43. *Involvement of "Thermal Interference" in the Multiple Working Strokes Per Hydrolyzed ATP Observed in Muscle Contraction*

By Kenichiro MOGI
Department of Physics, Faculty of Science, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku 113
and
*) Laboratory for Neural Networks, The Institute of Physical and
Chemical Research (RIKEN), 2-1 Hirosawa,
Wako-shi, Saitama 351-01

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Abstract: Recently, observed large values of 'step size' in *in vivo* muscle contraction[1),2)] and *in vitro* actomyosin motility assays[3)–5)] have raised new questions about the nature of coupling in biological systems. It has been experimentally demonstrated[7)] that multiple working strokes occur during the hydrolysis of one ATP molecule. It is difficult to explain these observations by conventional enzyme kinetics. In this paper, we propose a novel enzymatic mechanism (the 'coupling ratio constraint' mechanism). By 'free energy mixing' between the two coupled degrees of freedom, we can explain the mechanism underlying the large values of step size and the multiple working strokes per ATP hydrolyzed observed in muscle. We predict that myosin would behave in an unconventional manner during the multiple working strokes. This prediction can be tested experimentally. Finally, we propose that 'thermal interference', similar to quantum interference[20)] (but of different physical origin) is involved in the observed phenomenon.

Key words: Muscle contraction; multiple working strokes; thermal interference.

In many biologically important reactions, an otherwise unfavorable reaction is driven by 'coupling' it to another energetically favorable reaction. The exact nature of this important "coupling" mechanism, however, is not yet known. In this paper, we propose a novel principle of enzyme coupled reactions, which involves what we define in this paper as 'thermal interference'.

In muscle contraction, a relative sliding between the actin and the myosin filament is caused by the hydrolysis of ATP. The 'step size' is defined as the distance of sliding caused by the hydrolysis of one ATP molecule. The 'working stroke' is defined as the unit event of sliding conducted by myosin. It has been estimated that the distance of sliding caused by one working stroke is about 12 nm.[12)] Since it has been assumed that the hydrolysis of one ATP molecule causes one working stroke,[11)–13)] the sliding distance caused by the hydrolysis of one ATP molecule has been estimated to be about 12 nm. Yanagida *et al.*,[1)] however, observed that the step size in crab muscle under free load condition is > 60 nm.

The large values of 'step size' observed by Yanagida and others[1),2),4),5)] is very puzzling, although a smaller value of step size has not been completely excluded.[6)] Since at the scale of actin filament and myosin filament we can virtually neglect the inertia,[14),15)] actin filament can slide only during the working stroke. At the end of working stroke, actin filament is supposed to stop. As from structural limitations the sliding distance of actin filament caused by one working stroke cannot be larger than 40 nm,[18)] it has been concluded that observed large values of the step size must be the result of more than one

*) Corresponding address.

working strokes per ATP hydrolyed.[4] Recently, it has been experimentally shown that in contracting muscle several elementary working strokes occur per ATP molecule hydrolyzed.[7] It is therefore interesting to consider how multiple working strokes can occur during the hydrolysis of one ATP molecule.

An obvious explanation of the occurrence of multiple working strokes per ATP molecule hydrolyzed is to assume the existence of multiple intermediate states (multiple intermediate states hypothesis). Namely, we assume that the ATP hydrolysis cycle is composed of intermediate states as $S_1 \rightarrow S_2 \rightarrow \cdots \rightarrow S_N \rightarrow S_1$, with gradually decreasing free energy levels. If we then assume that one working stroke is caused by the transition from one intermediate state to the next state, N working strokes can in principle occur during the hydrolysis of one ATP molecule. However, it is difficult to make this scheme compatible with the repeated conformation change accompanying the multiple working strokes.[17] It is unlikely that the intermediate states can sequentially take the alternating conformations of myosin during one ATP hydrolysis cycle.

A second explanation is that the energy liberated by the hydrolysis of ATP is stored in some form in myosin molecule, and then used little by little in the multiple working strokes. The thermal ratchet models[8)-10)] postulate that the free energy liberated by the hydrolysis of ATP is stored as a temperature difference between the actin filament and myosin head. However, since the relaxation of the excitation of any mode of protein in water is very rapid,[19] this kind of temperature difference is unlikely to sustain sliding over time lasting for milliseconds unless 'temperature' in these models represent some previously unknown sustainable mode of excitation.

In summary, we are encountering a fundamental difficulty in understanding the multiple working strokes per ATP hydrolyzed observed in muscle contraction.

In what follows, we propose a novel coupling principle which can explain this interesting phenomenon.

