from the surroundings. They may utilize the energy from ATP in order to derive from thermal fluctuations the energy driving a unidirectionally macroscopic movement.

In the present article we try to examine whether the energy released from ATP in a myofibril actually regulates thermal fluctuations in such a way as to drive its unidirectional movement. For this purpose, we compare fluctuations of a length of sarcomere of a myofibril in its relaxed and rigor states. Measurements were carried out only for a single myofibril. The chemical features of each myofibril were considered to remain the same for all the similarly prepared samples, especially with regard to their protein components. We understand hereby that these chemical features may remain invariant even when the solution conditions are changed between relaxed and rigor states.

If myofibrils are in a rigor state, which is characterized by a complete cross-bridging in the absence of ATP, fluctuations in the length of sarcomere may completely obtain their driving energy from thermal fluctuations in the surroundings. On the other hand, if the myofibrils are in a relaxed state, with no appreciable cross-bridges but in the presence of ATP, it can be expected that an extremely slow hydrolysis of ATP proceeds on the myosin heads exposed to ATP or that the ATP changes the elastic modulus of the myofibrils. Accordingly, it is possible that the energy released from ATP may regulate or modulate fluctuations in a length of sarcomere in a different manner from the case of rigor states [9].

We shall in the following part of this article examine whether myofibrils in their relaxed states can exhibit fluctuations in their sarcomere length which are significantly different from the ones obtained for rigor states.

2. Materials and Methods

We sampled muscle fibers from rabbit psoas and treated them with 50% (v/v) glycerol solution having 5 mM potassium phosphate buffer (pH 6.8) and 10 mM DTT (dithiotheritol) at -20°C for 12 hours.

Measurement of fluctuations of the sarcomere length of rabbit psoas myofibrils in a rigor state was made with those myofibrils that were extracted from preserved psoas muscles by homogenizing them with rigor solution (50 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethansulfonic acid, pH 7.0) and 10 mM EDTA (ethylenediamine-N,N,N′,N′-tetraacetic acid)). Similarly, measurement of fluctuations in relaxed state was done by changing the rigor solution, after centrifugation of 3000 rpm for 1 min, to a relaxed one (100 mM KCl, 5 mM $MgCl_2$ 5 mM ATP, 5 mM EGTA (ethylene glycol bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid), and 10 mM HEPES (pH 7.0).

Examination of the fluctuations of the sarcomere length were made with a phase-contrast microscope Nikon Biophot with a Nikon oil-immersed, objective lens CF Plan DL 100X and a TV camera CT2400-07 (Hamamatsu Photonics). Images on the TV monitor screen (Sony SLV-R5) were retrieved into an image processing unit CT9800B (Cybertech, Co.). Images were taken every 1/60 s. The time interval for taking a time average was chosen to be 40 seconds.

Temperature control of our system was carried out by placing a large copper plate with a small hole at its center on a slide glass. The copper plate was connected to a copper pipe through which water was passed at a controlled temperature. Specimens were put on the hole at the center of the copper plate.

3. Results

We first examined the resolution power of our image processing system. For this purpose, a scale bar of 10 μm length was used as a reference. Under the microscope the 10 μm length was counted as 251 consecutive dots each of which was equally spaced along the length. The resolution power for reading a well-defined distance under a phase-contrast microscope [10] was about ± 3 nm in terms of its standard deviation, as is shown in the top of Fig. 1, in which an interpolation between adjacent sampled data was taken by assuming a Gaussian distribution. Although the resolution power of any optical microscope is limited by the wavelength of visible

light, a phase-contrast microscope can effectively enhance its resolution by appropriately adjusting the image phases. In particular, since each of our sample myofibrils had an oriented structure as exhibited by their definite polarity, their phase-contrast image was certainly available.

The results of the fluctuations in the length of sarcomere, whose mean value was roughly 2.6 μm, are presented for both relaxed and rigor states in Fig. 1 in which the temperature was 25°C. The time interval for the measurement is arbitrary, though the results represented here display only those obtained for an interval of 40 s. Fluctuations associated with rigor states originate only as thermal fluctuations in the ambient. In particular, as far as rapid fluctuations are concerned, we could not find any appreciable difference between relaxed and rigor states.

