The Molecular Mechanism of Force Generation in Striated Muscle*

Leepo C. Yu,[†] Robert M. Dowben, and Karl Kornacker

DIVISION OF BIOLOGICAL AND MEDICAL SCIENCES, BROWN UNIVERSITY, PROVIDENCE,
RHODE ISLAND, AND FACULTY OF BIOPHYSICS, OHIO STATE UNIVERSITY, COLUMBUS 43210

Communicated by Lorrin A. Riggs, May 13, 1970

Abstract. An electrostatic mechanism for force generation in muscle is proposed which does not require bond formation between thick and thin filaments nor movement of the cross bridges. The myosin heads, which project from the thick filaments and touch the thin filaments, possess a high negative surface charge density. Owing to their large dielectric increment, the thin filaments are polarized by the electric field generated by the myosin heads. The polarized thin filaments tend to move toward the center of the sarcomere. Myosin ATPase activity is increased in the overlap region to maintain the negative surface potential. Thus, ATP hydrolysis provides the energy for shortening. Calculations give estimated tensions generated by this model that are comparable to those observed experimentally for vertebrate striated muscle.

One of the spectacular achievements of cellular ultrastructure research has been the demonstration that skeletal muscle contains an array of two types of interdigitating filaments.^{1,2} Furthermore, it has been shown that the two types of filaments move with respect to one another during muscle shortening. Thus far there has not been an entirely satisfying explanation of the force that causes the filaments to slide during contraction. An electrostatic mechanism for the transduction of chemical energy liberated by ATP hydrolysis into mechanical energy is proposed in this communication. The proposed mechanism does not require any bond formation between the filaments nor movement of the cross bridges. The mechanism proposed in this paper differs from electrostatic models proposed in the past. It does not resemble, for instance, the model of Elliott³ or Shear,⁴ who propose a mechanism involving the mutual repulsion of thick filaments while the muscle volume remains constant.

According to electrostatic theory, a substance with a dielectric constant higher than that of the surrounding medium will displace the surrounding medium, moving into the region of greatest electric field as a result of increased polarization of the higher dielectric material. The principle is illustrated in Figure 1. In skeletal muscle (Fig. 2), we believe that a strong electric field is generated at the surface of myosin heads, located at the ends of cross bridges which project at regular intervals from the thick filaments; the myosin heads touch (a distance of approach smaller than the width of the double layer) the thin filaments. The thin filaments, composed of material with a dielectric constant higher than that

FIG. 1.—A model showing the principle that a rod of high dielectric will move into an electric field (generated between the two plates) minimizing the electrostatic free energy. The energy needed to polarize the dielectric material comes from the electric current of the battery.

of the medium, are drawn into the space between the thick filaments toward the center of the sarcomere by electrostatic forces. The energy for this process is supplied by ATP hydrolysis on the myosin heads.

The thin filaments are composed mainly of actin. It has been shown that solutions of F-actin extracted from rabbit muscle have a very large dielectric increment.⁵ In addition, electric birefringence studies indicate that the polarizability of an F-actin strand is greatest in a plane perpendicular to its long axis.⁶ It is of interest that the dipole moment of F-actin is markedly diminished upon the addition of stoichiometric amounts of heavy meromyosin and that the polarization effect is largely restored by the further addition of ATP. Thus, the thin

FIG. 2.—A diagram of striated muscle showing the arrangement of the cross bridges (myosin heads) as deduced by Huxley and Brown.⁵ Each thin filament receives cross bridges from three adjacent thick filaments. The cross bridges are coplanar on half of the thin filaments. The cross bridges on the other half of thin filaments form a helix. It is suggested that the high negative surface charge density on the surface of the myosin heads results in polarization of the thin filaments and movement toward the center of the sarcomere.

filaments are characterized by high polarizability and a capacity for strong electrostatic interaction with myosin heads.

The thick filaments are composed of myosin, a highly charged macromolecule. The cross bridges attached to the thick filaments project from the backbone of the thick filaments and end in the heads of myosin molecules which touch the thin filaments.⁷⁻⁹ It would seem that electrostatic forces across the large distance between the body of the thick filaments and the thin filaments would be severely limited by shielding from counterions in the medium. A strong and effective electrostatic interaction requires a small distance between the thick and thin filaments, a condition which is satisfied by the proximity of the myosin heads and thin filaments during contraction. On the basis of antibody studies, the myosin heads probably touch the thin filaments during relaxation as well as during contraction.⁸

According to our model, the electric field at the myosin heads (cross bridges) tends to draw the dielectric material (thin filaments) inward toward the center of the sarcomere. An analysis of the lattice reflections in the x-ray diffraction studies of Huxley and Brown⁷ suggests that in a given cross section, the cross bridges are not all oriented in the same direction. Rather, the cross bridges on the next-nearest thick filaments are in register, while the cross bridges projecting from adjacent thick filaments are rotated by $\pm 120^\circ$. Each thin filament receives cross bridges from three adjacent thick filaments; on one half of the thin filaments, the set of three cross bridges is coplanar, while on the other half of the thin filaments, the cross bridges form a helix. Iwazumi¹⁰ has shown that the helical electric field thus produced in half of the thin filaments stabilizes the filament array laterally.

