Electrostatic Forces as a Possible Mechanism Underlying Skeletal Muscle Contraction

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Abstract. A possible mechanism is put forward to explain the sliding of thin filaments during muscle contraction In our model, repulsion due to electrostatic forces is the mechanism which triggers crossbridges to cause the thin filaments to slide The mechanism proposed could operate regardless of whether the myosin heads rotate or bend, although recent experimental evidence seems to confirm the latter action In spite of its simplicity, the model prediction of the velocity of sliding of the thin filaments agrees well with experimental values from *in vitro* motility assays

Key words: Skeletal muscle — Electrostatic forces — Muscle contraction — Model

Introduction

It is well known that, during muscle contraction, thin filaments slide over thick filaments, ATP is hydrolyzed in the myosin heads and troponin-C binds Ca^{++} In the past, the most widely accepted contraction model was that of Huxley and Simmons (1971)

It has been proposed that rotation of the myosin head is due to the expulsion of the products of ATP hydrolysis (Morel and Bachouchi 1988) However, our analysis of the velocity of sliding of the thin filaments led us to propose a modification of some aspects of the assumptions made by these authors (Marin et al 1990) In fact,

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recent experimental studies of the molecular structure of myosin (Rayment et al 1993a,b) and EPR spectroscopy of spin labeled myosin (Roopnarine and Thomas 1995, Ostap et al 1995) have provided evidence that the myosin head does not rotate, as was previously assumed Instead, the molecule appears to bend in a pre-force step in which a weak binding of myosin and actin occurs Following on from the more recent experimental evidence we are here proposing a mechanism which explains the triggering of muscle contraction by invoking the involvement of electrostatic forces We also make a quantitative analysis of the model predictions of the velocity of sliding of the thin filament, and compare our values with those from *in vitro* studies of isolated filaments A preliminary report has already been published (Muñiz et al 1992)

The model

The Mg^{++}-ATP complex is attached at a specific site on the myosin head (S1) (Vibert and Cohen 1988 Highsmith 1990, Rayment et al 1993a) As the intracellular calcium concentration ($[Ca^{++}]_i$) increases, troponin-C (TnC) acts as a trapping site for Ca^{++}, causing noticeable conformational changes in TnI, TnT, actin and tropomyosin (Parmacek and Leiden 1991) This promotes the formation of the actomyosin complex as a tightly bound structure (Brozovich et al 1988 Highsmith and Eden 1990), and completes the hydrolysis of ATP When ATP hydrolysis is completed the products Mg^{++}-ADP^{-}, H_3O^{+} and P_i are ejected The expulsion of these products generates a positive charge center localized at the site at which Mg-ATP was bound (Hazard and Cusanovich 1986, Morel and Bachouchi 1988) Moreover, the presence of Ca^{++} in TnC increases its dipole moment (Maeda et al 1992 Salcedo et al 1994) At the time the positive charge center is formed on S1 a net electrostatic interaction appears between this center and Ca^{++} TnC

The unbending of the myosin head and the geometrical constraints provoke the movement of the thin filament toward the M-line (see Fig 1) During this step ADP is released and the strong actomyosin binding is inhibited by a new Mg-ATP attachment to S1 Then, the positive charge center is neutralized and the myosin head returns to its original shape This cycle occurs repetitively if the Ca^{++} concentration is maintained in the presence of ATP

Thus the energy obtained from ATP hydrolysis is used 1) in the production of conformational changes in myosin which allow a tight actin-to myosin binding (Brozovich et al 1988, Highsmith and Eden 1990, Highsmith 1990, Rayment et al 1993a,b Roopnarine and Thomas 1995), and 2) in the expulsion of the charges resulting from the splitting of ATP Fig 1 shows, schematically, the system involving the thin filament and S1

The total electrostatic interaction can be represented by the sum of the charge-charge and dipole-charge terms

