Published in final edited form as:
Adv Exp Med Biol. 2010 ; 682: 57–75. doi:10.1007/978-1-4419-6366-6_4.

A Strain-Dependency of Myosin Off-Rate Must Be Sensitive to Frequency to Predict the B-Process of Sinusoidal Analysis

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Abstract

Muscle force arises as the result of many myosin molecules, each producing a force discrete in magnitude and in time duration. In previous work we have developed a computer model and a mathematical model of many myosin molecules acting as an ensemble and demonstrated that the time duration over which myosin produces force at the molecular level (referred to here as "time-on") gives rise to specific visco-elastic properties at the whole muscle level. That model of the mechanical consequences of myosin-actin interaction predicted well the C-process of small length perturbation analysis and demonstrated that the characteristic frequency $2\pi c$ provided a measure of the myosin off-rate, which is equal to the reciprocal of the mean time-on. In this study, we develop a mathematical hypothesis that a strain-dependence of the myosin off-rate at the single molecule level can result in a negative viscous modulus like that observed at low frequencies, i.e., the B-process. We demonstrate here that a simple monotonic strain-dependency of the myosin off-rate cannot account for the observed B-process. However, a frequency-dependent strain-dependency, as may occur when visco-elastic properties of the myosin head are introduced, can explain the observed negative viscous modulus. These findings suggest that visco-elastic properties of myosin constitute the specific molecular mechanisms that underlie the frequency-dependent performance of many oscillatory muscles such as insect flight muscle and mammalian cardiac muscle.

Keywords

Oscillatory muscle; Oscillatory work; Insect fight muscle; Cardiac muscle; Sarcomere

1 Introduction

Pick up a pencil, and the muscles in your arm and fingers casually produce the forces necessary to achieve the required movement and stability. We generally experience muscle force as being easily controlled over a continuous range of forces. At the molecular level, however, muscle force is discrete in both magnitude and time duration (Finer et al. 1994; Spudich 1994; Tyska and Warshaw 2002). Myosin is the specific molecule that produces this discrete force as it goes through the following biochemical-mechanical cycle: (a) myosin binds to an actin filament, (b) myosin undergoes a discrete physical deformation, called the power stroke, thus generating a unitary force (F_{uni}) on the actin filament, (c) F_{uni} is maintained until inorganic phosphate (P_i) and adenosine-diphosphate (ADP) are released and adenosine-5′-triphosphate (ATP) is bound to the myosin thus initiating the detachment of myosin from actin, and (d) while detached from actin, myosin hydrolyzes the ATP thus

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providing the energy to recover the pre-force conformation of the myosin molecule (Geeves and Holmes 1999; Spudich 1994; Steffen and Sleep 2004; Stein et al. 1981). Myosin is now ready for another cycle. Many actin filaments exist in parallel in a muscle, and many myosin molecules act together, although not synchronously, on any one actin filament. The result is a seemingly continuous force generated by a muscle due to the many discrete forces summed over space and time.

Several properties of the myosin force-producing cycle have been studied at the level of the single molecule. Interestingly, the magnitude of F_{uni} for striated-muscle myosin does not vary much from myosin to myosin (Tyska and Warshaw 2002). On the other hand, the time duration over which myosin is bound to actin, referred to t_{on}, is inversely dependent upon ATP concentration and also varies greatly among different isoforms, species and patterns of post-translational modifications (Tyska and Warshaw 2002). The myosin t_{on} appears to bear the most significant influence on muscle performance (Spudich 1994; Tyska and Warshaw 2002). As an example of how t_{on} affects force production, consider that the force average over time generated by a single myosin molecule (F_{ave}) must be proportional to F_{uni} and the ratio $t_{on}/(t_{on}+t_{off})$, i.e., $F_{ave}=F_{uni}\times t_{on}/(t_{on}+t_{off})$, where t_{off} is the time duration when myosin is detached from actin (Fig. 1). The ratio $t_{on}/(t_{on}+t_{off})$ describes the fraction of time over which myosin is producing force and is often referred as the duty ratio. Changes in the duty ratio will result in changes in F_{ave}. For example, when the sum of t_{on} and t_{off} (t_{cycle}) does not change, a shorter t_{on} reduces F_{ave} and a longer t_{on} enhances F_{ave}, as illustrated in Fig. 1. If we now imagine a muscle containing N independent myosin molecules, then the total force (F_{total}) can be written as $F_{total}= \mathbf{N} \times F_{uni}\times t_{on}/(t_{on}+t_{off})$. Muscle performance furthermore depends upon the velocity of muscle shortening, which is inversely proportional to t_{on} (Tyska and Warshaw 2002). Muscles found throughout the animal kingdom tend to adapt myosin t_{on}, t_{off} and **N** to suit the force and velocity requirements of the muscle performance (Spudich 1994; Tyska and Warshaw 2002).

Measuring myosin t_{on} has emerged as an important component of any investigation into the molecular mechanisms that underlie muscle performance and dysfunction. Experiments in the laser trap probing the molecular performance of cardiac myosin heavy chain (MHC), for example, have shown that myosin t_{on} is significantly shorter in the α-MHC isoform compared to β-MHC (Palmiter et al. 1999). This result is important because, when α-MHC is inadequately expressed or absent as occurs in human heart failure, one consequence is an inability for the heart to perform effectively at high frequencies (Herron and McDonald 2002; Suzuki et al. 2009). As another example, some point mutations in MHC lead to shorter t_{on} compared to that of non-mutant myosin leading to greater velocities of shortening and ultimately to a hypertrophic cardiomyopathy (Debold et al. 2007; Palmiter et al. 2000; Tyska et al. 2000; Yamashita et al. 2000). The mal-adaptation of myosin t_{on} to the required performance of the heart appears to be a significant detrimental factor in some cardiomyopathies and heart failure.