Let us write the two degrees of freedom that are coupled as x (sliding of actin filament) and y (hydrolysis of ATP). We write the rates of transition in these degrees of freedom as V_x (number of working strokes per second) and V_y (number of ATP molecules hydrolyzed per second). In the mathematical model developed here, we approximate the ATP hydrolysis cycle with one step transition, which can be interpreted as the phosphate release step (A.M.ADPPi$\rightarrow$A.M.ADP). This approximation does not affect the essential feature of our model. The free energy change accompanying the working stroke and the ATP hydrolysis are written as E_x and E_y, respectively. We define 'coupling ratio' C as[16]

$$C = \frac{V_x}{V_y} = \frac{v_1 - v_2}{u_1 - u_2} \tag{1}$$

where (v_1, v_2) and (u_1, u_2) are the forward and backward rate constants for the working stroke and ATP hydrolysis, respectively. In other words, coupling ratio is the number of working strokes conducted per ATP molecule hydrolyzed.

We assume that through the interaction between actin filament and myosin coupling ratio C is determined as a function of the load (E_x). Namely, we assume that the essence of coupling through actomyosin interaction is the constraint imposed on coupling ratio C ('coupling ratio constraint' hypothesis) (Fig. 1).

Our hypothesis takes the form of

$$\frac{v_1}{u_1} = \frac{v_2}{u_2} = C \tag{2}$$

In order to satisfy relation (2), free energy changes E_x and E_y must be transformed. Let us write the transformed free energy changes as E_x' and E_y'.

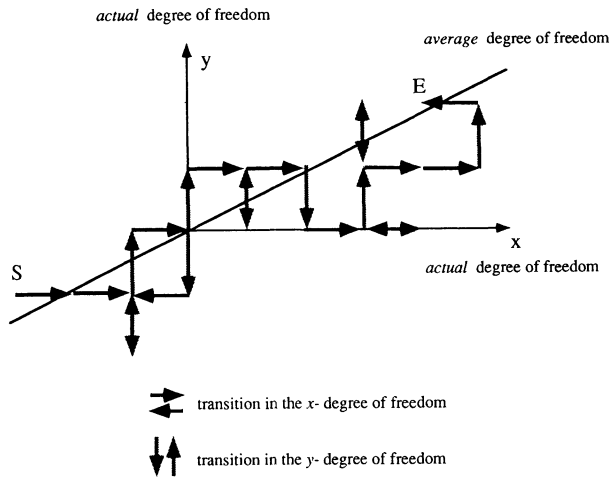

Fig. 1. Coupling ratio constraint mechanism. In the coupling ratio constraint mechanism, it is assumed that the coupling ratio (the ratio of the transition rate constants in the coupled degrees of freedom) is fixed at a constant value by the enzyme. The actual transitions, however, continue to occur in two dimensions. The discrepancy between the average and actual degrees of freedom leads to the thermal interference effect. In this figure, the average degree of freedom is shown by the bold line. The transitions of the system are restricted, *on average*, to this single degree of freedom. An example of a sequence of the actual transitions is represented by the directed edges.

We make a further assumption that the free energy change is invariant in the direction of vector $(C,1)$, which is the direction of the actual change under condition (2). This assumption is necessary for the conservation of energy. Namely,

$$CE_x+E_y = CE_x'+E_y' \tag{3}$$

In order to derive E_x' and E_y', we make use of the Arrhenius relations

$$\frac{v_1}{v_2} = \exp\left(-\frac{E_x'}{kT}\right) \tag{4}$$

$$\frac{u_1}{u_2} = \exp\left(-\frac{E_y'}{kT}\right) \tag{5}$$

From equations (2)–(5), we can derive the transformed free energy changes as

$$E_x' = E_y' = \frac{C}{1+C}E_x+\frac{1}{1+C}E_y \tag{6}$$

In particular, under the free load ($E_x=0$) condition, the transformed free energy levels can be given as

$$E_x' = E_y' = \frac{1}{1+C}E_y \tag{7}$$

We see that the free energy released by the hydrolysis of ATP (E_y) is used to drive the working stroke by a free energy gradient of E_x'. Multiple working strokes per ATP hydrolyzed is thus possible in this scheme. Moreover, since every working stroke is driven

by a free energy gradient of E_x', the sliding of actin filament is smooth.[5)]

It is interesting to consider implications of transformation (6). In our scheme, the two degrees of freedom are related through coupling ratio constraint of (2) and are no longer independent. As a result, the free energy changes E_x and E_y are 'mixed' into a single value. The hydrolysis of ATP drives the working strokes through this 'free energy mixing'. Note that mathematically, 'coupling ratio constraint' effectively reduces the dimension of the space in which transition occurs from two to one. The actual transitions of the state, however, continue to occur in two dimensions. It is this discrepancy that is the origin of the 'free energy mixing'.