The ATPase sites are located in the myosin heads (heavy meromyosin). Studies of the initial reaction of ATP with myosin indicate that a phosphorylated intermediate of myosin with a large negative charge probably is formed prior to appreciable ATP hydrolysis.^{11,12} Because live muscle contains about $2 \mu\text{mol/g}$ ATP, it is possible that *in vivo* most ATPase sites are normally phosphorylated. However, surface charge may consist of other charges in addition to those immediately involved in ATP hydrolysis. Additional charges can be created by ionization of substituent groups on the protein, the ionization energy coming from ATP hydrolysis. Thus, the myosin heads may well have a very large negative surface charge density during contraction. The surface charge density created will determine the magnitude of the electric field which polarizes the thin filaments. Assuming that the negative surface potential in the relaxed state is maintained by 20% of the free energy of ATP hydrolysis, a reasonable estimate of the surface potential is -100 mV , and the field at the surface of the myosin heads is of the order of 10^6 V/cm .¹³

The surface potential on the myosin heads tends to be lowered in the region of overlap between thick and thin filaments. Increased ATP hydrolysis occurs only in the overlap region. In our view, the rate of ATP hydrolysis raises the surface charge density of the myosin heads in the region of overlap so that the surface potential is kept constant along the whole length of the sarcomere. Thus, the myosin heads behave as though they were connected to a constant volt-

age generator. While there is no direct experimental data showing dependence of myosin ATPase activity upon electric field intensity, it is known that rate of ATP hydrolysis is markedly dependent upon the species of cationic counterions present, varying inversely with the hydrated ion diameter.¹⁴ It also has been observed that the acidity constant of an ionizable phenolic group on a myosin-azomercurial complex varies inversely with hydrated ion diameter of the salt.¹⁵

After the onset of increased ATP hydrolysis in the overlap region compared to the nonoverlap region, the negative charge density on the array of myosin heads is greater in the overlap region. Diffusion of ATP and potential determining counterions tends to redistribute the charges rapidly over the entire length of the thick filaments. In the calculations below, it is assumed that the field that polarizes the thin filaments is a linear function of overlap length. Consequently, the force generated is proportional to overlap length.¹⁶

Calculation of Isometric Tension during Tetanic Stimulation. It can be shown by means of a sample calculation using several simplifying assumptions that the force generated in terms of the model has a realistic magnitude.

The force responsible for the pulling occurs at the end of the rod of dielectric material where there are irregularities of field lines. A detailed analysis of the force in terms of field distortion is more complicated and less rewarding than an analysis in terms of free energy. The important quantities to be considered are the energy of the electric field which would be present if the dielectric material (thin filaments) were absent, E_1 , and the actual field in the thin filaments (dielectric body), E_2 . The change in electrostatic energy caused by the presence of the thin filaments is

$$\Delta U_{(\text{elec})} = U_2 - U_1 = - \frac{1}{8\pi} \int_V (\epsilon_2 - \epsilon_1) \mathbf{E}_1 \cdot \mathbf{E}_2 dV, \quad (1)$$

where ϵ_2 is the dielectric constant of the dielectric body and ϵ_1 is the dielectric constant of the medium. The expression is integrated over the volume occupied by the dielectric body. The force F in the z direction is given by

$$F_z = (\partial \Delta U / \partial z)_\psi. \quad (2)$$

The subscript ψ denotes a constant source potential at the heads of the cross bridges.

Let us assume that the myosin heads bearing a negative surface charge are distributed on the interior surface of a cylindrical tube of infinite length. The surface potential ψ is considered to be homogeneous over the inner surface of the tube and maintained constant throughout contraction. The cylinder is filled with cell sap (a solution of neutral salts, dipolar ions, and nonelectrolytes). Ions in the cell sap bearing charges opposite to that of the cylinder surface form an electric double layer. If we assume that the surface potential is small, the electrostatic field of the double layer can be evaluated by use of the Debye-Hückel equation. At the center of the cylindrical tube, the field intensity is zero.