In this paper, we describe the technique of small length perturbation analysis, which provides a measure of myosin t_{on} in muscle strips dissected to <150 μm diameter cross-section. This method permits the preservation of the myofilament lattice structure found in vivo, thus providing an appropriate structural context in which t_{on} is measured, and obviates the isolation of single myosin molecules as required for the laser trap. We will also explore here how data that arise during small length perturbation analysis may be interpreted beyond the measurement of myosin t_{on}.

2 Small Sinusoidal Length Perturbation Analysis

Visco-elastic properties of muscle can be measured at the macroscopic level using small amplitude sinusoidal length perturbation analysis (Abbott and Steiger 1977; Cheung and Gray 1983; Davis and Rodgers 1995; Kawai and Brandt 1980; Thomas and Thornhill 1995; Thorson and White 1969; White and Donaldson 1975; Zhao and Kawai 1993). Figure 2a depicts a sinusoidal length perturbation, which ensures that the force response is linear, and two example sinusoidal force responses, which lead or lag the length perturbation. Each force response can be constructed as one sinusoidal component that is in-phase with the perturbation and one cosine component that is 90° out-of-phase with the perturbation. The elastic and viscous moduli refer to the amplitudes of the in-phase and 90° out-of-phase responses, respectively, relative to the amplitude of the strain. Those force responses which lead the length perturbation result in a positive viscous modulus, while those that lag result in a negative viscous modulus (Fig. 2b). Taken together as respectively the real and imaginary parts of a complex number, the elastic and viscous moduli make up the complex modulus, $\tilde{Y}(\omega)$, which describes in the frequency domain the linear response of the muscle force to a length perturbation.

In practice the relative frequency characteristics of the elastic and viscous moduli, plotted as a Nyquist diagram in Fig. 2c, demonstrate a complex, yet systematically looped relationship which arises only when the muscle is activated and therefore must reflect temporal characteristics of the myosin force-producing cycle (Campbell et al. 2004;Kawai and Brandt 1980;Machin 1964;Maughan et al. 1998;Palmer et al. 2007;Pringle 1978). A mathematical expression, (1), describing the complex modulus has been used effectively to fit these data.

$$\tilde{Y}(\omega)=A(i\omega)^k - B\left(\frac{i\omega}{2\pi b+i\omega}\right)+C\left(\frac{i\omega}{2\pi c+i\omega}\right) \quad (1)$$

where the parameters *A*, *B* and *C* represent the magnitudes of three terms characterized by the frequency parameters *k*, 2π*b* and 2π*c*. The three terms of (1) correspond to three observed characteristics, often referred to as the A-, B- and C-processes, illustrated in Fig. 2c and d. When combined, these processes give rise to (a) the angular orientation of the data in the Nyquist diagram (A-process), (b) the negative viscous modulus observed at low frequencies (B-process) and (c) the semi-circular relationship between the viscous and elastic moduli observed at higher frequencies (C-process). It's important to note that the frequency characteristic 2π*b* is always observed to be lower than 2π*c*. The expression in (1), however, has evolved through empirical means (Kawai and Brandt 1980;Maughan et al. 1998), and it has been long recognized that an unambiguous physiological interpretation of the parameters would be useful.

Several mathematical models have been developed attempting to describe the frequency characteristics of the complex modulus and to provide physiological meaning to the parameters in (1) (Abbott and Steiger 1977;Campbell et al. 2004;Cheung and Gray 1983;Davis and Rodgers 1995;Kawai and Brandt 1980;Thomas and Thornhill 1995;Thorson and White 1969;White and Donaldson 1975). In one previously published study from our laboratory (Palmer et al. 2007), an analytical expression was derived and a corresponding computer model was developed to describe the mechanical consequences at the macroscopic level of many myosin-actin interactions occurring at the molecular level. Each myosin-actin interaction was characterized by (a) intermittent periods of t_{on} and t_{off} governed by independent stochastic processes described by single exponential probability functions, and (b) an elastic element engaged only during t_{on}. As illustrated in Fig. 3a, a frictional force resisting the length perturbation arises during t_{on} as the length perturbation elongates and

compresses the elastic element during t_{on}. This model predicted well the C-process of sinusoidal analysis (Fig. 3b) and provided a mathematical basis for estimating the mean myosin t_{on} as $(2\pi c)^{-1}$. We have since found, for example, that our estimates of myosin t_{on} for MHC isoforms and for some myosin point mutations are in qualitative agreement with those values reported using the laser trap to measure t_{on} (Palmer et al. 2004a,2007;Suzuki et al. 2009). We have furthermore been able to use this technique to highlight how myosin t_{on} adapts to suit the function of oscillatory muscles. For example, the respective frequencies of insect flight muscle, mouse heart muscle and human heart muscle are on the order of ~100 Hz, ~10 Hz and ~1 Hz, and the corresponding myosin t_{on} for each is estimated on the order of ~0.1 ms, ~1 ms and ~10 ms.

The friction model for the C-process, however, does not explain the other frequency characteristics of the elastic and viscous moduli that appear to bear physiological importance (Maughan et al. 1998; Palmer et al. 2007; Pringle 1978; Steiger 1977). In particular, the viscous modulus of activated muscle is invariably observed to be negative in value at the specific frequency of muscle operation, e.g., in the range ~1–3 Hz for human cardiac heart as shown in Fig. 2c (Fukagawa et al. 2005). The negative viscous modulus indicates that the myosin ensemble is not absorbing mechanical work as occurs with friction, but rather producing mechanical work at that specific frequency (Maughan et al. 1998). Clearly, a more complete model of the myosin-actin system must account for this phenomenon.

