A physical model of ATP-induced actin-myosin movement in vitro

Katsuhisa Tawada* and Ken Sekimoto‡
*Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Fukuoka 812; and ‡Department of Applied Physics, Nagoya University, Nagoya 464-01, Japan

ABSTRACT The nature of the mechanism limiting the velocity of ATP-induced unidirectional movements of actin-myosin filaments in vitro is considered. In the sliding proces two types of "cyclic" interactions between myosin heads and actin are involved, i.e., productive and nonproductive. In the productive interaction, myosin heads split ATP and generate a force which produces sliding between actin and myosin. In the nonproductive interaction "cycle," on the other hand, myosin heads rapidly attach to and detach from actin "reversibly," i.e., without splitting ATP or generating an active force. Such a nonproductive interaction "cycle" causes irreversible dissipation of sliding energy into heat, because the myosin cross-bridges during this interaction are passive elastic structures. This consideration has led us to prostulate that such cross-bridges, in effect, exert viscous-like frictional drag on moving elements. Energetic considerations suggest that this frictional drag is much greater than the hydrodynamic viscous drag. We present a model in which the sliding velocity is limited by the balance between the force generated by myosin cross-bridges in the productive interaction and the frictional drag exerted by other myosin cross-bridges in the nonproductive interaction. The model is consistent with experimental findings of in vitro sliding, including the dependence of velocity on ATP concentration, as well as the sliding velocity of co-polymers of skeletal muscle myosin and phosphorylated and unphosphorylated smooth muscle myosins.

INTRODUCTION

Muscle shortens as a result of sliding between thick and thin filaments within each sarcomere (Huxley, 1974). The sliding is powered by the splitting of ATP by myosin cross-bridge heads while interacting with actin.

Assay systems for directly observing unidirectional movements between actin and myosin in vitro have recently been developed (Sheetz and Spudich, 1983; Higashi-Fujime, 1985; Kron and Spudich, 1986; Toyoshima et al., 1987). Studies so far include the sliding of single actin-filaments on a myosin-coated substratum, single myosin-filaments on actin bundles, and (single) myosin-coated beads on *Nitella* actin.

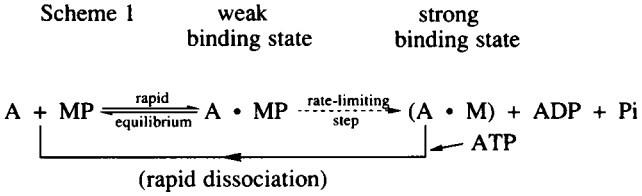
The following eight findings are particularly interesting with respect to the present study. (1) The velocity of filament sliding depends on the type of myosin used in the assay, not on the type of actin (Kron and Spudich, 1986). For example, the velocity of phosphorylated smooth muscle myosin is ~10% of that of skeletal muscle myosin (Sheetz et al., 1984). (2) The velocity of actin sliding is independent of the actin length, provided that actin filaments are longer than a lower limit (Takiguchi and Higashi-Fujime, 1988; Toyoshima et al., 1988). (3) The velocity of actin sliding is independent of the concentration of myosin heads, provided that their concentration is greater than a lower limit (Harada et al., 1987; Toyoshima et al., 1988). (4) The sliding velocity of single myosin filaments is independent of their filament length (Higashi-Fujime, 1986). (5) Sliding velocities of actin-, myosin-filaments, and myosin-coated beads are all similar to each other: ~5 μm/s with rabbit skeletal muscle myosin at room temperatures. This in vitro sliding velocity is similar to that calculated from the maximum velocity of shortening found in intact sarcomeres in vivo (Sheetz et al., 1984). (6) Sliding velocities of actin filaments and myosin-coated beads depend on the ATP concentration as does actomyosin ATPase activity. However, the ATP concentration for the half-maximal velocity of these movements is ~10 times greater than that for the half-maximal activity of actomyosin ATPase (Sheetz et al., 1984; Kron and Spudich, 1986; Harada et al., 1987; Toyoshima et al., 1988). (7) The addition of a small fraction of phosphorylated smooth muscle myosin to skeletal muscle myosin causes a drastic decrease in the velocity of sliding (Sellers et al., 1985). (8) Unphosphorylated smooth muscle myosin, unlike the phosphorylated form, does not produce the sliding, but its addition to phosphorylated smooth muscle myosin slows the velocity of the sliding (Sellers et al., 1985).

Dr. Sekimoto's present address is Groupe de Physico-Chimie Théorique, École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 75231 Paris Cedex 05, France.
Address correspondence to Dr. K. Tawada, Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka, Fukuoka 812, Japan.

What is the nature of the mechanism limiting the velocity of actomyosin sliding in vitro? This question is the main concern of this paper.

The ATPase activity of myosin is activated by actin. The actin-activated ATP splitting is the key reaction involved in the production of the contractile force (Taylor, 1979; Eisenberg and Hill, 1985). This ATPase activity, if measured in vitro, increases hyperbolically as a function of the concentration of added actin. The hyperbolic nature of this activity suggests the presence of a rapid equilibrium binding between actin and myosin which has nucleotide at its active site, followed by a rate-limiting ATP splitting step, as shown in Scheme 1 "of the barest kinetic essentials," where A is actin, M is myosin, and P is ATP or ADP · Pi. Because the step A +

Scheme 1



$MP \rightleftharpoons A \cdot MP$ is a rapid equilibrium, a single myosin head repeats, on the average, many "cycles" of attachment to and detachment from actin, before having a chance of going on through the rate-limiting step which leads to actin-activated ATP splitting. Thus, there are two types of cyclic interactions between myosin heads and actin: one accompanying and one not accompanying actin-activated ATP splitting. We will refer to them as productive and nonproductive cyclic interactions. Note that some fraction of weakly attached myosin cross-bridges completes the productive interaction cycle while the other fraction completes the nonproductive interaction cycle. The reason why the attachment/detachment of MP in the rapid equilibrium is called a cycle will be considered later.

The ATPase activity of unphosphorylated smooth muscle myosin is not activated by actin (Sellers, 1985) although the myosin weakly attaches to actin and is in a rapid equilibrium between detached and attached states in the presence of ATP (Sellers et al., 1982). As mentioned above, unphosphorylated smooth muscle myosin slows down the actin-myosin movement in vitro. Because the state of phosphorylation and unphosphorylation of each smooth muscle myosin does not change throughout a motility experiment in vitro and because the formation of latch bridges in smooth muscle, on the other hand, involves dynamic phosphorylation/dephosphorylation during attachment of myosin to actin (Dillon et al., 1981; Marston, 1989), the resistive effect of unphosphorylated myosin on the movement in vitro is not caused by the formation of latch bridges as postulated by Hai and Murphy (1988*a*, *b*). The resistive effect of the myosin must be instead caused by its simple attachment to, and detachment from, actin: a nonproductive cyclic interaction. These considerations have led us to postulate that the nonproductive cyclic interaction of myosin with actin in general exerts a resistive "frictional drag" on the moving elements such as actin filaments. In fact, a physical interpretation of the nonproductive interaction cycle shows that this interaction cycle, in effect, results in a viscous-like frictional drag as discussed in the Appendix B. Energetic considerations suggest that this frictional drag is much larger than the viscous drag of the surrounding solvent. We therefore postulate that the velocity of in vitro actomyosin sliding is limited by the frictional drag exerted by myosin cross-bridges in the nonproductive interaction, opposing the motive force generated by other myosin cross-bridges in the productive interaction.

Here we present a model which takes into account the frictional drag due to myosin cross-bridges in the nonproductive interaction. The model is consistent with the above-mentioned characteristics of the relative sliding in vitro. Our theoretical discussion developed for actomyosin sliding is also applicable to the relative sliding between microtubules and MAP 1C or kinesin or dynien (Vale et al., 1986; Paschal and Vallee, 1987; Paschal et al., 1987).