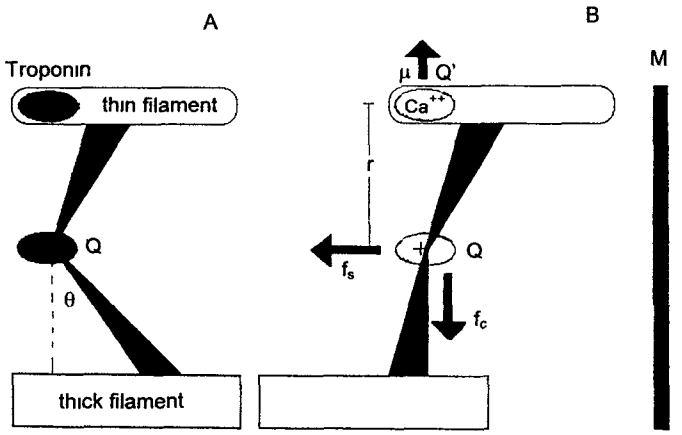

Figure 1. Schematic representation of Electrostatic Interaction model for cross-bridge action *A*) Representation of the system formed by thin and thick filaments before troponin traps Ca^{++} Q is the site for Mg^{++}-ATP at the myosin head *B*) Representation of the system after Ca^{++} is bound to troponin f_c is the force resulting from electrostatic interaction between 1) dipole (μ) and Q' (Ca^{++}-TnC) and 2) charge center Q f_s is the resistive force acting on the thin filament r is the distance from Q to μ and Q' The angle θ represents the angular displacement due to the unbending of the myosin head For simplicity only the S1 unit is drawn M represents the M-line

$$f_c = -\frac{2Q\mu}{4\pi\varepsilon\varepsilon_o r^3} + \frac{QQ'}{4\pi\varepsilon\varepsilon_o r^2} \tag{1}$$

Where Q is the charge on S1, Q' is the charge on Ca^{++}, μ the dipole moment of TnC-Ca^{++}, ε_o is the permittivity of vacuum, and ε is the dielectric constant of the medium

For simplicity, we have assumed that Ca^{++} and the dipole center are located at the same distance, r from Q (see Fig 1)

It is clear that f_c will cause the myosin head to unbend and that this, in turn, will exert a traction force on the thin filament However, once this traction force appears, a resistive force (f_s) will oppose the movement Hence, the required condition for a dynamic or static equilibrium of the thin filament is

$$f_c - f_s \geq 0 \tag{2}$$

At this stage, we would suggest that an inequality occurs and persists throughout the initial sliding of the filament that takes place during muscle shortening, whereas in the case of a static equilibrium, such as that existing during isometric contraction, the equality in Eq (2) remains unchanged

In this section we have described in general terms the mechanism through which muscle contraction could be generated, as summarized in Eqs (1) and (2) However, the exact nature of f_s is still a subject of discussions (Iwazumi 1989, Smith 1990) Accordingly, in this work we shall confine ourselves to myofilaments which have as the only resistance to their movement a viscous force

In such a situation, f_s can be identified as the Stokes drag force in Eq (2) This force depends upon the shape of the moving object, so if we consider the filament as a thin rod then (Lauffer 1989)

$$f_s = 6\eta l V \tag{3}$$

where η is the viscosity of the medium, l is the length of the filament and V is the velocity of sliding In our model the steady state for the velocity of sliding is attained very soon after the motion has been initiated (see Appendix A) In this model, the drag forces on S1 and the rod are not included since their contribution is small (see Appendix B)

Results

In order to compare the predictions made by this model with available experimental results we have evaluated the force (f_c) per myosin head and the velocity of sliding of the thin filament Hence, from equations 1–3

$$V = \frac{f_c}{6\eta l} \tag{4}$$

The dipole moment μ appearing in Eq 1 has a value of about 3.33×10^{-29} Cm (Salcedo et al 1994) In addition to the increased dipole moment due to the presence of Ca^{++}, a well-localized charge center should be formed around Ca^{++}

