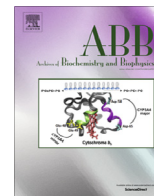




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The myofilament elasticity and its effect on kinetics of force generation by the myosin motor



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ABSTRACT

The half-sarcomere is the functional unit of striated muscle, in which, according to a “linear” mechanical model, myosin motors are parallel force generators with an average strain s acting between the opposing myosin and actin filaments that behave as a series elastic element with compliance C_f . Thus the definition of the mechanism of force generation by myosin motors in muscle requires integration of the crystallographic model of the working stroke with the mechanical constraints provided by the organization of motors in the half-sarcomere. The relation between half-sarcomere compliance and force ($C_{hs}-T$) during the development of isometric contraction deviates, at low forces, from that predicted by the linear model, indicating the presence of an elastic element in parallel with the myosin motors, which may influence the estimate of s . A working stroke model, kinetically constrained by the early phase of the isotonic velocity transient following a force step, predicts that the rate of quick force recovery following a length step is reduced to the observed value by a C_f of 12.6 nm/MPa. With this value of C_f , the fit of $C_{hs}-T$ relation during the isometric force rise gives $s = 1.8-1.9$ nm, similar to the values estimated using the linear model.

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Introduction

Force in the muscle sarcomere is generated by the head (Subfragment 1 or S1) of the motor protein myosin II extending from the thick filament and attaching to the thin actin filament to form cross-bridges that pull the thin filament toward the center of the sarcomere through an ATP driven structural working stroke. Crystallographic studies suggest that the working stroke consists of a 70° tilting of the light chain domain (LCD) of the myosin head about a fulcrum in the actin-attached catalytic domain (CD), corresponding to 11 nm movement at the junction between the LCD and the rod-like subfragment 2 (S2) that connects the cross-bridge to the thick filament (Fig. 1A).

However, the extent of the motor movement for force generation during isometric contraction cannot be defined with *in vitro* studies, as it depends on the stress experienced by the motor while it undergoes the structural transition. The question can be

addressed by determining the elastic characteristics of the half-sarcomere, the functional unit in which myosin motors and myofilaments are organized in a complex series-parallel network. According to the analysis by Ford et al. [1], this complex network can be reduced to a “linear” mechanical model, in which myosin motors are parallel force generators between the opposing myosin and actin filaments that act as elastic elements in series (model 1 in Fig. 1B).

The compliance of the actin and myosin filaments has been estimated in frog skeletal muscle (either whole muscle or single fibers) by measuring their force-extension relation with X-ray diffraction [2–6]. From these measurements, the filament compliance (C_f)² functionally in series with the array of myosin motors could be calculated [1] and was found to be more than 50% of the half-sarcomere compliance (C_{hs}). The average strain of the myosin motors (s) during

² Abbreviations used: C_{cb} , compliance of the myosin motor array at T_0 ; C_f , compliance of the myofilaments; C_{hs} , compliance of the half-sarcomere; C_p , compliance of the element in parallel to the myosin motor array; ϵ , stiffness of a myosin motor; ζ , structural transition between two consecutive states of the myosin motor; L , change in length of the half-sarcomere; M1–M5 states of the attached myosin motor; P_M , signal from the position of the lever connected to the motor-length transducer; τ_2 , rate of quick force recovery; s , average motor strain; T , force; T_0 , plateau force in an isometric tetanus; V_2 , the initial velocity of the isotonic velocity transient; x , relative position between the myosin motor and the actin filament; y , strain of the motor in a given state; Y , half-sarcomere strain; z , change in axial position of attached motors.

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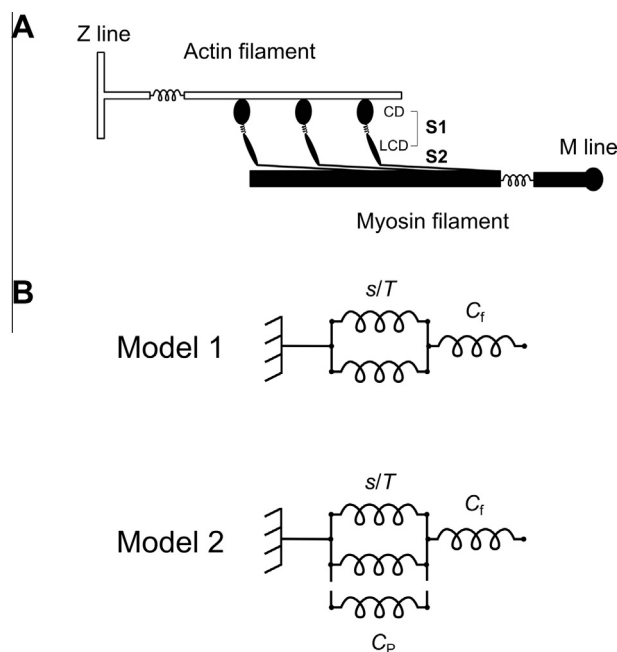