GLOSSARY

a	actin concentration in the kinetic scheme of actomyosin ATPase activity
A	actin
b	radius of an actin filament
e	concentration of myosin head in the kinetic scheme of actomyosin ATPase activity
E	elastic stiffness constant of a myosin head along the direction of actin movement
f_d	sliding force generated by single myosin head in the driving state
f_s	coefficient of hydrodynamic viscous drag on an ellipsoid of revolution
f_ζ	coefficient of generalized frictional drag
$F, F_d(t)$	sliding force per actin filament, where t is time
$F_h(t)$	total frictional drag exerted on an actin filament by myosin heads in the holding state, where t is time
k_i	rate constants of actomyosin ATPase activity ($i = 5 \ldots 1, -1 \ldots -4$)
K_i	$= k_{-i}/k_{+i}$ ($i = 1, \ldots, 4$)
K_m	Michaelis constant for actomyosin ATPase activity

K_{ms}	Michaelis constant for actin sliding
L	length of an actin filament
M	myosin
P	ATP or ADP · Pi
P_d	probability for a myosin head to be in the driving state
P_h	probability for a myosin head to be in the holding state
P_{hj}	probability for a myosin head to be in the holding state j (=1 or 2)
Pr	ADP + Pi
s	ATP concentration in the kinetic scheme of actomyosin ATPase activity
t	time
T_h	holding time: duration time of holding state
T_{hj}	holding time: duration time of holding state j (=1 or 2)
U	elastic energy stored in a myosin head during a holding time
$V, V(t)$	sliding velocity of actin filament, where t is time
V_{max}	maximum velocity of actin sliding
V(ATPase)	actomyosin ATPase activity
$V(\text{ATPase})_{max}$	maximum activity of actomyosin ATPase
W	rate of dissipation into heat of actin sliding energy by nonproductive interaction cycles
x	$= \rho/\rho_o$ or $\bar{\rho}/\rho_o$; or stretch length in APPENDIX B
$\bar{\epsilon}$	a term ϵ used for phosphorylated smooth muscle myosin, where ϵ is f_d, P_d, P_h, ζ, and ρ
$\underline{\epsilon}$	a term ϵ used for unphosphorylated smooth muscle myosin, where ϵ is as above
$[X]$	concentration of X such as M (= myosin)
α	fraction of weakly attached myosin cross-bridges which completes the productive interaction cycle
β	proportionality constant
γ	$=\zeta P_h/(\bar{\zeta}\bar{P}_h)$ or $\bar{\zeta}\bar{P}_h/(\underline{\zeta}\underline{P}_h)$ or $\zeta P_h/(\underline{\zeta}\underline{P}_h)$
Δ	$=(\zeta_2/\zeta_1)/(1-\alpha)$
ζ	coefficient of the molecular friction due to myosin heads in the holding state
ζ_j	molecular frictional coefficient of myosin heads in the holding state j (=1 or 2)
η	solvent viscosity
$\bar{\eta}_s$	coefficient of hydrodynamic viscous drag on an ellipsoid of revolution per unit length
$\Theta_d^{(i)}(t)$	driving-state function of ith myosin head: it is 1 when the myosin head is in the driving state; otherwise 0, where $i = 1, 2, \ldots, \rho L$ and t is time
$\Theta_h^{(i)}(t)$	holding-state function of ith myosin head: it is 1 when the myosin head is in the holding state; otherwise 0, where $i = 1, 2, \ldots, \rho L$ and t is time
$\Theta_{hj}^{(i)}(t)$	holding-state function of ith myosin head: it is 1 when the myosin head is in the holding state j (=1 or 2); otherwise 0, where $i = 1, 2, \ldots, \rho L$ and t is time
ρ	number of myosin heads per unit length of surface which are able to interact with a single actin filament
ρ_o	$=(\rho + \bar{\rho})$ or $(\bar{\rho} + \underline{\rho})$ or $(\rho + \underline{\rho})$

GENERAL THEORETICAL CONSIDERATIONS

We first consider the unidirectional movement of actin filaments on a myosin-coated surface in the presence of ATP (Fig. 1). Here we consider only lengthwise movement of actin. By ρ we will denote the number of myosin heads per unit length of the surface that are able to interact with single actin filaments.

Energy dissipation by the nonproductive interaction cycle

We may suppose that (*a*) productively interacting myosin heads generate an active force for actin sliding, but (*b*) nonproductively interacting heads do not: they are passive structures. This passive nature together with the elasticity of myosin cross-bridges (Ford et al., 1977) suggest that nonproductively interacting myosin heads are elastically strained by the moving actin filament before dissociation from actin. The elastic energy thus stored in the myosin heads dissipates as heat after their dissociation. Such nonproductive interaction "cycle" therefore causes irreversible dissipation of the sliding-movement energy into heat. (This is the reason why the myosin attachment/detachment in the rapid equilibrium is called a cycle here). This suggests that the nonproductive interaction cycle, in effect, results in a resistive, viscous-like "frictional drag" on moving actin, as treated mathematically in Appendix B. We incorporate this frictional drag effect of myosin into the following basic assumptions of our model.

Dantzig et al. (1988) have demonstrated the existence of such passive cross-bridges in skinned fibers in the presence of Ca^{++} by using the ATP analogue, ATP[γS]; the cross-bridges show viscous-like behavior in response

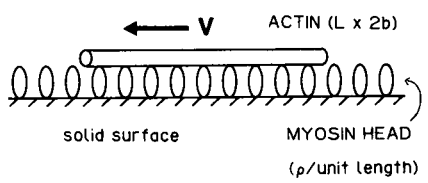


FIGURE 1 An actin filament sliding on a myosin-coated surface with velocity V.

to a rapid stretch of the fibers. Another evidence for the frictional effect of such nonproductive interaction is given by a recent study of the bidirectional Brownian movements of microtubules associated with dynein through a weak-binding interaction in the presence of vanadate, an ATPase inhibitor (Vale et al., 1989).

There can be other frictional drag forces opposing the motion of actin filaments, which are driven to slide by the active force by productively interacting myosin heads. These forces are drag by fluid viscosity and negative force due to delayed detachment of myosin cross-bridges after their power stroke, i.e., negatively strained cross-bridges after the power stroke (Huxley, 1957). The viscous drag by fluid is too small to limit the in vitro sliding velocity as will be shown below.

It has been traditionally assumed that the maximum shortening velocity of unloaded muscle contraction is attained when the positive force by myosin cross-bridges during their power stroke is balanced by the negative force by other cross-bridges after their power stroke (Huxley, 1957; Julian et al., 1974; Eisenberg et al., 1980). However, this assumption may not be applied to the explanation of the 8th finding, described in the Introduction, because solution studies (Sellers, 1985; Selers et al., 1985) suggest that unphosphorylated smooth muscle myosin does not produce positive force and therefore does not produce negative force after the power stroke. We hence assume that the viscous-like drag by myosin heads in the nonproductive interaction cycle limits the in vitro sliding by opposing the active force generated by other myosin heads in the productive interaction, and attempt to explain the observations about the in vitro sliding movement with this and additional simple assumptions.

It should be pointed out here, however, that our approach does not necessarily ignore the possible involvement of the drag by the "negatively strained cross-bridges after the power stroke" in the in vitro movement. As will be described below, our model assumes a constant force by myosin cross-bridge in the productive interaction, which drives the sliding of actin filament. This is an active force averaged over myosin heads in the productive interaction. The force could hence be equal to an average, over total productively interacting cross-bridges, of the difference between the positive force by cross-bridges during the power stroke and the negative force by other cross-bridges after their power stroke. Furthermore, there can be some kind of "internal friction" within the cross-bridge head as a force generator. When such internal friction is present, the force mentioned below (f_d in the assumption 3 = 2) is defined as a net force, which remains after the negative force due to the internal friction is subtracted.

Basic assumptions and equations

We assume: (1) Actin filaments are longitudinally stiff (Ford et al., 1977).

(2) Actin filaments have no inertia (see Berg, 1983).

(3) Myosin heads can take three states in the presence of ATP: detached, driving, and holding states (Table 1).

(3-1) Myosin heads detached from actin (=the detached state) have no effect on the movement of actin.

Myosin heads attached to actin in the presence of ATP are either in the driving state or in the holding state. We will denote the probabilities for a myosin head to be in the driving and holding states by P_d and P_h, respectively. Hence, the probability for the detached state is $(1 - P_d - P_h)$. P_d and P_h do not depend on the velocity of actin sliding.

(3-2) Myosin heads in the driving state complete the productive interaction cycle: they generate a sliding force by splitting ATP. We will denote the force generated by a single myosin head by f_d.

(3-3) Myosin heads in the holding state complete the nonproductive interaction cycle: they do not split ATP or generate sliding force, but exert resistive frictional drag on moving actin filaments. We will denote the coefficient of the frictional drag per myosin head by ζ. Thus, the frictional drag per myosin head is equal to $-\zeta V$, where V is the velocity of actin sliding.

Appendix B gives a possible physical model for the assumption 3-3. There we show that the frictional drag coefficient ζ consists of two physical quantities. One of them is the time a myosin head stays in the holding state: "holding time." (In Appendix B, we have assumed that the holding time is independent of the velocity of actin sliding. The significance of this velocity independence is also discussed there.) The other is the elastic stiffness constant of a myosin head along the direction of actin movement. There we show that the molecular frictional coefficient ζ is equal to half the product of the holding time mutliplied by the elastic stiffness constant of the myosin head. This elasticity may be within the myosin head and its subfragment-2 portion (Tawada and Kimura, 1986; Tsong et al., 1979).

TABLE 1 States of myosin heads

State		Probability	Function
attached	Driving	P_d	Translocating force (f_d)
	Holding	P_h	Frictional drag (coefficient ξ)*
Detached		$1 - P_h - P_d$	No effect

*see Appendix B.