Others have attempted to explain the negative viscous modulus as the result of a strain dependency on t_{on} and/or t_{off} (Abbott and Steiger 1977; Campbell et al. 2004; Cheung and Gray 1983; Thomas and Thornhill 1995; Thorson and White 1969; White and Donaldson 1975; White and Thorson 1972), however, without success in providing a mathematical representation of the negative viscous modulus. Kawai and Brandt (Kawai and Brandt 1980) offered a mathematical description of the negative viscous modulus based on a strain dependency of transition rates between biochemical states. The basis of their mathematical result was argued heuristically but not assigned mathematically. Nevertheless, a strain-dependency on t_{on} has been demonstrated at the single molecule level and should be included somehow in any model of the complex modulus (Kad et al. 2007). Below, we explore how a strain-dependence of myosin t_{on} at the single molecule level may result in the negative viscous modulus observed at the operating frequency of oscillatory muscles.

3 Analytical Results

In our previous study we used probability theory to arrive at analytical solutions describing the isometric force and frequency characteristics of the frictional force that arises from many myosin-actin interactions at the molecular level (Palmer et al. 2007). In the present study we opt not to use probability theory, but ordinary differential equations (ODEs) to explore the mechanical consequences of a strain-dependency on myosin t_{on} and a recorded muscle force. In using ODEs the mean t_{on} will be represented by its reciprocal, the myosin off-rate denoted as g by Huxley (Huxley 1957; Huxley and Simmons 1971).

We will first use ODEs to recapitulate the findings of our previous study as an illustration and validation of the use of ODEs for our main purpose here. We will then demonstrate that a strain-dependency on the myosin off-rate cannot alone result in a negative viscous modulus observed during small length perturbation analysis of oscillatory muscles. A frequency dependence on the strain-dependency on myosin off-rate does result in a negative viscous modulus like that observed as the B-process. Our analytical description of the B-process, however, relies upon a positive relationship between strain and myosin off-rate, which is contrary to observations from the laser trap (Kad et al. 2007). Nevertheless, a positive relationship between strain and myosin off-rate may arise under conditions of

relatively high P_i (Baker et al. 2002; Hibberd et al. 1985), and we offer the demonstration below as the basis of a hypothesis as to the molecular mechanisms that underlie the observed negative viscous modulus in skinned muscle preparations.

3.1 ODE Solution for Isometric Force and C-Process

Huxley proposed a two-state model of myosin attaching and detaching to actin with an apparent on-rate of attachment referred to as *f* and an apparent off-rate of detachment referred to as *g* (Huxley 1957; Huxley and Simmons 1971). The ODE used to describe the rate of change of the fraction of myosin attached to actin at any time, n(*t*), is the following:

$$\frac{d\mathrm{n}(t)}{dt} = f[1 - \mathrm{n}(t)] - g\mathrm{n}(t) \tag{2}$$

where the term [1 − n(*t*)] denotes the fraction of myosin detached from actin at any time. For the case when n(*t*) does not change with time, the left hand side of (2) is zero and the steady-state solution results: n(*t*) = *f*/(*f* + *g*). If each myosin attachment is now assigned a unitary force F_{uni}, then the total force of an ensemble of **N** myosin molecules is $F_{total} = \mathbf{N} \times F_{uni} \times f/(f+g)$. This particular result from (2) is reminiscent of the total force $F_{total} = \mathbf{N} \times F_{uni} \times t_{on}/(t_{on} + t_{off})$ stated in the Introduction. It should be noted that the rate constants, *f* and *g*, are first-order rate constants and are indeed equivalent to the reciprocals of mean t_{on} and mean t_{off}, respectively, when t_{on} and t_{off} are governed by independent stochastic processes described by single exponential probability functions. Under those conditions the ratios $f/(f + g)$ and $t_{on}/(t_{on} + t_{off})$ are equivalent and describe the duty ratio of the myosin crossbridge. Furthermore, the model parameter $2\pi c$ that emerges by fitting (1) to recorded data directly estimates the myosin off-rate *g* and therefore the mean t_{on} (Palmer et al. 2007).

Among the important contributions of Huxley's model was his use of the x-dimension to describe the length-dependence of *f* and *g*, i.e., $f = f(x)$ and $g = g(x)$. For our purposes, we will not adopt the specific $f(x)$ and $g(x)$ functions offered by Huxley. As illustrated in Fig. 4, we will assume an $f(x)$, which is reminiscent of that proposed by Hill (1974) and reflects the probability of myosin attachment to actin as being distributed along the x-dimension and symmetrical about zero displacement. For now, we will also assume $g(x) = G_0$, which is a constant and independent of the x-dimension. The function n(*t*) now also becomes a function of the x-dimension, n(*x*, *t*), which approaches zero value at $x = -\infty$ and $+\infty$.

The left hand side of (2) must now be revised according to the chain rule as follows:

$$\frac{d\mathrm{n}(x,t)}{dt} = \frac{\partial \mathrm{n}(x,t)}{\partial t} + v\frac{\partial \mathrm{n}(x,t)}{\partial x} \tag{3}$$

where *v* is the velocity, *dx*/*dt*, of any displacement of the attached myosin relative to the thick filament, as would occur with an externally driven length perturbation. Equation (2) can now be written as follows:

$$\frac{\partial \mathrm{n}(x,t)}{\partial t} + v\frac{\partial \mathrm{n}(x,t)}{\partial x} = f(x)\left[1 - \int_{-\infty}^{\infty} \mathrm{n}(x,t)dx\right] - G_0\mathrm{n}(x,t) \tag{4}$$

where the term $\left[1-\int_{-\infty}^{\infty} \mathrm{n}(x,t)dx\right]$ represents the fraction of myosin detached from actin at any time. It may be worthwhile to note that the definite integral over the x-axis leads to a function of time only.

