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# The interrelation between mechanical characteristics of contracting muscle, cross-bridge internal structure, and the mechanism of chemomechanical energy transduction

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**Abstract** The cross-bridge working stroke is regarded as a continuous (without jumps) change of myosin head internal state under the action of a force exerted within the nucleotide-binding site. Involvement of a concept of continuous cross-bridge conformation enables discussion of the nature of the force propelling muscle, and the Coulomb repulsion of like-charged adenosine triphosphate (ATP) fragments $ADP^{2-}$ and $P_i^{2-}$ can quite naturally be considered as the source of this force. Two entirely different types of working stroke termination are considered. Along with the fluctuation mechanism, which controls the working stroke duration $t_w$ at isometric contraction, another interrupt mechanism is initially taken into account. It is triggered when the lever arm shift amounts to the maximal value $S \approx 11$ nm, the back door opens, and $P_i$ crashes out. As a result, $t_w$ becomes inversely proportional to the velocity $v$ of sliding filaments $t_w \approx S/v$ for a wide range of values of $v$. Principal features of the experimentally observed dependences of force, efficiency, and rate of heat production on velocity and ATP concentration can then be reproduced by fitting a single parameter: the velocity-independent time span $t_r$ between the termination of the last and beginning of the next working stroke. $v$ becomes the principal variable of the model, and the muscle force changes under external load are determined by variations in $v$ rather than in the tension of filaments. The Boltzmann equation for an ensemble of cross-bridges is obtained, and some collective effects are discussed.

**Keywords** Muscle force · Chemomechanical transduction · Transient process

## Introduction

The conservation law states that the amount of energy taken by an engine minus losses is equal to the mechanical work that it performs. This quite trivial conclusion is equally valid for engines of all types and designs, and hence, in terms of energy solely, the difference between them consists only in the magnitude of their efficiency. Therefore, when investigating a machine of unknown type that performs mechanical work, of primary importance is the way the mechanical force is produced and applied to an object to be moved rather than the energy balance.

The overwhelming majority of human-made devices that perform mechanical work are driven by solely two kinds of forces. The first case is the pressure force onto a certain surface due to fast-moving molecules, and the second, ponderomotive forces due to the interactions of electric currents and/or magnetized cores. The former forces act on the blades of an ancient waterwheel and the walls of the combustion chamber in the newest jet engine. The latter forces work in virtually all electrical engines. It is absolutely evident, however, that the natural engine—muscle—works owing to forces of another type. Therefore, the first question to be asked while investigating the mechanism of muscle performance is: What kind of interaction is the source of the force generated by myosin head?

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It is natural to assume that the internal structure of any well-designed device should first of all correspond to the physical principle of its operation. In the case of myosin head, this means that its design must provide maximal efficiency for simultaneously functioning in two ways. First, inside it there must be a certain moveable object to which the mechanical force $F$ arising in the course of ATP hydrolysis is applied. To move this object over a distance $s$, mechanical work $Fs$ is performed and the myosin head structure must provide minimal losses in the course of chemomechanical energy transduction. Second, a mechanism must exist that provides application of the oppositely directed forces $+f$ and $-f$ to thin and thick filaments and the performance of work $fS$ upon their relative displacement by distance $S$. This mechanism must also be effective to a maximal extent, resulting in a minimal possible value of the difference $Fs - fS$. It can be stated for certain that the existence of such complex internal structure must lead to the appearance of different interrelations between the processes occurring at different structural levels, as mentioned in the title of this work.

First of all, these are relationships between the mechanical parameters that feature relative sliding of filaments, e.g., between force and velocity. In conventional macroscopic studies of muscles it is quite difficult to reveal relations conditioned by the gears which are built into the myosin head, since we observe the result of simultaneous work of a tremendous number of cross-bridges. However, one can try to pick out the above interrelations from the background of "many-particle" effects either theoretically or using experimental results for individual myosin molecules (see, for example, Moffitt et al. 2008; Greenleaf et al. 2007).

Further, with a more or less clear idea on the mechanical properties of myosin head and its internal structure, it is possible to assume that the mechanism of force generation occurs in the course of ATP hydrolysis. This is the main topic of our work. However, it is clear that, when solving this problem, it is impossible to take into account all the known details of myosin subfragment-1 (S1). Instead, the simplest model that still accounts for the main components of this structure should be used.

## The model

In the last half-century, the vast majority of attempts to quantitatively describe the process of muscle force generation have been based on different versions of the classical Huxley (1957) model. The very general phenomenological approach used in this model enables the description of a wide class of phenomena related to myosin-head functioning. This is achieved by fitting functions that define the probabilities of transitions between the attached and detached states of a cross-bridge versus its coordinate.

This initial model (Huxley 1957) is a classic example of black-box models, in this case representing the cross-bridge as a whole. In the early 1970s, Huxley and Simmons (1971) and Huxley (2000) made this model more sophisticated. Despite knowing almost nothing about the real cross-bridge structure, they nevertheless took into consideration some of its discrete internal states. In other words, they took into account several black boxes corresponding to different internal states of the cross-bridge rather than a single black box corresponding to a single internal state. The consideration of transitions between these states in the framework of statistical physics enables a formal description of the rapid transient behavior of a cross-bridge (see the discussion in "Relation to earlier theories").

However, models of this type are so general that they can be used for the description of quite different physical systems with the same number of degrees of freedom in an almost invariable form. Therefore, a corresponding disadvantage of such a framework is the impossibility of understanding the specificity of the physical processes that occur in the system under consideration.

### What features necessarily have to be taken into account

Of course, the great complexity of the processes conditioning and accompanying protein conformation is undeniable. However, we will fail to understand the principle of myosin motor performance until we are able, in a rough and simple approximation, to understand the essence of these processes. Consequently, the approach to this problem should be based on the desire to model just the processes occurring inside the myosin head rather than an arbitrary system with a given number of different states. The main features of the S1 structure and the principal interactions that occur within it should be initially considered in the model. One can expect that, if this is done correctly, the model should reproduce the main features of the system under consideration with a minimum number of adjustable parameters.

Under the discrete approach used by Huxley and his followers it is impossible to describe the process of the continuous change of coordinates of the cross-bridge during its transition from one state to another. At the same time, the stretching of the elastic link between the myosin head and the thick filament and the emergence of the force between actin and myosin are just consequences of this process. Hence, to understand the principle of myosin motor performance, one has to begin with a correct description of the process of the cross-bridge working stroke, taking into account the real internal structure of myosin head.

The principal features of this internal structure have been thoroughly investigated by now, and in some cases, we understand how the individual structural elements react upon each other (Rayment 1996; Geeves and Holmes 1999; Gordon et al. 2000; Cooke 2004). These already known elements of the structure and the relationships between them should be initially built into the model, while the rest can again be considered as a black box. I believe that there are three principal circumstances that necessarily have to be taken into account.

Firstly, an S1 conformation is merely a change of its shape, i.e., displacement of individual, fairly hard (Finkelstein and Ptitsyn 2002) fragments of a large molecule relative to each other. Consequently, for this displacement to arise and, moreover, in so doing, for mechanical work to be performed on external objects, forces must be born inside protein that put its separate parts into motion. The forces are born in the course of ATP hydrolysis, and this allows us to estimate the size of the region where this occurs. Actually, hydrolysis of a single ATP molecule results in an energy rise by say

$$E_{\mathrm{ATP}} \approx 8 \times 10^{-20}\,\mathrm{J} \approx 20\,k_{\mathrm{B}}T, \qquad (1)$$

where $k_{\mathrm{B}}$ is the Boltzmann constant, and $T$ is the absolute temperature, whose value in muscle experiments is about 300 K (see the discussion of the magnitude of $E_{\mathrm{ATP}}$ in Woledge et al. 1985, section 4.3.1.3). If this energy were uniformly distributed over all $3N$ degrees of freedom of the S1 fragment, consisting of $N \approx 10{,}000$ atoms, it would exert a vanishing effect equivalent to a temperature increase of the order of $\Delta T \approx {}^{2E_{\mathrm{ATP}}}/_{(3Nk_{\mathrm{B}})} \approx 0.4$ K.

Therefore, we have to assume that the process of transduction of chemical energy of the ATP molecule to mechanical energy should occur in the ATP binding site within a region that contains only a few atoms, i.e., within a region with representative size of the order of 1 nm or less. Moreover, the amount of ATP energy released must eventually be transferred to the single degree of freedom associated with the switch-2 movement (see, for example, Geeves and Holmes 1999, Fig. 6). At the expense of just this energy, the mechanical work is performed thereafter by lever arm rotation. Consequently, it is natural to assume that it is just the force arising in this active site that propels adjacent "hard" (Finkelstein and Ptitsyn 2002) protein structures ($\gamma$-phosphate-binding pocket, switch-2, converter domain, etc.), thus causing the conformation of cross-bridge as a whole.

Secondly, I proceed from an assumption that a cross-bridge can exert a force on the actin filament only while both hydrolysis products ADP and $P_i$ remain within the ATP binding site. If the cross-bridge is attached to a moving actin filament, the lever arm gradually rotates together with switch-2 until the displacement reaches its maximum value $S \approx 11$ nm, the back door opens, and $P_i$ fetches away. Bearing in mind the power generation process described below in "Coulomb interaction as the source of muscle force", I consider it necessary that the interruption of the working stroke immediately after the $P_i$ release be initially built into the model. The hypothesis that the working stroke is interrupted at the opening of the back door can hardly be considered an obvious assertion because the cross-bridge remains attached to the actin filament for some time after the $P_i$ and then ADP release (see, for example, Cooke and Bialek 1979). Nevertheless, this is the most important of the assumptions underlying the model, and only a direct experiment can unequivocally confirm or disprove it (see, for example, Dantzig et al. 1999).

Thirdly, the finiteness of the cross-bridge lifetime in the isometric contraction state means that there must be another mechanism of working stroke interruption, being independent of the filament sliding. Therefore, one would expect that it has a fluctuation nature. Such mechanism does not differ from its analog considered in Huxley's (1957) model, and it should be taken into account together with the mechanism of stochastic cross-bridge attachment to actin.

Finally, I would like to note that, in order to understand the nature of the processes occurring inside the myosin motor, one necessarily has to go beyond the framework of standard chemical thermodynamics. In this case the forces which change the cross-bridge configuration in the course of the working stroke are of our main interest, rather than the calculation of thermodynamic characteristics of contracting muscle. So, now the myosin head is no longer a member of a large ensemble and should be treated as a separate system evolving under the action of internal and random external forces (allowing for the effects of thermal motion). Since we are dealing with a device that contains a few atoms and that, in each working cycle, captures and splits a single ATP molecule, to describe the problem we should write the Hamiltonian of the system and investigate the temporal evolution of its density matrix. It is clear, however, that this is a problem of incredible complexity and its solution is currently impossible. So we have to use as radical simplifications as possible to facilitate the task.

First of all, to describe the myosin head conformation we have to use classical rather than quantum mechanics and neglect the influence of random external forces on the process. Consequently, in the simplest version of the analysis of myosin head performance as presented in this section, a simple mechanical model is employed which does not account for the action of thermal fluctuations in the course of the working stroke. This is a rough approximation, of course, but we need something to start with, and moreover, it does not ignore random external forces. It is not too difficult to take into account the effect of these

forces on the attachment and detachment processes, and the problems related to the role of thermal motion will be considered in "Fluctuations at low-velocity contraction".

## The simple mechanical model of S1 structure

Hereinafter, I use the following model directly based on a description of the cross-bridge operation as presented by Geeves and Holmes (1999, page 703): "Opening the switch-2 region destroys the $\gamma$-phosphate-binding pocket and … would appear to facilitate $\gamma$-phosphate release (a "back door enzyme"). …. The movement of the switch-2 in the closed form has other more far-ranging consequences, namely the rotation of the converter domain through about 60°. …. The end of the lever arm has moved through 11 nm along the actin helix axis between open and closed, which is about the expected magnitude of the power stroke. This large change is driven through molecular cogs and gears by a small (0.5 nm) change in the active site. Therefore, it now seems rather likely that the myosin power stroke works by switching between these two conformations."

The above-described internal structure of myosin head is reproduced in the model quite accurately (Fig. 1), the only difference consisting in the change in the cause–effect connection between the back door opening and the myosin head conformation. I proceed from the presumption that the "small change in the active site" arising from the appearance of the force within the $\gamma$-phosphate-binding pocket can be modeled as a shift of some kind of a piston analogous to the one shown in Fig. 1. The lever shown in this figure models all the intermediate molecular structures that transmit the force to the actin filament. Omitting for the time being the question of how this gear works, I merely assume that it operates according to the golden rule of mechanics. So, I want to emphasize again two basic provisions of the kinematic scheme: (1) opening the back door means pushing out the piston from the cylinder, and (2) the conformation of the cross-bridge arises under pressure from the moving door, rather than the back door opening due to the cross-bridge conformation.