(4) The coefficient of the viscous drag by surrounding solvent on a single actin filament, moving lengthwise, is given by a formula for an ellipsoid of revolution:

$$f_s = L\bar{\eta}_s \tag{1}$$

with

$$\bar{\eta}_s = 2\pi\eta/[\ln(L/b) - 0.5], \tag{2}$$

where L and b are the length and radius of an actin filament, respectively; η is the solvent viscosity.

From the above four assumptions, we have derived an equation for the velocity of actin sliding on a myosin-coated surface in Appendix A (Eq. A7). The equation is

$$V = f_d P_d \rho/(\bar{\eta}_s + \zeta P_h \rho). \tag{3}$$

If there are two types of holding states, the sliding velocity is (see Eq. A8)

$$V = f_d P_d \rho/(\bar{\eta}_s + \zeta_1 P_{h1}\rho + \zeta_2 P_{h2}\rho), \tag{4}$$

where P_{h1} and P_{h2} are the probabilities a myosin head is in the holding state-1 and holding state-2, respectively, and ζ_1 and ζ_2 are the corresponding frictional coefficients per myosin head. As shown in Appendix A, Eqs. 3 and 4 hold when $L\rho \gg 1$.

Multiplying both the numerators and the denominators of Eqs. 3 and 4 by L, and noting that $Lf_dP_d\rho$ is the active force translocating an actin filament, we see that Eqs. 3 and 4 take the form:

$$V = F/f_\zeta \tag{5}$$

with

$$f_\zeta = L(\bar{\eta}_s + \zeta P_h \rho) \quad \text{or} \quad L(\bar{\eta}_s + \zeta_1 P_{h1}\rho + \zeta_2 P_{h2}\rho), \tag{5a}$$

where F is sliding force and f_ζ is a coefficient of generalized frictional drag. The first term in Eq. 5a is the viscous drag coefficient due to solvent. The other term(s) is the coefficient of the frictional drag due to myosin heads in the holding state(s). Eq. 5 states that the velocity of actin sliding is limited by a balance between the sliding force and the generalized frictional drag.

Comparison of the frictional drag due to myosin with the viscous drag

Let us compare the size of the two terms in the generalized drag coefficient, f_ζ. To do so, we first estimate the value of f_ζ by an energetic consideration. Multiplying both sides of Eq. 5 by V and rearranging it, we have

$$f_\zeta = FV/V^2. \tag{6}$$

The numerator in Eq. 6 is the mechanical power required to move an actin filament at a velocity, V. The rate of ATP hydrolysis cycles on one 2.7-μm long actin-filament moving at a speed of 5 μm/s is 78/s (Yanagida et al., 1985), which is equivalent to the energy expenditure rate of $\sim 5 \times 10^{-11}$ erg/s. Assuming a 50% efficiency (Woledge, 1988) for the mechanical power, we obtain

$$f_\zeta = \sim 1 \times 10^{-4}\ \text{g/s}.$$

Note that this value may be an overestimation because if a drag by the negatively strained cross-bridges after their power stroke is involved in the in vitro sliding mechanism, this drag could also dissipate the energy derived from ATP hydrolysis into heat. In the following, however, we assume the above value for f_ζ.

The viscous drag coefficient can be computed from Eq. 1. It is $\sim 3 \times 10^{-6}$ g/s for an actin filament of 2.7 μm length, and only 3% of f_ζ. The viscous drag by surrounding solvent is too small to limit the actin sliding on a myosin-coated surface, as was noted by Kron and Spudich (1986). Instead, the drag force on actin filaments is mostly the frictional drag by myosin heads in the holding state(s). Neglecting $\bar{\eta}_s$ in Eqs. 3 and 4, we thus obtain

$$V = f_d P_d/(\zeta P_h), \tag{7}$$

and

$$V = f_d P_d/(\zeta_1 P_{h1} + \zeta_2 P_{h2}). \tag{8}$$

Eqs. 7 and 8 state that the velocity of actin sliding is limited by a balance between sliding force generated by myosin heads in the driving state and frictional drag due to myosin heads in the holding state(s).

All parameters in Eqs. 7 and 8 relate to a single myosin head; these equations contain neither myosin concentration nor actin length. The velocity of actin sliding is hence independent of both the actin-filament length and the myosin-head concentration on the substratum, as reported by Harada et al. (1987), Takiguchi and Higashi-Fujime (1988), and Toyoshima et al. (1988). To direct actin sliding, however, there must be lower limits for both the concentration of myosin heads and the length of actin. One should thus bear in mind that Eqs. 3, 4, 7, and 8 are good only when myosin-head concentrations and actin lengths are greater than these lower limits, in addition to the condition $L\rho \gg 1$.

Using similar arguments as developed above, we can derive Eqs. 3 and 4 for the directional movement of thick filaments and myosin-coated beads on actin bundles. Furthermore, we can derive Eqs. 7 and 8 for their movements, if we use the following estimates for the coefficient of viscous drag: 2.6×10^{-6} g/s for a single myosin thick filament (diam 0.02 μm and length 2 μm) and 6.6×10^{-6} g/s for a bead (radius 0.35 μm) and if we

assume that the generalized frictional coefficient (see Eq. 5a) for these movements is of the same order as that for actin sliding ($\sim 1 \times 10^{-4}$ g/s). Here, we used Eq. 1 and $f_s = 6\pi\eta r$, where r is the radius of a sphere, to calculate the viscous drag coefficients of a thick filament and a bead, respectively. Note that viscous drag is too small to limit the movement of myosin filaments and myosin-coated beads, as pointed out by Sheetz and Spudich (1983). The sliding velocity of thick filaments on actin bundles thus does not depend on the length of thick filaments as reported by Higashi-Fujime (1986). The velocities of directional sliding of beads, thick and actin filaments, which were all obtained with rabbit skeletal muscle myosin, are similar to each other as experimentally observed (Sheetz et al., 1984; Higashi-Fujime, 1985; Kron and Spudich, 1986).

ACTIN-SLIDING VELOCITY RELATED TO ACTOMYOSIN ATPase CYCLES

Actomyosin ATPase cycles

To explain the dependence of actin sliding velocity on the concentration of ATP, we relate recent biochemical concepts of actomyosin ATPase cycles to Eq. 8.

Myosin cross-bridge heads are either *strongly* or *weakly* attached to actin. Although the original kinetic models contain several biochemical intermediates of myosin heads that are weakly attached to actin (Taylor, 1979; Eisenberg and Hill, 1985), we lump them together and denote it simply by A · MP as illustrated in Fig. 2.

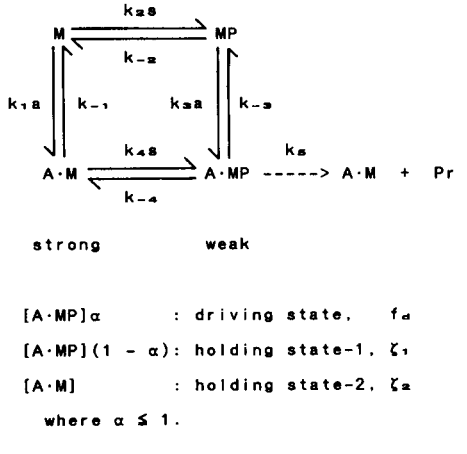


FIGURE 2 A simplified kinetic scheme of the actomyosin ATPase cycle. A: actin; M: myosin head; P: ATP or ADP · Pi; *Pr*: ADP + Pi; *a*: actin concentration; *s*: ATP concentration; k_i (i = 5..1,−1..−4,): rate constants.

Myosin heads in the rigor complexes are strongly attached to actin, and we denote them by A · M.

We assume: (5) A "rapid equilibrium" exists between pairs of M, MP, A · M, and A · MP, as shown in Fig. 2.

(6) A fraction of A · MP, ($\alpha \leqq 1$), is found in the driving state and completes the productive interaction cycle. In other words, myosin heads in this fraction go through the rate-limiting step of actin-activated ATP splitting and generate a sliding force.

(7) The other fraction of A · MP, $(1 - \alpha)$, is in the holding state-1. In other words, myosin heads in this fraction complete the nonproductive interaction cycle: they exert a frictional drag on moving actin filaments. By ζ_1 we will denote the coefficient of the frictional drag by the myosin head in the holding state-1.

(8) A · M is another holding state: holding state-2. By ζ_2 we will denote the coefficient of the frictional drag by the myosin head in holding state-2.

The "holding time" by A · M, rigor complexes, can be longer than the "holding time" by A · MP in holding state-1. There is evidence that no difference exists in the elasticity of myosin heads between A · M and A · MP (Tawada and Kimura, 1986). As is apparent from Eq. B5, therefore, A · M can exert a larger frictional drag on moving actin than A · MP in holding state-1: $\zeta_2 > \zeta_1$.

