Biophys. Struct. Mech. 7, 17–40 (1980)

Biophysics of Structure and Mechanism

A Quantitative Analysis of Elastic, Entropic, Electrostatic, and Osmotic Forces Within Relaxed Skinned Muscle Fibers

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Abstract. The elastic behavior of mechanically skinned skeletal muscle fibers in relaxing solution is modelled using equations developed by Flory (1953) for the elasticity of non-biological polymers. Mechanically, the relaxed skinned fiber is considered to be a semi-crystalline network of inextensible polymer chains, which are periodically cross-linked and which are bathed in an aqueous medium. We consider (1) configurational elastic forces in the network, (2) entropic forces due to mixing of polymer and water, (3) electrostatic forces due to fixed charges on the muscle proteins and mobile charges in the bathing solution, and (4) compressive forces due to large colloids in the bathing solution. Van der Waals forces are not considered since calculations show that they are probably negligible under our conditions. We derive an expression which relates known quantities (ionic strength, osmotic compressive pressure, and fiber width), experimentally estimated quantities (fixed charge density and volume fraction of muscle proteins), and derived quantities (concentration of cross-links and a parameter reflecting the interaction energy between protein and water).

The model was tested by comparison with observed changes in skinned fiber width under a variety of experimental conditions which included changes in osmotic compressive pressure, pH, sarcomere length, and ionic strength. Over a wide range of compressive pressure (0–36 atm) the theory predicted the nonlinear relation between fiber width and logarithm of pressure. The direction and magnitude of the decrease in width when pH was decreased to 4 could be modelled asssuming the fixed charge density on the protein network was 0.34 moles of electrons per liter protein, a value in accordance with the estimates of others. The relation between width and sarcomere length over the complete range of compressive pressures could be modelled with the assumption that the number of cross-links increases somewhat with sarcomere length. Changes of width with ionic strength were modelled assuming that increasing salt concentration both increased the

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0340-1057/80/0007/0017/$ 04.80

electrostatic shielding of fixed charges and decreased the number of cross-links. The decrease of fiber width in 1% glutaraldehyde was modelled by assuming that the concentration of crosslinks increased by some 10%. The theory predicted the order of magnitude but not the detailed shape of the passive tension-length relation which may indicate that, as with non-biological polymers, the theory does not adequately describe the behavior of semi-crystalline networks at high degrees of deformation.

In summary, the theory provides a semiquantitative approach to an understanding of the nature and relative magnitudes of the forces underlying the mechanical behavior of relaxed skinned fibers. It indicates, for instance, that when fibers are returned to near their in vivo size with 3% PVP, the forces in order of their importance are: | elastic forces | ~ | entropic forces | > | electrostatic forces | ~ | osmotic compressive forces |.

Key words: Muscle thermodynamics – Stiffness – Electrostatic force – Van der Waals force swelling – Polyvinylpyrrolidone

Introduction

Our previous work with stretch and radial compression of relaxed skinned muscle fibers of the frog suggested to us that the fibers behaved as elastic bodies (Maughan and Godt 1979). Anatomically, muscle is a complex arrangement of protein filaments, which makes the problem of describing its elastic behaviour fraught with difficulty. In order to reduce the problem to a tractable form, we put forward a simple though quantitative biophysical model for the relaxed skinned fiber, based upon the classical statistical thermodynamic model of polymer elasticity provided by Flory (1953), with modifications appropriate for skinned fibers under our conditions.

For our purposes, we shall consider the elastic behavior of relaxed skinned fibers to be that of a random collection of long-chain polymers, periodically cross-linked, which bear a net negative charge when bathed in physiological ionic solutions at neutral pH. The size of this polymeric network can be influenced by osmotic compressive forces arising from the inclusion in the bathing medium of large random-coil polymers which cannot penetrate this network. This simple model provides a semi-quantitative explanation for the behavior of relaxed skinned fibers under a wide variety of experimental conditions. Moreover, it gives insight into the nature and the relative contribution of the primary forces involved in the elastic behavior of relaxed skinned fibers.