## The force and work

We shall proceed from the following relationship, valid for any mechanical system:

$$F(x) = -\partial E(x)/\partial x, \tag{2}$$

where $E$ is the potential energy of a system which depends on the generalized coordinate $x$, and $F$ is the corresponding generalized force which performs work upon changing $x$. In our case $x$ is naturally taken as a coordinate for switch-2, which changes as the cleavage advances. The value $x = 0$ (switch-2 in the closed form) corresponds to the initial

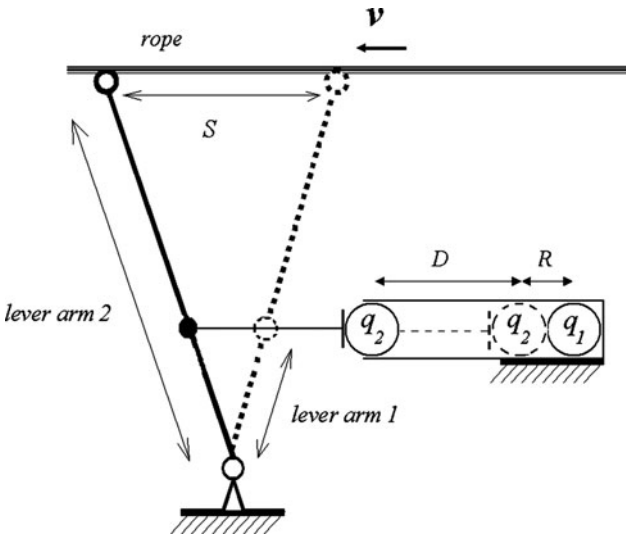


**Fig. 1** Key scheme of an engine moving a rope (actin filament) at velocity $v$. In the initial position (*dashed lines*) switch-2 is closed—the piston depicting it is slid into the cylinder depicting the active site. This is the moment of the working stroke beginning: lever arm 2 is attached to the rope, and the distance between the centers of the spherules $q_1$ and $q_2$, depicting ADP and $P_i$, respectively, is equal to $R$ ($x = 0$). Then, the repulsive force displaces the piston, and the distance between $q_1$ and $q_2$ increases up to $R + D$ ($x = D$) while the corresponding rope displacement is $S = \lambda D$. The working stroke is terminated now: switch-2 is opened (the back door opens—the piston is pushed out of the cylinder), and lever arm 2 detaches from the rope after ADP and $P_i$ fetched away. The ratio of the lever arms is equal to $\lambda =$ (lever arm 2):(lever arm 1), and at any point $v = \lambda dx/dt$

moment of the ATP cleavage, while $x = D$ corresponds to interruption of the working stroke (switch-2 in the open form). It is worth mentioning that this choice of the point of origin differs from the one used in Huxley's (1957) model, while $E$ still means the potential energy of an ATP molecule or, maybe, of some complex of the ATP molecule and proximal structures within the ATP binding site. There are no restrictions on the shape of the $E(x)$ dependence in the model. In the course of numerical calculations, any suitable form of $E(x)$, or equivalently, $F(x)$, can initially be postulated; for example, one can use the dependence $T_2(y)$ obtained in the experiments by Huxley and Simmons (1971) or Ford et al. (1977) to obtain the function $F(x)$. Alternatively, $E(x)$ can be fitted to provide the best agreement between the theory and some other experimental data available.

In this paper we investigate the effect of the internal structure of the S1 fragment on the physical characteristics of muscle. It is useful therefore to simplify the consideration, suppressing if possible the influence of other factors. So, in what follows we consider mainly the simplest linear dependence

$$E(x) = E_0 - Fx, \quad F = \text{const}. \tag{3}$$

Otherwise, a dependence of $F$ on $x$ would lead to changes in the shape of the curves in Fig. 4 at low speeds,

and, taking into account the results of Ritchie and Wilkie (1958) and Edman (1979), this problem requires separate detailed consideration (see also "Force, work, and efficiency for a single cross-bridge"). Besides, the usage of the simplest linear dependences for $E(x)$ and some other functions allows the majority of formulas to be obtained in analytical form. This significantly facilitates the analysis of the results of further numerical calculations. This, however, in no way reduces the generality of the complete system of equations describing the model. Similarly, rough estimations of various parameters that are given in the text, which employ the experimental data for particular muscles, do not imply the need to use just these values in the model.

The work

$$\mathrm{Work}(s) = \int_0^{s/\lambda} F(x)\,\mathrm{d}x \equiv E_{\mathrm{ATP}} - E\left(s/\lambda\right) \tag{4}$$

is evidently performed by the system in Fig. 1 in the course of the rope displacement by a distance $s$. According to the above quotation from Geeves and Holmes (1999), the change of the switch-2 coordinate after the completion of the working stroke is equal to $\Delta x = D \approx 0.5$ nm. We denote the working stroke duration as $t_{\mathrm{w}}$, and the displacement of the actin (rope) during this time period, $S$, as

$$S = \lambda D, \quad \lambda \approx 22. \tag{5}$$

Consequently, the maximal work performed by the system in the course of the working stroke is equal to Work($S$). Depending on the efficiency of the chemomechanical transduction process, which is approximately equal to 1/2, this work is about half the energy of hydrolysis, i.e., $\mathrm{Work}(S) \approx E_{\mathrm{ATP}}/2$. The residual energy must be dissipated as heat

$$Q = E(D); \tag{6}$$

i.e. in particular, it is spent to thermalize ADP and $P_i$ when they are fetched away from the active site (see "Efficiency and heat production" for further detail). Correspondingly, the efficiency of the single myosin head with no account of friction depends only on $S$ and is equal to

$$\eta_0(S) \equiv \mathrm{Work}(S)/E_{\mathrm{ATP}}. \tag{7}$$

Using the lever (Fig. 1), the work Work($S$) should be converted into work corresponding to the displacement of the rope. The average force $\langle f \rangle$ applied to the rope by the lever is equal to the work performed divided by the rope displacement. The force averaged over the working stroke is equal to

$$\langle f \rangle_{\mathrm{ws}} \equiv \mathrm{Work}(S)/S \approx 0.5E_{\mathrm{ATP}}/\lambda D \approx 3.6\,\mathrm{pN}. \tag{8}$$

This value does not depend on the velocity $v$ of filaments sliding past one another (in the model, the rope velocity relative to the cylinder) and fits well with the usual evaluation of the force per cross-bridge in the course of isometric contraction (4 pN; see Woledge et al. 1985, p. 20).

Actually, to find the experimentally measurable average force that a single cross-bridge exerts on the actin filament, one has to determine the force $\langle f \rangle(v)$ averaged over the period of the back and forth motion of the cross-bridge $T(v)$ (in what follows, $T$ without the multiplier $k_{\mathrm{B}}$ means the period rather than temperature). As long as $v$ does not change, one can easily find the working stroke duration [see also (40), "Force, work, and efficiency for a single cross-bridge"] as

$$t_{\mathrm{w}}(v) = S/v. \tag{9}$$

Now, to obtain the complete duration of the cross-bridge turnover it is necessary to merely add another parameter, namely the recovery time $t_{\mathrm{r}}$, i.e., the time span between the completion of the previous and the beginning of the next working stroke. Naturally, this time depends basically on the kinetics of the chemical reactions and to a much lesser degree on the velocity of the sliding filament. Therefore, we assume $t_{\mathrm{r}}$ to be independent of $v$ and obtain the following expression for the time of cross-bridge turnover:

$$T(v) \equiv t_{\mathrm{r}} + t_{\mathrm{w}}(v) = t_{\mathrm{r}} + S/v. \tag{10}$$

This equation obviously contradicts experimental fact: in the case of isometric contraction the average duration of cross-bridge turnover $T(v \to 0) \equiv T_0 \approx 450$ ms (Woledge et al. 1985, p. 25). The reason is that the formulas discussed in this section should be replaced at low speeds by others (see "Fluctuations at low-velocity contraction").

The force $\langle f \rangle(v)$ is somewhat less than $\langle f \rangle_{\mathrm{ws}}$, since it performs the same work Work($S$) but over a longer time $T(v) > t_{\mathrm{w}}$, i.e. in the course of actin displacement over a longer distance $vT(v) > S$. In addition, unlike $\langle f \rangle_{\mathrm{ws}}$, this force is a function of $v$:

$$\langle f \rangle(v) = \frac{\mathrm{Work}(S)}{vT(v)} = \langle f \rangle_{\mathrm{ws}} \frac{t_{\mathrm{w}}(v)}{t_{\mathrm{r}} + t_{\mathrm{w}}(v)} = \frac{\langle f \rangle_{\mathrm{ws}}}{1 + vt_{\mathrm{r}}/S}. \tag{11}$$

When $\langle f \rangle_{\mathrm{ws}} = \mathrm{const.}$, the dependence on the velocity is solely through the ratio $t_{\mathrm{w}}(v)/T(v)$. As regards the ensemble, this ratio represents the fraction of cross-bridges attached to actin at any moment, and this is another possible interpretation of the physical meaning of (11). Anyway, for isovelocity contraction we obtain the hyperbola (11) shifted along the $v$-axis, which is fundamental for the future consideration.

It should be noted that a model based on similar assumptions was already proposed more than 30 years ago by Cooke and Bialek (1979). The authors of the model

experimentally investigated the dependence of isometric force and force–velocity curves on ATP concentration and tried to describe the results in the framework of Huxley's (1957) model. They concluded that "the data is best fit by models in which head attachment occurs rapidly at the beginning of a power stroke, head detachment occurs rapidly at the end of the power stroke, and the force produced by a myosin head in a power stroke is independent of velocity" (Cooke and Bialek 1979, Abstract). As a result, in the course of any working stroke, the overwhelming majority of cross-bridges perform the same work, and hence some formulas obtained by Cooke and Bialek are similar to the corresponding formulas in this paper (compare, for example, Eq. 11 herein with Eq. 7 from Cooke and Bialek 1979). Nevertheless, all the results obtained in these approaches are by no means identical, and the reasons why they differ are analyzed further, when needed.

## Hill's force–velocity curve

Now, to obtain the standard Hill force–velocity curve

$$\frac{P}{P_0}=\frac{1-v/v_{\max}}{1+\left(P_0/a\right)v/v_{\max}}, \tag{12}$$

it is merely necessary to shift the hyperbola (11) along the $P$-axis, taking into account the "hindering force" $F_{\rm h}$. This is the only parameter we use to describe all the elements of the myosin head structure that are not explicitly considered in the model (a new black box).

Since we want the hyperbola (11) to move without changing its shape [so that Hill's law (12) is successfully approximated], the choice of the dependence $F_{\rm h}(v)$ is greatly narrowed; we can add either a linear-in-$v$ term to the numerator of (11) or a constant to the whole expression. If the hindering force operates inside the myosin head, it must operate only in the course of the working stroke. Consequently, in this case $F_{\rm h}(v)$ should be included in the numerator of (11) and therefore be proportional to velocity:

$$\begin{aligned} &F_{\rm h}=\gamma v \quad \Rightarrow \quad P(v)=\frac{\mathrm{Work}(S)-F_{\rm h}S}{vT(v)}=\frac{\langle f\rangle_{\rm ws}-\gamma v}{1+vt_{\rm r}/S};\\ &P_0=\langle f\rangle_{\rm ws}, \quad v_{\max}=\frac{P_0}{\gamma}, \quad a=\gamma\frac{S}{t_{\rm r}}. \end{aligned} \tag{13}$$

here, $P$ is the average force per single cross-bridge, applied to an actin filament and performing useful work.

Second, the hindering force operating continuously outside a cross-bridge should be added to the entire expression (11) and therefore must be independent of velocity:

$$\begin{aligned} &F_{\rm h}=\mathrm{const} \;\Rightarrow\; P(v)=\frac{\langle f\rangle_{\rm ws}}{1+vt_{\rm r}/S}-F_{\rm h}\\ &P_0=\langle f\rangle_{\rm ws}-F_{\rm h}, \quad v_{\max}=\frac{P_0S}{t_{\rm r}F_{\rm h}}, \quad a=F_{\rm h}. \end{aligned} \tag{14}$$

The elucidation of these and some other (not yet discussed) notations and parameters used in the model can be found in Table I in the Electronic Supplementary Material.