Dependence of actin-sliding velocity on the ATP concentration

From assumptions 6, 7, and 8, we have

$$P_d\rho = \beta\alpha[\mathrm{A \cdot MP}], \tag{9}$$

$$P_{h1}\rho = \beta(1 - \alpha)[\mathrm{A \cdot MP}] \tag{10}$$

and

$$P_{h2}\rho = \beta[\mathrm{A \cdot M}], \tag{11}$$

where [A · MP] and [A · M] are the concentrations of A · MP and A · M, respectively, and β is a proportionality constant.

From assumption 5, we have

$$[\mathrm{A \cdot M}] = a[\mathrm{M}]/K_1, \tag{12}$$

$$[\mathrm{A \cdot MP}] = a[\mathrm{M}]s/(K_2K_3) \tag{13}$$

and

$$[\mathrm{M}] = \bar{e}/(K_m + s), \tag{14}$$

where [M] is the concentration of M, $K_i = k_{-i}/k_i$ (i = 1, 2, 3, 4; thus, $K_2K_3 = K_1K_4$), $\bar{e} = eK_2/(1 + a/K_3)$,

$$K_m = K_4(K_1 + a)/(K_3 + a), \tag{15}$$

and a, s, and e are the concentrations of actin, ATP and total (=bound and unbound) myosin head, respectively.

Eq. 8 together with Eq. 9–14 leads to an expression for the actin-sliding velocity:

$$V = V_{max}s/(K_{ms} + s), \tag{16}$$

where

$$V_{max} = f_d\alpha/[\zeta_1(1 - \alpha)], \tag{17}$$

and

$$K_{ms} = K_4(\zeta_2/\zeta_1)/(1 - \alpha). \tag{18}$$

With the limits $s \to \infty$, V is V_{max} (Eq. 17). Eq. 17 states that the maximum velocity is limited by a balance between the sliding force by myosin heads in the driving state and the frictional drag due to myosin heads in the holding state-1.

Eq. 17 is the same as Eq. 7; only P_d and P_h have been replaced by α. By definition, $\alpha = P_d/(P_d + P_h)$. One should hence bear in mind that Eq. 7 gives the velocity of actin sliding with the limits $s \to \infty$, practically when the ATP concentration $\gg K_{ms}$. When the ATP concentration is large enough, the concentration of A · M is virtually insignificant, so that A · M does not contribute to limiting the actin sliding. At lower ATP concentrations, on the other hand, the concentration of A · M does become significant. Myosin heads in the A · M form thus exert an additional drag on moving actin. The smaller the concentration of ATP, the larger the concentration of A · M and therefore the slower the velocity of actin sliding.

Dependence of actomyosin ATPase activity on the ATP concentration

Assumption 6 requires that actomyosin ATPase activity is proportional to α[A · MP]. From this together with Eq. 13 and 14, we have an expression for the ATPase activity:

$$V(\text{ATPase}) = V(\text{ATPase})_{max}s/(K_m + s), \tag{19}$$

where $V(\text{ATPase})_{max} = ek_5a/(K_3 + a)$. See Eq. 15 for K_m.

Comparison of actin-sliding velocity with actomyosin ATPase activity

Let us compare Eqs. 16 with Eq. 19. The dependence of actin-sliding velocity on ATP concentration obeys an equation of the Michaelis · Menten type as does that of actomyosin ATPase activity. However, the ATP concentration for the half-maximal velocity of actin sliding, K_{ms}, is different from that for actomyosin ATPase activity, K_m. On comparison of Eq. 18 with Eq. 15, we have

$$K_{ms} = K_m\Delta(K_3 + a)/(K_1 + a), \tag{20}$$

where

$$\Delta = (\zeta_2/\zeta_1)/(1 - \alpha). \tag{21}$$

If $a \gg K_3$ and $a \gg K_1$, $K_{ms} \simeq K_m\Delta$ with $K_m \simeq K_4$.

Note that a difference in a physical property of the myosin heads between the two holding states, ζ_1 and ζ_2, contributes to the difference between K_m and K_{ms}. As ζ_2 can be larger than ζ_1 as pointed out above and because $\alpha \leqq 1$, Δ can be larger than 1. As K_3 is also larger than K_1, K_{ms} can be larger than K_m. This means that the half-maximal velocity of actin sliding is expected to occur at an ATP concentration greater than the ATP concentration necessary for half-maximal ATPase activity.

Fig. 3 shows that Eqs. 16 and 19 are a good fit to the experimental data of Harada et al. (1987). The half-maximal velocity of actin sliding occurs at 81 μM ATP, whereas the half-maximal ATPase rate occurs at 4 μM ATP. Kron and Spudich (1986) and Toyoshima et al., (1988) have also reported similar values of ATP concentration necessary for the half-maximal velocity of actin sliding.

SLIDING MOVEMENT OF MYOSIN-COATED BEADS ON ACTIN BUNDLES

Directional movement of beads coated with copolymers of skeletal muscle myosin and phosphorylated smooth muscle myosin

Phosphorylated myosin of smooth muscle generates the movement of beads but the velocity of the movement is

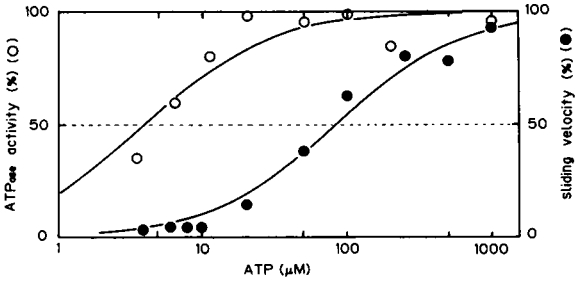


FIGURE 3 Relative velocities of actin filaments (●) moving on double-headed myosin and the actomyosin ATPase activity (○) as a function of the ATP concentration. These data are taken from Harada et al. (1987). Eqs. 16 and 19 were respectively fitted to the sliding data and the ATPase data by nonlinear regression using simplex method (Press et al., 1986); the solid lines represent the best fit. This gives a value for K_{ms} in Eq. 16 of 81 μM and a value for K_m in Eq. 19 of 4 μM.

~ one-tenth of that of beads coated with skeletal muscle myosin (Sheetz et al., 1984). Mixing phosphorylated smooth muscle myosin with skeletal muscle myosin before the formation of thick filaments results in the formation of their copolymers. Mixing with phosphorylated smooth muscle myosin slows the movement of skeletal muscle myosin (Sellers et al., 1985).

To describe the movement of a bead coated with the copolymers of these two types of myosins, we may modify Eq. 8, to obtain

$$V = (f_d P_d \rho + \bar{f}_d \overline{P}_d \bar{\rho})/(\zeta P_h \rho + \overline{\zeta P}_h \bar{\rho}), \quad (22)$$

where all variables with and without upperlines refer respectively to those of smooth and skeletal muscles and $\rho + \bar{\rho} = \rho_o$ (= constant). Rewriting Eq. 22, we obtain

$$V = [V_{sk}\gamma(1 - x) + V_{sm}x]/[\gamma(1 - x) + x], \quad (23)$$

where $V_{sk} = f_d P_d/(\zeta P_h)$, $V_{sm} = \bar{f}_d \overline{P}_d/(\overline{\zeta P}_h)$, $\gamma = \zeta P_h/(\overline{\zeta P}_h)$ and $x = \bar{\rho}/\rho_o$. Fig. 4 shows that Eq. 23 is a good fit to the experimental data of Sellers et al. (1985).

Movement of beads coated with copolymers of unphosphorylated and phosphorylated myosins of smooth muscle

Unphosphorylated myosin of smooth muscle does not generate the movement of beads but slows the movement of phosphorylated myosin of smooth muscle (Sellers et al., 1985). The ATPase activity of unphosphorylated myosin is little activated by actin (Sellers, 1985), although the myosin weakly attaches to actin and is in a rapid equilibrium between detached and weakly attached states in the presence of ATP (Sellers et al., 1982). We therefore assume that (1) unphosphorylated myosin does not generate a sliding force but (2) it does exert a frictional drag due to the nonproductive interaction cycle. To describe the movement of a bead coated with copolymers of phosphorylated and unphosphorylated myosins, thus, we may modify Eq. 8 to obtain

$$V = \bar{f}_d \overline{P}_d \bar{\rho}/(\overline{\zeta P}_h \bar{\rho} + \underline{\zeta P}_h \underline{\rho}), \quad (24)$$

where $\bar{\rho} + \underline{\rho} = \rho_o$ (= constant), and all variables with upper and under lines refer respectively to those of phosphorylated and unphosphorylated myosin. Rewriting Eq. 24, we obtain

$$V = V_{sm}\gamma x/[1 + (\gamma - 1)x], \quad (25)$$

where $x = \bar{\rho}/\rho_o$, $V_{sm} = \bar{f}_d \overline{P}_d/(\overline{\zeta P}_h)$ and $\gamma = \overline{\zeta P}_h/(\zeta P_h)$. Fig. 5 shows that Eq. 25 is a good fit to the experimental data of Sellers et al. (1985).