Instead of solving (4) for the function n(x, t), which has two dimensions, we can choose instead to solve for the moments of the distribution n(x, t) along the x-axis, thus effectively simplifying to a one-dimension problem. Specifically, we can integrate (4) over the x-dimension, $\int_{-\infty}^{\infty} x^i(\text{Eq.4})dx$ which results in a series of one-dimensional equations. Each of these resulting equations represents an important characteristic of the distribution n(*x*, *t*) along the x-axis. Specifically, when i = 0 the total fraction of myosin attached to actin is described; when i = 1 the mean length displacement of myosin attached to actin is described; when i = 2 the variance of the length displacement is described, etc.

The proposed integration transforms (4) into the following:

$$\int_{-\infty}^{\infty} x^i \frac{\partial \mathrm{n}(x,t)}{\partial t}dx + v\int_{-\infty}^{\infty} x^i \frac{\partial \mathrm{n}(x,t)}{\partial x}dx = \left[1-\int_{-\infty}^{\infty} \mathrm{n}(x,t)dx\right]\int_{-\infty}^{\infty} x^i f(x)dx - G_0\int_{-\infty}^{\infty} x^i \mathrm{n}(x,t)dx \tag{5}$$

Let's focus on evaluating the integral in the second term of (5) and perform its integration by parts.

$$\int_{-\infty}^{\infty} x^i \frac{\partial \mathrm{n}(x,t)}{\partial t}dx = \left(x^i \mathrm{n}(x,t)\right)\Big|_{-\infty}^{\infty} - \int_{-\infty}^{\infty} i x^{i-1} n(x,t)dx \tag{6}$$

Because the function n(*x*, *t*) goes to zero at $x = -\infty$ and $x = +\infty$, only the last term of (6) is necessary to represent the second term of (5), which can now be written as follows:

$$\int_{-\infty}^{\infty} x^i \frac{\partial \mathrm{n}(x,t)}{\partial t}dx - v\int_{-\infty}^{\infty} i x^{i-1} \mathrm{n}(x,t)dx = \int_{-\infty}^{\infty} x^i f(x)dx\left[1-\int_{-\infty}^{\infty} \mathrm{n}(x,t)dx\right] - G_0\int_{-\infty}^{\infty} x^i \mathrm{n}(x,t)dx \tag{7}$$

We can also make the following definitions for $\mathrm{N_i}(t)$ and F_i:

$$N_i(t) = \int_{-\infty}^{\infty} x^i \mathrm{n}(x,t)dx \tag{8a}$$

and

$$F_i = \int_{-\infty}^{\infty} x^i f(x)dx \tag{8b}$$

Equation (7) then becomes the following:

$$\frac{d\mathrm{N}_i(t)}{dt} - vi\mathrm{N}_{i-1}(t) = F_i[1 - \mathrm{N}_0(t)] - G_0\mathrm{N}_i(t) \tag{9}$$

The above equation, (9), represents a series of equations for all values of *i*. We will use the cases $i = 0$ and $i = 1$ to describe the force recorded from an ensemble of myosin. Specifically, force due to the myosin force-producing cycle is $\mathbf{N} \times \mathrm{F}_{\mathrm{uni}} \times \mathrm{N}_0(t)$, and frictional force that arises due to strain on the myosin with a stiffness k_{stiff} is $\mathbf{N} \times \mathrm{k}_{\mathrm{stiff}} \times \mathrm{N}_1(t)$ (Palmer et al. 2007).

Referring to (8a), the function $\mathrm{N}_0(t)$ represents the fraction of myosin molecules bound to actin at any time. Equation (9) then reduces to the following:

$$\frac{d\mathrm{N}_0(t)}{dt} = F_0[1 - \mathrm{N}_0(t)] - G_0\mathrm{N}_0(t) \tag{10}$$

which has the same form and steady-state solution as (2), namely:

$$\mathrm{N}_0(t) = F_0/(F_0+G_0) \tag{11}$$

If each myosin of **N** molecules bears a unitary force $\mathrm{F}_{\mathrm{uni}}$, then $\mathrm{F}_{\mathrm{total}} = \mathbf{N} \times \mathrm{F}_{\mathrm{uni}} \times F_0/(F_0 + G_0)$, which is the same result found from (2).

Equations (10) and (11) do not offer anything new compared to (2) and its solution, but our arriving at (10) does offer an important check as to the validity of (9). Equation (10) also provides the interpretation of *f* in the two-state model of (2) as the integration of any spatially-dependent $f(x)$ over the entire x-dimension. Accordingly, the parameter *g* of (2) is furthermore interpreted as a constant value of the off-rate without any dependence on the x-dimension.