Fig. 1. Mechanical model of the half-sarcomere. *A.* Schematic representation of the half-sarcomere in which actin and myosin compliances are lumped into two springs in series with the array of myosin motors arranged in parallel. *B.* Mechanical models of the elements contributing to the half-sarcomere compliance. Model 1: actin and myosin compliances are represented as a spring with compliance (C_f) in series with the array of parallel elastic elements with a constant strain s , representing the attached myosin motors (two only are shown for simplicity). The compliance of the array is s/T , and is inversely proportional to the level of isometric force exerted by the array that increases linearly with the number of attached motors. Model 2: an elastic element with compliance C_p , independent of the isometric force T , is added in parallel with the array of motors.

isometric contraction (force T_0) was therefore less than half of the half-sarcomere strain (<2 nm). If the isometric force developed by a myosin motor is 5 pN, as suggested by a series of mechanical, structural and energetic studies on single fibers and by single molecule experiments [6–9], then the stiffness (ε) of the individual cross-bridge that links the thick and thin filaments is $>(5/2)=2.5$ pN/nm.

Here following we show how the mechanical parameters defined in single muscle fibers from frog skeletal muscle constrain the model of force generation and how an independent estimate of the filament compliance, obtained with the analysis of mechanical transients, supports the view that myosin motors in isometric contraction generate force with an average strain <2 nm, due to a distribution of cross-bridge states biased to the beginning of the working stroke.

The elastic properties of the half-sarcomere constrain the model of force generation by myosin motors

The hypothesis that the isometric force is generated by a mechanism based on the all-or-none 11 nm movement implies that the fraction of attached motors responsible for force is less than $(2/11)=0.18$, while the remaining fraction of the attached motors (larger than 0.82) remains in a pre-working stroke conformation.

However this mechanism seems unlikely on thermodynamic grounds, because the mechanical energy implied in the generation of isometric force by an actin-attached motor ($E_m = \frac{1}{2}\varepsilon\zeta^2$, where ζ is the size of the force generating step) at 4 °C would be larger than 150 zJ, or $40 k_B\theta$ (where k_B is the Boltzmann constant and θ is the absolute temperature). This is almost twice the free energy released during the hydrolysis of one ATP molecule (83 zJ, [10]), and the equilibrium constant of the force generating transition would

be too low to account for the isometric force. Moreover an all-or-none 11 nm working stroke implies a dispersion of conformations of the myosin heads attached in the isometric contraction larger than that indicated by X-ray diffraction studies [5,6].

As far as the kinetics of the working stroke is concerned, a foundation stone is represented by the experimental and theoretical work of Huxley and collaborators who first developed a model of force generation based on stepwise structural changes in the actin-attached myosin head controlled by strain-dependent rate functions inferred from the force responses to sudden stretches and releases [11]. In the original model it was assumed that the myosin motors are the only source of compliance in the half-sarcomere, so that, according to the relatively large value of the half-sarcomere strain estimated at that time, the working stroke could be explained by a single state transition in the attached head. Improving the performance of single fiber mechanics, it became evident that the half-sarcomere strain (and thus the motor strain) is at least three times smaller than the sliding accounted for by the working stroke [1,12]. This suggested that the working stroke could be composed of more than one step, though the concept of a tight coupling between mechanical steps and biochemical steps (essentially the release of ATP hydrolysis products orthophosphate and ADP) of the chemo-mechanical cycle was maintained [13]. A further reduction of the myosin motor compliance by a factor of two became evident following X-ray diffraction demonstration that the compliance of the myofilaments could account for more than $\frac{1}{2}$ of the half-sarcomere compliance [2,3]. The progressive reduction in motor compliance was taken into account in an extension of the original model, in which, following previous modeling work [14,15], three states, separated by two structural steps of 5 nm, were assumed [16].

Eventually on the basis of energetic, structural and biochemical studies [4–7,17] the alternative idea emerged that the biochemical steps and the mechanical steps are not tightly coupled [18,19] and the ATP hydrolysis cycle can terminate and the motor detach from actin at an intermediate stage of the 11 nm working stroke. According to this idea, the small average strain of the cross-bridges responsible for the isometric force ($s < 2$ nm) reflects a limited dispersion of motor conformations characterized by a narrow distribution of LCD angles biased towards the beginning of the working stroke. In other words, the force developed by the half-sarcomere during isometric contraction is the sum of the relatively constant force contributions by the actin-attached myosin heads which have undergone a structural change that is a small fraction ($<1/5$) of the 11 nm working stroke. During steady shortening against a load $<T_0$, the structural change increases but only to ~ 6 nm, while the force of the motors remains high and the number of motor reduces in proportion to the load. The 11 nm working stroke expected from crystallographic model can be observed only during the early rapid shortening following a stepwise drop to near zero force [4,6].

The compliances of the myofilaments and myosin motors according to the linear mechanical model of the half-sarcomere

Strong support for the idea that isometric force in a muscle fiber is proportional to the number of attached motors, in which the strain and thus the force is constant, came from sarcomere-level mechanical experiments in both rabbit skinned fibers and frog intact fibers. In these experiments, the relation between half-sarcomere stiffness and isometric force was determined while force changed either with $[Ca^{2+}]$ (skinned fibers, [20]) or during the development of isometric tetanus (intact fibers, [21,22]). Under these conditions it was found that the half-sarcomere strain (Y) increases with force (T) in proportion to the increase of myofilament

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