From the analysis of the model we presented in this paper, we can make the following predictions about the nature of coupling between the hydrolysis of ATP and the sliding of actin filament.

(i) ATP is not necessarily hydrolyzed when one working stroke occurs.
(ii) When ATP is not hydrolyzed, the chemical state of myosin is the same before and after the working stroke.
(iii) The sliding of actin filament is uniform regardless of the particular time at which the hydrolysis of ATP is completed.
(iv) The multiple working strokes during the hydrolysis of ATP is driven by exactly the same amount of free energy change.
(v) The number of intermediate states involved in the coupling can be much smaller than the number of working strokes conducted per ATP hydrolyzed.
(vi) One myosin molecule is sufficient to sustain multiple working strokes per ATP hydrolyzed.

These predictions can be tested in future experiments to verify the validity of our model. In particular, it would be interesting to test the above predictions under conditions where large values of coupling ratio is realized.[21)]

The 'coupling ratio constraint' hypothesis that we introduced in this paper has several novel features.

Firstly, our model predicts an unconventional behavior of myosin during the multiple working strokes. If the coupling ratio is C, C working strokes are conducted during the hydrolysis of one ATP molecule. In C-*1* out of C cases, one working stroke would be conducted without the completion of the hydrolysis of ATP. Therefore, in our model the state of myosin before and after the working strokes remains the same in C-*1* out of C cases (Fig. 2).

Secondly, a 'thermal interference effect' is involved in our model. The 'coupling ratio constraint' is imposed between two events, where the first event is the event 'working stroke is not done and ATP is hydrolyzed', and the second event is the event 'working stroke is done and ATP is not hydrolyzed'. These two events are mutually inconsistent. However, the free energy changes accompanying these two inconsistent events are 'mixed' into a single value $E_x' = E_y'$ through the 'thermal interference' effect. It should be noted here that one myosin molecule is sufficient to induce the 'thermal interference' effect. Interaction between multiple myosin molecules is not necessary.

We can summarize the situation in which a thermal interference effect occurs as follows.

(i) There is a stochastic process, which occurs in two degrees of freedom.
(ii) Through a constraint imposed by the enzyme (myosin), the stochastic transition is restricted *on average* to one degree of freedom.
(iii) The *actual* transitions, however, continue to occur in two degrees of freedom.
(iv) The discrepancy between the *average* and *actual* behaviors of the system leads to the 'thermal interference' effect.

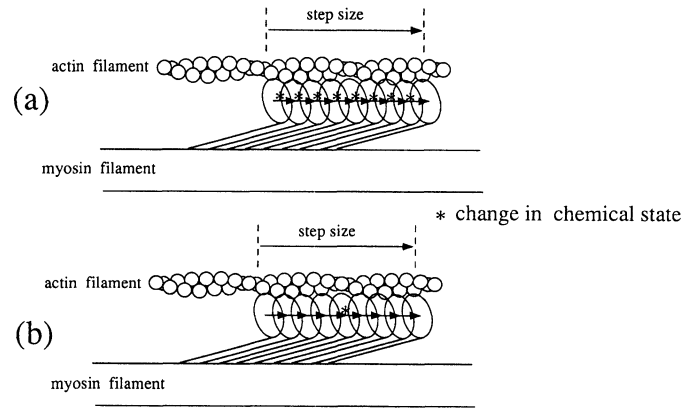

Fig. 2. Change in myosin states during the multiple working strokes. In this figure, coupling ratio is taken to be 8. (a) If we assume multiple intermediate states, every working stroke must be accompanied by a change in the chemical state of myosin, e.g., A.M.ADPPi→A.M.ADP (phosphate release step). (b) In the 'coupling ratio constraint' mechanism, the change in chemical state occurs *on average* in 1 out of 8 working strokes. In 7 out of 8 cases, working strokes occur without the hydrolysis of ATP. In such cases, the working strokes are driven by the mixing of free energy changes which is the result of the 'thermal interference' effect. This unconventional behavior of the myosin molecule can be tested in future experiments.

It is interesting to note that the above situation is similar to the situation leading to the quantum interference effect.[20] However, the underlying physical principles are quite different. In the quantum interference effect, the scale of the system is characterized by the Planck's constant, which is orders of magnitude smaller than the characteristic energy scale of $\sim kT$ (where k is the Boltzmann's constant, and T is the absolute temperature) for enzyme coupled reactions.

At present, it is not clear how an enzymatic protein can impose the 'coupling ratio constraint' in the form of (2). It would be interesting to reconsider the mechanism of enzymatic coupling from this point of view. Since myosin is expected to modulate the coupling ratio in order to work effectively under conditions of various load, there must be a corresponding molecular mechanism involved in muscle contraction.

Mathematical details of the model we presented here will be reported elsewhere.

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