Referring again to (8a), the function $\mathrm{N}_1(t)$, i.e., $i = 1$, represents the mean length displacement of the ensemble of myosin molecules bound to actin at any time. For $i = 1$ (9) becomes the following:

$$\frac{d\mathrm{N}_1(t)}{dt} - v\mathrm{N}_0(t) = F_1[1 - \mathrm{N}_0(t)] - G_0\mathrm{N}_1(t) \tag{12}$$

It's important to note the F_1 will have a value of zero when $f(x)$ is an even function symmetric about $x = 0$, which we have assumed here (Fig. 4a). We will also assume steady-state conditions and use the relationship $\mathrm{N}_0(t) = F_0/(F_0 + G_0)$ to get the following equation:

$$\frac{d\mathrm{N}_1(t)}{dt} - v\left(\frac{F_0}{F_0+G_0}\right) = -G_0\mathrm{N}_1(t) \tag{13}$$

We can now utilize the Fourier transform and recognize that *v* represents the time derivative of the externally driven length perturbation, which we call $L(t)$. Specifically, we will define the following:

$$\tilde{N}_1(\omega)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} N_1(t)e^{-i\omega t}dt \tag{14a}$$

$$\tilde{L}(\omega)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} L(t)e^{-i\omega t}dt \tag{14b}$$

Equation (13) then becomes

$$i\omega\tilde{N}_1(\omega) - i\omega\,\tilde{L}(\omega)\left(\frac{F_0}{F_0+G_0}\right) = -G_0\tilde{N}_1(\omega) \tag{15}$$

The solution is

$$\tilde{N}_1(\omega)=\left(\frac{F_0}{F_0+G_0}\right)\frac{i\omega}{G_0+i\omega}\,\tilde{L}(\omega) \tag{16}$$

Equation (16) represents in frequency space the mean length displacement, i.e., the mean strain, of actin-attached myosin when an external perturbation has been applied to one filament relative to the other. If we assign a stiffness coefficient, k_{stiff}, to each myosin, then we would have a description of the frictional force that arises when an external length perturbation is applied while myosin intermittently and repeatedly attaches to actin. Equation (16) has the same form as the C-process of (1) and suggests, as previously (Palmer et al. 2007), that the displacement of the myosin due to the thin and thick filaments sliding past each other results in that portion of the complex modulus represented as the C-process. It should be noted that this interpretation of the C-process does not require a strain dependence on the myosin off-rate.

3.2 Simple Strain Dependency on Myosin Off-Rate

We will demonstrate in this section that a simple monotonic strain dependency on $g(x)$ cannot account for the B-process observed in small amplitude sinusoidal length perturbation analysis. As mentioned above, the use of small amplitude length perturbation analysis is restricted to systems assumed to be linear. If we were to consider (9) for the case $i = 2$ or greater, the second term of the resulting equation would be non-linear. Furthermore, a result for higher order $N_i(t)$ is not related to force. For example, $N_2(t)$ describes the amount of potential energy stored in the elastic elements of the acto-myosin crossbridge. We will therefore not consider the cases $i = 2$ or greater, but we will retain focus on the cases $i = 0$ and $i = 1$. We can still ask whether a monotonic strain dependency on the myosin off-rate can result in the negative viscous modulus at low frequencies as observed during sinusoidal analysis. The measure of a negative viscous modulus can occur only if the recorded force measurement lags in time behind the length perturbation as illustrated in Fig. 2a and b. Our fundamental understanding of friction suggests that this is not possible as a direct result of friction, i.e., friction gives rise to a force proportional to the velocity of a length perturbation and therefore leads the perturbation as suggested by the positive sign in the description of $\tilde{N}_1(\omega)$ in (16).

Considering that others have demonstrated a monotonic strain-dependency on g (Kad et al. 2007), it would be reasonable to consider how a strain dependency on g may affect the number of myosin attached, $N_0(t)$, and the resulting recorded force. As illustrated in Fig. 5, an enhanced off-rate during the lengthening phase of a perturbation would shorten t_{on} and depress F_{ave}. A reduced off-rate during the shortening phase would prolong t_{on} and enhance F_{ave}. Conceivably, a monotonic strain-dependency on g could then result in a force signal lagging the perturbation like that shown in Fig. 5. As the result for $N_1(t)$ describes the mean strain of actin-attached myosin, we will consider below whether $N_1(t)$ given in (16) may be used to affect g and in turn $N_0(t)$ to produce a force lagging the length perturbation and therefore a negative viscous modulus.

If g were given a monotonic strain dependence like that shown in Fig. 3b, i.e., $g(x) = G_0 + G_1x$ in (4), then (10) would have the following form:

$$\frac{dN_0(t)}{dt} = F_0[1 - N_0(t)] - G_0N_0(t) - G_1N_1(t) \tag{17}$$

If we now use the solution of (16) to represent the last term of (17) and ignore any transient response from equilibrium, we have the following result for the steady-state response of the fraction of actin-bound myosin due to the length perturbation L(t).

$$\tilde{N}_0(\omega) = -G_1\left(\frac{F_0}{F_0+G_0}\right)\left(\frac{i\omega}{G_0+i\omega}\right)\left(\frac{1}{F_0+G_0+i\omega}\right)\tilde{L}(\omega) \tag{18}$$

Equation (18) describes in frequency space the change in the number of attached myosin due to an externally applied length perturbation. Again, if each myosin of **N** molecules bears an F_{uni}, we could use (18) to describe the total force due to the myosin force-producing cycle.

The form and sign of (18) are reminiscent of the B-process term of (1). However, the two rates embedded in the denominator terms of (18), namely G_0 and $F_0 + G_0$, are respectively equal to or greater than the myosin off-rate, g, which would be detected as $2\pi c$. Recall that the rate constant associated with the B-process, $2\pi b$, is consistently observed to be lower than $2\pi c$. We conclude that the steady-state response described in (18), and therefore a simple monotonic strain dependency on $g(x)$ in a two state model, cannot account for the B-process observed in small amplitude sinusoidal length perturbation analysis.