In both cases (13) and (14) the average force $P$ applied to the actin filament vanishes when the work performed by the hindering force during the period becomes equal to Work($S$). The same condition determines the value of $v_{\max}$ in the model by Cooke and Bialek (see Eqs. 8 and 11 in Cooke and Bialek 1979). However, there are also significant differences. In the cases (13) and (14) $F_{\rm h}$ acts exactly similar in all the cross-bridges, and its value does not change during the working stroke or the total cycle. However, in the model of Cooke and Bialek (1979) the hindering force acts only in a few cross-bridges which did not detach in a timely fashion by ATP binding. These cross-bridges must be mechanically dissociated from actin at the very end of the working stroke by the movement of the filaments. Therefore, the hindering force has a clear physical meaning in a model such as the latter, which is a great advantage. It is not difficult to use in the model considered here the $F_{\rm h}(v)$ dependence obtained in Cooke and Bialek (1979) (see the expression for $f_{\rm u}$ preceding Eq. 11). However, the real nature of the hindering force can be ascertained only by further experimental research. At least, until this problem be solved, the model in which Hill's equation (12) is obtained in analytical form may be preferable.

## Recovery stroke

The recovery time $t_{\rm r}$ determines the part of the period $T(v)$ (10) of the cross-bridge back and forth motion that does not depend on the velocity $v$. This is why in the denominators of the two formulas (13) and (14) the term $vt_{\rm r}/S$ plays exactly the same role as the term $\left(P_0/a\right)v/v_{\max}$ in the Hill equation (12). It is precisely these terms in the denominators that determine the degree of deviation of the hyperbolic dependence (12) from the linear one appearing in the numerator. Hence, one can now obtain for both cases (13) and (14) the same simple equality bounding the parameters $v_{\max}$, $t_{\rm r}$, and $P_0/a$:

$$t_{\rm r}=\frac{P_0}{a}\frac{S}{v_{\max}}. \tag{15}$$

Inasmuch as this formula appears immediately after rewriting Eq. (11) using the notations of Eq. (12) it does not depend on the nature of the hindering force and allows

better understanding of the physical meaning of Hill's parameter $P_0/a$. Indeed, we have two basic parameters of myosin under consideration—$S$ and $v_{max}$—with dimensions of length and velocity, respectively. It is just these parameters that define a characteristic length and characteristic velocity of the system. Obviously, the ratio of these parameters determines the characteristic time $S/v_{max}$. Thus, it follows from (15) that Hill's parameter $P_0/a$ determines the relation between $t_r$ and this characteristic time, or, if you will, between the characteristic length $S$ and the value of $v_{max}t_r$.

Now $t_r$ appears to be an unambiguous parameter which imposes the shape of the force–velocity curve (change of $v_{max}$ simply corresponding to a scaling). Therefore, $t_r$ becomes the principal fitting parameter of the model, and it makes sense to carefully analyze the conclusions that follow from formula (15).

We begin with the estimation of the order of magnitude of $t_r$ directly from this formula. For muscles of various types, the magnitude of the ratio $S/v_{max}$ amounts to a few milliseconds at low (about 10 °C) temperature, and an order of magnitude smaller at a temperature of about 30–40 °C (see Table 2.II in Woledge et al. 1985). In contrast, as can be seen from the same table, the ratio $P_0/a$ amounts to a few units at all temperatures. Therefore, with decreasing temperature, $t_r$ should increase from a few units to a few tens of milliseconds. This conclusion seems quite reasonable, although I am not aware of experimental data which could definitely confirm or refute it.

Next, we obtained formula (15) using only the principles of the model and the laws of mechanics. In reality, however, biochemical events occur alternately with mechanical ones, and a particular stage of the biochemical cycle permits the next stage of the mechanical cycle and vice versa (see Huxley 1980). Therefore, it is hardly possible to assert a priori that the relationship (15) between $v_{max}$ and $t_r$ should be completely universal. Nevertheless, if the model adequately describes the cross-bridge operation, this formula should correctly reproduce the influence of biochemical processes on the change of $t_r$ (see also "Force, work, and efficiency for a single cross-bridge").

In the course of the recovery stroke, a number of processes of both (biochemical and mechanical) types take place. We confine our discussion to only three of them: (1) the ADP release process, whose duration we denote by $t_{-ADP}$, (2) the ATP binding process (duration $t_{ATP}$), and (3) the detachment and the new myosin head attachment to actin filament (duration $t_{da}$). Ignoring the probability of counterreactions, one can write

$$t_r = t_{-ADP} + t_{ATP} + t_{da};$$
$$t_{-ADP} = \frac{1}{k_{-ADP}}, \quad t_{ATP} = \frac{1}{k_{ATP}\cdot[ATP]}, \tag{16}$$

and $t_{da}$ does not depend on the ATP concentration [ATP]. Now we obtain from (15)

$$v_{max} = \frac{P_0 S}{a}\frac{k_{-ADP}k_{ATP}\cdot[ATP]}{k_{-ADP} + k_{ATP}\cdot[ATP] + t_{da}k_{-ADP}k_{ATP}\cdot[ATP]}. \tag{17}$$

Assuming that the ATP binding and myosin head detachment and new attachment processes are fast, i.e.,

$$t_{-ADP} \gg t_{ATP} \quad \text{and} \quad t_{da}, \tag{18}$$

we obtain from (17) that

$$\frac{v_{max}}{k_{-ADP}} \approx \frac{P_0}{a} S. \tag{19}$$

This implies that, at constant $P_0/a$, the speed $v_{max}$ is directly proportional to $k_{-ADP}$. This fact is well known experimentally (see, for example, Fig. 4 in Siemankowski et al. (1985), where $k_{min} \leftrightarrow v_{max}/S$, $k_{-AD} \leftrightarrow k_{-ADP}$). In view of Eq. (15), such a connection seems quite natural for a system in which the ADP release turns out to be the rate-limiting step, so that $t_r \approx t_{-ADP}$. It should be emphasized that this conclusion is by no means grounded on the strain sensitivity of the rate of ADP release, since in this model the strain disappears after $P_i$ release.

By the way, formula (15) describes a more general regularity: $v_{max}$ is proportional to the rate constant of just the slowest process occurring in the course of the recovery stroke. If the ATP concentration decreases, the time $t_{ATP}$ increases and can exceed the time $t_{-ADP}$ (see 16). In this case, it is easy to obtain also the standard (see, for example, Fig. 5 in Cooke and Bialek 1979) hyperbolic dependence of the speed of unloaded contraction $V_{max}$ on [ATP]. Since $t_{-ADP}$ does not explicitly depend on the ATP concentration and at high [ATP] the inequality (18) holds true, we can put $t_r \equiv t_r([ATP] \to \infty) \approx k_{-ADP}^{-1}$ and assume that the value of $t_{da}/t_r$ is negligible. Then we obtain

$$V_{max}([ATP]) \approx v_{max}\frac{t_r k_{ATP}\cdot[ATP]}{1 + t_r k_{ATP}\cdot[ATP]},$$
$$v_{max} \equiv V_{max}([ATP] \to \infty). \tag{20}$$

The coefficient preceding [ATP] is equal to $t_r k_{ATP}$ here, and hence, $V_{max}$ reaches half its asymptotic value $v_{max}$ if

$$[ATP]|_{V_{max}=v_{max}/2} \approx (t_r k_{ATP})^{-1}, \tag{21}$$

(compare with Eq. 48).

Efficiency and heat production

We define the efficiency $\eta$ as the ratio of the useful mechanical work performed per cross-bridge turnover to the ATP cleavage energy $E_{\mathrm{ATP}}$. From (13) and (14) we obtain for the case $v = \mathrm{const}$.

$$\eta(v) \equiv \frac{P(v)vT(v)}{E_{\mathrm{ATP}}} = \frac{P_0 S}{E_{\mathrm{ATP}}}\left(1 - {}^{v}\!/_{v_{\max}}\right). \tag{22}$$

This definition of efficiency, being standard for a usual mechanical system, is not completely correct for the description of the cleavage of a single molecule within an atomic-size system. In fact, it is impossible to totally convert the $E_{\mathrm{ATP}}$ energy to mechanical work since the temperature of the sarcomere as a whole is constant. Actually, if we do not take into account the internal and rotatory degrees of freedom, their total number is equal to 3 for the ATP molecule and 3 + 3 for its fragments ADP and $P_i$. Hence, energy of no less than ${}^{3}\!/_{2}k_{\mathrm{B}}T$ must be spent to accelerate them to thermal velocities (thermalize). This energy dissipated to heat by no means can be converted to work, although one cannot assert that it is lost irreversibly. I suggest that the kinetic energy of ADP and $P_i$ takes a noticeable part in the course of ATP synthesis during metabolic processes. Thus, at least part of the heat should be used again. This reasoning is in agreement with the arguments of Wilkie (1974), who suggested that one should calculate the ratio of useful work to the free rather than total energy, as in (22). Nevertheless, knowing that this is a mere renormalization of the efficiency value and the heat loss is barely about 10 % of the total hydrolysis energy, we can neglect these corrections to a first approximation.

One can easily see that the dependence $\eta(v)$ (22) is linear in $v$. The velocity dependence of efficiency is traceable solely to the presence of "friction", and this dependence is linear in $v$ because in both cases (13) and (14) the friction losses (the work performed by the force $F_{\mathrm{h}}$ per cycle) increase in proportion to $v$. However, just as for Eq. (10), Eq. (22) is in sharp contradiction to experiment since $\eta(v)$ must fall to zero at $v \to 0$. The reason is the same—the formulas of this section cannot be used at $v \to 0$.

The part of the energy released in the course of ATP hydrolysis which was not converted to mechanical work eventually dissipates to heat. Hence, the rates of work (WR) and heat production (HR) and their sum (heat + work rate, HWR) are defined by the equations

$$\begin{aligned}
\mathrm{HR}(v) &\equiv \frac{E_{\mathrm{ATP}}}{T(v)}\left[1 - \eta(v)\right] \\
&= (P_0 v_{\max})\frac{E_{\mathrm{ATP}}}{P_0 S}\frac{1 - \eta(v)}{1 + \left({}^{P_0}\!/_{a}\right){}^{v}\!/_{v_{\max}}}\,{}^{v}\!/_{v_{\max}}, \\
\mathrm{WR}(v) &\equiv \frac{E_{\mathrm{ATP}}}{T(v)}\eta(v) = (P_0 v_{\max})\frac{{}^{v}\!/_{v_{\max}}\left(1 - {}^{v}\!/_{v_{\max}}\right)}{1 + \left({}^{P_0}\!/_{a}\right){}^{v}\!/_{v_{\max}}}, \\
\mathrm{HWR}(v) &\equiv \mathrm{WR}(v) + \mathrm{HR}(v)
\end{aligned} \tag{23}$$

(the value $P_0 v_{\max}$ is a convenient unit for power and therefore is factorized). Again, these formulas, as for (10) and (22), do not work at $v \to 0$. However, we know that work is not done for $v = 0$ and so

$$\mathrm{HR}(v \to 0) \equiv \mathrm{HR}_0 = {}^{E_{\mathrm{ATP}}}\!/_{T_0}. \tag{24}$$

Conversely, in the case of contraction at maximal velocity we obtain from (23) and both (13) and (14) that

$$\mathrm{HR}(v_{\max}) = \frac{E_{\mathrm{ATP}}}{t_{\mathrm{r}} + {}^{S}\!/_{v_{\max}}} = (P_0 v_{\max})\frac{E_{\mathrm{ATP}}}{P_0 S\left(1 + {}^{P_0}\!/_{a}\right)}. \tag{25}$$

The straight line $\mathrm{SL}(v)$ connecting the initial $(0, HR_0)$ and the final $(v_{\max}, \mathrm{HR}(v_{\max}))$ points of the curve $\mathrm{HR}(v)$ is determined by an equation of the form

$$\begin{aligned}
\mathrm{SL}(v) &= \mathrm{HR}_0 + (P_0 v_{\max}) \cdot q \cdot \left(\frac{a}{P_0}\right){}^{v}\!/_{v_{\max}}, \\
q &= \frac{E_{\mathrm{ATP}}}{P_0 S}\frac{t_{\mathrm{r}}}{T(v_{\max})} \cdot \left[1 - {}^{T(v_{\max})}\!/_{T_0}\right].
\end{aligned} \tag{26}$$

According to the above estimations, the ratio ${}^{T(v_{\max})}\!/_{T_0} = {}^{\mathrm{HWR}(v=0)}\!/_{\mathrm{HWR}(v_{\max})}$ should be about 1/10 or less, so that it can be neglected as compared with 1. Then, the not too rough approximation $q \approx \left[\eta(0)\left(1 + {}^{S}\!/_{(v_{\max} t_{\mathrm{r}})}\right)\right]^{-1}$ can be used. Since $T(v_{\max})$ is only slightly greater than $t_{\mathrm{r}}$, the $q$ value in (26) must be about unity and the slope of $\mathrm{SL}(v)/P_0$ is about $(a/P_0)$ [compare with Hill's (1938) assumption]. Next, the second derivative of $\mathrm{HR}(v)$ is proportional to

$$\mathrm{HR}''(v) \sim \frac{\eta(0)}{1 - \eta(0)} - \frac{v_{\max} t_{\mathrm{r}}}{S},$$

and therefore the function is a convex curve (i.e., $\mathrm{HR}''(v) < 0$), if $\eta(0)$ (see 22) is not too large. Consequently, the function $\mathrm{HR}(v)$ (23) beyond the low-velocity region is similar to the standard experimental curves (see Fig. 4.13 in Woledge et al. 1985).

