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A highly non-ideal solution: the contractile system of skeletal muscle

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Abstract The contractile system is a highly non-ideal solution. The activities of its components must be determined in order to achieve a meaningful representation of cross-bridge kinetics and of chemio-mechanical transduction. Osmotic techniques may help in this respect. A few examples are presented. Protein osmotic pressure influences cross-bridges by determining (1) their free energy minimum, (2) their stiffness and (3) their contractile force.

Key words Contractile system · Skeletal muscle · Non-ideal solution

Introduction

Almost half a century ago the "two Huxleys" (Huxley and Hanson 1954; Huxley and Niedergerke 1954) showed that, in isotonic contraction, thin filaments slide past thick filaments towards the centre of the sarcomere, without a length change of the two types of filaments. At the molecular level the event was explained by cyclic interaction and concomitant conformational change of the heads of myosin with the monomers of actin, coupled, somehow, with the hydrolysis of adenosine triphosphate (Huxley 1969).

Nowadays the proposal is still completely plausible. It is grounded on the intrinsic features of the proteins of the contractile system and assumes a conformational change, which, actually, must follow cross-bridge association. We say plausible because we are not sure that two basic questions were answered: (1) what structures are involved in the conformational change and (2) how chemio-mechanical coupling takes place.

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So far, muscle fibre mechanics has been profitably investigated: the kinetics of myosin and actomyosin in dilute solutions was unravelled (Lymn and Taylor 1971; Eisenberg and Greene 1980; Geeves 1991), the structures of the actin-DNAse complex (Kabsch et al. 1990), of myosin subfragment-S1 (Rayment et al. 1993), and of the profilin-actin complex (Schutt et al. 1993) were solved, and fascinating models of the actin filament were proposed (Holmes et al. 1990). A number of important details were defined but the overall picture of the contraction of skeletal muscle is still lacking. Perhaps a part of the story was overlooked.

The investigation of a non-ideal solution

Ideal and non-ideal solutions

The contractile system is a highly non-ideal solution, where proteins strongly compete for water. This representation, which ignores any structural detail, is probably more adequate than a representation which takes care of the morphology but overlooks the non-ideality of the system. Structure and non-ideality are interconnected. The change of the structure implies a change of the non-ideality of the solution and vice versa. The formation of thin and thick filaments by spontaneous association of actin and myosin, their assembly in the sacomere, the cyclic interaction of cross-bridges, all these events are accompanied by concomitant changes of the non-ideality of the solution. A correct evaluation of the non-ideality of the solution implicitly includes the recognition of the structural detail.

A convenient way to investigate the properties of a solution is to study its osmotic pressure. In fact the change of the chemical potential of the solute is obtained by combining Eqs. (1) and (2):

$$\delta\mu_1 = \mu_1 - \mu_1^0 = -\pi V \tag{1}$$

$$(n_1 \mathrm{d}\mu_1 + n_2 \mathrm{d}\mu_2)_{T,P,n2} = 0 \tag{2}$$

where n_1 and n_2 are the number of moles of water and of the solute, μ_1 and μ_2 are the chemical potentials of water and of the solute, V is the volume of one mole of water (18 cm^3), and π is the osmotic pressure. In an ideal solution the molality of the solute, m_2, is related to the osmotic pressure by the equation:

$$\frac{1000\pi}{RT \times m_2} = 1 \tag{3}$$

Equation (3) holds only for dilute solutions. In more concentrated solutions the ratio deviates from unity and equals the function θ, the molal osmotic coefficient:

$$\frac{1000\pi}{RT \times m_2} = \theta \tag{4}$$

These solutions are defined as non-ideal solutions.

Reasoning on the energetics of a non-ideal solution requires knowledge of the activities and not only of the concentrations of the system's components. In a binary solution the activity coefficient of the solute, α_2, is given by (Edsall and Wyman 1958):

$$\ln \alpha_2 = \int_0^k \frac{(\theta - 1)}{m_2}\, dm_2 + (\theta - 1) \tag{5}$$

Thus, by measuring the osmotic pressure, the free energy change and the activity of the solute can be determined. In particular cases these rules can be extended to systems more complex than binary solutions.