Theory

Consider the skinned fiber in relaxing medium to be a three-dimensional network of charged polymers. Following closely Flory's thermodynamic theory

for the swelling of such networks under constant external pressure, the Gibbs free energy change between the initially pure, unstrained polymeric network of muscle proteins and the final swollen network in equilibrium with the solvent water, consists of four parts: 1) the free energy of initial mixing of pure solvent and polymer, ΔF_m, 2) the elastic free energy, ΔF_{el}, arising from subsequent expansion of the network, 3) the electrostatic free energy, ΔF_{es}, which arises since the network is charged and bathed in an ionic solution and, finally, 4) the osmotic free energy, ΔF_{os}, which must be considered when the volume of the network is constrained by a constant osmotic force (this latter term is important for modelling the behavior of skinned fibers in solutions containing large longchain polymers). Therefore,

$$\Delta F = \Delta F_m + \Delta F_{el} + \Delta F_{es} + \Delta F_{os} .$$

The chemical potential of the solvent in the swollen network is given by

$$\mu_1 - \mu_1^0 = \left(\frac{\partial \Delta F_m}{\partial n_1} + \frac{\partial \Delta F_{el}}{\partial n_1} + \frac{\partial \Delta F_{es}}{\partial n_1} + \frac{\partial \Delta F_{os}}{\partial n_1} \right)_{T,P} , \tag{1}$$

where n_1 is the number of moles of solvent molecules in the solution, T is absolute temperature and P is the total pressure on the system. From Flory (1953):

$$\left(\frac{\partial \Delta F_m}{\partial n_1} \right)_{T,P} = RT[\ln(1-v_2) + v_2 + \chi_1 v_2^2] , \tag{2a}$$

where v_2 is the volume fraction of solute (i.e., polymer) in the swollen network in equilibrium with pure solvent (i.e., water), χ_1 is a dimensionless quantity reflecting the first-neighbor interaction free energy, and R is the gas constant. Expression 2a is derived from a statistical thermodynamic treatment of polymer solutions according to the Flory-Huggins liquid lattice theory. The quantitiy $RT\chi_1$ represents the difference in energy of a solvent molecule immersed in pure polymer ($v_2 \simeq 1$) compared with one surrounded by molecules of its own kind; i.e., in the pure solvent (Flory 1953).

Also from Flory (1953),

$$\left(\frac{\partial \Delta F_{el}}{\partial n_1} \right)_{T,P} = RTV_1 \cdot \frac{v_e}{V_0} [v_2^{1/3} - v_2/2] , \tag{2b}$$

where v_e is the effective number of moles of polymer chains in the network, V_0 is the volume of the unswollen (undeformed) network, and V_1 is the molecular volume of water. This expression comes from consideration of the entropy change associated with the configurational change of the network.

Finally, from Flory (1953),

$$\left(\frac{\partial \Delta F_{es}}{\partial n_1} \right)_{T,P} \simeq - RTV_1 \cdot \left(\frac{i}{V_u} \right)^2 \cdot \frac{v_2^2}{4I} , \tag{2c}$$

where i/Vu is the number of moles of electronic charges per polymer unit, V_u is the molecular volume of the polymer unit, and I is the ionic strength of the bathing medium. This expression comes from a consideration of the osmotic pressure due to the counter-ions associated with the fixed charges on the polymer bathed in a 1:1 electrolyte. The approximation given above assumes that the charge on the polymer is not high; i.e., $ic_2 < I$, where c_2 is the molar salt concentration within the network. This criterion is easily satisfied in muscle (Elliott 1973).

In the presence of an osmotic constraining pressure, we have the additional term

$$\left(\frac{\partial \Delta F_{os}}{\partial n_1}\right)_{T,P} = V_1 \Pi, \tag{2d}$$

where Π is the colloid osmotic pressure exerted on the network by the medium bathing the fiber.

In the fully swollen network at equilibrium, $\mu_1 = \mu_1^0$. Therefore, Eq. (1) with rearrangement becomes

$$RTV_1 \cdot \left(\frac{i}{V_u}\right) \cdot \frac{v_2^2}{4I} - V_1 \cdot \Pi - RT \cdot [\ln(1-v_2) + v_2 + \chi_1 v_2^2]$$
$$-RT\, V_1 \cdot \frac{v_e}{V_0} \cdot (v_2^{1/3} - v_2/2) = 0. \tag{3}$$

In order to assess whether this expression describes the behavior of relaxed skinned fibers as a function of the explicit parameters given, we have conducted the series of experiments described in the following sections. We have not considered the contribution of Van der Waals forces since calculations (given in Discussion) indicate that, under our conditions, Van der Waals forces are neglible relative to the osmotic compressive forces.

