FEBS Letters 387 (1996) 101–104

Hypothesis

The stiffness of the crossbridge is a function of the intrinsic protein osmotic pressure generated by the crossbridge itself

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Received 26 February 1996; revised version received 26 April 1996

Abstract A model is presented that makes it possible to determine the stiffness of the crossbridge from protein osmotic stress experiments. The model was elaborated while studying the osmotic properties of F-actin and of myosin subfragment-1 F-actin. These studies showed that the elastic modulus by bending of the monomer is directly related to the intrinsic protein osmotic pressure of the system. At a protein osmotic pressure of 1.8×10^5 dynes/cm^2, the physiological protein osmotic pressure of frog skeletal muscle, it was found that the elastic moduli by bending of the monomer in F-actin and in the myosin subfragment-1 decorated F-actin are 6.5×10^7 and 3.3×10^8 dynes/cm^2, respectively. The value of the elastic modulus by bending of the monomer in the myosin subfragment-1 decorated F-actin compares favorably with the values of the elastic modulus by stretching determined in skeletal muscle fibres.

1. Introduction

Even though it is clear that muscle contractility depends on the properties of the crossbridge proteins and that force generation is associated with the entire crossbridge and not with its separate component proteins, actin is not usually considered to take an active role in muscle contraction. Among the few exceptions are Oosawa, who proposed that conformational changes within the actin monomer might be needed for force generation [1,2], and Schutt et al., who suggested that the actin monomer could be involved actively in muscle contraction through local, reversible, nucleotide dependent, cooperative twist and stretch of the actin filament [3]. More recently, Grazi et al. found evidence that, at the protein physiological osmotic pressure, the interaction of myosin subfragment-1 with F-actin produces a conformational change of any single decorated actin monomer. The conformational change was estimated to account for a 4 nm displacement of the actin filament in relation to the myosin filament [4].

As the natural development of those osmotic stress studies we present here a model that predicts a direct relationship between the intrinsic protein osmotic pressure of the contractile structures and the elastic modulus by bending of the crossbridge. The model also predicts that the contractile force is a function of the intrinsic protein osmotic pressure of the contractile structure itself.

2. Protein osmotic stress as water withdrawal

The main effect of osmotic stress is the transfer of water between two competing compartments, until osmotic equilibrium is reached. As a result of the water withdrawal from one compartment, the concentration of the macromolecular species in that compartment increases. In the case of F-actin, the increase of the concentration leads to a change of phase: i.e. to the organization of the actin filaments into hexagonally packed bundles of filaments [5]. If we now assume that the formation of the bundles occurs without interpenetration of the actin filament (this point will be discussed in the Appendix), the hexagonal packing of the filaments relates the molal concentration of the protein, m, to the average radius of the actin filament, R [6,7] (Fig. 1):

$$R = \sqrt{\frac{[(v\times M_r\times m)+1000+5]}{m\times N\times 2.73\times10^{-7}\times2\sqrt{3}}}\ \text{cm} \qquad (1)$$

where 2.73×10^{-7} is the number of cm of filament per actin monomer [8]; N is the number of Avogadro; v is the partial specific volume, 0.718 cm^3/g for F-actin [9], 0.723 cm^3/g for the myosin subfragment-1 decorated F-actin; M_r is the molecular mass, 42 000 for actin and 157 000 for the myosin subfragment-1 decorated F-actin. Eq. 1 is not valid below the protein osmotic pressure of 10^5 dynes/cm^2. This is because, below this pressure, actin filaments are not homogeneously organized into bundles [7].

Eq. 1 implies, of course, that the radius of the actin filament is a function of the protein osmotic pressure.

3. Protein osmotic stress and the contractile proteins

Osmotic pressure (P) is the rate of change of energy in relation to the volume of all the exchangeable species. Thus changing the volume fraction or concentration of the macromolecular species by applying osmotic pressure is physical work done on that species. This work can be expressed as the chemical potential of the macromolecules subject to stress at the fixed values of the intensive thermodynamic variables pertaining to the particular preparation (temperature T, hydrostatic pressure p, and activities n_i of small molecules):

$$\Delta(T,p,n_i) = -P\Delta V \ \text{(erg)} \qquad (2)$$

where V is the total volume (essentially the water volume) that moves to or from the phase of interest [10].