In order to observe their characteristics over a longer time-scale, we also measured the autocorrelation function of the fluctuations. The autocorrelation function of the fluctuating intensity of the length of sarcomere $A(t)$ at time t was estimated by measuring the time average $\overline{A(t+\tau)A(t)}$ of the product of $A(t+\tau)$ and $A(t)$, in which the normalization

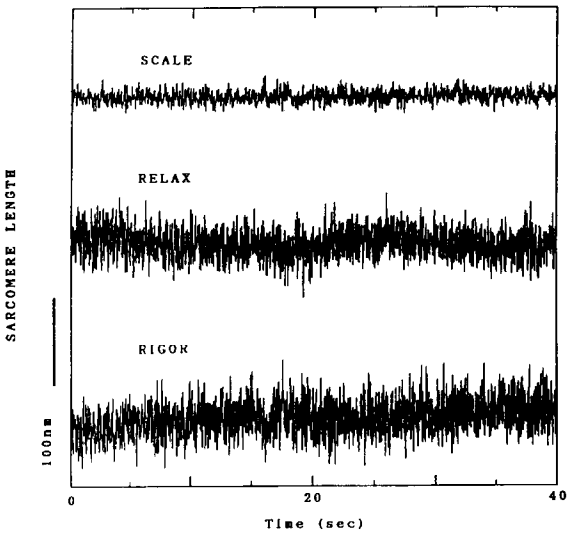

Fig. 1. Development in the fluctuations of a sarcomere length for relaxed (in the middle) and rigor conditions (at the bottom). As a reference, fluctuations occurring in the measurement of the scale bar with a definite length are shown at the top. These fluctuations, associated with the measurement of a definite scale bar, set the resolution power of the measurement equipment.

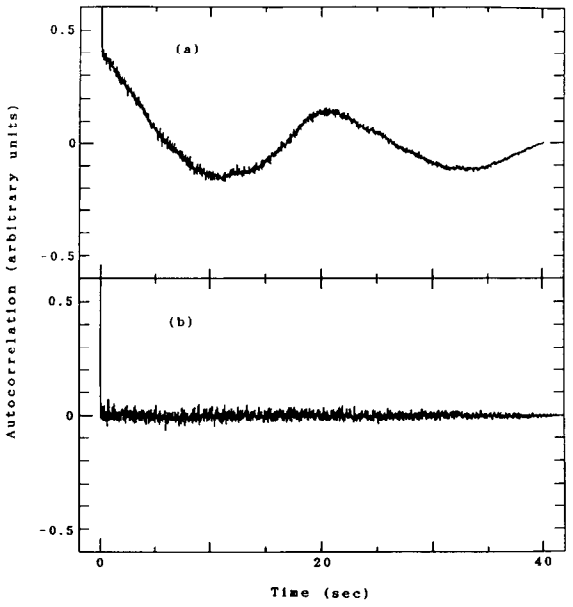

Fig. 2. The autocorrelation function of fluctuations in the length of sarcomere for relaxed (above) and rigor conditions (below).

condition $\overline{A(t)=0}$ was taken and the value of $\overline{A^2(t)}$ was counted in arbitray units. The results are presented in Fig. 2, in which the time interval for taking a time average to estimate the autocorrelation function was chosen to be 40 s. We thus observed that the fluctuations for relaxed states clearly exhibited a much longer correlation time when compared to those for rigor states.

No significant difference was observed in the fluctuations of the relaxed and rigor states during short periods of time. The absence of an appreciable difference suggests that fluctuations in the relaxed states may be equivalent to those found for rigor states with the exception that the former are slightly macroscopically modulated with time. In order to examine the possibility of modulating thermal fluctuations, we obtained the power spectra for both the fluctuations for the relaxed and rigor states presented in Fig. 2. The results of the spectral representation of $\overline{A(t+\tau)A(t)}$ averaged over 40 s are shown in Fig. 3. Actually, the spectral representation is simply the Fourier transform of the the autocorrelation function. Fluctuations in the relaxed states were found to exhibit a large spectral component near to a frequency of 0.05 Hz. These results remained un-

changed even when the time interval for averaging was changed from 20 and 50 seconds.