GENERAL DISCUSSION

The main feature of this paper is our postulate that the nonproductive interaction cycle of myosin heads with actin, in effect, exerts a viscous-like frictional drag on moving elements: actin or myosin filaments, or myosin-coated beads. Hydrodynamic viscous drag on these elements is very small compared with the frictional drag exerted by the myosin heads.

It is impossible to directly measure the viscous response of an active muscle fiber during rapid stretching because elastic responses of force-producing cross-bridges during the stretching interfere with the measurement (Ford et al., 1977). Nonetheless, there are two reports which show viscous-like behavior of cross-bridges under somewhat unphysiological conditions (Brenner et al., 1982; Dantzig et al., 1988). We could compare our estimate of the viscous-like frictional drag

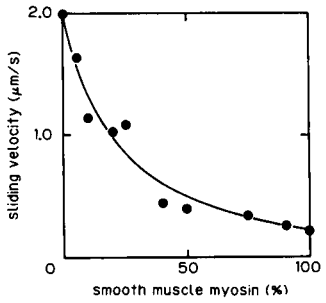


FIGURE 4 Sliding velocity of mixtures of skeletal muscle myosin and phosphorylated smooth muscle myosin. Experimental data (●) are taken from Sellers et al. (1985). Eq. 23 was fitted to the data by the simplex method, by assuming V_{sk} = 1.97 μm/s and V_{sm} = 0.22 μm/s; the solid line represents the best fit. This gives a value for γ of 0.19.

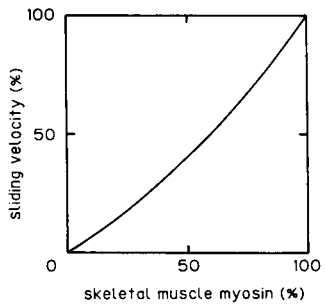


FIGURE 5 Relative velocity of mixtures of phosphorylated and unphosphorylated smooth muscle myosins. Experimental data (●) are taken from Sellers et al. (1985). Eq. 25 was fitted to the data by the simplex method; the solid line represents the best fit. This gives a value for γ of 3.6.

force due to nonproductively interacting myosin heads with these available data, although such a comparison might not have straightforward meaning. Our estimate of the coefficient of this drag force acting on an actin filament of 2.7 μm is $\sim 1 \times 10^{-4}$ g/s. Suppose that a segment of muscle fiber containing 1-μm long thin filaments is stretched at a speed of 5 μm/s per half sarcomere. If the fiber contains $\sim 1 \times 10^{11}$ thin filaments per square centimeter of its cross-secional area, the resulting force only due to this viscous-like drag in response to the stretching may be 2×10^3 dyn/cm^2.

As cited above, Dantzig et al. (1988) reported such viscous-like behavior of weakly attached cross-bridges in response to rapid stretching of skinned fibers treated with Ca^{++} and ATP[γS] at 200 mM ionic strength. Muscle fibers do not produce active force with this ATP analogue. They observed a drag force of ~ 150 KN/m^2 ($= 1.5 \times 10^6$ dyn/cm^2) in response to stretching with a speed of ~ 20 μm/s per half sarcomere. If the stretching speed is 5 μm/s, the force response would be 4×10^5 dyn/cm^2: this value is much larger than our estimate, probably reflecting a smaller detachment rate of myosin cross-bridges from actin in ATP[γS].

Brenner et al. (1982) reported similar viscous-like behavior of weakly attached cross-bridges in skinned fibers treated with ATP at an extremely low ionic strength in the absence of Ca^{++}. With a stretching speed of ~ 2 μm/s per half sarcomere, they observed a force response of $\sim 10^4$ dyn/cm^2. Their value is larger than our estimate. At physiological ionic strength (170 mM), such weakly attached cross-bridges as demonstrated in the absence of Ca^{++} by Brenner et al. (1982) are almost nonexistent, whereas Schoenberg (1988) reported that the fraction of such cross-bridges in the absence of Ca^{++} is 0.07 at 160 mM ionic strength. At such physiological ionic strengths, however, the amount of weakly attached cross-bridges may increase with the increase of Ca^{++}, as shown by Dantzig et al. (1988) with ATP[γS]. Thus, our estimate for the viscous-like frictional drag force due to nonproductive interaction cycle is not too large when compared with available data.

The mechanism limiting the maximum shortening velocity (V_{max}) of muscles

The velocity of in vitro sliding at a saturating ATP concentration is close to the maximum speed of relative sliding of thick and thin filaments in muscles shortening under no external load, when comparing the same type of myosin (Sheetz et al., 1984). This suggests that the mechanism limiting the shortening velocity of unloaded muscles (V_{max}) is the same as that limiting the sliding of actin and myosin in vitro at a saturating ATP concentration.

The traditional explanation for V_{max} is derived from the theory proposed by Huxley (1957). Models later developed by Julian et al. (1974) and Eisenberg et al. (1980) adopt similar mechanisms for the explanation of V_{max}. In the Huxley theory, when myosin heads attach to the thin filament, they always generate a "positive" force for the power stroke. When muscles are shortening, such attached cross-bridges are carried forward as "negative distortions" by the relative sliding of the myofilaments past each other. Consequently, they produce a negative force. The faster the muscle shortens, the larger the fraction of cross-bridges supporting the negative force. Eventually, the total positive and negative forces are balanced, then, the velocity reaches a maximum, V_{max} with zero force production by muscle. Thus, V_{max} is inherent to the characteristics of a single myosin cross-bridge in the Huxley model, as in our model.

Concerning the mechanisms limiting V_{max}, our model shares the following features with the Huxley model. The resistive force against the relative movement of actin and myosin filaments is generated by myosin cross-bridges: those during their negative distortions in the Huxley model and those in the holding state in our model. While these two models are thus operationally similar, however, there is a difference between them. In the Huxley model, cross-bridges produce negative force only *after* their power stroke. In contrast, weakly attached cross-bridges in the holding state, which have not made power stroke, exert frictional drag in our model. As pointed out already in the General Theoretical Considerations section, however, our model could include a role of the negatively strained cross-bridges after their power stroke in the sliding mechanisms.

One may ask why features of the Huxley model are not sufficient to explain actomyosin sliding motion in vitro. We have also applied the Huxley model for the explanation of the in vitro sliding movement but found it not as simple as the current model because to explain the sliding motion of myosin copolymers, an inordinately large number of modeling parameters are necessary due to the position dependence of cross-bridge attachment/detachment rate constants. Also, the explanation for the suppressive effect of unphosphorylated smooth muscle myosin on the sliding of phosphorylated smooth muscle myosin requires additional assumptions (other than the "negative distortions after the power stroke"), if the frictional drag effect of nonproductive interaction cycle is not assumed, as pointed out in General Theoretical Considerations. On the other hand, with the new model presented here we can explain characteristics of in vitro sliding motion in a simple way, by assuming the viscous-like frictional drag effect of nonproductive interaction

cycle. However, our success does not necessarily mean that the "negative distortions" of the cross-bridges after the power stroke are not operating in muscle contraction as pointed out already. It could be that both negative distortions after the power stroke and the frictional drag effect of the nonproductive interaction cycle are involved in the muscle contraction mechanism.

A proposed experiment to test our model

Fig. 4 shows that the addition of phosphorylated smooth muscle myosin to skeletal muscle myosin causes a decrease in the velocity of sliding. Fig. 5 shows that the addition of unphosphorylated smooth muscle myosin to phosphorylated smooth muscle myosin slows the velocity of sliding. How will the addition of unphosphorylated smooth muscle myosin affect the sliding velocity of skeletal muscle myosin? As is described below, these three experiments are not mutually independent if analyzed by our model. The effect of unphosphorylated smooth muscle myosin on the velocity of skeletal muscle myosin is predictable from the other two experiments.

As unphosphorylated smooth muscle myosin does not produce bead movement, we can express the velocity of the copolymers of skeletal muscle myosin and unphosphorylated smooth muscle myosin by the following equation:

$$V = f_d P_d \rho / (\zeta P_h \rho + \underline{\zeta P_h} \underline{\rho}), \tag{26}$$

where $\rho + \underline{\rho} = \rho_o$ (= constant), and all variables with and without underlines refer respectively to those of unphosphorylated smooth muscle myosin and skeletal muscle myosin. Rewriting Eq. 26, we obtain,

$$V = V_{sk} \gamma x / [1 + (\gamma - 1)x], \tag{27}$$

where $x = \rho/\rho_o$, $V_{sk} = f_d P_d/(\zeta P_h)$ and $\gamma = \zeta P_h/(\underline{\zeta P_h})$.