The physical meaning of the non-ideality

Non-ideal behaviour arises when solutes compete for water. This competition, especially in protein solutions, favours in some cases the association between the molecules of the solute, in all cases perturbs their hydration shell, influences their conformation and possibly their function. Thus changing the concentration of a protein solution significantly influences the physico-chemical properties of that protein.

The macromolecular osmotic pressure

Macromolecular osmotic pressure is the fraction of total osmotic pressure generated by the macromolecular components of the system. Macromolecular osmotic pressure is easily measured by secondary osmometry, i.e. by equilibrating through a dialysis membrane a small volume of the protein solution of unknown macromolecular osmotic pressure against a large volume of the reference solution of known macromolecular osmotic pressure. Since small solutes diffuse freely through the dialysis membrane, osmotic stress (the macromolecular osmotic pressure) is due only to the macromolecular components. At equilibrium the macromolecular osmotic pressure of the unknown and of the test solution are the same. Furthermore, since the volume of the unknown solution is much smaller than that of the test solution, the macromolecular osmotic pressure of this latter is essentially equal to its own pressure at the beginning of the experiment. Thus the macromolecular osmotic pressure of the equilibrated protein solution is known.

The limits of the osmotic technique

The weight of the osmotic experiments is that they are at equilibrium; this allows us to relate, directly and without any limiting assumption, the changes of protein concentration to the changes of protein chemical potential. The weakness is that they are at equilibrium; thus they do not allow us to investigate the power stroke. We are confined to investigating the interaction of actin with myosin in the presence and in the absence of non-hydrolysable ligands. Nevertheless, we are able to do this under conditions approaching those of muscle in vivo, and thus to set a borderline for an educated guess on muscle energetics.

The free energy minimum of attached cross-bridges

In the previous section we have shown that, because of the competition for water, rearrangements of structural water and conformational changes are coupled to the increase of protein concentration. We remind here that protein concentration and protein elastic energy are also coupled: to apply an elastic deformation to a protein molecule in a concentrated solution costs much more than applying the same deformation to the same protein molecule in a dilute solution. As an example we will consider an attached cross-bridge, formed by the interaction of actin and myosin.

Both actin and myosin filament suspensions display a non-ideal behaviour. Myosin, 1.67 mM (as monomer), displays a molal osmotic coefficient of 90. The molal osmotic coefficient of actin decreases from 1 to 0.19, at 5 mM actin (as monomer), then increases to ~2.27 at 22 mM actin.

By increasing the protein osmotic pressure from 1.7 to 18 kPa the free energy of F-actin (as actin monomer) increases by $\sim 0.23 k_b T$; that of the myosin filament (as myosin monomer) increases by $\sim 11 k_b T$. The values of 1.7 and of 18 kPa were selected on purpose: 1.7 kPa is the highest value of the protein osmotic pressure, which can be associated with the "dilute" solutions of actin and myosin so far employed in kinetic studies; 18 kPa is the value of the physiological protein osmotic pressure in frog skeletal muscle (Maughan and Gorman 1987). To appreciate the relevance of a free energy change of $\sim 11 k_b T$, we must consider that this is approximately the increase of the free energy of an attached cross-bridge, deformed by 10 nm with respect to the position of minimum free energy (Brenner 1990). So, the level of the

free energy minimum is determined both by the actomyosin association constant and by the protein osmotic pressure. This means that, even in the position of minimum free energy, the cross-bridge is subjected to an elastic stress:

$$dG = nf\,dl - \pi\,dV_w \quad (6)$$

where n is the total number of cross-bridges, f is the elastic force, dl is the deformation, π is the change of the water chemical potential in pressure units, and V_w is the volume of water released or taken up by the system.