Table 1

Quantity	Value	Reference
μ	3.33×10^{-29} Cm	Salcedo et al 1994
Q	$+2e$	Hazzard and Cusanovich 1986
l	1 μm	Pollack 1990
θ	45°C	Rayment et al 1993b
$1/4\pi\varepsilon_o$	9×10^9 Nm^2/C^2	
r	13 nm	
Q'	$+2e$	

e is the elementary charge (1.6×10^{-19} C)

Table 1 displays the values for the remaining quantities used in our estimations For ε and η, we took the nominal values for water at 25 °C

Using the values given in Table 1 we found a stroke force per myosin head of the order of 0 1 pN a value closely coinciding with experimental values reported by other authors (Ishijima et al 1991, Finer et al 1994)

The velocity of sliding obtained from this model is approximately 10 μm/s a value similar to that reported from motility assays by Sellers and Kachar (1990) Their value was 8 8 $\pm$ 1 4 μm/s for the thin filament moving toward the center of the thick filament whereas Ishijima et al (1991) reported a value of 9 μm/s at zero load

Discussion

The real life system is bound to be more complex than that described in the present model For this reason the electrical and geometrical factors assumed in the present calculations should be considered effective parameters

Despite its simplicity we believe that the model proposed here constitutes one possible mechanism among others through which thin filament displacement might occur Recent experimental observations of the motion of actin filaments in the presence of myosin heads and ATP have confirmed the role of ATP hydrolysis in increasing the velocity of sliding of the thin filaments (Burlacu and Borejdo 1992) Moreover the bending and unbending of myosin heads during muscle contraction has been experimentally observed (Rayment et al 1993a b Roopnarine and Thomas 1995 Ostap et al 1995) These two findings might be thought to constitute some support for the main assumptions made in the present study

Since our treatment seems to give rise to a reasonable working hypothesis, an experiment is proposed to evaluate the role of electrostatic forces as precursors of thin filament movement Such an experiment would be similar to that performed by Sellers and Kachar (1990) using complete thin and thick filaments with native and modified myosin (Sellers et al 1985, Warshaw et al 1990) However, it would also involve the presence of an external electric field with an intensity of

$$\frac{f_c}{e} \cong 10^5 \frac{V}{cm} \tag{5}$$

where e is the elementary charge If our hypothesis is correct the velocity of sliding should be modulated as a function of the direction and intensity of the external electric field In addition these experiments would provide information about the strength of the bond between actin and myosin (Suda 1990 Mann et al 1994), and also about the role of viscoelastic effects in this phenomenon (Bagni et al 1992)

Finally we have to mention two other models that have been put for forward in attempts to explain the process of contraction these are the Helix-Coil melting

model (Harrington 1979, Tsong et al 1979, Pollack 1990), and the electrostatic dipole array model (Iwazumi 1989). The main difference between these models and the present one is that in them the myosin head would not be bent. In addition, a stochastical model has recently been proposed to explain muscle contraction in terms of a cooperative molecular motor (Julicher and Prost 1995). In this model the process is viewed as a large ensemble of motors (myosin) rigidly attached to a backbone that can move along a track (actin), all working in a cooperative fashion. Each motor can be either in a strongly bound state or in a weakly bound state, its corresponding energy being represented by a periodic potential (with the periodicity of the actin filaments). An important result of this model might be the generation of a directed force and directed motion, as observed in muscle contraction. However, the exact strength of such a periodic potential has not been specified at all. In any case, the relevance of electrostatic forces still needs to be addressed, since all three models assume that Ca^{++}-TnC interaction and ATP hydrolysis play an important role in the process of mechanical force generation.

In conclusion, we believe that electrostatic forces may be important for the regulation by Ca^{2+} and ATP hydrolysis of muscle contraction.