Methods

Skinned fibers were prepared from semitendinosus muscle of the frog *Rana pipiens*. Details of the skinning and mounting procedures as well as the experimental apparatus and techniques for monitoring fiber width and sarcomere length are described in a previous paper (Maughan and Godt 1979). We took care to select only those fibers which, after skinning, had a cylindrical appearance. The standard relaxing solution used contained (in mM): 61.2 KCl, 5 EGTA, 20 imidazole, 15 creatine phosphate, 8.06 $MgCl_2$, 3.04 Na_2ATP (yielding 3 Mg^{2+} and 3 MgATP, using MgATP and MgHATP binding constants given by Phillips et al. 1966, and Khan and Martell 1966), pH 7.00, ionic strength (I) 0.15 M. Some early experiments were conducted with similar solutions which contained instead: 54 KCl, 7 EGTA, 20 imidazole, 15 creatine phosphate, 2.80 $MgCl_2$, 2.14 Na_2ATP (yielding 0.5 Mg^{2+} and 2 MgATP), pH 7.00, I 0.15 M. Further details on calculation of ionic composition of solutions

are given in Godt (1974). In some of the experiments with variation of pH, imidazole was replaced by tris maleate buffer with 20 mM maleate and (in mM) 39.8, 29.4, 21.6, 20.5, and 20.0 tris for pH values of 7, 6, 5, 4.5, and 4, respectively. Total concentrations of $MgCl_2$, $NaCl_2$, Na_2ATP and KCl were altered to maintain 1 mM Mg^{2+}, 3 mM MgATP (or 0.5 mM Mg^{2+}, 2 mM MgAtP) and ionic strength 0.15 M in these solutions. In some cases, ionic strength was altered by changing the concentration of KCl. Two fibers were fixed by immersion in standard relaxing solution containing 1% glutaraldehyde. The osmotic pressure, Π, of solutions containing polyvinyl-pyrrolidone (PVP-40) was estimated from the osmometric data of Vink (1971). The volume fraction of polymeric protein network, v_2, was estimated in the following way: if length is kept constant then, assuming that the fiber has a circular cross-section, (cf. Maughan and Godt 1979), v_2 is equal to D_0^2/D^2, where D is the width of the fiber at a given point in solution and D_0 is the width at that same point of the dehydrated fiber. The dehydrated width was determined by first transferring the fiber to a relaxing solution containing 20% PVP. This caused the fiber to shrink and to become rigid without change of sarcomere length. The fiber was then transferred to air and allowed to dehydrate for a period of 15–30 min, until there was no further change in fiber width. At this time the fiber was photographed. Our confidence that fibers were nearly completely dehydrated by this procedure was bolstered by the observation that one fiber (# 11–17–78) which was taken through this dehydration procedure did not change diameter upon immersion in 100% ethanol or, subsequently, in high vacuum over a one hour period in a scanning electron microscope. It is noteworthy that the dehydration procedure appeared to be very nearly reversible, since fibers rehydrated in PVP-containing relaxing solutions generally resumed their former shape, with orderly striations similar to that observed previously. Furthermore, transmission electron micrographs of rehydrated fibers appeared normal. Rehydrated fibers could be repeatedly activated in calcium-containing solutions (10^{-4} M Ca^{2+}), although the force was appreciably less than the force developed by fibers which had not been dehydrated. For example, when fiber # 11–17–78 was rehydrated, it could be repeatedly activated with 10^{-4} M Ca^{2+} to generate 3.5×10^4 N/m^2 tension at approximately 2.6 μm, a force level which is roughly 30% of that expected for fresh skinned fibers at the same sarcomere length (Schoenberg and Podolsky 1972).

In 16 fibers, we found that, at a given sarcomere length, D_0 was 0.63 ± 0.03 (SD) of the width of the fiber bathed in 20% PVP relaxing solution ($D_{20\%}$). Therefore, for those cases in which we had no direct measure of D_0, we took as a nominal estimate $D_0 = 0.63\ D_{20\%}$.

Results

Changes in Polymer Concentration and Sarcomere Length

Our aim was to examine the adequacy of Eq. (3) to describe the relation between fiber width and changes in osmotic pressure, ionic strength, network charge (as

influenced by pH) and the number of cross-links. In Eq. (3), the fiber width, D, appears as v_2 which we have assumed to be equal to D_0^2/D^2, where D_0 is the width of the dehydrated fiber. Of the variables in the equation, we know the ionic strength of the solution (I), the colloid osmotic pressure (Π), the molar volume of water (V_1), the temperature (T) and the gas constant (R). We can experimentally determine v_2. We do not know the interaction paramter (χ_1) or the concentration of chains in the network (ν_e/V_0) referred to the dehydrated volume. We also do not know the ratio i/V_u, that is, the number of fixed charges per polymer unit.