3.3 A Frequency Dependency on the Strain-Dependence of Myosin Off-Rate

We will demonstrate in this section that a frequency dependency on the effects of strain on myosin off-rate can result in the B-process. It would appear that a strain sensitivity on myosin off-rate would be a reflection of the mechanical attributes of the myosin molecule, i.e., affecting either the myosin interface with actin and/or the myosin affinity for ATP (Kad et al. 2007). For example, if the myosin head were the most mechanically compliant portion of the myosin molecule, then the myosin head would experience the majority of the strain that had been applied during the laser trap experiment. With this in mind, we would hypothesize that the visco-elastic characteristics of the myosin molecule, which could affect the myosin interface with actin and/or the myosin affinity for ATP, bear a characteristic rate constant that is lower than the off-rate of myosin. In other words, the intra-molecular strain of myosin must possess a frequency dependency that gives rise to significant physical distortion of myosin at frequencies of perturbation lower than the off-rate of myosin and not at higher frequencies. Such would be the case if the visco-elastic characteristics of myosin

head (including the lever arm) were reasonably modeled as a spring and dashpot in series, i.e., a Maxwell model, and the myosin rod were modeled as a spring in series (Fig. 6). The mean physical distortion of the spring element in the myosin head, call it $\tilde{D}(\omega)$, would be proportional to the compliance of the elastic element divided by the total compliance of the mechanical system and multiplied by the mean strain applied to the system:

$$\tilde{D}(\omega)=\frac{\left(\frac{k_{rod}}{k_{rod}+k_{head}}\right)i\omega}{(k_{head}/\eta_{head})\left(\frac{k_{rod}}{k_{rod}+k_{head}}\right)+i\omega}\tilde{N}_1(\omega) \tag{19}$$

where k_{head} = the stiffness of the elastic element, η_{head} = the viscosity of the dashpot and k_{rod} = the stiffness of elastic element of the myosin rod in series with the Maxwell element.

If we use this distortion $\tilde{D}(\omega)$ instead of $\tilde{N}_1(\omega)$ to influence $\tilde{N}_0(\omega)$ in (17), then we have a steady-state solution given as:

$$\tilde{N}_0(\omega)=-G_1\left(\frac{F_0}{F_0+G_0}\right)\left(\frac{\xi i\omega}{R\xi+i\omega}\right)\left(\frac{i\omega}{G_0+i\omega}\right)\left(\frac{1}{F_0+G_0+i\omega}\right)\tilde{L}(\omega) \tag{20}$$

where $\xi = k_{rod}/(k_{rod} + k_{head})$ and $R = (k_{head}/\eta_{head})$. Note that the value of ξ is always less than 1. The rate constant represented by $R\xi$ describes the characteristic rate of the myosin head deformation due to a length perturbation. This rate constant depends on the mechanical attributes of the myosin head and may well be lower than the myosin off-rate, G_0, which would be detected as $2\pi c$. It should be noted that the form of (20) does not strictly comply with the analytical expression for the B-process provided in (1). Nevertheless, if we assume the values $F_0 = 4.44\ s^{-1}$ and $G_0 = 40.0\ s^{-1}$, which represent a duty cycle = 0.1 (Harris and Warshaw 1993) and t_{on} = 25 ms similar to that in human cardiac muscle, $G_1 = 1\ (nm\ s)^{-1}$, $\xi = 0.8$ and $R = 5\ s^{-1}$, we find that (20) predicts negative values for the elastic and viscous moduli at low frequencies (Fig. 7a) as would be expected from a B-process shown in Fig. 2d. The result of (20) also predicts a positive viscous modulus at higher frequencies, which has not been modeled previously or included in (1) but is also not contrary to observation. The addition of the A- and C-process as given in (16) results in a loop in the Nyquist diagram (Fig. 7b) that is qualitatively similar in shape to that observed in practice and shown in Fig. 2c.

4 Discussion

This paper demonstrates that a frequency dependent, positively monotonic strain-dependency on the myosin off-rate can result in negative values for the elastic and viscous moduli, which could account for the observed B-process of sinusoidal analysis of oscillatory muscles. The frequency dependency utilized here was modeled as the result of the visco-elastic properties of the myosin molecule. Although the actual physical distortion of myosin that occurs with an imposed length perturbation is not known, the frequency characteristic of myosin strain may well be less than that of the C-process, $2\pi c$, and could therefore underlie the frequency characteristics of the B-process. A similar concept of visco-elastic properties leading to changes in acto-myosin kinetics has been demonstrated for smooth muscle myosin (Kad et al. 2007) and has been forwarded to explain the processivity of myosin-V (Veigel et al. 2005).

We showed in our development to (20) that, if the myosin off-rate is positively related to the physical distortion of the visco-elastic elements of myosin, then negative values for the

elastic and viscous moduli would arise. The form of the model presented here also offers an explanation how mutations in the myosin rod, like those that lead to cardiomyopathies (Debold et al. 2007;Palmiter et al. 2000), can affect myosin off-rate. According to our results, a mutation that results in a stiffer myosin rod would raise the characteristic frequency of distortion in the myosin head (Miller et al. 2009). A more compliant rod would lead to a lower characteristic frequency.

There is, however, one significant caveat to the present model that must be addressed. The B-process developed here relies upon the myosin off-rate being positively related to the positive strain of myosin, i.e., a physical elongation of myosin. Studies of the strain dependency of the myosin off-rate using the laser trap have demonstrated a negative relationship between the myosin off-rate and the imposed load or strain, i.e., off-rate is enhanced with a negative or assistive load and is reduced with a positive or resistive load (Harris and Warshaw 1993; Kad et al. 2007). We reconcile these experimental findings with our analysis by suggesting that the negative relationship between myosin off-rate and strain observed in the laser trap is restricted to those myosin t_{on} which reflect only an ATP-dependent force-producing cycle without thin filament regulatory proteins. According to Kad et al. (2007) the positive strain of the myosin head due to a resistive load inhibits the release of ADP and/or the binding of ATP, thus prolonging the myosin t_{on}. If we recognize that myosin t_{on} may also be governed via a P_i-dependent cycle (Hibberd et al. 1985), which is much shorter in duration than that of the ATP-dependent cycle (Baker et al. 2002), then we have the possibility of a positive relationship between myosin off-rate and strain. Considering that P_i release and rebinding occur at different sites of the myosin head relative to the nucleotide binding pocket where ADP is released and ATP is bound (Geeves and Holmes 1999), it is conceivable that the P_i-dependent cycle bears a positive relationship between myosin off-rate and strain. Indeed, we find in practice that P_i concentration will enhance the amplitude of the B-process (Palmer et al. 2004b; Swank et al. 2006). In the scheme that we propose here, a rise in the P_i concentration would effectively raise the myosin off-rate, G_0, and the sensitivity of the strain-dependency, G_1.