A comparison of the fine details of the power spectra between the relaxed and rigor states near the major spectral component at 0.05 Hz are presented in Fig. 4. In order to eliminate the likelihood of the intervention of any experimental artifacts we examined the power spectrum of the average of the ensemble of the time-averaged autocorrelation function $\langle \overline{A(t+\tau)A(t)} \rangle$. Ten statistically independent samples were chosen and the interval for the time average was 40 s. Again, the results presented in Fig. 4 remained unchanged even when the number of the independent samples was varied from 10 to 20 and the time interval for averaging was varied from 20 to 50 s. The observed results show that as far as the side band of the spectrum in the lower frequency region is concerned, the power spectrum for the relaxed states seems to be shifted upwards by a frequency of approximately 0.13 Hz when compared to that for the rigor states.

In order to improve upon the likelihood of the frequency shift, we estimated the power spectrum of the cross-correlation between the correlation function for the rigor state $C_{\text{rigor}}(\tau') = \overline{A_{\text{rigor}}(t+\tau')A_{\text{rigor}}(t)}$ and a similar function for the relaxed state $C_{\text{relax}}(\tau'') = \overline{A_{\text{relax}}(t+\tau'')A_{\text{relax}}(t)}$. In fact, this power spectrum of the cross-correlation points to the spectral representation of the cross-correlation between the power spectra of the sarcomere lengths in rigor and relaxed states as displayed in Figs. 3 and 4. The cross-power spectrum was evaluated by the spectral analysis of the ensemble average of $\langle C_{\text{rigor}}(t+\tau)C_{\text{relax}}(t) \rangle$, in which we prepared twenty statistically-independent samples. The result is shown in Fig. 5. The significant spectral peak at 0.13 Hz compared to other minor ones in the cross-power spectrum suggests that an appreciable macroscopic frequency shift of approximately 0.13 Hz may exist between the fluctuations in the rigor and the relaxed state. The present shift in the frequency domain of the power spectrum suggests that these fluctuations in the relaxed states are effectively equivalent to the ones for the rigor states modulated at a frequency of approximately 0.13 Hz to such an extent that we can read the observed spectral representation of the fluctuations. Modulating thermal fluctuations is unique to the fluctuations observed for relaxed states. The presence of other minor modulations, in addition to

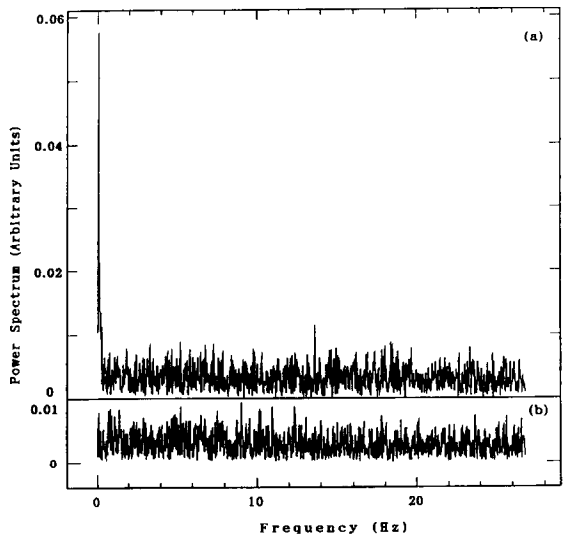

Fig. 3. The power spectrum of fluctuations in the length of sarcomere for relaxed (above) and rigor conditions (below).

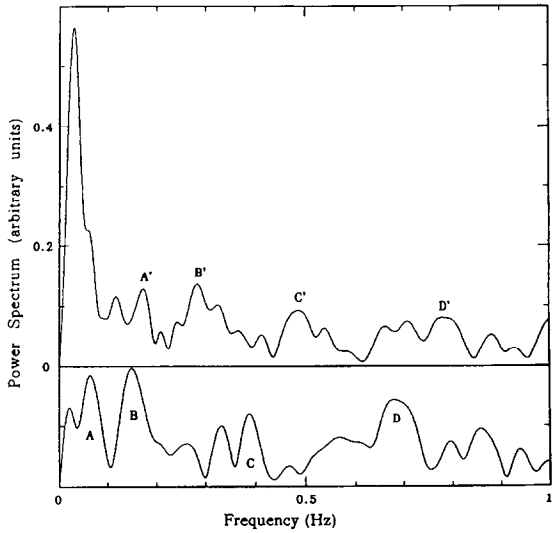

Fig. 4. Fine details of the power spectrum of fluctuations in the sarcomere length in the low-frequency region for relaxed (above) and rigor conditions (below). Several spectral peaks of the power spectrum for rigor conditions denoted as A, B, C, and D are seen to be shifted to A′, B′, C′, and D′ in the power spectrum for relaxing conditions, in which the frequency shifts are almost the same for all of these peaks with their value equal to approximately 0.13 Hz.