Consider first the number of moles of fixed charges per polymer unit, i/V_u. The key assumptions are that the charges are uniformily distributed on the polymer network and that the charge concentration on the network is equal to the concentration of fixed charges on the myofilaments; i.e., that myofilaments are typical network elements. From amino acid analysis of myofilament proteins and myofilament concentration in intact muscle, Elliott (1973) has estimated that the concentration of charge in intact muscle due to the myofilaments is 40 meq of negative charge per liter. Total muscle protein is approximately 70% contractile protein (Elliott 1973), so we have provisionally taken total fixed charge concentration to be 40/0.7 = 57 meq/l. In order to apply this value to skinned fibers, we note from our previous work (Maughan and Godt 1979) and from the work of Magid (unpublished, cf. Maughan and Godt 1979) that fibers returned to their in situ size in a solution containing approximately 3% PVP. Thus the concentration of fixed charges referred to the dehydrated volume can be simply determined from the ratio of skinned fiber width in 3% PVP ($D_{3\%}$) to its dehydrated width, i.e., i/V_u = (57 meq/l) $D_{3\%}^2/D_0^2$, treating the fiber as having a circular cross-section (cf. Methods). In 10 fibers we found that $D_0/D_{3\%}$ is 0.41 ($\pm$ 0.04). Thus we have used a tentative estimate of 57 meq/l/$(0.41)^2$ = 0.34 eq/l for i/V_u at near neutral pH.

Focussing next on the problem of determining χ_1, we can use information obtained from the interaction of polymer and solvent for other polymer systems. Experimental observations indicate that χ_1 is a linear function of the volume fraction (v_2) of polymer (Flory 1953, Fig. 111). We shall assume that the polymer network of muscle behaves similarly. Therefore, our approach was to rearrange Eq. (3) and to solve explicitly for χ_1 as a function of v_2, at various values of ν_e/V_0. The parameter v_2 is derived experimentally from data on fibers at constant sarcomere length in solutions with varying colloid osmotic pressure. The procedure was to guess a value of ν_e/V_0 and to calculate χ_1, for each v_2. Then the χ_1 vs. v_2 data was then fitted by linear regression to the equation:

$$\chi_1 = a_0 + a_1 v_2 \,. \tag{4}$$

That range of values of ν_e/V_0 which gave a correlation coefficient (r) greater than 0.99 was chosen for further analysis. We returned to Eq. (3) using the linear relation for χ_1 and a value of ν_e/V_0 from this range. To arrive at a final value of ν_e/V_0, we chose from the range that ν_e/V_0, and the associated a_0 and a_1, which gave the best fit of the experimental data with a plot of fiber diameter (D) versus ln Π/Π_0, as determined from Eq. (3). An example of the linearization procedure is given in Fig. 1 and the final fitting of theory and experiment in Fig. 2.

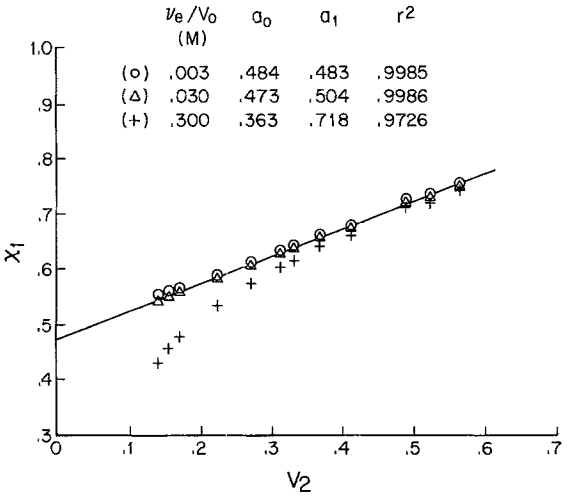


Fig. 1. Values of the interaction parameter χ_1 plotted against the volume fraction v_2 of muscle protein polymer, according to Eq. (3). Data (symbols) given at three different assumed values of polymer chain concentration v_e/V_0; with i/v_u = 0.34 M, I = 0.15 M, D_0 = 27.3 μm, sarcomere spacing = 3.05 μm, and temperature 21.0° C. Fiber 10/11/78. Volume fraction $v_2 = (D_0/D)^2$, where D_0 is the width of the dehydrated fiber and D the width of the hydrated at any given PVP concentration. The straight line is drawn according to the relation $\chi_1 = a_0 + a_1 v_2$, for the case of v_e/V_0 = 0.03 M, where the values of a_0 and a_1 are given in the figure. Note how much poorer the fit is to the linear relation at v_e/V_0 = 0.3 M