Appendix A

Analysis of the time needed by the thin filament to reach terminal velocity

When electrostatic repulsion occurs, there is an instantaneous acceleration expressed by

$$f_e - 6\eta l V = m\frac{dV}{dt} \tag{1A}$$

On the basis of the molecular weight of the components of a thin filament 1 μm in length, m is approximately 4.2×10^{-20} kg. Integrating equation (1A), the velocity can be written as a function of time:

$$V(t) = \frac{f_e}{6\eta l}\left[1 - \exp(-6\eta l t/m)\right] \tag{2A}$$

where $V(0) = 0$ is assumed to be the initial condition. The terminal velocity is defined as

$$V_T = \lim_{t\to\infty} V(t) = \frac{f_e}{6\eta l} \tag{3A}$$

The time needed to reach 67% of V_T (relaxation time) is given by

$$t_r = \frac{m}{6\eta l} = 7 \times 10^{-12}\,\text{s} \tag{4A}$$

and thus, for all practical purposes, on the time-scale of ATP hydrolysis, V_T is reached instantaneously.

Appendix B

Contribution of the drag forces on S1 S2 and LMM to the velocity of sliding

According to current knowledge of the myosin thick filament the portion that participates in the formation of crossbridges consists of three subunits S1 (head) S2 (rod) and LMM In order to make an assessment of the part played by these subunits in determining the velocity of sliding via drag forces S1 is considered as a sphere of radius $R \approx 9$ nm, and S2 and LMM as thin rods of lengths L_2 and L_m respectively As a first approximation we can assume that each subunit contributes independently to the total drag force that is

$$f_s = [6\eta\pi R + 6\eta(l + L_2 + L_m)]\, V_s \tag{1B}$$

when η is the viscosity V_s is the velocity of sliding of the thin filament and l is its length

On the other hand if we neglect the contributions of S1 S2 and LMM the drag force is simply given as

$$f_s' = 6\eta l V_s' \tag{2B}$$

In either case this force produces a moment around Q (see Fig 1) which opposes the one produced by the electrostatic interaction between TnC-Ca^{++} and the charge center on S1 so that

$$f = f' \tag{3B}$$

since in both situations the force due to the electrostatic interaction is the same

Combining Eqs (1*B*) and (2*B*) we can obtain

$$V_s = \frac{l}{[\pi R + (l + L_2 + L_m)]} V' \tag{4B}$$

From Table 1 $R \approx 9$ nm, $l = 1$ μm and $L_2 + L_m$ can be taken as 0 15 μm (Garcia de la Torre and Bloomfield 1980 Highsmith et al 1977) so that

$$V_s \cong 0\,85\, V_s' \tag{5B}$$

Thus the value of the velocity of sliding changes by about 15% when we take into consideration the contributions of S1 S2 and LMM subunits to the drag force In conclusion the contributions of S1 S2 and LMM to the analysis of the drag forces do not change the overall results obtained with the present model when the filaments slide with a constant velocity

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References

Bagni M. A., Cecchi G., Colomo F., Garzella P. (1992) Are weakly binding bridges present in resting intact muscle fibers? Biophys. J. **63**, 1412—1415

Brozovich F. V., Yates L. D., Gordon A. M. (1988): Muscle force and stiffness during activation and relaxation: Implication for actomyosin ATPase. J. Gen. Physiol. **91**, 399—420

Burlacu S., Borejdo J. (1992): Motion of actin filaments in the presence of myosin heads and ATP. Biophys. J. **63**, 1471—1482

Finer J. T., Simmons R. M., Spudich J. A. (1994): Single myosin molecule mechanics: piconewton forces and nanometre steps. Nature **368**, 113—119

García de la Torre J., Bloomfield V. A. (1980): Conformation of myosin in dilute solution as estimated from hydrodynamic properties. Biochemistry USA **19**, 51518–51523

Harrington W. F. (1979): Origin of the contractile force in skeletal muscle. Proc. Natl. Acad. Sci. USA **76**, 5066—5070

Hazzard J. O., Cusanovich M. A. (1986): Binding and hydrolysis of ATP by cardiac myosin subfragment-1: Effect of solution parameters on transient kinetics. Biochemistry USA **25**, 8141—8149

Highsmith S. (1990): Electrostatic contributions to the binding of myosin and myosin-MgADP to F-actin in solution. Biochemistry USA **29**, 10690—10694