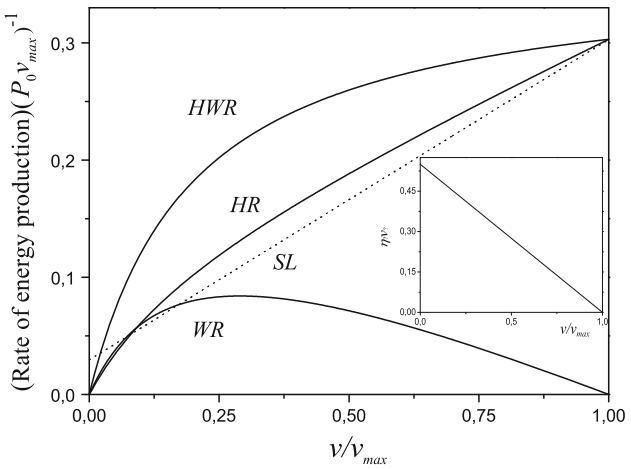


**Fig. 2** Dependence of the rates of heat (HR), work (WR), and heat + work output (HWR) on the relative contraction velocity $v/v_{\max}$ (see 23) in the framework of the simple mechanical model. The corresponding parameter values are listed in (27) and (28) or (29). *Inset* dependence of the cross-bridge efficiency (22) on $v$. Note the values of the functions HR($v$), HWR($v$), and $\eta(v)$ at $v = 0$ (compare with Fig. 4)

For purely illustrative purposes, Fig. 2 shows the dependence of the efficiency (22) and of the rates of heat, work, and (heat + work) production on the contraction velocity. The following parameter values were chosen:

$$S = 11\,\text{nm}, \quad P_0 = 4\,\text{pN}, \quad v_{\max} = 1.5\,\mu\text{m}\,\text{s}^{-1}, \quad T_0 = 450\,\text{ms}, \quad a/P_0 = 0.2, \tag{27}$$

(see also (1)), and the values

$$\eta_0 = 0.55, \quad \gamma = 2.67 \times 10^{-6}\,\text{kg}\,\text{s}^{-1}, \quad t_r = 37\,\text{ms}, \quad q \approx 1.37 \tag{28}$$

or

$$\eta_0 = 0.66, \quad F_h = 0.8\,\text{pN}, \quad t_r = 37\,\text{ms}, \quad q \approx 1.37 \tag{29}$$

were obtained from (27) and (13) or (14), respectively.

In the low-velocity region, the difference between the curves shown in Fig. 2 and experimental ones is not fundamentally important. The problem is solved in the next section (compare Fig. 2 and Fig. 4). However, there also arises a problem in the high-speed region $v \approx v_{\max}$. In accordance with some data (see, for example, Hill 1964), the rate of (heat + work) production might decrease when $v$ approaches $v_{\max}$. Naturally, it is impossible to explain this fact in a model in which the cross-bridge cycle duration decreases monotonically with increasing velocity of muscle contraction. This circumstance forced Huxley (1973) to make his model additionally complicated.

One of the main purposes of this work is the creation of an extremely simple model with a minimal number of parameters but which is still capable of explaining the basic laws governing the process of muscle contraction. It seems to me that, although ATP hydrolysis by the actomyosin complex appears to be the unique source of energy for the work which a muscle performs, it is not the unique source of heat. In fact, heat fluxes of various capacity and character arise in the course of any chemical reactions in muscle. In particular, a significant amount of heat is released during the operation of calcium pumps (see Woledge et al. 1985, p. 25), and there are no grounds to think that the intensity of these processes does not depend on the speed of muscle contraction. There are some uncertainties concerning the heat fluxes connected with metabolic processes. Their velocity should by no means necessarily be proportional to the muscle contraction velocity (i.e., the rate of growth of the concentration of ATP hydrolysis products). In such a situation, reliable experimental estimation of just the amount of heat released during ATP hydrolysis by actomyosin complex seems more urgent than the introduction of amendments to the model of this process. *Frustra fit per plura quod potest fieri per pauciora*.

## Fluctuations at low-velocity contraction

The above-considered mechanism of the working stroke interruption can hardly account for the reason for cross-bridge detachment from a thin filament in the case of isometric contraction. Under this mechanism, the cross-bridge attached to actin at the moment $t = 0$ with coordinate $x = 0$ can detach from it only at $x = D$, no matter how much time $t$ it takes until this moment. In fact, upon isometric contraction, the cross-bridges do not change their coordinates, rather remaining attached to actin only for a limited time, on average, equal to $T_0$. Taking account of the fact that we are dealing with objects with characteristic size of about a few nanometers, the assumption about the leading role of thermal fluctuations seems dominant in this case. These fluctuations can manifest themselves in our description as a random force large enough to pass switch-2 from the "closed" to "open" state. Besides, one cannot exclude that some other fluctuation mechanism is essential in the cross-bridge detachment process. Anyhow, by analogy with Huxley's (1957) model, we have to introduce a probabilistic description for these effects.

### Probability function for a single cross-bridge

Considering the evolution of a cross-bridge which is attached to actin at the moment $t = 0$ with coordinate $x = 0$, we must introduce a function $W(x,t)$—the probability for the cross-bridge, being still attached to the actin

filament, to reach the state with coordinate $x$ at the moment $t$. This probability satisfies a general equation

$$\frac{\mathrm{d}}{\mathrm{d}t}W(x,t) \equiv \left(\frac{\partial}{\partial t} + \frac{\mathrm{d}x}{\mathrm{d}t}\frac{\partial}{\partial x}\right)W(x,t), \quad W(0,0) = 1. \tag{30}$$

The specific form of the partial derivatives with respect to time and coordinate depends on which processes are responsible for changing the state of the cross-bridge.

To describe our mechanism of back door opening, the partial derivative with respect to $x$ should be of the form

$$\frac{\partial}{\partial x}W(x,t) = -\delta(x-D)W(D,t). \tag{31}$$

In this case, the state of the cross-bridge does not change at any $x < D$ and the cross-bridge necessarily detaches from actin; i.e., $W$ vanishes as soon as $x$ increases up to $D$.

To describe the action on a cross-bridge of random forces, the partial derivative with respect to $t$ should be of the form of the equation of radioactive decay

$$\frac{\partial}{\partial t}W(x,t) = -\frac{W(x,t)}{\tau_{\mathrm{w}}(x)}. \tag{32}$$

Here, $\tau_{\mathrm{w}}(x)$ is the lifetime of the working stroke of the cross-bridge residing in the state with coordinate $x$. To determine this value experimentally, it is necessary to shift quite a number of cross-bridges after attachment to actin to the state with coordinate $x$ and to measure the average time prior to their detachment. Consequently, the function $\tau_{\mathrm{w}}(x)$ can be determined from experiments of the type described by Huxley and Simmons (1971), Ford et al. (1977), Piazzesi and Lombardi (1995), and Piazzesi et al. (2002), although I know nothing about such studies.

Being controlled by the fluctuations of the force applied to the back door, the lifetime $\tau_{\mathrm{w}}(x)$ should be smaller, the closer $x$ is to $D$. Indeed, the closer the piston is to the exit from the cylinder (Fig. 1), the easier it is to knock it out with one blow. We suppose here that

$$\tau_{\mathrm{w}}(x) = \tau_{\mathrm{w}}^{(0)}\exp(-\kappa x), \tag{33}$$

where $\tau_{\mathrm{w}}^{(0)}$ and $\kappa$ are the model parameters [compare with $g(x)$ in Duke (2000, Eq. 9)]. The choice of the function type in (33) is convenient since, on the one hand, it enables the form of the $\tau_{\mathrm{w}}(x)$ dependence to be changed over a wide range and, on the other hand, the $W(x,t)$ dependence with this choice can be found analytically, at least in the case of isovelocity contraction. However, in the case of numerical calculations, this form of the $\tau_{\mathrm{w}}(x)$ dependence is not compulsory; see the text relating to (3).

Thus, we obtain the following equation for $W(x,t)$:

$$\frac{\mathrm{d}}{\mathrm{d}t}W(x,t) = -\left[\frac{1}{\tau_{\mathrm{w}}(x)} + \delta(x-D)\frac{\mathrm{d}x}{\mathrm{d}t}\right]W(x,t), \quad W(0,0) = 1. \tag{34}$$

It depends on coordinate $x$ and on the time span $t$ between hydrolysis onset (coordinate $x = 0$) and the moment under consideration (coordinate $x$). To solve (34), these two variables should be linked by a functional dependence $x(t)$ which determines the law of motion (the type of muscle contraction). In other words, the time span $t$ obviously must depend on the velocity of sliding filaments $v(t) = \lambda \mathrm{d}x/\mathrm{d}t$, because the relative displacement of filaments is $\lambda$ times as large as the displacement of switch-2 ($x$) owing to the lever arm (Fig. 1). Below we consider only the simplest case of isovelocity contraction in which the relationship between $x$, $v$, and $t$ takes the form

$$t = \lambda x/v, \quad v = \mathrm{const}. \tag{35}$$

The characteristic scale of measurement for this time is set by the value

$$\tau_{\mathrm{w}}^{(0)} = T_0 - t_{\mathrm{r}}, \tag{36}$$

which is on the order of a few tenths of a second. This is the average duration of the working stroke at isometric contraction, and the cross-bridge certainly detaches from actin under the action of fluctuations before its coordinate reaches the value $x$, if it takes a time span substantially exceeding $\tau_{\mathrm{w}}^{(0)}$. On the contrary, a cross-bridge attached to slowly moving actin is unlikely to be detached from it for a time span much smaller than $\tau_{\mathrm{w}}^{(0)}$.

In the case of isovelocity contraction, these conclusions are supported by direct analytical calculation. The coordinate $x$ becomes a single-valued function of time $t$ (see 35), and $W$ turns out to be a function of a single variable:

$$W(x,t) = W\left(vt/\lambda, t\right) \equiv \tilde{W}(t). \tag{37}$$

Now, we obtain an ordinary differential equation instead of (34):

$$w(t) \equiv -\frac{\mathrm{d}}{\mathrm{d}t}\tilde{W}(t) = \left[\frac{1}{\tau_{\mathrm{w}}\left(vt/\lambda\right)} + \delta\left(t - \lambda D/v\right)\right]\tilde{W}(t), \quad \tilde{W}(0) = 1, \tag{38}$$

and, integrating it, obtain the result

$$\tilde{W}(t) = \exp\left\{-\frac{t}{\tau_{\mathrm{w}}^{(0)}}\frac{\mathrm{e}^z - 1}{z}\right\}\theta\left(S/v - t\right), \quad z \equiv \kappa v t/\lambda;$$
$$w(t) = \frac{\tilde{W}(t)}{\tau_{\mathrm{w}}\left(vt/\lambda\right)}\theta\left(S/v - t\right) + \tilde{W}\left(S/v\right)\delta\left(t - S/v\right), \tag{39}$$

where $\theta(x)$ is the step function. This solution satisfies both the natural initial condition $\tilde{W}(0) = 1$ (the cross-bridge is

attached to actin filament at the initial moment) and the condition that $\tilde{W}$ vanishes if the cross-bridge displacement reaches one pace $S$ at the moment $t = S/v$ (working stroke interruption because of back door opening at $x = D$).