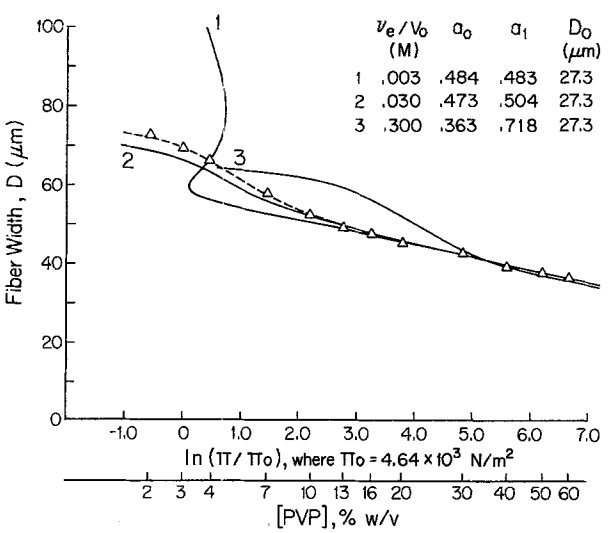


Fig. 2. Plot of fiber width D vs. ln (Π/Π_0), computed from Eqs. (3) and (4), given the values of the parameters listed. The osmotic pressure $\Pi_0 = 4.64 \times 10^3$ N/m^2; i.e., the colloid pressure exerted by a 3% PVP solution (Vink 1972). Curves 1, 2, and 3 correspond to the three different values of v_e/V_0 of Fig. 1, where i/V_u = 0.34 M, I = 0.15 M, and temperature = 21.0° C. The dashed line refers to the case where v_e/V_0 = 0.028 M, with the value of the other constants being those given for curve 2. The symbols are data points from fiber (10/11/78) at a sarcomere spacing of 3.05 μm. Note the good correspondence between data and theory for v_e/V_0 = 0.030 M, and especially 0.028 M, compared with wide departures at the other two values.

Note that the plot of χ_1 vs. ν_2 in Fig. 1 is a coarse measure of ν_e/V_0. Much finer criteria for ν_e/V_0 come from the D vs. ln Π/Π_0 plot in Fig. 2, where the correspondence of theory and data is especially sensitive to the choice of ν_e/V_0 at low and intermediate values of ln Π/Π_0.

Having fitted the equation to data at one sarcomere length, how well does it fit at other sarcomere lengths? The only variable which we will allow to change is the dehydrated width, D_0, which we know experimentally does vary with sarcomere length. Figure 3 shows an example for three sarcomere lengths, including the intermediate sarcomere length shown in Figs. 1 and 2. Note the good agreement at the longest sarcomere length. The fit, however, was not as good at the shortest sarcomere length, departing especially at lower osmotic pressure. The fit could be made better at shorter sarcomere lengths by decreasing ν_e/V_0 slightly, i.e., by assuming that the number of chains decreases with decreasing sarcomere length. Similar results were obtained for 13 other fibers, whose values used for fitting are given in Table 1.

In our previous paper, we examined the relation between D and ln Π and found that over a limited range of Π (that is, for 2–10% PVP), the relationship was approximately linear. In this study, we carried out the experiment over a much greater range of concentrations of PVP (up to 60%) and found a distinctly nonlinear relationship (cf. Figs. 2 and 3). Over the larger range our theoretical expression fits the overall shape of the experimental data better than the empirical linear expression.

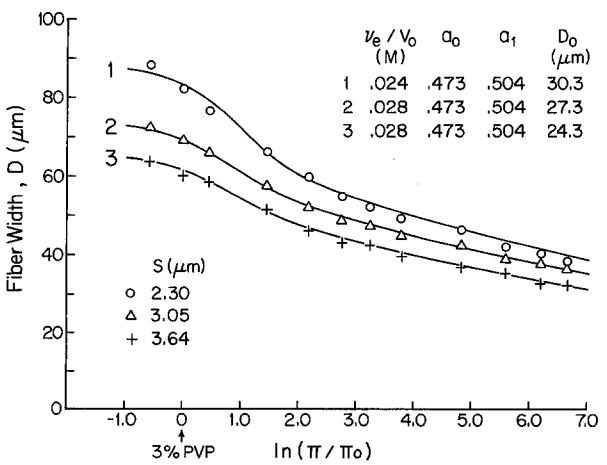


Fig. 3. Fiber width D vs. ln Π/Π_0 at three sarcomere spacings (s) for the fiber of Figs. 1 and 2. Symbols, experimental data points; curves, computed from Eqs. (3) and (4) using the values of the parameters listed and $i/V_u = 0.34$ M, $I = 0.15$ M, and temperature = 21.0° C. Note the good fit of curves to data assuming that ν_e/V_0 increases slightly with sarcomere spacing.