Curve fitting in Fig. 4 has given a value for the ratio of $\zeta P_h/(\overline{\zeta P_h})$ in Eq. 23, while that in Fig. 5 has given a value for the ratio of $\overline{\zeta P_h}/(\zeta P_h)$ in Eq. 25. From these two ratios, we can calculate the ratio of $\zeta P_h/(\underline{\zeta P_h})$, the value of which is unknown in Eq. 27. We thus obtain $\zeta P_h/(\underline{\zeta P_h}) = 0.19 \times 3.6 = 0.68$.

Fig. 6 shows Eq. 27 with $\gamma = 0.68$, which predicts the effect of the addition of unphosphorylated smooth muscle myosin on the sliding velocity of skeletal muscle myosin. Testing this prediction experimentally is one way to check our model.

After submission of the manuscript of our paper, we noticed new results on copolymer experiments by Warshaw et al. (1990) using skeletal muscle myosin, unphosphorylated and phosphorylated smooth muscle myosins, employing an actin motility assay method. Their Fig. 5 *A* corresponds to Fig. 6 in our paper, a prediction based on the data of Sellers et al. (1985). Unfortunately, we cannot directly compare these two figures because of the different experimental conditions used by Warshaw et al. (1990) and Sellers et al. (1985). However, it can be shown that our theory is consistent with the data of Warshaw et al. by independently analyzing their data as shown here for the data of Sellers et al. (1985). The consistency can also be shown by the following comparison, although it is not quantitative. Warshaw et al. reported that the velocity curve for the copolymer of skeletal muscle myosin and phosphorylated smooth muscle myosin is similar to that for the copolymer of skeletal muscle myosin and unphosphorylated smooth muscle myosin (see their Figs. 5, *A* and *B*). Likewise, the corresponding figures in the present paper, i.e., Figs. 4 and 6, are similar to each other (invert the *X*-axis in Fig. 4 for the comparison). Because Fig. 6 shows a prediction, these similarities suggest that the present theory is consistent with the new experimental data of Warshaw et al.

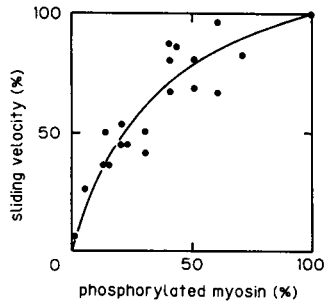


FIGURE 6 Relative velocity of mixtures of skeletal muscle myosin and unphosphorylated smooth muscle myosin. (*Solid line*) Eq. 27 with $\gamma = 0.68$, which was predicted by our model after analyzing the data shown in Figs. 4 and 5 (see text).

Warshaw et al. (1990) also showed that a weak-binding analogue of myosin, which was prepared by chemical modification of skeletal muscle myosin with *N*,*N*′-*p*-phenylenedimaleimide (pPDM-myosin), slows down the actin movement driven by unmodified myosin. This finding provides additional evidence for our central assumption that the nonproductive cyclic interaction exerts resistive friction to the movement in vitro. In their paper they developed a theory which fits their data of myosin copolymers, by assuming three different hyperbolic force-velocity curves for three different myosins including unphosphorylated smooth muscle myosin and that the force-velocity curves can be extended in negative-force region for slow or nonmotile myosin. Because their model assumes that phosphorylated smooth muscle myosin in a copolymer with skeletal muscle myosin

produces only negative force whereas the smooth muscle myosin produces positive force in a copolymer with unphosphorylated smooth muscle myosin, their model is essentially different from our model.

Estimation of the "holding times"

Let T_{h1} and T_{h2} be the holding times of holding state-1 and state-2, respectively. Let E_1 and E_2 be the elastic stiffness constants of the myosin head in holding state-1 and in holding state-2, respectively. Using Eq. B5, we have $T_{h2} = T_{h1}(\zeta_2/\zeta_1)(E_1/E_2)$. As Young's modulus of elasticity for a myosin head with and without nucleotide is the same (Tawada and Kimura, 1986), the elastic stiffness constant of a myosin head in holding state-1 may be the same as that in holding state-2: $E_1 = E_2$. If so, we have

$$T_{h2} = T_{h1}(\zeta_2/\zeta_1). \tag{28}$$

If $a \gg K_3$ and $a \gg K_1$ in Eq. 20, we have $\Delta = K_{ms}/K_m$. In Fig. 3 we had $K_{ms}/K_m \simeq 20$, so that $\Delta \simeq 20$. Assuming $0.1 \sim 0.8$ for α in Eq. 21, we have $4 \sim 18$ for ζ_2/ζ_1. From Eq. 28 we thus have

$$T_{h2} \simeq (4 \sim 18) \times T_{h1}. \tag{29}$$

Myosin heads in the rigor state stay bound to actin, in the presence of nonsaturating ATP, about 10 times longer than the weakly attached heads in the holding state.

Let us estimate the holding time (T_h) at a saturating ATP concentration. T_h is equivalent to T_{h1}. From Eq. B5 with $f_\zeta = L\zeta P_h \rho$, we have

$$T_h = 2f_\zeta/(L\rho E P_h). \tag{30}$$

As estimated above, $f_\zeta = \sim 1 \times 10^{-4}$ g/s. $L\rho$ is the number of myosin heads facing a single thin filament, and it is ~ 80 per thin filament of 2.7 μm length (Yanagida et al., 1985). When a force of $\sim 1.3 \times 10^{-7}$ dyn is applied to a single myosin head along the long axis of the muscle fibers, the head is strained by 7.7×10^{-7} cm (Tawada and Kimura, 1986). Thus, E is 1.3/7.7 dyn/cm. Using these values in Eq. 30, we have $T_h \simeq 1.5 \times 10^{-5}/P_h$ s. Assuming $P_h = 0.2 \sim 0.8$, we have $20 \sim 75$ μs for T_h. The T_h value is much shorter than the duration of a single power stroke by the myosin cross-bridge, which is in the millisecond range (Huxley, 1974). The short T_h substantiates our central assumption that myosin heads during the nonproductive interaction, rather than the negatively strained myosin cross-bridges after their power stroke, exert the viscous-like frictional drag. Furthermore, the inverse of T_h, which gives the dissociation rate of the myosin head in the weak-binding interaction, is in the range of 10^4 s^{-1}, which is consistent with the value reported by Schoenberg (1988). If T_h is such as estimated above, a single myosin head weakly attached to actin that is sliding at a speed of 5 μm/s is strained by $0.1 \sim 0.4$ nm along the direction of actin movement during a single holding state. This magnitude of strain is well within that of thermal fluctuations of the myosin-head structure (Tawada and Kimura, 1986; see Appendix B for its significance).

The primary concern of the present paper is with the nature of the mechanism limiting the velocity in the sliding movement in vitro. To discuss it, we postulated that the nonproductive cyclic interaction of myosin with actin exerts a viscous-like frictional drag. This has been useful for explaining, in a unified way, the ATP-concentration dependence of the sliding velocity of the movement in vitro, the velocity of copolymers with various myosins, and other characteristics of the sliding movement in vitro. This postulate can also give a physical molecular interpretation of the Brownian movement of microtubules weakly associated with dynein, which was found by Vale et al. (1989) (Tawada and Sekimoto, 1990). The present model with this postulate and other simple assumptions may be used for theoretical studies such as the interpretation of the tension fluctuation in vitro, which can be measured by the system described by Kishino and Yanagida (1988) for the measurement of tension development by actomyosin in vitro. We hence believe that such theoretical studies along the line developed here would contribute to elucidating physical mechanism of the force generation by biological motors as well.

APPENDIX A

Equations of ATP-dependent sliding velocity of an actin filament on a myosin-coated substratum

From our four basic assumptions described in General Theoretical Considerations, we can describe the sliding velocity $V(t)$ of an actin filament of length L at time t by

$$-f_s V(t) + F_d(t) + F_h(t) = 0. \tag{A1}$$

The first term on the left hand side of Eq. A1 shows the force from viscous drag by the surrounding solvent (see Eqs. 1 and 2 for the definition of f_s). The second term $F_d(t)$ shows the total sliding force generated by myosin heads in the driving state. The third term $F_h(t)$ shows the total frictional-drag force exerted on actin by myosin heads in the holding state(s). This frictional drag by myosin is the working hypothesis of the present model. In Appendix B we propose a molecular mechanism that can effectively produce the frictional drag due to myosin heads.

We first consider the case with a single holding state. Generalization to the case with two holding states is straightforward, and will be described later.