Highsmith S., Kretzschmar K. M., O'Konski C. T., Morales M. F. (1977): Flexibility of myosin rod, light meromyosin and myosin subfragments-2 in solution. Proc. Natl. Acad. Sci. USA **74**, 4986—4990

Highsmith S., Eden D. (1990): Ligand-induced myosin subfragments global conformational change biochemistry. Biochemistry USA **29**, 4087–4093

Huxley A. F., Simmons R. M. (1971). Proposed mechanism of force generation in striated muscle. Nature **233**, 533–538

Ishijima A., Doi T., Sakurada K., Yanagida T. (1991): Sub-picoNewton force fluctuation of actomyosin *in vitro* Nature **352**, 301–306

Iwazumi T. (1989): Molecular mechanism of muscle contraction. Physiol. Chem. Phys. Med. NMR **21**, 187–219

Jülicher F., Prost J. (1995): Cooperative molecular motors. Phys. Rev. Lett. **75**, 2618–2621

Lauffer M. A. (1989) Motion in Biological Systems. Alan R. Liss Inc., USA

Máeda Y., Popp D., Stewart, A. A. (1992): Time-resolved X-ray diffraction study of the troponin. Associated reflexions from the frog muscle. Biophys. J. **63**, 815–822

Marin J. L., Muñiz J., Huerta M. (1990): Further analysis of the molecular jet hypothesis during muscle contraction. J. Theor. Biol. **147**, 373–376

Marin J. L., Huerta M., Muñiz J. (1994): Modulation of molecular friction in actomyosin complex by an external electric field. J. Theor. Biol. **167**, 167—168

Muñiz J., Marin J. L., Yeomans L., Acuña H., Del Castillo L. F., Cruz S. A. (1992). Electrostatic repulsion model for skeletal muscle contraction. Biophys. J. **61**, A296

Morel J. E., Bachouchi N. (1988). Muscle contraction and movement of calcium organelles Are these two different types of mechanics for their generation? J. Theor. Biol. **132**, 83—96

Ostap E. M., Barnett V. A., Thomas D. D. (1995). Resolution of three structural states of spin-labeled myosin in contracting muscle. Biophys. J. **69**, 177—188

Parmacek M. S., Leiden J. M. (1991) Structure, functionand regulation of troponin C. Circulation **84**, 991—1003

Pollack G H (1990) Muscles & Molecules pp 1–300 Ebner & Sons Publ

Rayment I, Rypniewski W R, Schmidt-Base K, Smith R, Tomchick D R, Benning M M, Winkelmann D A, Wesenberg G, Holden H M (1993a) Three dimensional structure of myosin subfragment-1: A molecular motor Science **261**, 50—58

Rayment I, Rypniewski W R, Schmidt-Base K, Smith R, Tomchick D R, Benning M M, Winkelmann D A, Wesenberg G, Holden H M (1993b) Structure of the actin-myosin complex and its implications for muscle contraction Science **261**, 58–65

Roopnarine O, Thomas D D (1995) Orientational dynamics of indane dione spin-labeled myosin heads in relaxed and contracting skeletal muscle fibers Biophys J **68**, 1461–1471

Salcedo R, Del Castillo L F, García M, Cogordan J A, Muñiz J, Marin J L (1994) Molecular configuration of the active site of TnC-Ca^{2+} complex Biophys J **66**, A311

Sellers J R, Spudich J A, Sheetz M P (1985) Light chain phosphorylation regulates the movement of smooth muscle myosin on actin filaments J Cell Biol **101**, 1897—1902

Sellers J R, Kachar B (1990) Polarity and velocity of sliding filaments: Control of direction by actin and of speed by myosin Science **249** 406–408

Smith D A (1990) The theory of sliding filament models for muscle contraction II: Biochemically-based models of the contraction cycle J Theor Biol **146** 433—466

Suda H (1990) Molecular friction in an actomyosin molecular machine J Theor Biol **146**, 341–346

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

Vibert P, Cohen C (1988) Domains, motions and regulation in the myosin head J Muscle Res and Cell Motil **9**, 296–305

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

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