Table 1. Parameters a_0 and a_1 of the interaction variable χ_1, where $\chi_1 = a_0 + a_1 v_2$, for 14 fibers. The volume fraction of muscle protein $v_2 = (D_0/D)^2$ (cf. text). The corresponding sarcomere spacings and chain concentrations (ν_e/V_0) are also given. The symbol r refers to the Pearson product-moment correlation coefficient (Ott et al. 1978, p. 412) which measures the strength of the linear relationship between χ_1 and v_2. In each case, the parameter χ_1 is calculated from Eq. (3), using $i/V_u = 0.34$, $I = 0.15$ M, mean $T = 22°$ C, and the appropriate values of D_0 and ν_e/V_0. The asterisk (*) indicates that the D_0 was taken as the observed dehydrated width, determined in the manner described in the Methods section; otherwise, D_0 was taken as 0.63 of the width of the fiber in 20% PVP solution (cf. Methods section). The dagger (†) indicates fibers bathed in relaxing solution with 0.5 mM Mg^{2+} and 2 mM MgATP. Note the excellent fit of the linear relationship to the data

Fiber	Sarcomere Spacing(s) [μm]	ν_e/V_0 [mM]	a_0	a_1	r
10/11/77†	2.55	40 40	0.472	0.506	0.9998
10/20/77b†	2.41 2.80	29 34	0.473	0.501	0.9996
10/20/77c†	2.41 3.39	40 40	0.464	0.533	0.9996
10/27/77†	3.01 3.51	42 42	0.467	0.526	0.9994
11/3/77†	2.34 2.99	41 41	0.465	0.533	0.9999
11/17/77†	2.28 2.54 2.87 3.39	40 42 45 46	0.454	0.554	0.9998
11/30/77†	2.87	40	0.464	0.535	0.9998
12/14/77†	2.26 2.88 3.31	40 42 42	0.464	0.535	0.9998
7/27/78	2.12 3.87	40 60	0.461	0.539	0.9993
10/5/78	2.44 2.85 3.60	38 40 42	0.467	0.521	0.9942
10/11/78	2.30 3.05* 3.64*	24 28 28	0.473	0.504	0.9993
10/26/78	4.08*	40	0.463	0.542	0.9986
3/27/79	2.46* 3.23* 4.48*	40 40 44	0.467	0.522	0.9965
5/2/79	2.45 3.08 3.87*	50 56 62	0.458	0.549	0.9999
Mean		41.1	0.465	0.529	
SD		8.2	0.005	0.016	
n		31	14	14	

Changes in Solution pH

By changing solution pH we should be able to alter the electrostatic contribution to Eq. (3), and thereby test the model. Our assumption is that pH affects only the ionization of charged groups; i.e., pH only alters the i/V_u term. Further, when the pH is lowered to the isoelectric point of the network, i/V_u equals zero. Figure 4 demonstrates the relation between width and osmotic pressure at pH 7 and 5.5 (near the isoelectric points of myosin and actin; cf. Discussion). Note that a decrease in pH causes the fiber to shrink (Godt and Maughan 1977) but that as osmotic pressure increases, pH has a diminished effect upon width and at higher pressure there is little or no influence of pH. The curves in Fig. 4 are the theoretical predictions for varying values of i/V_u. The lowest curve refers to the case where all the charge is neutralized; i.e., where $i/V_u = 0$. The central curve is the case where $i/V_u = 0.19$ M and is fitted to the data points by trial and error. At this point, 44% of the charge has been neutralized.

Note from Eq. (3) that decreasing pH below the isoelectric point should lead to fiber swelling since the i/V_u term is squared; i.e., the sign of the charge is irrelevant. Further tests with skinned fibers demonstrate, however, that decreasing pH to 4 causes fibers to shrink more rather than to swell (Fig. 5). Changes in fiber width with pH were reversible for excursions of pH from 6.0–7.5 but became somewhat irreversible when pH was decreased to 5.5 or below. When a fiber was returned from these low pH test solutions to the pH 7.0 control solution fiber width was always less than before the test. In an attempt to compensate for this irreversibility, we have compared the fiber width in the test solutions to the average of the widths in the control solution before and after the

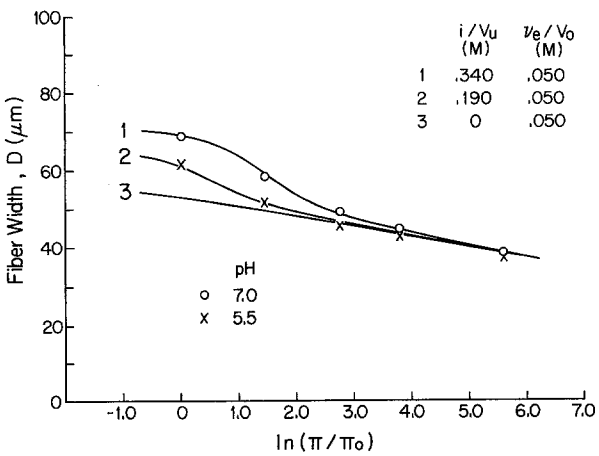


Fig. 4. Changes in fiber width with alterations in solution pH over a range of osmotic pressures. Symbols, data obtained at pH 7.0 (0) and 5.5 (×) at a sarcomere spacing of 2.45 μm; fiber 5/2/79. The curves are calculated from Eqs. (3) and (4) using the values of the parameters listed and letting I = 0.15 M, $a_0 = 0.458$, $a_1 = 0.549$, $D_0 = 27.0$, temperature 20.2° C. Curve 1 refers to the case where i/V_u = 0.34 M; curve 2, $i/V_u = 0.19$ M; curve 3, $i/V_u = 0$, i.e., where the polymer network has a net charge of zero