From Assumption 3, we may express the two forces, $F_d(t)$ and $F_h(t)$, by

$$F_d(t) = f_d \sum_{i=1}^{\rho L} \Theta_d^{(i)}(t) \tag{A2}$$

and

$$F_h(t) = -\zeta V(t) \sum_{i=1}^{\rho L} \Theta_h^{(i)}(t), \tag{A3}$$

where the suffix i distinguishes myosin heads facing an actin filament, and ρL is their total number. The functions $\Theta_d^{(i)}(t)$ and $\Theta_h^{(i)}(t)$ ($i = 1, 2, \ldots, \rho L$) represent the state of the ith myosin head. We define these functions as $\Theta_d^{(i)}(t) = 1$ when the ith myosin head is in the driving state, and otherwise $\Theta_d^{(i)}(t) = 0$; similarly, $\Theta_h^{(i)}(t) = 1$ when the ith myosin head is in the holding state, and otherwise $\Theta_h^{(i)}(t) = 0$.

We suppose that each of the myosin heads facing an actin filament spontaneously changes its state among the three states described in Assumption 3: the driving, the holding, and the detached states. We suppose that the state of the myosin head changes frequently with a short time scale, say τ. Then, from the statistical point of view, we can regard the right-hand side of Eq. A2 or A3 as the sum of random variables obeying a stationary independent identical distribution (IID) with a short correlation time in the order of τ. If $\rho L \gg 1$, which we assume here, we can approximate the summations on the right-hand side of Eqs. A2 and A3 by their respective statistical averages. These averages are equal to their respective time averages over a time interval much larger than τ. This is so because of the stationary IID. Therefore, from Eqs. 1 and A1–A3 the velocity of an action filament is given in the crudest approximation as follows

$$V(t) \simeq f_d \langle D \rangle \rho / (\bar{\eta}_s + \zeta \langle H \rangle \rho), \tag{A4}$$

with

$$D = \sum_{i=1}^{\rho L} \Theta_d^{(i)}(t)/(\rho L) \text{ and } H = \sum_{i=1}^{\rho L} \Theta_h^{(i)}(t)/(\rho L),$$

where the bracket $\langle \cdot \rangle$ denotes the time average just mentioned, and the right-hand side of Eq. A4 is therefore independent of time.

Because the $\Theta_d^{(i)}(t)$'s are statistically identical to each other, we obtain

$$\langle D \rangle = \langle \Theta_d^{(1)}(t) \rangle = P_d \tag{A5}$$

and similarly

$$\langle H \rangle = \langle \Theta_h^{(1)}(t) \rangle = P_h. \tag{A6}$$

In this calculation, we have used the fact that the probability P_d (or P_h) of finding a myosin head in the driving state (or in the holding state) is equal to the fraction of time a myosin head stays in the driving state (or the holding state, respectively).

From Eqs. A4–A6, we obtain

$$V(t) \simeq V \equiv f_d P_d \rho / (\bar{\eta}_s + \zeta P_h \rho), \tag{A7}$$

which is independent of time but slightly dependent on the actin filament length L through $\bar{\eta}_s$ (see Eq. 2).

From the theory of statistics we can estimate the relative magnitude of the fluctuations of the quantities D and H in Eq. A4:

$$\langle (D - \langle D \rangle)^2 \rangle^{1/2} / \langle D \rangle \text{ and } \langle (H - \langle H \rangle)^2 \rangle^{1/2} / \langle H \rangle,$$

where the bracket $\langle \cdot \rangle$ means the time average, and we can show that their magnitude is in the order of $(\rho L)^{-1/2}$ ($\ll 1$). From this fact we can show that the correction term to the expression of the velocity in Eq. A7, $V(t) - V$, is a rapidly fluctuating small quantity, the magnitude of which is smaller by a factor of $(\rho L)^{-1/2}$ than the mean value V given by Eq. A7. We can also show that the correction term fluctuates with a time constant in the order of τ. Therefore, observations of the sliding velocity, which usually take much longer time, would not detect this fluctuation.

When there are two types of holding states, each with a different frictional-drag cofficient ζ_1 or ζ_2, the equation for motion is the same as Eq. A1, but the definition of $F_h(t)$ has to be modified:

$$F_h(t) = -V(t) \sum_{i=1}^{\rho L} \left[\zeta_1 \Theta_{h1}^{(i)}(t) + \zeta_2 \Theta_{h2}^{(i)}(t) \right],$$

where $\Theta_{hj}^{(i)}(t)$ ($j = 1$ or 2) takes unity or zero, depending upon whether the ith myosin head is in the holding state j or not. Following the same argument that has led to Eq. A7, we obtain the following approximated expression for the sliding velocity of an actin filament:

$$V(t) \simeq V \equiv f_d P_d \rho / (\bar{\eta}_s + \zeta_1 P_{h1} \rho + \zeta_2 P_{h2} \rho), \tag{A8}$$

where we have used the fact that

$$\langle \Theta_{h1}^{(i)}(t) \rangle = P_{h1}, \text{ and } \langle \Theta_{h2}^{(i)}(t) \rangle = P_{h2};$$

the bracket $\langle \cdot \rangle$ again denotes the time average mentioned above (see Eq. 4 for the definition of P_{h1} and P_{h2}).

APPENDIX B

A molecular mechanism for frictional drag exerted on moving actin-filaments by myosin heads in the holding state

Here we propose a possible molecular mechanism for the frictional drag exerted by myosin heads in the holding state, based on the transfer of elastic energy, stored in strained myosin heads, into heat.

We start with the following two assumptions. (*a*) Strain/relaxation cycle of the myosin head. Myosin heads during the holding state are passive structures; they are elastically strained along the direction of actin sliding, the motion of which is driven by other myosin heads in the driving state. This strain occurs because the myosin molecule has elasticity within its head and its subfragment-2 portion (Tawada and Kimura, 1986; Tsong et al., 1979). The holding state ends with the dissociation of the strained cross-bridge from actin. Soon after the dissociation, the strain induced in the myosin molecule "relaxes." On this "relaxation," the elastic energy stored in the molecule, which we denote by U, irreversibly dissipates as heat. We assume that before the myosin head reattaches to actin, the stored elastic energy almost completely dissipates as heat.

(*b*) The duration time of the holding state (= holding time) is independent of the velocity of actin sliding. We denote the holding time by T_h. This second assumption becomes significant only when we consider experiments in which the velocity of actin sliding is varied.

We define by E the elastic stiffness constant of a myosin head along the direction of actin movement. E is equal to the ratio of the elastic restoring force to the size of the stretch of a myosin head along the direction of actin movement.

Suppose an actin filament of the length L sliding on a myosin-coated substratum with a given velocity V (hereafter we neglect the fluctua-

tions of V, see Appendix A). Consider a single myosin head in the holding state which is attached to the actin filament. The myosin head is stretched by the moving actin. As the size of a stretch during a single holding state is VT_h, the elastic energy stored in the myosin head is given by

$$U = \int_0^{VT_h} E \cdot x dx = E(T_h V)^2/2, \quad \text{(B1)}$$

where x stands for the length of stretch. The stored energy eventually dissipates as heat before the myosin head enters into a new cycle of the interaction with actin.

The probability a myosin head stays in the holding state is P_h. While an actin filament slides for a unit-time interval ($\Delta t = 1$), a myosin head facing the actin filament therefore experiences P_h/T_h cycles of the strain/relaxation described in *a*. Because the number of myosin heads facing the actin filament is ρL on the average, there occur $\rho L P_h/T_h$ cycles of the stain/relaxation in total during the actin sliding for a unit-time interval. Because each cycle dissipates the energy U, the energy dissipation rate W due to all these cycles during this sliding process is

$$W = U\rho L P_h/T_h. \quad \text{(B2)}$$

Substituting Eq. B1 into Eq. B2, we obtain

$$W = (ET_h/2)\rho L P_h V^2. \quad \text{(B3)}$$

We can interpret the right-hand side of Eq. B3 as the product of the sliding velocity V by the total frictional force $(ET_h/2)\rho L P_h V$, which is proportional to the sliding velocity V. This is possible as long as we consider the sliding process on a time scale much longer than the microscopic time T_h. (The time scale τ introduced in Appendix A was the typical time interval for a single myosin head to change its state among the three states defined in Assumption 3. Therefore, τ is equal to or larger than T_h by definition.)

Let us relate T_h and E, both introduced in the present microscopic model, to the molecular frictional coefficient, ζ, defined in Assumption 3 described in the General Theoretical Considerations section. Remembering that ζ is the coefficient of the frictional drag per single myosin head in the holding state, the total frictional drag force exerted on a single actin filament of length L, which is sliding with a given velocity V, is $\zeta\rho L P_h V$. Hence, we have another expression for the energy dissipation rate W as follows,

$$W = \zeta\rho L P_h V^2. \quad \text{(B4)}$$

Equating these two expressions for W, we obtain

$$\zeta = ET_h/2. \quad \text{(B5)}$$

Schoenberg (1985) has developed a mathematical model to describe viscous-like behavior of cross-bridges in relaxed fibers in response to a rapid stretch. An equation in his modeling (see his Eq. 12) contains a term, which is equivalent to Eq. B5.