## Force, work, and efficiency for a single cross-bridge

Let us consider once more the dependence of the average duration of the working stroke on the contraction velocity. This dependence is now determined by the integral

$$t_{\mathrm{w}}(v) \equiv \int_0^{S/v} tw(t)\mathrm{d}t = \int_0^{S/v} \tilde{W}(t)\mathrm{d}t, \quad \lim_{v\to 0} t_{\mathrm{w}}(v) = \tau_{\mathrm{w}}^{(0)}. \tag{40}$$

If $\kappa = 0$ and hence $\tau_{\mathrm{w}}$ in (33) does not depend on $x$, this can be easily integrated:

$$\begin{aligned} \tilde{W}(t)\big|_{\kappa=0} &= \exp\left(-t/\tau_{\mathrm{w}}^{(0)}\right), \\ t_{\mathrm{w}}(v)\big|_{\kappa=0} &= \tau_{\mathrm{w}}^{(0)}\left[1 - \exp\left(-S/\left(v\tau_{\mathrm{w}}^{(0)}\right)\right)\right]. \end{aligned} \tag{41}$$

It is seen that the function $t_{\mathrm{w}}(v)$ varies very differently in two different regions of $v$ values: $v < v_{\mathrm{ch}}$ and $v > v_{\mathrm{ch}}$, where $v_{\mathrm{ch}} = S/\tau_{\mathrm{w}}^{(0)}$. In the first case $t_{\mathrm{w}}$ is almost unchanged $t_{\mathrm{ws}}(v < v_{\mathrm{ch}}) \approx \tau_{\mathrm{w}}^{(0)}$, while in the second case it decreases in inverse proportion to the speed $t_{\mathrm{ws}}(v > v_{\mathrm{ch}}) \approx S/v$. A similar situation holds for $\kappa \neq 0$, but in this case it is better to use the approximate formula

$$v_{\mathrm{ch}} \approx S/\tau_{\mathrm{w}}(D). \tag{42}$$

In the case $v < v_{\mathrm{ch}}$ fluctuations dominate and the probability for a cross-bridge to be kept attached to actin filament up to completion of the step ($x = D$) is negligible. In the contrary limiting case $v > v_{\mathrm{ch}}$ fluctuations turn out to be too slow, so that the majority of cross-bridges detach from actin filament just at the moment when the displacement reaches $S$, and we return to (9) for $t_{\mathrm{w}}$.

The work done by the force $F(x)$ (2) on the movement of filaments in a distance $s$ is determined by (4). Averaging this work over the duration of the working stroke (it is not permanent now on account of the fluctuations) in the case of isovelocity contraction $s = vt$ one obtains

$$\begin{aligned} v = \mathrm{const}: \langle s\rangle_{\mathrm{ws}} &= vt_{\mathrm{w}}(v), \\ \langle \mathrm{Work}\rangle_{\mathrm{ws}} &= \int_0^{S/v}\left[\int_0^{vt/\lambda} F(x)\,\mathrm{d}x\right]\cdot w(t)\,\mathrm{d}t \\ &= \frac{v}{\lambda}\int_0^{S/v} F\left(vt/\lambda\right)\cdot\tilde{W}(t)\,\mathrm{d}t. \end{aligned} \tag{43}$$

In contrast to Work($S$) (see "The simple mechanical model of S1 structure") this expression depends on the contraction velocity and vanishes at $v \to 0$.

Now, we want determine the average force exerted on actin by a single cross-bridge during its working stroke. The result will depend on the conditions of the corresponding experiment. If the forces exerted by a single cross-bridge (i.e., work/displacement) are sequentially measured and then averaged, one obtains

$$\langle f\rangle_{\mathrm{ws}} = \int_0^{S/v}\left[\frac{1}{vt}\int_0^{vt/\lambda} F(x)\,\mathrm{d}x\right]\cdot w(t)\,\mathrm{d}t. \tag{44}$$

Otherwise, if a sarcomere or a group of sarcomeres is tested, one at best can measure only the total work and average pace $\langle s\rangle_{\mathrm{ws}}$ so that

$$\langle f\rangle_{\mathrm{ws}} = \langle \mathrm{Work}\rangle_{\mathrm{ws}}/\langle s\rangle_{\mathrm{ws}} \tag{45}$$

In general, the expressions (43–45) are functions of $v$. There is some experimental evidence that the force generated per cross-bridge really varies with velocity (Barclay et al. 2010), and using data of this sort one can try to determine the true $E(x)$ dependence. However, in this paper, we restrict ourselves to the case $F = \mathrm{const}$ (see 3). The following very simple expressions appear in this case:

$$\begin{cases} \langle f\rangle_{\mathrm{ws}} = F/\lambda, \\ \langle \mathrm{Work}\rangle_{\mathrm{ws}} = \langle f\rangle_{\mathrm{ws}} vt_{\mathrm{w}}(v). \end{cases} \quad \begin{pmatrix} F = \mathrm{const.} \\ v = \mathrm{const.} \end{pmatrix} \tag{46}$$

Substituting these expressions into Eq. (11), we obtain the same formulas (13–25) in the region $v > v_{\mathrm{ch}}$. At the same time, the situation is completely different in the region $v < v_{\mathrm{ch}}$. Here, the functions $\langle f\rangle(v)$ (11) and $P(v)$ (13, 14) decrease very slowly with increasing $v$, just as $t_{\mathrm{w}}(v)$ (40). This effect is very similar to that discussed by Ritchie and Wilkie (1958) and Edman (1979), but with the condition $F = \mathrm{const.}$ and reasonable values of the parameters, the region where the deviations from Hill's curve (12) emerge turns out to be too narrow.

To conclude this section it remains to discuss the dependence of the isometric force on the ATP concentration. Such a dependence should occur at small values of [ATP] when the ATP binding process turns out to be the rate-limiting step instead of the $P_i$ release process, i.e., $t_{\mathrm{w}}(0)$ is less than or approximately $t_{\mathrm{ATP}} = (k_{\mathrm{ATP}}[\mathrm{ATP}])^{-1}$. Putting $t_{\mathrm{r}} \ll T_0$ and therefore $t_{\mathrm{w}}(0) \approx T_0$, by analogy with (20) we obtain from (45) and (46) that

$$P([\mathrm{ATP}])\big|_{v\to 0} = \frac{F}{\lambda}\frac{\tau_{\mathrm{w}}(0)}{\tau_{\mathrm{w}}(0) + t_{\mathrm{r}}} \approx \frac{F}{\lambda}\frac{T_0 k_{\mathrm{ATP}}[\mathrm{ATP}]}{1 + T_0 k_{\mathrm{ATP}}[\mathrm{ATP}]}. \tag{47}$$

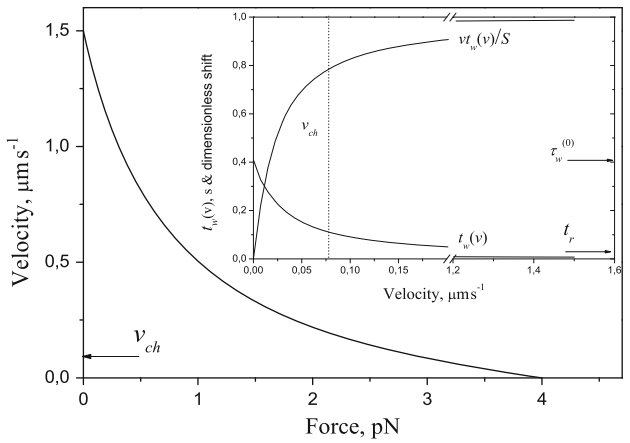


**Fig. 3** Force–velocity curve at the parameter values specified in (50) or (51); the value of $v_{ch}$ is indicated by an *arrow*. The curve coincides with the hyperbola (12) at $a/P_0 = 0.2$ (this value was not fitted). *Inset* the dimensionless ratio $vt_w(v)/S$ of the actin filament shift averaged over the working stroke to the maximal pace, and the dependence $t_w(v)$ (in seconds) at the same parameter values are shown. The value of $v_{ch}$ is indicated by a *vertical dashed line* here; note the break on the abscissa

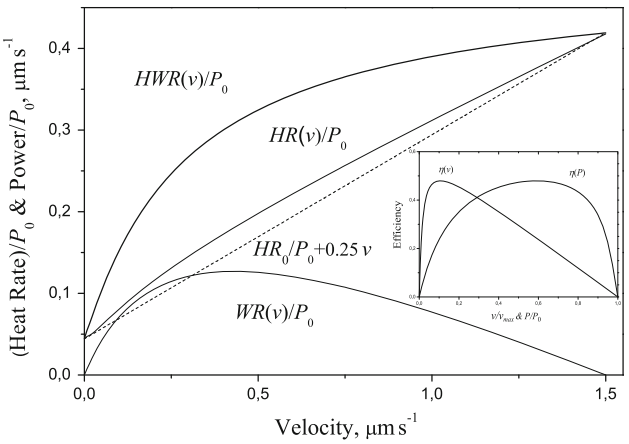


**Fig. 4** Dependence of the rates of heat (HR), work (WR) and heat + work (HWR) output on contraction velocity $v$ (see 23), taking into account the contribution of fluctuations. Corresponding parameter values are listed in (27) and (50). *Inset* dependence of the cross-bridge efficiency (22) on $v/v_{max}$ and $P/P_0$. Note the values of the functions HR($v$), HWR($v$), and $\eta(v)$ at $v = 0$ (compare with Fig. 2)

The coefficient preceding [ATP] is now equal to $T_0 k_{ATP}$ [compare with Eq. (20)]. This is why this hyperbole rises with increasing [ATP] an order of magnitude steeper than hyperbola (20):

$$\frac{[\mathrm{ATP}]|_{V_{\max}=v_{\max}/2}}{[\mathrm{ATP}]|_{P([\mathrm{ATP}])|_{v=0}=\frac{F}{2\lambda}}} \approx \frac{T_0}{t_r}. \tag{48}$$

In the main, these equations correctly describe the experimental curves [compare (47) and (48) with (20) and (21) and Figs. 2 and 5 in Cooke and Bialek (1979)]. However, it should be noted that equation (47) does not describe a slight isometric force decrease at high [ATP] (see Fig. 2 in Cooke and Bialek 1979). It might be that this decline is associated with factors that are not accounted for in the model under consideration; For instance, according to Karatzaferi et al. (2004), the isometric force decreases sharply with increasing $P_i$ concentration, which should rise with [ATP].

## Numerical calculations

Since $\langle \mathrm{Work} \rangle_{ws} \to 0$ at $v \to 0$, the efficiency (22) also vanishes in this limit while the rate of heat release (23) becomes equal to $E_{ATP} \cdot T_0^{-1}$. The results of numerical calculations performed using these formulas are shown in Figs. 3 and 4. The computations again were done for the two types of dependences of hindering force on velocity: the linear one with $F_h = \gamma v$ operating within a cross-bridge, and the constant one with $F_h = \text{const.}$ operating outside it. The numerical values of the five model fitting parameters $t_r$, $F$, $\tau_w^{(0)}$, $\kappa$, and $F_h$ (or $\gamma$) were determined to reproduce the values of the parameters (27). The only difference was that the slope of the curve HR($v$) was selected as close to the value 0.25 as possible instead of the value of ($a/P_0$) determined preliminarily:

$$\mathrm{HR}(v) \approx \mathrm{HR}_0 + 0.25 P_0 v. \tag{49}$$

To fit $\mathrm{HR}_0$, the value of $\tau_w^{(0)}$ was specified to satisfy the first condition in (10): $\tau_w^{(0)} = T_0 - t_r$. It must be emphasized that, in the approximation $F(x) = \text{const.}$, the shape of all the curves depends chiefly on $t_r$ and nearly does not depend on $\kappa$. This is quite natural because, in a wide range $v > v_{ch}$, the curvature of the function $\langle f \rangle(v)$ (11) (or, what is the same, the magnitude of the parameter $P_0/a$, which regulates the curvature of the force–velocity curve) is mainly determined by the $t_r$ value (see 15). The dependence of the curvature on $\kappa$ is weak because this parameter plays an important role for the function $t_w(v)$ only in the narrow interval $v < v_{ch}$. Finally, the $P_0$ value depends mainly on $F$, while $v_{max}$ is fixed by a finer fitting of $F$ and $F_h$.

In Fig. 3, the dependence $P(v)$ of the force exerted by a single cross-bridge on the contraction velocity is shown only for the case $F_h = \gamma v$ for the following parameter values:

$$F = 97\,\mathrm{pN}, \quad \gamma v_{\max} = 4.41\,\mathrm{pN}, \quad t_r \approx 0.04\,\mathrm{s}, \quad \kappa = \lambda \times 10^8\,\mathrm{m}^{-1}. \tag{50}$$

At this scale, the distinction between this case and the case $F_h = \text{const.}$ at

$$F = 116\,\mathrm{pN}, \quad F_h = 0.8\,\mathrm{pN}, \quad t_r \approx 0.04\,\mathrm{s}, \quad \kappa = \lambda \times 10^8\,\mathrm{m}^{-1} \tag{51}$$

is negligible, similarly to the distinction between the force–velocity curve and the hyperbola (12) at $(a/P_0) = 0.2$. The dependence $vt_w(v)/S$ shown in the inset illustrates the correctness of the approximation $t_w \approx S/v$ and of expression (42) for $v_{ch}$. The dependence $t_w(v)$ and the value of $t_r$ are shown in the inset as well.