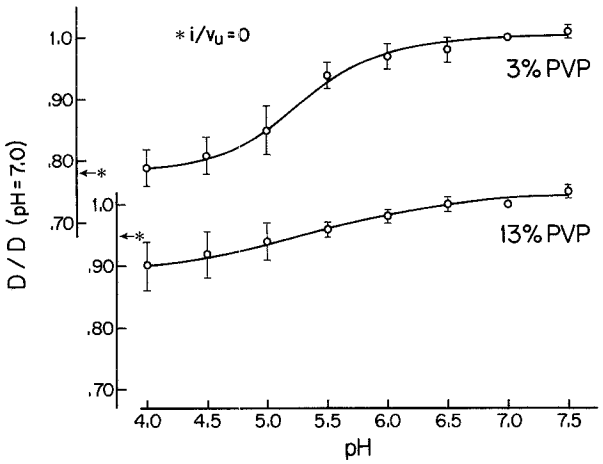


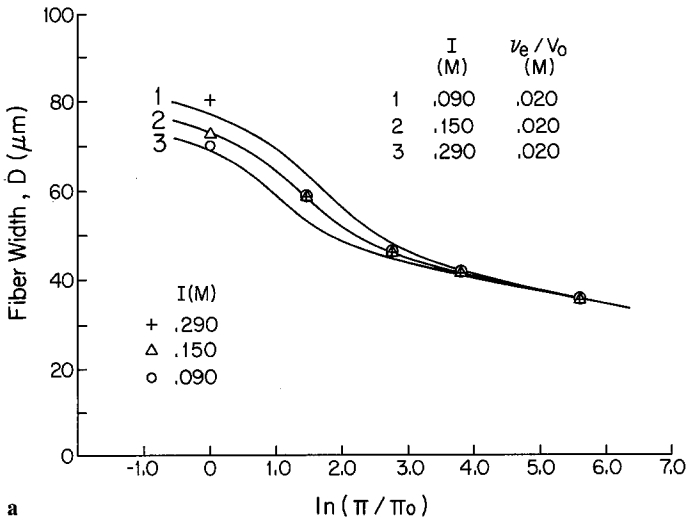
Fig. 5. Changes in fiber width D with solution pH, at two concentrations of PVP (3 and 13%). Mean and standard deviations (vertical bars) of fiber width relative to width at pH 7.0. Range of sarcomere spacings, 2.24–3.89 μm. Mean diameter of fibers in 13% PVP relaxing solution was 0.73 ± 0.07 of that in 3% PVP. Mean temperature, 22° C. Experiments were carried out in solutions buffered using either 20 mM imidazole or 20 mM maleate with appropriate amounts of Tris, as described in the Methods. All solutions at pH 4.0 were buffered with Tris maleate. Curves drawn by eye. The asterisks (*) indicate the mean value to which the fiber widths decrease (as a fraction of the initial width at pH 7) for the case $i/V_u = 0$ (fibers of Table 1)

test. Note that if the total network charge concentration is 0.34 M, as we assume in the model, the maximal decrease in fiber width in 3% PVP, where the charge is completely neutralized ($i/V_u = 0$), is 22% (indicated by the asterisk), which is close to the observed decrease of 21% in pH 4 solutions. On the other hand, this value of charge concentration of 0.34 M yields a decrease in diameter of only 5% when fibers in 13% PVP are exposed to pH 4 solutions (cf. other asterisk) whereas fibers under these conditions shrink nearly 10%. We have no explanation for this discrepancy.

In order to determine if the fractional decrease of width with pH depends on sarcomere length, we compared the fractional shrinkage of fibers of Fig. 5 whose sarcomere lengths varied from 2.2–3.9 μm. In the eight fibers examined, the fractional decrease of diameter with pH did not depend significantly on sarcomere length, with either the same fiber at various sarcomere lengths or different fibers at various sarcomere lengths.