We can now state that the position of minimum free energy of a single, attached cross-bridge, embedded in the sarcomere, i.e. at the protein osmotic pressure of 18 kPa, is by $\sim 11 k_b T$ higher than the position of minimum free energy of a single, attached cross-bridge alone, i.e. at the protein osmotic pressure < 1.7 kPa. Furthermore, as we will show rigorously below, the compliance is different in the two cases because the same deformation of 10 nm increases the free energy of the attached cross-bridge by $\sim 11 k_b T$ at 18 kPa (Brenner 1990) and by only $\sim k_b T$ below 1.7 kPa (Fig. 1). Quoting from Duke (1999), albeit in a different context: "the molecule will be an ineffective motor either if the spring were too compliant (because the unitary force will be weak), or if it were too stiff (because the power stroke would fail)". In conclusion, protein osmotic pressure is an additional but important tool for the regulation of muscle contraction.

Sarcomere stretching and free energy of detached cross-bridges

It is generally accepted that stretching a sarcomere in rigor increases the free energy of attached cross-bridges and does not affect the free energy of detached cross-bridges. This opinion stems from the formalism, first introduced by Hill (1974) that:

1. Cross-bridge states are defined by their free energy levels.
2. For an attached cross-bridge state the free energy level is the sum of its free energy without deformation plus the energy due to elastic deformation.

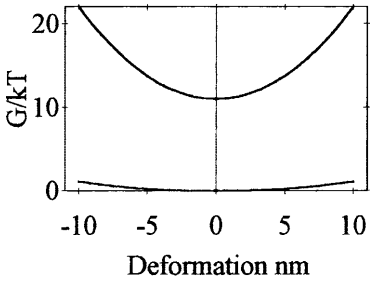

Fig. 1 Free energy minimum and free energy change of a deformed attached cross-bridge as a function of protein osmotic pressure. Linear cross-bridge elasticity assumed. Deformation (x), force (kx), elastic energy ($kx^2/2$) (Brokaw 1976). *Upper curve*: single attached cross-bridge in the sarcomere (18 kPa): $G/k_bT = 11 + k'x^2/2$. The free energy minimum is set to $11RT$. *Lower curve*: single attached cross-bridge (< 1.7 kPa): $G/k_bT = k''x^2/2$. The free energy minimum is arbitrarily set to zero. $k' = 0.11$ nm^{-2}, $k'' = 0.011$ nm^{-2}

According to this definition the free energy of a detached cross-bridge does not change when the sarcomere is stretched [Fig. 4 of Eisenberg and Hill (1978); Figs. 3 and 4 of Eisenberg and Greene (1980); Fig. 3 of Eisenberg et al. (1980); Figs. 4.13, 4.14 and 4.15 of Brenner (1990)]. Unfortunately, this formalism ignores the contribution of water.

Let us consider a volume of the A band of the sarcomere, containing, n_{AT} moles of attached cross-bridges, n_{DE} moles of detached cross-bridges, n_A moles of actin monomers not bound to myosin and n_W moles of water. At constant temperature and hydrostatic pressure the system is defined by:

$$\frac{\delta G}{\delta f} df = n_{AT}\,d\mu_{AT} + n_{DE}\,d\mu_{DE} + n_A\,d\mu_A + n_W d\mu_W \quad (7)$$

where, G, f, n and μ, indicate free energy, force, the number of moles and the chemical potential, respectively. It is also assumed that, while the force is applied, attached cross-bridges do not dissociate, so that the number of moles of the system components does not change.

Now, if we assume that stretch influences only the free energy of associated cross-bridges ($d\mu_W = d\mu_{DI} = d\mu_A = 0$), Eq. (7) becomes:

$$\frac{\delta G}{\delta f} df = n_{AT}\,d\mu_{AT} \quad (8)$$

The mixing of concentrated protein solutions at constant protein osmotic pressure (constant water chemical potential) perturbs water equilibria, as is revealed by the change of the volume of the solutions:

1. At the protein osmotic pressure of 18 kPa, $CaCl_2$ (0.2 mM) induces the release of 38.7 L of water per mol (as actin monomer) of the calcium-regulated actin filament (Schwienbacher et al. 1995).
2. Under the same conditions, 1 mM MgADP induces the release of 187 L of water per mol (as myosin) from a solution of myosin filaments, while the further increase of MgADP to 2 mM induces the uptake of 118 L of water per mol of myosin (Grazi et al. 1998).
3. The formation of actomyosin from myosin filaments and F-actin is accompanied by the uptake of 287 L of water per mol of myosin (Magri et al. 1996).