The Debye-Hückel equation for an infinite cylinder is

$$\frac{d^2\psi}{dr^2} + \frac{1}{r} \frac{d\psi}{dr} - \kappa^2\psi = 0, \quad (3)$$

where $\kappa^2 = 4\pi e^2 \sum n_i \nu_i^2 / \epsilon_1 kT$, $1/\kappa$ is the Debye shielding length, r is the distance from the center, e is the electronic charge, n_i is the number of ions of the i th type, ν_i is the valence of ions of the i th type, and ϵ_1 is the dielectric constant of the solution. The solution to Eq. (3) is

$$\psi_r = AI_0(\kappa r) + BK_0(\kappa r), \quad (4)$$

where $I_n(r)$ and $K_n(r)$ are modified Bessel functions of the first and second kind of the n th order.

For the boundary conditions $\psi = \psi_0$ at $r = a$ (a is the radius of the cylindrical tube), and $d\psi/dr = 0$ at $r = 0$, the following explicit solutions are obtained:

$$\psi_r = \psi_0 [I_0(\kappa r)/I_0(\kappa a)], \quad (5)$$

$$-d\psi/dr = \mathbf{E}_1 = -\kappa\psi_0 [I_1(\kappa r)/I_0(\kappa a)]. \quad (6)$$

In the overlap region, the field $\mathbf{E}_1$ is altered by the presence of the thin filaments composed of high dielectric material. In the absence of more precise data, the thin filaments are considered to be cylinders of infinite length with a dielectric constant ϵ_2 . Due to the symmetry of cylinders, it must be assumed that there is a concentration of charges in the core of the thin filaments in order to account for a field at the surface. With the exception of cytochrome *c*, the charged groups of most proteins appear to be on the outer surface. F-actin may have an anomalous charge distribution. If the distribution of myosin heads on the actin filament is helical instead of cylindrical, the above assumption is not necessary. In any event, a different charge distribution will not result in a markedly different calculated value.

A cylindrical dielectric with charges in the center satisfies the Poisson equation

$$\frac{\partial^2\psi}{\partial r^2} + \frac{1}{r} \frac{\partial\psi}{\partial r} = -\frac{\Lambda \delta(r)}{\epsilon_2}, \quad (7)$$

where $\delta(r)$ is the Dirac delta function, and Λ is the charge per unit length. For the boundary conditions $\psi = \psi_0$ at $r = a$, and $-\partial\psi/\partial r = \mathbf{E} = 4\pi\sigma/\epsilon_2$ at $r = a$ (where σ is the surface charge density), the solution is

$$\psi = \psi_0 - 4\pi\sigma a \ln(r/a), \quad (8)$$

$$\mathbf{E} = \frac{4\pi\sigma a}{\epsilon_2} \frac{1}{r} \quad r > 0. \quad (9)$$

Let us equate the free energy released by ATP hydrolysis with the change in electrochemical energy of the myosin heads

$$-\Delta G_{(\text{ATP})} = \Delta\mu + \Delta m \mathcal{F} \psi \approx \Delta m \mathcal{F} \psi, \quad (10)$$

where $\Delta\mu$ is the change in myosin cyemical potential, Δm is the change in charge

number, $\mathcal{F}$ is the Faraday. Because the ATP concentration is maintained constant by transphosphorylation of phosphorylcreatine, the chemical potential of ATP is almost constant, and $\Delta\mu \ll \Delta m\mathcal{F}\psi$.⁴ The surface density is

$$\sigma = [n\Delta m\mathcal{F}/\text{surface area of myosin heads}], \quad (11)$$

where n is the number of active ATPase centers. Owing to the fact that only the enzyme centers in the region of overlap are active, n is proportional to the overlap length.

The free energy change associated with the insertion of the thin filaments now can be evaluated. Integrating Eq. (1) over the overlap region and substituting $\mathbf{E}_1$ and $\mathbf{E}_2$ from Eqs. (6) and (9) gives

$$\Delta\mu = -\frac{(\epsilon_2 - \epsilon_1)}{8\pi} \int_V \left(-\kappa\psi \frac{I_1(\kappa r)}{I_0(\kappa a)} \right) \left(\frac{4\pi\sigma a}{\epsilon_2} \right) \frac{1}{r} r \, dr \, d\phi \, dz \quad (12)$$

$$= \frac{1}{2} \left(1 - \frac{\epsilon_1}{\epsilon_2} \right) \frac{\kappa\psi\sigma a}{I_0(\kappa a)} 2\pi \Delta z \frac{1}{\kappa} (I_0(\kappa a) - I_0(0)) \quad (13)$$

$$= \left(1 - \frac{\epsilon_1}{\epsilon_2} \right) \pi\psi\sigma a \Delta z \left(1 - \frac{I_0(0)}{I_0(\kappa a)} \right), \quad (14)$$

where Δz denotes the overlap length. The tension developed, F_z (from Eq. 2) is

$$F_z = \frac{\Delta U}{\Delta z} = \left(1 - \frac{\epsilon_1}{\epsilon_2} \right) \pi\psi\sigma a \left(1 - \frac{I_0(0)}{I_0(\kappa a)} \right). \quad (15)$$