Thus, when a protein osmotic stress is applied to F-actin [6,7] or to the myosin subfragment-1 decorated F-actin [4], osmotic work is transformed into mechanical work that compresses the filament. The free energy change of the solution in the protein compartment accompanying the simultaneous

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PII S0014-5793(96)00481-4

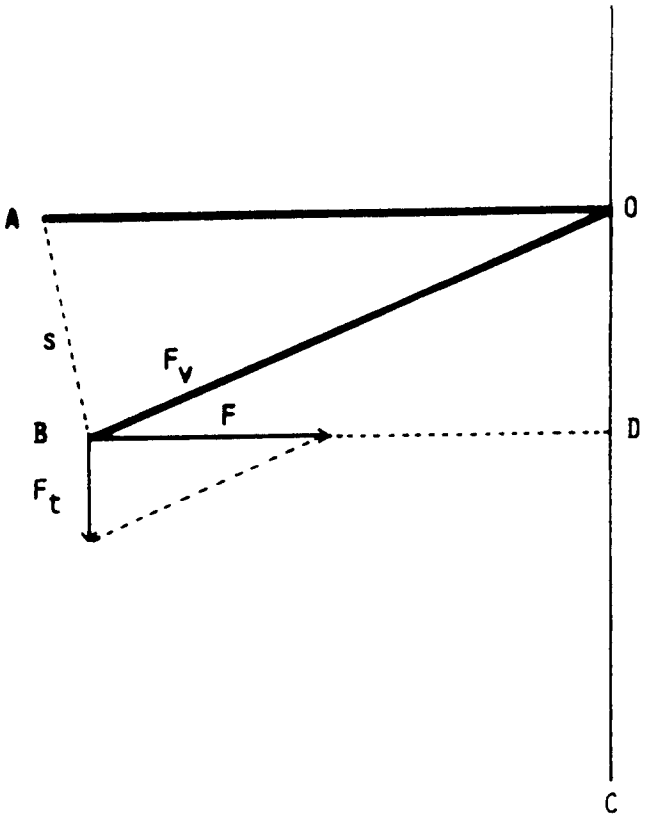

Fig. 1. The orientation of the monomer in the actin filament. The axis of the actin monomer (OA=OB=*l*) is indicated by the boldface line, OC is the axis of the filament; C is the pointed end of the filament; angle (α): AÔC = 90° in the Heidelberg filament, BÔC = 61.5° at 1.8×10^5 dynes/cm^2; OB sin(α) = OD = *R*; AB = *s* is the flexure; *F* is the force orthogonal to the filament axis, acting on each actin monomer; F_v is the component directed toward the constraint; F_t is the component parallel to the filament axis.

compression and water movement is:

$$dG = nFdR - \Delta PdV_w \text{ (erg)} \quad (3)$$

[11], where $n=m\times N$ is the total number of molecules of actin in the filaments; *F* is the force orthogonal to the filament axis, acting on each actin monomer; *R* is the radius of the filament; Δ*P* is the chemical potential difference of water in pressure units and V_w is the water volume.

At the equilibrium:

$$nFdR = \Delta PdV_w \text{ (erg)} \quad (4)$$

Owing to the hexagonal packing of the actin filaments, the water volume, V_w, can be calculated [6,7]:

$$V_w = (2\sqrt{3}\times R^2\times 2.73\times10^{-7}\times m\times N) - (v\times M_r\times m) \text{ (cm}^3\text{)} \quad (5)$$

The force acting on each actin monomer (or decorated actin monomer) thus is:

$$F = 4\sqrt{3}\times 2.73\times10^{-7}\times R\times\Delta P \text{ (dynes)} \quad (6)$$

4. The Young's moduli by bending of the monomer in F-actin and in the myosin subfragment-1 decorated F-actin rigor complex

The force *F* is split into two components: F_v, directed toward the constraint (i.e. the ideal point where the actin monomer meets the filament axis) and F_t, parallel to the filament axis and directed toward its pointed end:

$$F_t = F\times\tan(90-\alpha) \text{ (dynes)} \quad (7)$$

where α is the acute angle formed between the long axis of the monomer and the pointed end of the filament axis. At the equilibrium, the force F_t is balanced by the elastic reaction of the monomer or of the decorated monomer. By making use of Hooke's law [12] it is possible to calculate the Young's moduli by bending, *M*, of the actin monomer and of the myosin subfragment-1 decorated actin monomer:

$$M = \frac{4\times l^3}{3\pi r^4}\times\frac{dF_t}{ds} \quad (8)$$

where the actin monomer and the decorated actin monomer are treated as cylindrical bars supported at one end (i.e. at the filament axis) and where *l* is the length of the particle and *r* is the radius of the particle. The flexure *s* is given by:

$$s = 2\times l\times\sin\frac{(90-\alpha)}{2} \quad (9)$$

To estimate the values of *l* and *r* we first calculated the volume of the actin monomer and of the myosin subfragment-1 decorated actin monomer by making use of their equivalent masses and of their partial specific volumes. The particles were then shaped as cylinders of length (*l*), equal to the radius assumed by the filament when the protein osmotic pressure in the outer compartment is close to zero. Our actin monomer is, therefore, represented by a cylinder of length (*l*) 4.75 nm [13] and of radius (*r*) 1.83 nm, and the decorated actin monomer by a cylinder of length (*l*) 11.15 nm [14] and of radius (*r*) 2.318 nm.

On the basis of these premises, the Young's moduli by

Table 1
Concentration of F-actin and of the myosin subfragment-1–F-actin rigor complex as a function of protein osmotic pressure

Log *P*	F-actin mmolality	S1-decorated F-actin mmolality
4.93	10.6	1.70
4.97	11.9	1.70
5.02	11.85	1.73
5.20	13.8	1.74
5.32	16.9	1.77
5.51	14.6	1.70
5.62	14.6	1.75
5.67	19.0	1.80
5.82	17.1	1.85
5.94	18.0	1.89
6.02	20.7	1.91
6.35	22.2	2.19
6.65	24.0	2.82
6.82	28.2	2.87
6.95	27.7	3.02

Experiments were performed as described in [4]. Protein osmotic pressure is expressed in dynes/cm^2. Molal concentrations are related to the actin monomer and to the 1:1 myosin subfragment-1–actin complex.

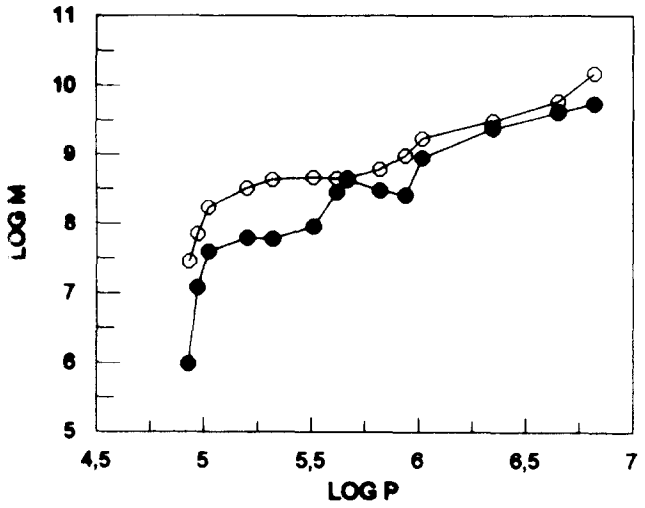

Fig. 2. Elastic modulus by bending of F-actin (●) and of the myosin subfragment-1 decorated F-actin (○) as a function of protein osmotic pressure. P and M are in dynes/cm^2.

bending of the actin monomer and of the myosin subfragment-1 decorated actin monomer are calculated.

5. The elastic moduli by bending of the monomer in F-actin and in the myosin subfragment-1 decorated F-actin increase with protein osmotic pressure

Osmotic stress experiments were performed as previously described [4]. The molal concentrations of F-actin and of the myosin subfragment-1 decorated F-actin, associated with protein osmotic pressures ranging from 8.5×10^4 to 8.9×10^6 dynes/cm^2, were determined Table 1.

To calculate the elastic moduli by bending, F_t and s were first calculated according to Eqs. 7 and 9. The $F_t(s)$ was then searched by a least square fitting program of Mathematica 15] and the dF_t/ds were calculated. A plot of M as a function of the protein osmotic pressure is presented in Fig. 2.

As is shown in Fig. 2, the elastic modulus by bending of the monomer, between the protein osmotic pressures of 10^5 and 5.82×10^6 dynes/cm^2, increases from 4×10^7 to 5.6×10^9 dynes/cm^2 in F-actin and from 1.76×10^8 to 1.48×10^{10} dynes/cm^2 in the myosin subfragment-1 decorated F-actin.