The dependences of the efficiency (22) on the relative velocity $v/v_{max}$ in the course of isovelocity contraction and on the relative force $P/P_0$ in the course of isotonic contraction are shown in the inset to Fig. 4. The maximal $\eta$ value amounts to about 0.5 and was not fitted. The same Fig. 4 shows the dependences of the rates of work and heat output on the contraction velocity per half-sarcomere (compare Fig. 4 and Fig. 2). It is clear from (26) that the slope of the $HR(v)/P_0$ curve is about $a/P_0$. Therefore, by fitting the parameters to satisfy (49), we actually determine the parameter $a/P_0$. We see that the curves shown in Figs. 3 and 4 and those usually obtained in experiments on frog sartorius muscle at low temperatures coincide in outline. It should be emphasized that this reasonably good agreement between theory and experiment was in fact obtained by fitting a single parameter. Indeed, the changes of $F$ and $F_h$ [i.e., $P_0$ and $v_{max}$, see (13), (14)] merely change the scale and do not alter the shape of the curves, while the role of $\kappa$ is negligible. As a result, a single parameter—the recovery time $t_r$ (see 15)—is now in the forefront.

## Boltzmann equation and collective effects

The solution (39) describes the probability that a single cross-bridge which at the moment $t = 0$ attaches to actin filament moving uniformly in $v$ will stay attached to it up to the moment $t$. Now, we consider the whole ensemble consisting of a large number $N$ of identical myosin heads which attach to actin filaments and detach from them with random phases. Apparently, there are no grounds to think that some direct interaction exists between these myosin heads. Hence, for all the cross-bridges, alteration in the velocity of filament sliding is the only means for a single cross-bridge to feel the change in external load.

In the model discussed above, just the contraction velocity is the principal parameter determining the average force generated by each cross-bridge. Consequently, the decrease in the velocity of contraction, which necessarily arises upon an increase in the load on the muscle, causes a synchronic increase of the average force generated by each cross-bridge. In other words, it is just this muscle response to the changes in the contraction velocity that causes any weight to be lifted by a muscle uniformly.

This explanation is very simple and transparent compared with the assumption suggested by Huxley and Simmons (1971) and now underlying a number of models from those of Duke (1999) to Walcott and Sun (2009); see the discussion in "Relation to earlier theories." Its meaning is that the load change results in the change in tension of the cross-bridge neck; this in turn modifies the energies and population of different S1 states and, eventually, leads to an alteration of the force which is generated by the cross-bridge. It seems, however, that even the author of this theory, Huxley himself, did not quite believe in its correctness: "The fact … implies that the molecular events are directly affected by the longitudinal displacement of filaments rather than by the tension in them" (Huxley 2000, p. 435).

### Distribution function and Boltzmann equation

Let us still suppose that after the instant $t = 0$ the contraction velocity $v$ does not change, and look at a much longer time span comprising a series of cross-bridge cycles. We now want to examine the evolution of this ensemble for $t > 0$, i.e., to ask:

1. What is the average number $N_d$ $(t)$ of myosin heads detached from actin filaments?
2. What is the average number $N_a$ $(x,\delta x,t)$ of cross-bridges which are attached to actin and have coordinates between $x$ and $x + \delta x$?

It is convenient to introduce the distribution function $\rho(x,t)$ satisfying the equation

$$N_a(x, \delta x, t) = N\rho(x, t)\delta x \tag{52}$$

for any $x$, $t$ and rather small $\delta x \ll D$. Since any myosin head should either be attached to actin filament with some value of $x$ or be detached from it, this distribution function satisfies the normalization condition

$$n_d(t) + \int_0^D \rho(x, t)\,\mathrm{d}x \equiv 1, \quad n_d(t) \equiv N_d(t)/N. \tag{53}$$

Taking into account that the filaments slide at velocity $v$, after an interval $\delta t$ the states of $N_a(x,\delta x,t)$ cross-bridges that had coordinates between $x$ and $x + \delta x$ at the moment $t$ change as follows:

1. $N_a(x,\delta x,t)\cdot(\delta t/\tau_w(x))$ of them detach from actin owing to the fluctuations according to (32) if $x + \delta x < D$;
2. All of $N\rho(D,t)v\delta t/\lambda$ cross-bridges with coordinates from $D - v\delta t/\lambda$ up to $D$ detach from actin because of the forced opening of their back doors;
3. Coordinates of all other cross-bridges increase by $v\delta t/\lambda$.

This means that the decrease of the function $\rho(x,t)$ due to cross-bridge detachment depends on time according to the equation

$$\left(\frac{\partial}{\partial t}+\frac{v}{\lambda}\frac{\partial}{\partial x}\right)\rho(x,t)\Big|_{\text{dec}}=-\left[\frac{1}{\tau_{\text{w}}(x)}+\frac{v}{\lambda}\delta(x-D)\right]\rho(x,t). \tag{54}$$

Consequently, the equation for $n_{\text{d}}(t)$ has the form

$$\frac{\text{d}}{\text{d}t}n_{\text{d}}(t)=\frac{v}{\lambda}\rho(D,t)+\int_0^D\frac{\rho(x,t)}{\tau_{\text{w}}(x)}\,\text{d}x-\frac{n_{\text{d}}(t)}{\tau_{\text{r}}}. \tag{55}$$

Here, the last term is written based on the hypothesis that the rate of cross-bridge transition from the detached to attached state is determined by an equation of the type (32) with lifetime $\tau_{\text{r}}$ independent of $v$. These cross-bridges become attached to actin with some values of coordinate $x$, and this is the only contribution leading to an increase in $\rho(x,t)$. Therefore, we have to define the last term which describes the increase of $\rho(x,t)$ in the equation

$$\frac{\text{d}}{\text{d}t}\rho(x,t)=\left(\frac{\partial}{\partial t}+\frac{v}{\lambda}\frac{\partial}{\partial x}\right)\rho(x,t)\Big|_{\text{dec}}+\frac{\partial}{\partial t}\rho(x,t)\Big|_{\text{inc}}. \tag{56}$$

We shall presume that the working stroke of any cross-bridge always begins at the state with $x=0$. On the one hand, this assumption seems natural because the waste of energy is minimal in this case. On the other hand, this assumption does not seem well established because a cross-bridge can attach to actin filament only at certain points and thus should bend somehow to do so. Anyway, this assumption does not seem improbable and simplifies the calculations, although at any moment it can be rejected in favor of a function of the type $f_{\pm}$, $g_{\pm}$ (Huxley 1957).

Thus, we reach the conclusion that, during the time span $\delta t$, all cross-bridges with coordinate values from $x=0$ up to $\delta x=v\delta t/\lambda$ leave this band while $N_{\text{d}}(t)(\delta t/\tau_{\text{r}})$ of the newly attached cross-bridges fill their places. Consequently,

$$\rho(0,t)=\lambda n_{\text{d}}(t)/v\tau_{\text{r}}, \tag{57}$$

and now instead of (56) we must solve Eq. (54) with the boundary condition (57).

The set of equations (53–57) enables us to find the distribution function $\rho(x,t)$ if the initial one $\rho(x,0)$ and the value $v$ are known. One can expect that the same system of equations will describe the evolution of $\rho(x,t)$ in the case when $v(t)$ changes sufficiently slowly with time. At least this means that the velocity change during a single cross-bridge cycle should be negligible, i.e., $|v(t+T(v))-v(t)|\ll v(t)$.

Actually, the system (53–57) represents one of the simplest versions of the Boltzmann equation, specifically that in which the velocities of all the "particles" are equal to $v$ and unusual boundary conditions are used. Consequently, we can try to convert the system to a single integrodifferential equation. This is indeed possible in the case of isovelocity contraction, at least. In this case, a cross-bridge can turn out to be in the state with coordinate $x$ at moment $t$ only if it has attached to the actin earlier at the moment $t$ - $\lambda x/v$ in the state with $x=0$ and has not detached as yet. This means that

$$\begin{aligned}\rho(x,t)|_{v=\text{const.}}&\equiv\rho\left(0,t-\lambda x/v\right)\tilde{W}\left(\lambda x/v\right)\\&=\lambda/v\tau_{\text{r}}n_{\text{d}}\left(t-\lambda x/v\right)\tilde{W}\left(\lambda x/v\right)\end{aligned} \tag{58}$$

[see (39) for $\tilde{W}$ and (57)]. Therefore, it can be said that the distribution function $\rho$, determined at any moment $t$ in the phase space within the interval $0\le x\le D$, is in one-to-one correspondence with the function $n_{\text{d}}(t')$ related to the instants $t-\lambda D/v\le t'\le t$. We obtain now the desired integrodifferential equation in $n_{\text{d}}(t)$ by substituting (58) into (55):

$$\frac{\text{d}}{\text{d}t}n_{\text{d}}(t)=\frac{1}{\tau_{\text{r}}}\left\{-n_{\text{d}}(t)+\int_0^{\lambda D/v}w(t')n_{\text{d}}(t-t')\,\text{d}t'\right\}. \tag{59}$$

According to (58), specifying the distribution function $\rho(x,0)$ at an initial moment $t=0$ within the interval $0\le x\le D$ results in an unambiguous determination of fictitious values of $n_{\text{d}}(t')$ at "earlier" moments $-\lambda D/v\le t'\le 0$, which is just the initial condition for (59).

## Calculation of experimentally observed parameters

In a nonequilibrium system, the above-obtained equations could hardly be solved analytically, but the computation can easily be performed. If we restrict ourselves to the case of $v=$ const., the results of this type of calculations should be compared with the results of experiments on the stepwise transition of muscle from a definite initial state to isovelocity contraction at $t=0$. Even more interesting may be the analysis of experiments in which the contraction velocity changes stepwise twice or more times in relatively short intervals, for example, the experiments by Edman (1980) or Irving and Woledge (1981).

Providing that $\rho(x,t)$ is found, the force, heat rate, and other physical data per cross-bridge can be obtained by a standard averaging procedure:

$$\begin{aligned}P(t)&=-\frac{1}{\lambda}\int_0^D\frac{\partial E(x)}{\partial x}\rho(x,t)\text{d}x-\gamma v[1-n_{\text{d}}(t)],\\\text{HR}(t)&=\int_0^D E(x)\frac{\rho(x,t)}{\tau_{\text{w}}(x)}\text{d}x+E(D)\frac{v}{\lambda}\rho(D,t)\\&\quad+\gamma v^2[1-n_{\text{d}}(t)],\ldots\end{aligned} \tag{60}$$

Here, relation (2) instead of (3) was used and the case $F_{\text{h}}=\gamma v$ was chosen.

## Echo effect

Detailed numerical treatment of concrete experiments lies beyond the scope of this paper, and I confine myself to the examination of twitch shortening of a muscle following isometric tetanus. Some curious phenomena would occur in this case if the above model is adequate. According to the assumptions used in deriving equation (57), the distribution function in the isometric contraction state should resemble a Dirac $\delta$-function; that is, $\rho(x)$ at the isometric tetanus represents a narrow peak because the coordinates of all the attached cross-bridges have nearly the same time-independent value $x \approx 0$. Let us trace the evolution of this narrow peak if the state of the muscle changes stepwise from isometric to isovelocity contraction.

The evolution of $\rho(x,t)$ at the velocity $v = 0.15v_{\max}$ with $\tau_r = t_r$ and the magnitudes of all the other parameters specified in (27) and (50) is shown in Fig. 5. It is seen that the initial peak lowers and smears, shifting right with time, while its square (the total number of cross-bridges attached to actin filaments) decreases relatively slow. It is worth noting that, in accordance with (54), the peak of the function $\rho(x,t)$ must lower, shifting right, but no smearing should be observed in the interval $0 < t < S/v$, since the equation is linear and all the cross-bridges move with the same velocity $v$. In the calculations which I made, the real differential equation (54) was changed to a finite-difference one, and this resulted in the appearance of smearing shown in Fig. 5. I would expect similar smearing to occur in real muscle also, coming from spatial inhomogeneities of the contraction velocity within the bulk.