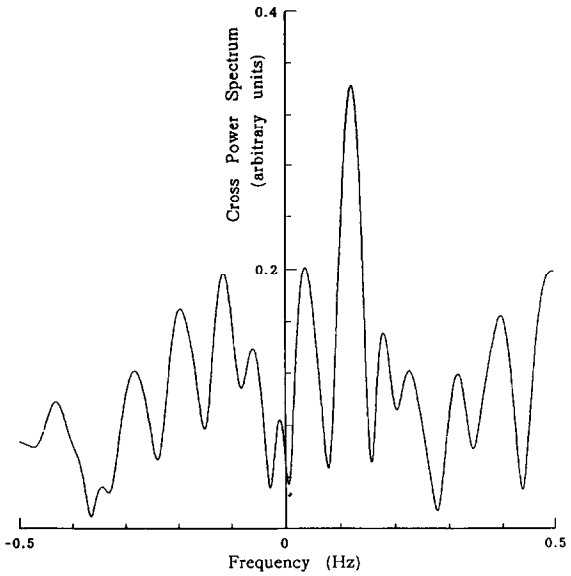

Fig. 5. Cross-power spectrum comparing fluctuations in the sarcomere length in a rigor state with those in a relaxed state.

the major one at 0.13 Hz, also suggests that the slow and macroscopic modulation of thermal fluctuations takes place at many different frequencies at the same time.

4. Discussion

The differences in the fluctuations of a length of sarcomere during relaxed and rigor states has been found to be due to whether a slow and macroscopic modulation of the thermal fluctuations is present or absent. In fact, the modulation of thermal fluctuations poses an intriguing problem in its own light. The possibility of modulating thermal fluctuations macroscopically comes to mean whether it is possible to regulate thermal fluctuations in a controlled manner. What we have observed in the measurements made above is that myofibrils in their relaxed states may modulate thermal fluctuations originating in the ambient. The present modulation exhibits a marked contrast to the case of rigor states having no capacity to influence and act upon thermal fluctuations.

One of the likely interpretations for the occurrence of the macroscopic modulation of thermal fluctuations may be found in how the energy stored in ATP is released during its hydrolysis. There seems to be at least two possibilities for energy dissipation from ATP. One is that the energy stored in an ATP molecule is dissipated simply by adding to the available thermal fluctuations without modulation. The energy dissipation occurs in one direction from the energy source within an ATP molecule to its ambient. Another possibility is to dissipate the stored energy while temporarily taking in the thermal energy in the ambient. One of the contributions of the thermal energy from the ambient to the ATP hydrolysis in myofibrils may be to change their stiffness. Consequently, the possibility of modulating thermal fluctuations could reside in a two-way energy transaction between releasing the stored energy in an ATP molecule through hydrolysis and then being acted upon by thermal energy from the ambient. Underlying the two-way energy transaction is maintenance of, or equilibration for, energy flow continuity between the available energy sources [2].

The modulation of thermal fluctuations provides a possibility of facilitating a heat engine. This possibility may materialize if thermal fluctuations really are modulated especially with regard to their amplitude. By associating the amplitude with temperature, one can conceive of the possibility that a certain amount of work can be achieved and extracted by allowing the energy dissipation to take place between a higher and a lower temperature region actualized within the modulated thermal fluctuations themselves. However, the relationship between the energy released and utilized for modulating the thermal fluctuations and the work carried out by the engine heat feeding on these modulated thermal fluctuations are at most indirect. Even if no work is extracted from the heat engine, energy for modulating the thermal fluctuations would still be required.

The energy released from ATP in myofibrils in their relaxed conditions can serve as a factor to regulate and modulate thermal fluctuations originating in the ambient, and these modulated fluctuations can now induce fluctuations in the sarcomere length even if there is no cross-bridge between actin filaments and myosin heads. In particular, no appreciable cross-bridges have been identified for myofibrils in their relaxed states. Slowly modulating fluctuations in the sarcomere length of myofibrils in relaxed

conditions suggests that the transduction from chemical to mechanical energy due to ATP hydrolysis is indirectly responsible for incorporating into itself the intervening process of modulating thermal fluctuations.

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