Before concluding this Appendix, we briefly discuss the implication of assumption *b*.

We may suppose that a myosin head in the holding state dissociates from actin as soon as the strain accumulated in the head exceeds a threshold. We then expect that the maximum strain in the head, rather than the duration of the holding state, is the intrinsic quantity characterizing the duration of the holding state, and therefore should be independent of the sliding velocity V. If so, T_h is inversely proportional to V, and then we can easily show that the total frictional force is independent of V. This is analogous to the mechanical friction between dry solid surfaces.

In contrast, our assumption *b* of the velocity-independent holding time implies that the dissociation of the myosin head in the holding state from actin is a nonmechanical process such as a chemical or a thermal process, or possibly the combination of both, which occurs well below the mechanical breakup threshold. This appears to be the case because the magnitude of the strain induced in myosin heads during a single holding state is within that of thermal fluctuations of the myosin-head structure as discussed in General Discussion.

We thank Profs. H. Matsuda and L. J. Berliner, and Drs. K. Yoshida, M. Schoenberg, and J. Sellers for helpful comments, Dr. Suzanne Phillips and Prof. Daniel Walz for reading our manuscript.

This work was supported by grants from the Ministry of Education, Science and Culture in Japan to Dr. Tawada.

Received for publication 7 May 1990 and in final form 14 September 1990.

REFERENCES

Berg, H. C. 1983. Random Walks in Biology. Princeton Univ. Press, Princeton, NJ. 75–77.

Brenner, B., M. Schoenberg, J. M. Chalovich, L. E. Greene, and E. Eisenberg. 1982. Evidence for cross-bridge attachment in relaxed muscle at low ionic strength. *Proc. Natl. Acad. Sci. USA.* 79:7288–7291.

Dantzig, J. A., J. W. Walker, D. R. Trentham, and Y. E. Goldman. 1988. Relaxation of muscle fibers with adenosine 5′-[γ-thio]triphosphate (ATP[γS]) and by laser photolysis of caged ATP[γS]: evidence for Ca^{2+}-dependent affinity of rapidly detaching zero-force cross-bridges. *Proc. Natl. Acad. Sci. USA.* 85:6716–6720.

Dillon, P. F., M. O. Aksov, S. P. Driska, and R. A. Murphy. 1981. Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle. *Science (Wash. DC).* 211:495–497.

Eisenberg, E., T. L. Hill, and Yi-D. Chen. 1980. Cross-bridge model of muscle contraction. *Biophys. J.* 29:195–227.

Eisenberg, E., and T. L. Hill. 1985. Muscle contraction and free energy transduction in biological systems. *Science (Wash. DC).* 227:999–1006.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibers near slack length. *J. Physiol. (Lond.).* 269:441–515.

Hai, C. M., and R. A. Murphy. 1988*a*. Regulation of shortening velocity by crossbridge phosphorylation in smooth muscle. *Am. J. Physiol.* 255:C86–94.

Hai, C. M., and R. A. Murphy. 1988*b*. Crossbridge phosphorylation and regulation of latch state in smooth muscle. *Am. J. Physiol.* 255:C99–106.

Harada, Y., A. Noguchi, A. Kishino, and T. Yanagida. 1987. Sliding movement of single actin filaments on one-headed myosin filaments. *Nature (Lond.).* 326:805–808.

Higashi-Fujime, S. 1985. Unidirectional sliding of myosin filaments along the bundle of F-actin filaments spontaneously formed during superprecipitation. *J. Cell Biol.* 101:2355–2344.

Higashi-Fujime, S. 1986. In vitro movements of actin and myosin filaments from muscle. *Cell Motil. Cytoskeleton.* 6:159–162.

Huxley, A. F. 1957. Muscle structure and theory of contraction. *Prog. Biophys. Biophys. Chem.* 7:255–318.

Huxley, A. F. 1974. Muscular contraction. *J. Physiol. (Lond.)*. 243:1–43.

Julian, F. J., K. R. Sollins, and M. R. Sollins. 1974. A model for the transient and steady-state mechanical behavior of contracting muscle. *Biophys. J.* 14:546–562.

Kishino, A., and T. Yanagida. 1988. Force measurements by micromanipulation of a single actin filament by glass needles. *Nature (Lond.)* 334:74–76.

Kron, S. J., and J. A. Spudich. 1986. Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc. Natl. Acad. Sci. USA.* 83:6272–6278.

Marston, S. B. 1989. What is latch? New ideas about tonic contraction in smooth muscle. *J. Muscle Res. Cell Motil.* 10:97–100.

Paschal, B. M., and R. B. Vallee. 1987. Retrograde transport by the microtubule-associated protein MAP 1C. *Nature (Lond.)*. 330:181–183.

Paschal, B. M., S. M. King, A. G. Moss, C. A. Collins, R. B. Vallee, and G. B. Witman. 1987. Isolated flagellar outer arm dynein translocates brain microtubules *in vitro*. *Nature (Lond.)*. 330:672–674.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1986. Numerical Recipes. Cambridge Univ. Press, Cambridge. 289–293.

Schoenberg, M. 1985. Equilibrium muscle cross-bridge behavior: theoretical considerations. *Biophys. J.* 48:467–475.

Schoenberg, M. 1988. Characterization of the myosin adenosine triphosphate (M · ATP) crossbridge in rabbit and frog skeletal muscle fibers. *Biophys. J.* 54:135–148.

Sellers, J. R. 1985. Mechanism of the phosphorylation-dependent regulation of smooth muscle heavy meromyosin. *J. Biol. Chem.* 260:15815–15819.

Sellers, J. R., E. Eisenberg, and R. S. Adelstein. 1982. The binding of smooth muscle heavy meromyosin to actin in the presence of ATP: Effect of phosphorylation. *J. Biol. Chem.* 257:13880–13883.

Sellers, J. R., J. A. Spudich, and M. P. Sheetz. 1985. Light chain phosphorylation regulates the movement of smooth muscle on actin filaments. *J. Cell Biol.* 101:1897–1902.

Sheetz, M. P., and J. A. Spudich. 1983. Movement of myosin-coated fluorescent beads on actin cables *in vitro*. *Nature (Lond.)*. 303:31–35.

Sheetz, M. P., R. Chasan, and J. A. Spudich. 1984. ATP-dependent movement of myosin *in vitro:* characterization of a quantitative assay. *J. Cell Biol.* 99:1867–1871.

Takiguchi, K., and S. Higashi-Fujime. 1988. In vitro sliding movement of F-actin with HMM and S-1. *Cell Motil. Cytoskeleton.* 10:347.

Tawada, K., and M. Kimura. 1988. Stiffness of carbodiimide-crosslinked glycerinated muscle fibers in rigor and relaxing solutions at high salt concentrations. *J. Muscle Res. Cell Motil.* 7:339–350.

Tawada, K., and K. Sekimoto. 1990. Protein friction exerted by motor enzymes through a weak-binding interaction. *J. Theor. Biol.* In press.

Taylor, E. W. 1979. Mechanism of actomyosin ATPase and the problem of muscle contraction. *CRC Crit. Rev. Biochem.* 6:103–164.

Toyoshima, Y. Y., S. J. Kron, E. M. McNally, K. R. Niebling, C. Toyoshima, and J. A. Spudich. 1987. Myosin subfragment-1 is sufficient to move actin filaments *in vitro*. *Nature (Lond.)*. 328:536–539.

Toyoshima, Y. Y., S. J. Kron, and J. A. Spudich. 1988. Observation of *in vitro* movement of actin filaments directed by myosin fragments bound to a nitrocellulose surface. *Cell Motil. Cytoskeleton.* 10:347.

Tsong, T. Y., T. Karr, and W. F. Harrington. 1979. Rapid helix-coil transitions in the S-2 region of myosin. *Proc. Natl. Acad. Sci. USA.* 76:1109–1113.

Vale, R. D., J. M. Scholey, and M. P. Sheetz. 1986. Kinesin: possible biological roles for a new microtubule motor. *TIBS (Trends Biochem. Sci.) Trends Biochem. Sci.* 11:464–468.

Vale, R. D., D. R. Soll, and I. R. Gibbons. 1989. One-dimensional diffusion of microtubules bound to flagellar dynein. *Cell.* 59:915–925.

Warshaw, D. M., J. M. Desrosiers, S. S. Work, and K. M. Trybus. 1990. Smooth muscle myosin cross-bridge interactions modulate actin filament sliding velocity in vitro. *J. Cell Biol.* 111:453–463.

Woledge, R. C. 1988. Forced dilemma to come in muscle contraction. *Nature (Lond.)*. 334:655.

Yanagida, T., T. Arata, and F. Oosawa. 1985. Sliding distance of actin filament induced by a myosin cross-bridge during one ATP hydrolysis cycle. *Nature (Lond.)*. 316:366–369.