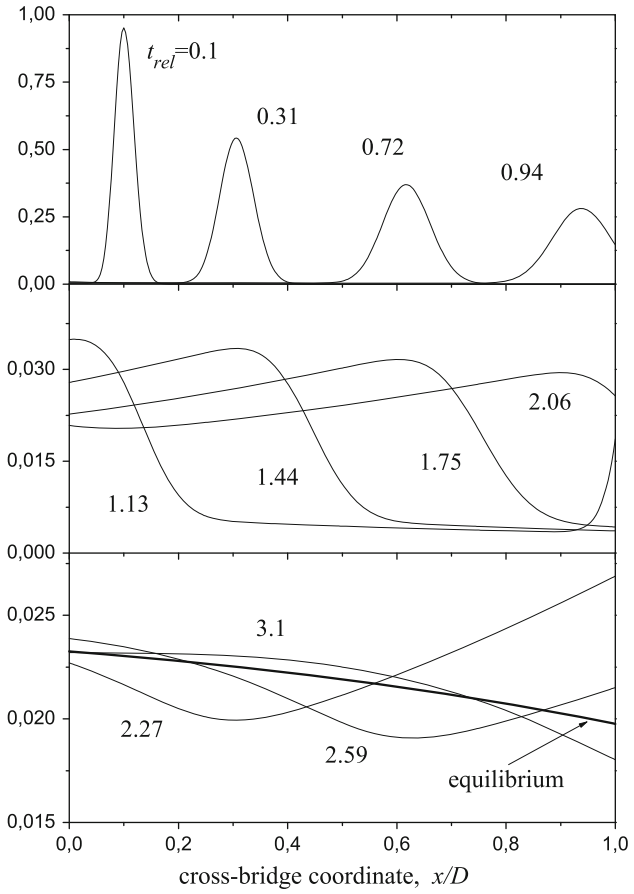


**Fig. 5** Dependence of the distribution function $\rho(x,t)$ on relative coordinate $x/D$ at $v = 0.15v_{\max}$ for successive instants shown in the graphs (*panels* from *top downward*, note the scale changes). The initial state is isometric contraction, so $\rho(x,0)$ is analogous to a narrow peak. The final state corresponds to the equilibrium at this velocity distribution function; see the *lowermost panel*. Time is represented in dimensionless units $t_{\text{rel}} = {}^{vt}/_{S}$. Note the sharp change of $\rho(x,t)$ at $t \approx 0.05$ s $(t_{\text{rel}} \approx 1)$ and the weaker one at $t \approx 0.1\,s$ $(t_{\text{rel}} \approx 2)$

A dramatic change in the distribution function takes place at $t \approx \lambda D/v$. Here, the attached cross-bridges detach from the actin filaments like an avalanche; the faster it is, the narrower the $\rho(x, \lambda D/v)$ peak. This leads to an abrupt decrease of the force exerted by the muscle and an equally abrupt peak of the rate of heat production (Fig. 6).

Now, most of the cross-bridges are detached from actin; i.e., $n_d\left({}^{\lambda x}/_{v}\right)$ is about unity. During the time span of the order of $\tau_r$, the majority of them should attach to actin again. If the velocity of filament sliding is low and the cross-bridge shift for this period is insignificant, the new narrow peak bulges on the $\rho(x, t)$ dependence and then disappears at $t \approx 2\lambda D/v$, etc. Thus, at rather low shortening velocity, an echo peculiarity repeated with period $\lambda D/v$ appears in the form of drops of force and overshoots of heat output. In the case shown in Figs. 5 and 6, the sliding velocity is quite large (about $0.15v_{\max}$) and instead of the new peak, a prolonged step forms on the distribution

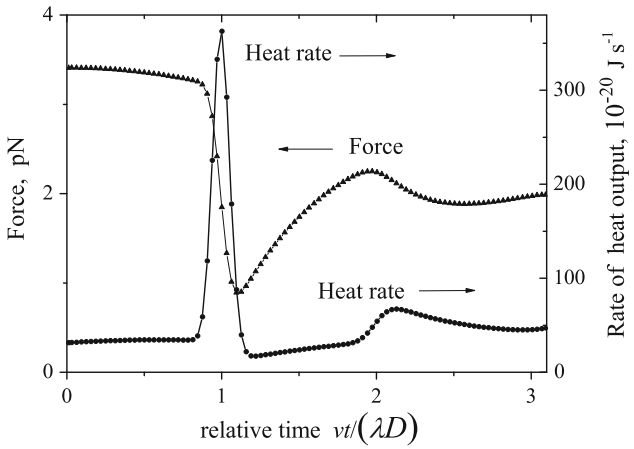


**Fig. 6** Time dependences of tension (*triangles*) and rate of heat production (*circles*); on the abscissa, time is represented in dimensionless units $t_{\text{rel}} = {}^{vt}/_{S}$. The zero time reference on the abscissa is related to the initial moment of isovelocity contraction with velocity $v = 0.15v_{\max}$; see Fig. 5. The force and heat rate values at $t_{\text{rel}} = 3$ ($t \approx 0.15$ s) already almost coincide with equilibrium at this velocity value

function after $t \approx \lambda D/v$ (middle panel in Fig. 5). As a result one can see in Fig. 6 only faint traces of even the second echo, and the relaxation time of the distribution function is very short (about $3\lambda D/v$) (lowermost panel in Fig. 5). It can also be observed that, at low contraction velocity $v < v_{ch}$, the initial $\rho(x,0)$ peak does not last even until the instant $t = \lambda D/v$, so the echo disappears in this region.

I failed to find experimental studies that would report on the observation of a similar echo. If such a phenomenon does exist, the lack of this information may be related to the necessity for a special choice of experimental parameters for its observation. Presumably, an analogous peak of heat production should also be observed after a sudden cease of muscle stimulation in an isometric tetanus. The corresponding experiment was performed by Hill (1961). He observed a fall adjacent to the last shock [presumably due to the sarcoplasmic reticulum calcium pumps cutting out] and then a much more prominent peak of the heat production rate. It should be emphasized that experimentally the beginning of the peak coincides nearly with the onset of tension decrease. One can expect that the beginning of the tension fall provokes the appearance of inhomogeneity: any relaxation of a separate region must lead to an abrupt contraction of other regions in series with the first one. As noted above, this results in a torrent of cross-bridge detachments accompanied by a heat outburst (the work done in the course of this local contraction is converted to heat as well). Since the myosin heads cannot attach to the actin again, the new region turns out to be relaxed, enabling contraction of the neighboring one, etc. One can draw an analogy between this process and the principle of laser operation. In an isometric tetanus, the stimulation, similar to laser pumping, transfers most of the cross-bridges to the same state with high potential energy, which they leave almost simultaneously, performing work and producing heat during avalanche-type relaxation (compare also with the "synchronization effect" in Duke 2000).

## Coulomb interaction as the source of muscle force

The force that causes a muscle to move arises during ATP splitting, which is why the problem on the origin of this force is directly related to the question of why ATP is the energy source for muscular contraction. In other words, one should consider what it is in the structure of the ATP molecule that provides a capacity to generate force and perform mechanical work in the course of its hydrolysis.

The only interaction that completely controls the course of chemical reactions is the electromagnetic interaction. Its pure magnetic part is almost always negligibly small, and consequently the only force that can perform mechanical work during a chemical reaction, i.e., upon rearrangement of atomic electron shells, is the Coulomb force. Therefore, direct conversion of chemical to mechanical energy corresponds to utilization of the mechanical work performed by Coulomb forces when rearranging the electrical charges in the course of the chemical reaction.

Thus, to understand the mechanism of the force generation, one should ascertain which charges shift upon ATP cleavage and in which directions. In this connection the work of Lampinen and Noponen (2005) is worth mentioning. In that work, the electric-dipole theory of storing and transforming ATP chemical energy in the actomyosin molecular motor is presented. The authors of this work suppose that the cross-bridge conformation is its response to the change in the dipole electric field of the ATP molecule, which occurs when the phosphate tail shortens. However, the dipole field represents the major part of the electric field of some system only in the case when the system as a whole does not have electrical charge. Otherwise, the common Coulomb interaction becomes dominant, and it is just this interaction that must be taken into consideration for the ATP molecule, which is a tetraanion in the cell milieu. Upon the ATP $\rightarrow$ ADP + $P_i$ hydrolysis, the molecule decomposes into like-charged fragments $ADP^{2-}$ and $P_i^{2-}$ (Bendall 1969; Bohinski 1983), and the Coulomb repulsion of these pieces can quite naturally be considered as the force propelling the myosin motor.

This principle is quite similar to the one underlying firearm (or combustion engine) performance. In the course of a shot, the cartridge breaks up into the bullet and the cartridge box (cf. ATP cleavage). The force due to the hot powder-gas pressure (cf. Coulomb force) acts upon these pieces in opposite directions, performing mechanical work. The energy transferred to the bullet is higher, the longer the distance it passes inside a trunk (see 4), and when the bullet leaves the trunk, the residual powder-gas energy dissipates (cf. spherule $q_2$ moving in the cylinder in Fig. 1).

To assure ourselves of the rationality of this point of view, we should first of all evaluate the potential energy of a charged molecule and the repulsive force between its parts. Returning to Fig. 1, let us suppose that $q_1 = -n_1 e$ and $q_2 = -n_2 e$ are the charges of ADP and $P_i$, respectively, whose centers are at a distance $R$ away from each other just after breaking the P–O–P chemical bond. $e \approx 1.6 \times 10^{-19}$ C is the electron charge modulus. Using the standard Coulomb law we obtain now instead of (2) and (3)

$$E(x) = \frac{1}{4\pi\varepsilon_0\varepsilon}\frac{n_1 n_2 e^2}{R+x} \simeq 2.3 \times 10^{-28}\frac{n_1 n_2/\varepsilon}{R+x}\ \mathrm{J},$$
$$\mathrm{F(x)} \simeq \frac{\mathrm{E(x)}}{\mathrm{R+x}}\ \mathrm{N}, \qquad (61)$$

where $\varepsilon$ is the permittivity of the milieu. To meet the conditions $E(R) = E_{ATP} \approx 2E(R + D)$ (the second equality

signifies that the maximal efficiency value is about $\eta_0 \approx 1/2$) we have to consider the problem under the assumptions $R \approx D \approx 0.5$ nm, $n_1 n_2/\varepsilon \approx 0.17$. The order of magnitude of the distance between the phosphates in the phosphate tail $R$ is correct. Regarding the charges, their product $n_1 n_2$ is determined by the permittivity, which for protein structures in water falls in the range from units up to many tens, depending on the form, dimensions, and relative positions of the proteins (Finkelstein and Ptitsyn 2002). Therefore, the nominal charge values $n_1 = n_2 = 2$ corresponding to $\varepsilon \approx 25$ are possible just as well as the lower values $n_1 = n_2 = 1/2$ corresponding to $\varepsilon \approx 1.5$. Anyhow, the values of the energy and repulsive force are of the correct order of magnitude $F(R) \approx 8 \times 10^{-20}/5 \times 10^{-10} = 1.6 \times 10^{-10}$ N, compared with (50) and (51). [One can find a thorough analysis of these and related questions in arXiv q-bio.BM/0703014. This is the only text that I was able to publish after 3 years of disputes with referees from different biological and biophysical journals.]

It should be noted that a discussion of the Coulomb contribution to the overall energy balance, and accentuation of the important role of the Coulomb interaction, can be found in biochemical literature (see, e.g., Ross 2006; Nath and Nath 2009). Furthermore, the Coulomb forces are explicitly taken into account in the course of computer modeling of biochemical reactions by the methods of quantum mechanics and molecular dynamics (QM/MD) (see, e.g., Kamerlin et al. 2009). It may seem, therefore, that the simple ideas set forth in this section are so trivial that, in accordance with the suggestion of one of the reviewers, they should be removed from the text.

Indeed, the fact of the existence of Coulomb repulsion between like-charged ions within a single molecule is quite evident, but it is well known that it is obvious things that particularly easily pass out of sight and seldom become the object of careful analysis. The motivation for this analysis may be either the absence of any other way to find an answer to some important issue or the existence of serious arguments in favor of the productivity of such study. We resorted to scrutiny of the Coulomb repulsion between ADP and $P_i$ after having exhausted other possibilities to find a physical cause of myosin head conformation. The structural peculiarities of the S1 fragment and the impossibility of explaining its conformation involving many-body effects forced us to consider the presence of a charge on the ATP molecule. On the other hand, the results obtained here give some grounds to continue research in this direction.

I believe that a clear understanding of the physical grounds for the conformation is particularly important for QM/MD calculations. Indeed, if we want to explore the working stroke by simply comparing the energies of different conformations, the error of its calculation should be substantially less than the value of $E_{ATP}$. However, the free-energy error increases rapidly with increasing number of atoms, which imposes rigorous restrictions on the size of the system under consideration. It is also pointless to try to analyze the wavefunction of a many-body system if you do not know exactly what you want to find. Therefore, already while stating the problem, it is desirable to clearly identify relatively small subsystems, the state and the movement of which must first be analyzed. If the above considerations are correct, it would be most interesting to analyze: (1) the initial ATP charge redistribution between different groups of atoms in the course of hydrolysis, (2) the forces acting on these groups and the dynamics of their movement, and (3) the presence of rigid elements connecting the neck with the active site.