The experiments reported above were performed at the protein osmotic pressure of 18 kPa, the putative protein osmotic pressure in mucle (Maughan and Gorman 1987). It is thus reasonable to assume that, at least for experiment 3, the chemical potentials of the interacting species were of the same order of magnitude of those of muscle in rigor. The same experiment indicates that in vivo cross-bridge attachment and the subsequent

strain must be accompanied by a significant perturbation of the water solvation layers equilibria of both actin and myosin and, consequently, by the perturbation of the water chemical potential. Thus, a "water" term must be introduced into Eq. (8), which becomes:

$$\frac{\delta G}{\delta f}\mathrm{d}f = n_{\mathrm{AS}}\ \mathrm{d}\mu_{\mathrm{AS}} + n_{\mathrm{W}}\ \mathrm{d}\mu_{\mathrm{W}} \tag{9}$$

There is a basic difference between Eqs. (8) and (9). Equation (8) predicts that stretch is completely taken up by the attached cross-bridges. Equation (9), on the other hand, allows mechanical energy to be in part taken up by the water molecules of the solvation layers of the attached cross-bridges, thus promoting the desorption of water molecules and the increase of their chemical potential. If stretch influences water chemical potential, it influences also the chemical potential of detached cross-bridges and of the actin monomer of the thin filament, not bound to myosin. All these components are stabilised by the interaction with the newly released water molecules, with the decrease of their free energy (Fig. 2). Thus the system is described by Eq. (7), where stretching influences the chemical potential of all four components.

As a conclusion, it is worth pointing out that stretching a protein in dilute solutions certainly has negligible effects on the water chemical potential. The event, on the other hand, is relevant in muscle, where protein concentration is of the millimolar order and the competition between proteins for water is very strong.

Protein osmotic pressure and the elastic Young's modulus by bending of the myosin-S1-decorated actin monomer

In this section we show that protein osmotic pressure determines the elastic properties of the myosin-S1-decorated actin filament. By making use of a simple model, which represents the elastic stress on the complex as a function of the bending of S1 with respect to the longitudinal axis of the actin filament, we calculate the Young's modulus by bending of this distortion and show that it increases from 13.3 MPa to 2.05 GPa when protein osmotic pressures increases from 9 kPa to 890 kPa. That is to say that the compliance of the actin-S1 complex (attached cross-bridge) decreases with the increase of protein osmotic pressure, a feature that bears a direct relationship to the mechanical properties of the sarcomere. We are reminded (see above sections) that changing the protein osmotic pressure does not necessarily require changing the total protein concentration; it is also achieved in the formation and dissociation of the actomyosin (cross-bridge attachment-detachment process).

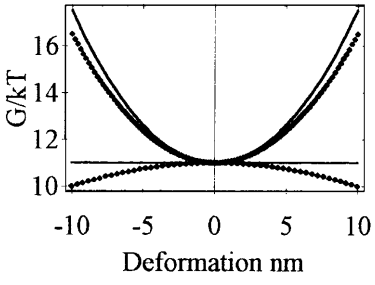

Fig. 2 Free energy of attached and detached cross-bridges as a function of the sliding of the thin past the thick filament. Linear cross-bridge elasticity assumed. Deformation (x), Force (kx), elastic energy ($kx^2/2$) (Brokaw 1976). Hill's formalism (*solid line*): attached cross-bridge (Eq. 7): $G/k_{\mathrm{b}}T = 11 + kx^2/2$; detached cross-bridge: $G/k_{\mathrm{b}}T = 11$. Formalism including water effects (*dotted line*): attached cross-bridge (Eq. 6): $G/k_{\mathrm{b}}T = 11 + k'x^2/2$; detached cross-bridge: $G/k_{\mathrm{b}}T = 11 - k'x^2/2$; free energy at $x = 0$ is the free energy of myosin at 18 kPa. $k = 0.13$ nm^{-2} is taken from Brenner (1990); $k' = 0.11$ nm^{-2} is selected to provide a small free energy difference between the representation by Hill's formalism and the representation by the formalism including water effects; $k'' = k - k'$