At 1.8×10^5 dynes/cm^2, the physiological protein osmotic pressure in frog muscle [16], the elastic modulus by bending of the monomer is 6.5×10^7 dynes/cm^2 for F-actin and 3.3×10^8 dynes/cm^2 for myosin subfragment-1 decorated F-actin. The latter value is of the same order of magnitude as the elastic modulus by stretching for skinned fibers in frog muscle, 2×10^8 dynes/cm^2 [17], and for glycerinated rabbit psoas fibers in rigor, 2.5×10^8 dynes/cm^2 [18].

Recent X-ray diffraction measurements in contracting muscle have shown filament extensions totalling 2–3 nm per half sarcomere [19,20]. It is thus clear that a crossbridge component (equivalent to our elastic modulus by bending) and a filament component must be considered in stiffness measurements. This finding does not affect the general plan of our hypothesis; however, it does affect the matching between the figures of the elastic modulus by bending and the elastic modulus by stretching of the fiber. These figures, however, will still be of the same order of magnitude since the crossbridge component is estimated to be about 50% of the overall stiffness.

The agreement between the values found in vitro, starting from mixtures of actin and of myosin subfragment-1, and those found in fibers from skeletal muscle is not surprising: the phenomena investigated are in fact the same. Stretching in skeletal muscle involves, beside the sign, the same challenge of the crossbridge as the bending of the monomer in the protein mixture in vitro. Thus bending of the monomer and stretching of the fiber are exactly the same thing. Furthermore, the fact that similar values of the elastic moduli are displayed by such a different systems indicates: (a) that the spontaneous association of the proteins of the contractile structures is a very stringent event; (b) that the mechanical properties of the muscle are essentially determined by the properties of the contractile protein themselves.

We conclude by saying that, as long as the contractile apparatus maintains its integrity, the elastic modulus by bending of the crossbridge is determined by the intrinsic protein osmotic pressure and, consequently, that the contractile force itself is also a function of the protein osmotic pressure.

This conclusion seems to be challenged by the in vitro experiments on single molecule mechanics, where the interaction of a single actin filament with one (or more) molecules of myosin, even though taking place at very low protein concentration (thus at very low protein osmotic pressure), develops a force in the order of magnitude of that developed by a single crossbridge in vivo [21].

The challenge is only apparent since, under the conditions employed above, no force is developed by the interaction of myosin with *native* actin for the very simple reason that F-actin depolymerizes. In an attempt to overcome the drawback, phalloidin F-actin was used [21]. It turns out, however, that decoration with phalloidin dramatically alters the osmotic properties of F-actin and is accompanied by an extensive rearrangement of the F-actin protein solvation water [22]. It is thus hopeless, at least at the present state of our knowledge, to compare meaningfully the behavior of native actin with that of phalloidin-decorated F-actin.

Acknowledgements: This work was supported by the grants 94/40/05/016 and 94/60/05/029 of the Italian Ministero dell'Università e della Ricerca Scientifica.

Appendix

Our model is formulated on the assumption that filaments do not interpenetrate, while being subjected to osmotic stress. The contrary assumption, that filaments do indeed interpenetrate, would make it impossible to relate the change of molal concentration to the change of the elastic modulus by bending of the monomer. We assume in fact that, beyond a given pressure, the change in volume of the solution is accompanied by a change in shape of the filaments. This shape change is modeled by the decrease of the angle α formed between the long axis of the monomer and the pointed end of the filament axis. Other representations of the shape change could be used as well without significantly affecting the results of the calculations.

We know from electron microscope observations that, even at the highest protein osmotic pressure employed in our experiments, the filamentous structure is conserved. We know

also that filaments run parallelly in the bundles. Since the filaments are helicoidal we expect that significant interpenetration would be accompanied by supercoiling of the filaments, a feature incompatible with the observed parallelism of the filaments. We admit, nevertheless, that we cannot, on this basis, exclude a partial interpenetration of the filaments.

The real proof that interpenetration of the filaments is not a significant phenomenon lies, actually, in the physical untenability of the contention that osmotic stress is not accompanied by a change of the shape of the filaments. If this were true, the stiffness of the monomer in the filaments would tend to infinity. This is excluded both by good sense and by the experiments. The elastic modulus by stretching of skeletal muscle fibers has finite values [17,18], which are of the same order of magnitude as the values of the elastic modulus by bending calculated according to our model.

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