Finally in this section, one should note the article by Ross (2006), in which the idea of producing mechanical work by means of Coulomb repulsion between ATP and $P_i$ was clearly formulated. Some consequences of this idea were briefly discussed in the paper as well, but neither myosin head structure nor consistent patterns of its operation were considered. This might explain why even Ross's assertions which really are true do not seem well established, and the presence of clear logical relationships between different pieces of the work is far from obvious. For these or other reasons, the article unfortunately cannot give a much needed boost towards enhanced research into myosin head conformation.

## Relation to earlier theories

The similarity of the proposed model to the model after Cooke and Bialek (1979) was adequately considered in previous sections. There is another class of models that have much in common with the one considered in this work, namely the phenomenological models of meccano type, the first of which was, apparently, suggested by Hill (1938). In these models, the muscle is considered as a set of elements which are combined in a certain order and possess specific properties: force-generating active element (AE), damping element (PDE) in parallel to AE, elastic element (SEE) in series to AE, etc.; for discussion of some advanced versions of this model and review, see Gunther and Schmitt (2010). In theories of this type, a muscle can be modeled by a single unit consisting of AE, SEE, and PDE (Hill 1938), as well as by a set of such units which simulate individual sarcomeres and interact with each other (Denoth et al. 2002; Telley and Denoth 2007). In a sense, the above-presented theory can also be seen as a phenomenological model of the minimal-in-size unit of this

type. Indeed, many results delivered in "The model" to "Boltzmann equation and collective effects" can be obtained under a simple assumption that cross-bridges operate with random phase displacements and for each of them (1) fluctuations specify the average lifetime $\tau_w$, (2) AE works by generating a constant force $F$ only in the range of displacements $0 \leq s \leq S$, (3) the recovery time between working strokes makes up $t_r$, and (4) $F_{PDE} = -\gamma v$. However, presenting opportunities for a formal description of experiments such an approach in itself does not reveal any new perspectives for advances of understanding of the principles of the work of myosin motors. It is consideration of a particular internal structure of myosin head that allows somehow grounded suggestions to be made on the nature of the force that produces its conformation (see "Introduction" and "Coulomb interaction as the source of muscle force").

The above-mentioned theories, I think, exhaust the list of models in some respects similar to the model proposed here. Therefore, in this section we now focus on the fundamental differences between this model and the theories most widely used today.

In the response of a muscle to stepwise shortening (Huxley and Simmons 1971; Ford et al. 1977; Piazzesi and Lombardi 1995) one can clearly trace three main types of processes taking place inside it. On the one hand, upon slow changes of the force, the main part is taken by the cross-bridge attachment and detachment processes with a characteristic time of tens to hundreds of milliseconds. To describe these, Huxley's (1957) model is quite appropriate, and in one form or another, this model should probably be a part of any other theory. On the other hand, upon very sharp (fractions of a millisecond) changes of length, the response is as quick, which testifies to the existence of SEE. However, having rapidly arisen, these changes in the force generated by the muscle then start to relax with a characteristic time on the order of a millisecond. Such a duration cannot feature cross-bridge attachment and detachment processes, which means that the internal structure of myosin head (for example, PDE) has to be involved. In this case, quick relaxation processes are due to some kind of retardation of changes in the internal state, while Huxley's (1957) mechanism becomes activated later.

Seemingly, the internal degrees of freedom of a myosin head were explicitly introduced into a model for the first time in the classical work by Huxley and Simmons (1971). The only information about the myosin head available at that time was that it is connected with the thick filament by an elastic neck. Therefore, a particular form of the other elements of internal structure introduced in the model must necessarily be defined based on the concepts of the physical processes that give rise to the myosin head conformation. One can think that quite an important part in the formation of these conceptions was taken by an obvious analogy with the abrupt first-order transitions that arise in other proteins upon, say, temperature changes (Finkelstein and Ptitsyn 2002). Besides, these conceptions were formed taking account of the then common and up to now firm principle, which had not yet been clearly outlined in Huxley and Simmons (1971). I present here its formulation from the later work by Duke (2000, Sect. 2a): "Since the transduction of chemical energy to mechanical work by motor proteins occurs via a series of biochemical reactions, the most natural theoretical description is a kinetic one."

One way or another, in 1971 Huxley and Simmons suggested a new model based on the assumption that the cross-bridge can occur only in a small number of discrete states. Such a model made it possible, using a standard procedure of calculating concentrations of reaction products, to determine instead populations for the cross-bridge states. As a result, the rate of fast relaxation is controlled now by the rate constants of reactions which, as in Huxley's (1957) model, should be somehow specified.

Certainly, the simplest and formally faultless approach would be to duplicate Huxley's (1957) model, sorting out ad hoc necessary forms of dependences on coordinates of the energies and rate constants for these new states and transitions between them. However, this would result in an unacceptable increase of the number of fitting functions; for example, despite the additional limitations which were imposed by the authors of the well-known work (Piazzesi and Lombardi 1995), graphs of these dependences take a whole page.

A second possibility, proposed for the first time by Huxley and Simmons (1971), consists not simply in that different stages of the chemical reaction of ATP hydrolysis are placed in correspondence with certain spatial configurations of the myosin head; rather, now the energies of these configurations are considered equal to the free energies of the corresponding reagents and intermediate and/or finite products of the reaction. From the viewpoint of classical thermodynamics this is quite unexpected, since the free energy turns out to be a function of coordinates of individual molecules (myosin heads), whose occasional migrations from state to state (thermal fluctuations) now must take place with a frequency that depends on the characteristic time of fast relaxation of a muscle. However, nowadays (Walcott and Sun 2009), microscopic models of this type are widely used for treatment of experimental data.

Without getting into a discussion on the rigor of the deduction of the results in the framework of these models, I would again underline one principally important circumstance. These microscopic models are based on the assumption that possible spatial configurations of the myosin head are discrete for no other reason than that

40 years ago nothing was known about the real internal structure of myosin head. Until today, this assumption has not been questioned, despite the fact that no real mechanisms for the transformation of a cross-bridge into an analog multiposition switch with a small number of stable states have so far been proposed. All the problems connected with the determination of rate constants arise in these models just because of the postulate on the existence of discrete states. Moreover, the thus-based models, being very useful for the description of experimental results, do not allow any advances into understanding of the working principle of the myosin motor. In a system with discrete spatial configurations, it is by definition impossible to introduce the very concept of force. Force is the derivative of the energy (see 2), and it is impossible to differentiate a function defined only at a few points.

In other words: under this approach it is impossible to describe the process of continuous transition from one cross-bridge state to another. At the same time, the stretching of the SEE and therefore the emergence of the force between actin and myosin are just a consequence of this process. If we want to understand the operation principle of myosin motor, we need to determine the nature of the force which propels the *continuous* cross-bridge conformation.

The model presented in the above sections is based on direct experimental data on the structure of myosin head. It deals with nondiscrete modification (changes without jumps) of the internal cross-bridge state, and, in this regard, is diametrical to the already existing theories. It is quite curious to note that, at the same time, this model is to some extent a logical accomplishment of the ideas presented by Huxley and Simmons. The number of different states now tends to infinity, while the number of parameters determining the rate of transitions between them decreases to one.

This parameter is a hindering force whose emergence in the model is connected with the main advantage of the latter: The model presents an opportunity to explicitly introduce into consideration forces acting upon the lever arm and compelling it to rotate. Now, in the state of rest, the lever arm simply takes a position in which the pressure force $F$ exerted by inorganic phosphate compensates (accounting for the lever rule) the external tension $P$. When the external force deviates from this equilibrium value, the lever arm starts to rotate with a velocity determined by the equation $P = {}^{F}/_{\lambda} - \gamma v$. Since the maximal rate of changes of its coordinate is on the order $v_{\max}$, upon a sharp change of the muscle length by $\delta l$ per half-sarcomere, the quick equilibrium state is reestablished for the time period on the order of $\tau \approx \delta l / v_{\max}$. At $v_{\max} \approx 1.5$ μm s$^{-1}$ and $\tau \approx 1$ ms we obtain $\delta l \approx 1.5$ nm, which is in reasonable agreement with experiment (Huxley and Simmons 1971; Ford et al. 1977; Piazzesi and Lombardi 1995).

However, not wishing to preempt results which I hope to publish in the near future, I would like to note two circumstances. First, herein I have not taken into account the existence of the SEE for a very simple reason: if the SEE is absent, the main ideas of the model become essentially simplified. It will be rather easy to introduce SEE into consideration in a next step. Second, to satisfactorily describe the bulk of quick muscle response to stepwise changes in its length or force acting on it, in the framework of the "nondiscrete" model, it is sufficient to only suppose that inside the myosin head there acts a hindering force. It can be considered as the only parameter describing a black box that contains all elements of the internal structure of myosin head which are not covered by the model.

Last but not least, only in the framework of conceptions about the indiscrete conformation does there appear the possibility to discuss the nature of the force propelling muscle.

To conclude this section, I note that the suggested and already existing models, in particular the Duke (1999, 2000) model, along with drastic differences, reveal some common features. Thus, the main parameter of the "nondiscrete" model, i.e., $t_r$, is inversely proportional to the parameter $f$, and the fluctuation mechanisms determining the dependences $g(x)$ and $\tau_w(x)$ on coordinates are in essence indistinguishable. Moreover, the ways of description of the attachment–detachment processes in this work and in Huxley's (1957) model also coincide.

## Discussion

As one can see from Figs. 3, 4, and 6 and equations (20), (21), and (47, 48), the proposed model can semiquantitatively explain and even in part predict a rather wide range of relations between the physical characteristics of contracting muscle. In addition, all parameters (except $F_h$) used in the model [see (27), (28)] have clear physical meaning, and most of them have already been determined experimentally. Among them, the most important role is played by the single parameter $t_r$, whose value determines mainly the shape of the curves in Figs. 2–4. Apparently, more detailed features of contracting muscle behavior can be reproduced by picking out (or experimental determination of) two functions: $E(x)$ and $\tau_w(x)$, which in the model are merely "adjustable functions." Specifying the form of these functions, the time dependence of the contraction velocity, and the initial state of the ensemble of myosin heads, one can determine its further evolution from the system of kinetic equations (54–57).

It should again be noted that there are two main reasons why the form of the whole series of curves is determined by a few numerical parameters:

- The working stroke interruption at the $P_i$ release moment is initially built into the model;
- The existence of a special type of hindering force was assumed.

The central point here is the former hypothesis that the working stroke should be interrupted by the back door opening just at the very instant when the displacement of the actomyosin complex reaches the maximal pace value $S$. Therefore, the results obtained provide indirect evidence of the correctness of this assumption, and it seems appropriate to raise the question of its experimental verification.

The second most important assumption is the availability of an approximately linear in $v$ "friction loss." In the absence of a hindering force, the stimulated back door opening would lead to the following situation: (1) the force hyperbolically diminishes but does not vanish as the contraction velocity rises, and (2) the efficiency does not depend on the velocity. Consequently, to attain the appearance of $v_{max}$, the linear-in-$v$ decline of $\eta(v)$ in (22), etc., we have to take into account the hindering force. Since usual viscous friction is negligibly small at such velocities, we should emphasize the problem of the physical nature of two forces, namely the force applied to the back door and propelling muscle, and the hindering force counteracting it.

Since the suggested model is to a large degree phenomenological, the question on the nature of the above forces oversteps its framework. Nevertheless, although we know almost nothing about the latter of these forces (see, however, Cooke and Bialek 1979), based on very general considerations (see "Introduction" and "Coulomb interaction as the source of muscle force"), we can make some suggestions about the former of them. It is known that the ATP molecule is a tetraanion in the cell milieu and upon $ATP \rightarrow ADP + P_i$ hydrolysis decomposes into like-charged fragments $ADP^{2-}$ and $P_i^{2-}$. So it is natural to assume that the force which pushes $\gamma$-phosphate and eventually moves switch-2 is the force of Coulomb repulsion between $ADP^{2-}$ and $P_i^{2-}$. Although the simplest evaluations do not contradict this assumption, only straightforward numerical calculations using the QM/MD methods could clarify this problem. From the experimental point of view it would be very interesting to find a means to change the charge of the ATP molecule in the cell milieu while conserving muscle efficacy, and determine its force in this case.

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