By increasing the macromolecular osmotic pressure, water is withdrawn and the volume of the solution decreases. Above a give pressure, most of the volume of the solution is occupied by the decorated filaments, which are forced to stack, their lengths paralleling each other. Under these conditions, simple trigonometric considerations (Grazi et al. 1993; Schwienbacher et al. 1995; Grazi et al. 1996b) relate the volume of the solution to the radius, R, of the S1-decorated actin filament as follows:

$$V = n_{\mathrm{A}} \times N \times 2\sqrt{3} \times R^2 \times 2.73 \times 10^{-9}\ \mathrm{m}^3 \tag{10}$$

where n_{A} is the number of moles of actin, N is the Avogadro number and 2.73×10^{-9} m is the axial repetition of the actin monomer along the genetic helix (Hanson and Lowy 1963).

In the model the S1-decorated actin monomer is shaped as a cylinder of length l = 11.15 nm (Milligan et al. 1990). The radius of the cylinder, $r = 2.318$ nm, is calculated from the length, the mass of the monomer and its partial specific volume. In the model the effects of protein osmotic pressure on the shape of the filament are explained by changes of the angle, α, formed between the S1-decorated monomer and the pointed end of the longitudinal axis of the filament (Fig. 3). At a protein osmotic pressure below 1.7 kPa, the angle $\alpha = \mathrm{AOC}$ is assumed to be 90° and the radius of the decorated filament, R, is assumed to be equal to l. By increasing the protein osmotic pressure, the angle α is assumed to decrease from AOC to BOC and the radius of the decorated actin filament to decrease from $R = l = \mathrm{AO}$ to $R = \mathrm{BD} = l \sin \alpha$.

Protein osmotic pressure is related to the elastic force applied to each S1-decorated actin monomer by:

$$\mathrm{d}G = nf\ \mathrm{d}R - \pi\ \mathrm{d}V_w \tag{11}$$

where $n = n_{\mathrm{A}} \times N$ is the total number of actin monomers in the filaments, f is the force applied to each monomer, orthogonally to the axis of the filament, and R is the radius of the filament. At equilibrium, $nf\mathrm{d}R = \pi \mathrm{d}V_{\mathrm{w}}$; thus:

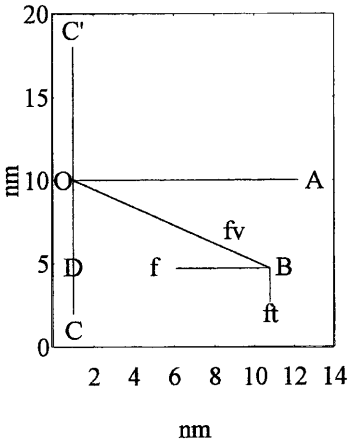

Fig. 3 Orientation of the myosin-S1-decorated actin monomer in the actin filament. CC′, longitudinal axis of the filament; C, pointed end; $l = OA = OB = 11.15$ nm, longitudinal axis of the myosin-S1 decorated actin monomer (Milligan et al. 1990); angle α, $AOC = 90°$ at a protein osmotic pressure < 1.7 kPa (Holmes et al. 1990), $BOC = 61.5°$ at 18 kPa; $OB \times \sin \alpha = BD = R$, radius of the filament; f, force, orthogonal to the axis of the filament, applied to each monomer; f_v, component directed against the constraint; f_t, component to the axis of the filament

$$f = (\pi/n) \times dV_w/dR \tag{12}$$

Since the volume of the solution is $2\sqrt{3}R^2 \times 2.73 \times 10^{-9} \times n_A \times N$ and the volume of the protein is $v \times n_A \times N$, where v is the partial specific volume of the S1-decorated F-actin, 0.723×10^{-3} m^3/kg, the water volume is:

$$V_W = \left(2\sqrt{3}R^2 \times 2.73 \times 10^{-9} \times n_A \times N\right) - (v \times n_A \times N) \tag{13}$$

and

$$\frac{dV_W}{dR} = 4\sqrt{3} \times 2.73 \times 10^{-9} \times N \times n_A \times R \tag{14}$$