We suggest then that a positive strain-dependency on the myosin off-rate could arise if both ATP- and P_i-dependent cycles are considered. We furthermore hypothesize that (20) underlies the observed B-process of sinusoidal analysis of oscillatory muscles in a manner that relies upon the release rates of P_i and ADP and the binding rates of P_i and ATP. We believe the hypothesis raised in (20) is important because it suggests a molecular mechanism by which myosin can regulate temporal characteristics of force production necessary for the effective and efficient function of oscillatory muscles like insect flight muscle and cardiac muscle (Maughan et al. 1998). We expect to proceed with these investigations with analytical solutions using probability theory and a computer model that will test the validity of (20) based on data available in the literature and the assumptions outlined in the present work.

Acknowledgments

This paper is dedicated in honor of Dr. Mark R. Hilty. This work was supported by a grant from the NIH P01-HL59408.

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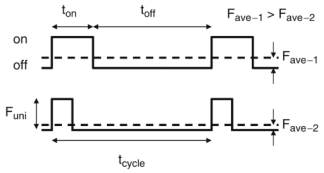

Fig. 1.

Two diagrams of force production and time durations at the molecular level. The time duration over which myosin is attached to actin is indicated by as the 'on' position and referred to as time-on, t_{on}. The time duration when myosin is detached is indicated as the 'off' position and referred to as time-off, t_{off}. The time average force (F_{ave}) of an acto-myosin crossbridge is proportional to the unitary force (F_{uni}) produced by the myosin molecule during t_{on} and the crossbridge duty ratio, $F_{ave} = F_{uni} \times t_{on}/(t_{on} + t_{off})$. When t_{on} is shortened relative to t_{cycle}, the duty ratio and F_{ave} are reduced. When t_{on} is prolonged relative to t_{cycle}, the duty cycle and F_{ave} are enhanced

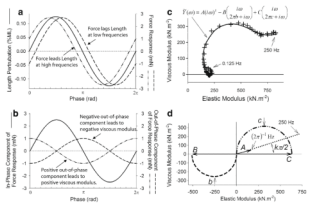

Fig. 2.

Sinusoid analysis and resultant Nyquist diagram. (**a**) The application of a small amplitude sinusoidal length perturbation, i.e., 0.125% of muscle length (ML), to a muscle strip will result in a recorded force response that either leads or lags the length perturbation in time. (**b**) The force response is made up of two components, one in-phase with the length (*solid line*) and one out-of-phase (*dashed lines*) with the length. A force response that leads the length contains an out-of-phase component that is positive (shown here with amplitude +1 at time zero), which corresponds to a positive viscous modulus as observed at high frequencies. A lagging force response contains an out-of-phase component that is negative (shown here with amplitude −1), which corresponds to a negative viscous modulus as observed at low frequencies. (**c**) A plot of the viscous modulus vs. elastic modulus, i.e., Nyquist diagram, recorded from human cardiac muscles demonstrates a looped relationship between the two moduli. A negative viscous modulus occurs in the range of 1–3 Hz, and a positive viscous modulus occurs at frequencies greater than ~3 Hz. The complex modulus often conforms to the mathematical model, $\tilde{Y}(\omega)$, containing the so-called A-, B- and C-processes. (**d**) The processes describe the angular orientation of the data within the diagram (A-process), the negative viscous modulus at low frequencies (B-process) and the semicircular relationship between the elastic and viscous moduli at higher frequencies (C-process)

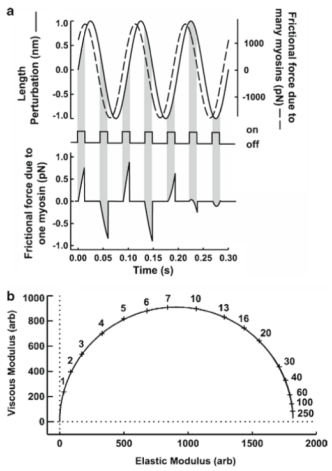

Fig. 3.

The C-process arises from the frictional force due to actin-myosin interactions. (**a**) The intermittent and repeated attachment of myosin to actin results in a force that is proportional to the velocity of the length perturbation during the time of attachment, t_{on}. The net force is a frictional force that resists and leads the length perturbation. (**b**) The elastic and viscous moduli are positive due to this friction between the thick and thin filaments, and the frequency responses of the moduli conform to the mathematical expression used to describe the C-process

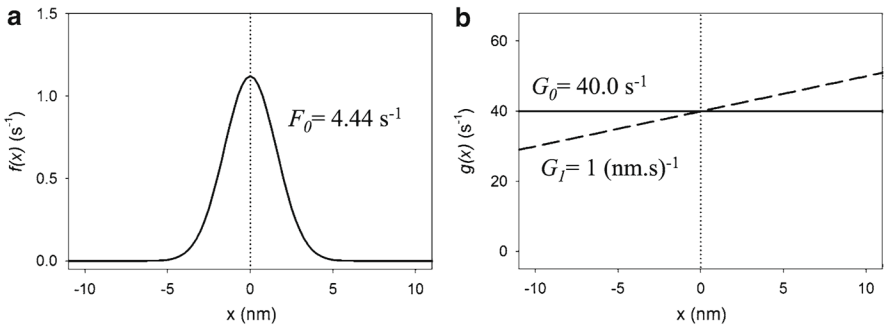

Fig. 4.