An approximate value for F_z can now be estimated by assuming $1/\kappa \approx 10 \text{ \AA}$; $a \approx 50 \text{ \AA}$; and $I_0(0)/I_0(\kappa a) \ll 1$ and can be neglected. Because the polarizability of F-actin is very much greater than water, $\epsilon_1/\epsilon_2 \ll 1$, and can be neglected. From Eqs. (11) and (10), the surface charge is approximately

$$\sigma \approx [n(-\Delta G_{(\text{ATP})})/\psi(2\pi ah)], \quad (16)$$

where n is the number of active centers (located in the overlap region) and a is the radius of the thin filament. The quantity h is taken to be half the length of the thick filament because the sarcomere consists of two symmetric halves which are mirror images. The quantity σ can be expressed in terms of the fraction of overlap up to the plateau region¹³ by using the relation

$$n/h = z/\lambda l, \quad (17)$$

where z/l is the fraction of overlap and λ is the distance between neighboring active centers. From Eqs. (15), (16), and (17), the expression for the tension now reduces to

$$F_z \approx \pi\sigma a\psi \quad (18)$$

$$= \pi a \frac{z/l}{\lambda} \left(\frac{-\Delta G_{(\text{ATP})}}{2\pi a} \right) \quad (19)$$

$$= \frac{z/l}{2\lambda} (-\Delta G_{(\text{ATP})}). \quad (20)$$

Assuming $-\Delta G_{(ATP)} \approx 10$ kcal/mole $\approx 10^{-19}$ joule/molecule, and on the basis of two active centers per myosin head $\lambda \approx 150$ Å, then $F_z \approx (4 \times 10^{-12}$ newton) (z/l) per thin filament. If we take the area of a unit cell to be about 10^{-15} m²,

$$F_z \approx (4 \times 10^3 \text{ newton/m}^2)(z/l). \quad (21)$$

The actual tension F_z found experimentally for vertebrate muscle ranges from 1.5 to 4×10^4 newton/m².

* This work was supported by grant GB-8320 from the National Science Foundation and grant AM-13438 from the National Institute of Arthritis and Metabolic Diseases.

† This work was performed during the tenure of a fellowship from the National Institute of Child Health and Development. Address inquiries concerning this communication to Dr. R. M. Dowben, Division of Biomedical Sciences, Brown University, Providence, R.I. 02912.

¹ Huxley, A. F., and R. Niedergerke, *Nature*, **173**, 971 (1954).

² Huxley, H. E., and J. Hanson, *Nature*, **173**, 973 (1954).

³ Elliott, G. F., *J. Theoret. Biol.*, **21**, 71 (1968).

⁴ Shear, D. B., *Physiol. Chem. Phys.*, **1**, 495 (1969).

⁵ Kobayasi, S., H. Asai, and F. Oosawa, *Biochim. Biophys. Acta*, **88**, 528 (1964).

⁶ Kobayasi, S., *Biochim. Biophys. Acta*, **88**, 541 (1964).

⁷ Huxley, H. E., and W. Brown, *J. Mol. Biol.*, **30**, 383 (1967).

⁸ Pepe, F. A., *J. Mol. Biol.*, **27**, 203 (1967).

⁹ Huxley, H. E., *Science*, **164**, 1356 (1969).

¹⁰ Iwazumi, T., personal communication.

¹¹ Imamura, K., T. Kanazawa, M. Tada, and Y. Tonomura, *J. Biochem. (Tokyo)*, **57**, 627 (1965).

¹² Imamura, K., M. Tada, and Y. Tonomura, *J. Biochem. (Tokyo)*, **59**, 280 (1966).

¹³ Overbeek, J. T. G., in *Colloid Science*, ed. H. R. Kruyt (Amsterdam: Elsevier, 1952), vol. 1, pp. 115ff.

¹⁴ Seidel, J. C., *Biochim. Biophys. Acta*, **189**, 162 (1969).

¹⁵ Mattocks, P. W., Jr., G. B. Keswani, and R. M. Dowben, *Biochemistry*, **6**, 3751 (1967).

¹⁶ Gordon, A. M., A. F. Huxley, and F. J. Julian, *J. Physiol.*, **184**, 170 (1966).