The force (in N), f, acting on each monomer is:

$$f = 4\sqrt{3} \times 2.73 \times 10^{-9} \times R \times \pi \tag{15}$$

The force f is divided into two components: the first, f_v, is directed toward the constraint and the second, f_t,

$$f_t = f \times \frac{\cos \alpha}{\sin \alpha} \tag{16}$$

is balanced by the reaction of the S1-decorated actin monomer and is directed toward the pointed end of the filament (Fig. 3).

To calculate the elastic modulus by bending, the S1-decorated actin monomer is treated as a cylindrical bar held at one extreme. According to Hooke's law the Young's modulus by bending, M, is:

$$M = \frac{4 \times l^3}{3\pi r^4} \times \frac{\Delta f_t}{\Delta s} \tag{17}$$

where

$$\Delta s = 2 \times l \times \sin \frac{\Delta \alpha}{2} \tag{18}$$

The $\frac{\Delta f_t}{\Delta s}$ are calculated numerically for $\Delta \alpha = 0.1°$. The elastic modulus by bending of the S1-decorated actin monomer increases almost linearly ($\sim$2500 Pa/Pa) as a function of protein osmotic pressure, being 13.3 MPa at 9 kPa and 2.05 GPa at 890 kPa. This indicates that the stiffness of the S1-decorated actin monomer is severely controlled by the increase of protein osmotic pressure, i.e. by the increase of the competition for water.

At the protein physiological osmotic pressure, 18 kPa, the elastic modulus by bending of the S1-decorated actin monomer is 22–24 MPa, a value comparable to those of 20 MPa for frog muscle (Yamamoto and Herzig 1978) and of 25 MPa for rabbit muscle (Tawada and Kimura 1984) obtained for the elastic modulus by stretching of muscle fibre: good evidence for the correctness of our model (Grazi et al. 1996a, b).

Protein osmotic pressure and the "contractile force" developed by the S1-decorated actin monomer

As was made clear above, osmotic pressure techniques, being at equilibrium, do not allow direct study of the power stroke and the cyclic attachment-detachment process. Nevertheless, the attachment of S1 to F-actin to form the rigor complex is somewhat similar to a single power stroke. At the protein osmotic pressure of 18 kPa the equilibrium position of the angle α is 61.5° in the actin filament and 84° in the S1-decorated actin filament. The formation of the S1-actin complex thus involves the shift of α from 61.5°, the value at time zero, to 84°, the value at equilibrium (Grazi et al. 1994). This shift produces a force of the same orientation and of the same order of magnitude as that of the power stroke (Grazi et al. 1996b).

At a given protein osmotic pressure, each structure is elastically compressed and the deformation depends on its own compliance. In our system the deformation is produced by the force f_t (Eq. 16) parallel to the longitudinal axis of the filament (Fig. 3) and is balanced by the elastic reaction of the body.

When the S1-actin complex first forms (cross-bridge attaches), the initial deformation is $\alpha = 61.5°$ and corresponds to the elastic reaction, Er_1. The deformation then decreases to the equilibrium value ($\alpha = 84°$) and corresponds to the elastic reaction, Er_2, balanced by the experimental protein osmotic pressure of 18 kPa. The "contractile force", at the constant protein osmotic pressure of 18 kPa, is thus given by:

$$\text{Contractile force} = Er_1(\alpha = 61.5°) - Er_2(\alpha = 84°) = \sim 4.66 \text{ pN} \tag{19}$$