The rate functions $f(x)$ and $g(x)$ used in calculations. (**a**) The function $f(x)$ describes the spatial-dependence of the probability of myosin attaching to actin along the length thin filament. The position x = 0 indicates the central position of a myosin head and distribution about x = 0 reflects the possible reach of the head in both the negative and positive directions. The term F_0 refers to the integral of $f(x)$ along the x-axis (see (8b)) and its value, 4.44 s^{-1}, is used to assure a duty cycle of 0.1 (see text). (**b**) The function $g(x)$ describes the spatial-dependence of the probability of myosin detaching to actin along the length thin filament. When $g(x) = G_0$, a constant, there is no spatial dependence. A monotonic strain-dependence on $g(x)$ is provided by the added term G_1x. A positive value for G_1 reflects a faster myosin off-rate, or shorter t_{on}, when myosin molecules are experiencing a positive strain, as occurs during a lengthening phase of the length perturbation

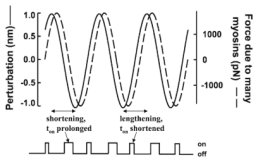

Fig. 5.

A force response lagging the length perturbation may arise if t_{on} is prolonged during a shortening phase of a length perturbation and alternately shortened during a lengthening phase. The changes in F_{ave} due to changes in t_{on} would result in a lower recorded force during lengthening and higher recorded force during shortening, i.e. lagging the length perturbation. The result would be a negative viscous modulus

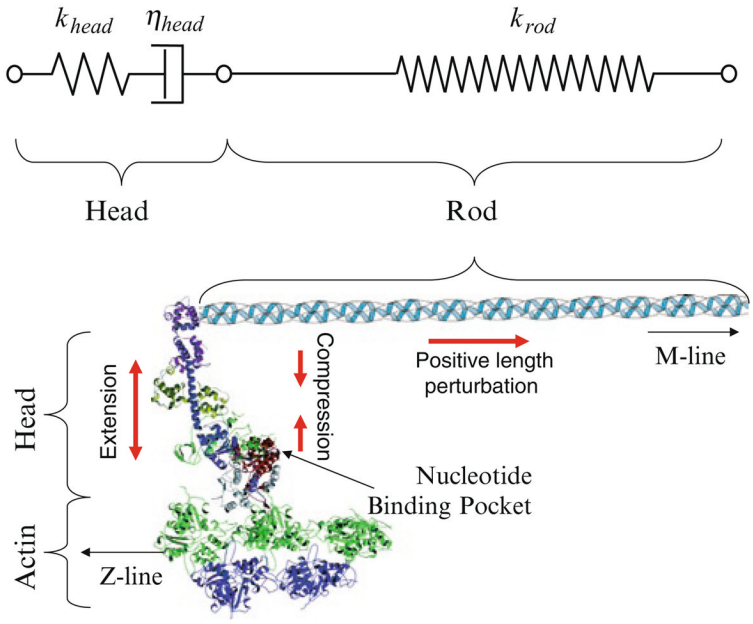

Fig. 6.

A Maxwell model of the myosin head including lever arm in series with an elastic element representing the myosin rod. The nucleotide binding pocket is shown in *red*. The structure of the myosin head and lever arm shown here depicts the myosin post-power stroke state. The force generated by the power stroke and a positive length perturbation results in an extension of the myosin along the Z-line side of the myosin head and a compression of the myosin along the M-line side. A negative length perturbation would result in less extension and possibly compression along the Z-line side of the myosin head and less compression and possibly extension along the M-line side. The physical distortion of the elastic element, k_{head}, of the myosin head, as would occur during a length perturbation, is proposed as the specific strain that affects myosin off-rate, $g(x)$, via the effects on P_i and ADP release and/or P_i and ATP binding. Structure adapted from Geeves and Holmes (1999)

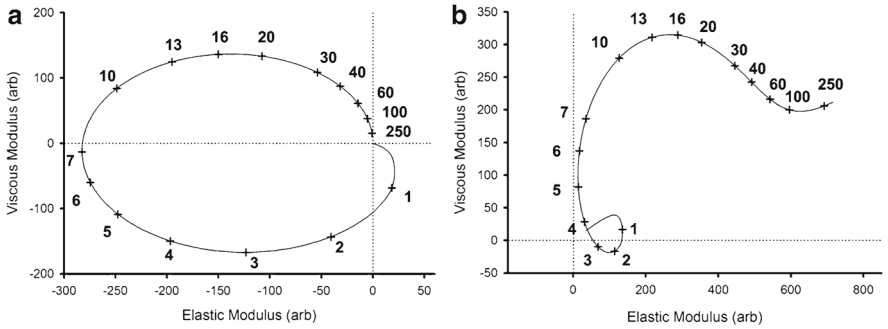

Fig. 7.

Example result of frequency-dependence and strain-dependence on myosin off-rate. (**a**) Equation (20) predicts a B-process within a recorded complex modulus that includes a negative elastic modulus and a negative viscous modulus at low frequencies according to the parameter values described in the text. (**b**) When illustrative examples of the A- and C-processes are added, the Nyquist diagram qualitatively resembles that of actual recorded data like that shown in Fig. 2c