The "contractile force" is estimated by rearranging Eq. (17), on the assumption that the elastic Young's

modulus by bending, 22–24 MPa, calculated at the protein osmotic pressure of 18 kPa and $\alpha = 84°$, holds also for $\alpha = 61.5°$. The value so obtained, ~4.66 pN, is probably underestimated because it is likely that the Young's modulus by bending changes with the deformation. Nevertheless, the force we have estimated is comparable to that developed by a single cross-bridge in skeletal muscle: 2.4–4.7 pN (Oplatka 1972) or 8 pN (Merah and Morel 1993). In situ the force would be directed toward the barbed end of the actin filament and would cause the sliding of the filament toward the centre of the sarcomere (Grazi et al. 1996b).

The in vitro motility assay

We have provided evidence that water chemical potential, alias protein osmotic pressure, alias protein concentration, determines the elastic properties of the contractile proteins, thus influencing the mechanic behaviour of the contractile structure. A corollary of this view is that disrupting the sarcomere structure harms the functioning of the contractile apparatus. Our conclusion seems to be contradicted by the in vitro motility assays, which are performed at extremely low protein osmotic pressure. These assays, by measuring piconewton forces and nanometer steps, raise the hope of understanding the mechanics of the interaction of single myosin molecules with single actin filaments (Harada et al. 1987; Toyoshima et al. 1989; Harada et al. 1990; Tanaka et al. 1992; Finer et al. 1994; Molloy et al. 1995; Dupins et al. 1997). We too are fascinated by these techniques. However, demonstration is still awaited that what is going on at the single molecule and filament level bears any relationship with what is going on in muscle. The basic weakness of the in vitro motility assay is the use of phalloidin F-actin. Using phalloidin-decorated F-actin to study the actin filament is like using silk to study wool. Phalloidin F-actin and F-actin display distinctly different osmotic properties (Cuneo et al. 1995). The critical concentration of phalloidin F-actin is at least one order of magnitude lower than that of F-actin (Kishino and Yanagida 1988). Since the critical concentration (i.e. the dissociation constant of the elongation reaction) is the main determinant of the free energy of the monomer-monomer interaction, it must also influence the tensile strength of the actin filament. This is in fact the case: critical concentration and tensile strength are inversely related (Adami et al. 1999), and the tensile strength of phalloidin F-actin is much larger than that of F-actin. Since the behaviour of F-actin and of phalloidin F-actin are different it does not seem productive to discuss why phalloidin F-actin displays good performances at very low protein osmotic pressure. We are, on the other hand, ready to discuss the performance of F-actin in the in vitro motility assay. Unfortunately, nobody is using F-actin, for the very simple reason that it does not work in these assays.

Conclusion

Contractile force is measured directly and precisely. The concomitant displacement of protein masses is measured by X-ray diffractometry, sometimes with ambiguity. The partition of free energy among contractile proteins cannot be determined, unless the non-ideality of the contractile system is properly recognized. Reasoning on non-ideal systems requires the knowledge of activities. Determination of activities requires an osmotic approach to the system. Somewhat primitively, but mimicking the non-ideality of skeletal muscle, we have followed this pathway. Protein osmotic pressure was found to influence cross-bridges by determining: (1) their free energy minimum, (2) their stiffness and (3) their contractile force. Even though working with protein mixtures, the values of the elastic modulus by bending and of the "contractile force", calculated according to our model, match those determined directly on muscle fibres.

Osmotic pressure measurements are slow, hold at the equilibrium, apply to binary solutions and, in some cases, to ternary solutions. Certainly they cannot solve directly a complex system such as the contractile apparatus. Nevertheless, the skilled application of this technique may help to obtain a reasonable sketch of the free energy changes in vivo. The pathway is narrow but is a forced one since predictions in non-ideal systems are made a posteriori. The study of the thermodynamics of muscle fibre must supplement the study of muscle fibre mechanics. The activities of the components of the system must be determined, to allow the study of cross-bridge kinetics and the unravelling of chemio-mechanical transduction.

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