Effects of Ionic Strength

By changing ionic strength, I, we should directly influence fiber width since ionic strength appears explicity in Eq. (3). As ionic strength is increased, there should be increased electrostatic shielding of fixed changes and the fiber should shrink. Conversely if ionic strength is decreased the decreased shielding should lead to an increase of fiber width. In fact, experiment shows exactly the opposite effects



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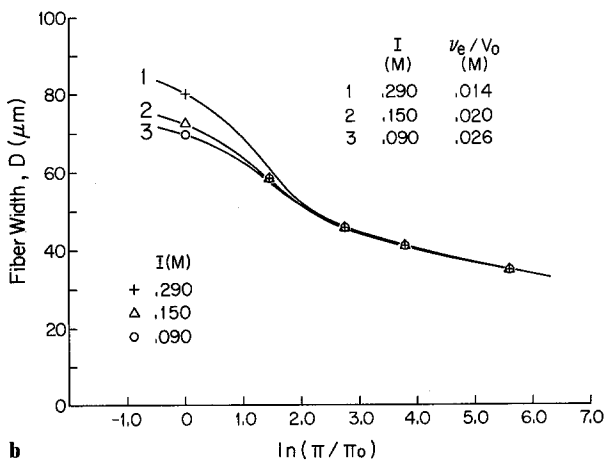

b

Fig. 6. Effect of solution ionic strength I on fiber width over a range of osmotic pressures. Symbols, experimental data points obtained at $I = 0.09, 0.15, 0.29$ M at a sarcomere spacing of 2.28 μm. Fiber 12/4/78. The curves are computed from Eqs. (3) and (4) using the following values: $i/V_u = 0.34$ M, $a_0 = 0.471$, $a_1 = 0.498$, $D_0 = 24$ μm, temperature = 22° C, and the indicated values of ν_e/V_0. **a,** curves computed at same value of ν_e/V_0 (0.020 M); **b,** curves computed at various indicated values of ν_e/V_0 ranging from 0.016–0.024 M

of changes in ionic strength (Fig. 6a, see also Godt and Maughan 1977). Figure 7 shows that in 3% PVP, the fiber widths increase by an average of 7% when ionic strength is raised to 0.29 M with addition of KCl, whereas theory predicts a 4% decrease. Additionally, in 3% PVP, fiber width decreases by 3% when ionic strength is lowered to 0.09 M by deletion of KCl, whereas theory predicts an increase of 4%. Similar results were obtained at 13% PVP, although the magnitude of the effect was correspondingly less.

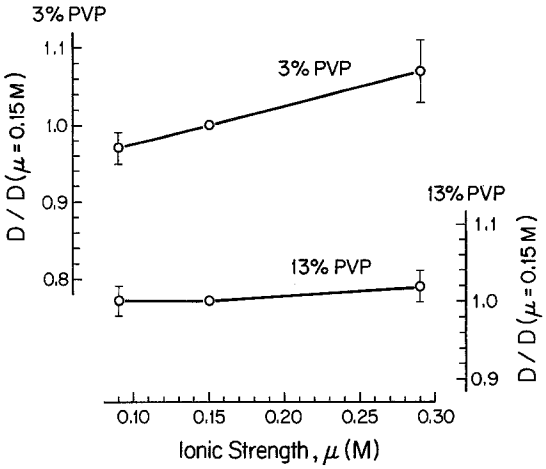

Fig. 7. Change in fiber width D with solution ionic strength I at two concentrations of PVP (3 and 13%). Mean and standard deviations of fiber width relative to width at I = 0.15 M. Range of sarcomere spacing, 2.21–3.85 μm. At I = 0.15 M, mean width of fibers in 13% PVP, 0.77 ± 0.11 of that in 3% PVP. Mean temperature, 22° C. Solution ionic strength was adjusted by adding or subtracting appropriate amounts of KCl, as described in the Methods

However, ionic strength is likely to have other effects besides those due to simple electrostatic shielding of the charges on the network. One plausible additional effect of ionic strength might well be upon the structural integrity of the network or upon the strength or number of chains. This is not unexpected since, for example, at high ionic strength, myosin is extracted. In three fibers studied, assuming that ionic strength influences the number of chains, some 2–4 mmol/l of chains must be formed (a 4–8% increase) when ionic strength is decreased to 0.09 M (cf. Fig. 6b). Similarily, some 4–8 mmol/l of chains must be broken (a 8–16% decrease) when ionic strength is increased to 0.29 M (cf. Fig. 6b).

Changes Resulting from Fixation

By fixing the fiber with glutaraldehyde added to the bathing medium, one might expect to increase the number of cross-linkages between elements of the network. The theory would predict that an increase of the concentration of cross-linkages, $\nu_e/2\ V_0$ (i.e., half the concentration of chains), would lead to a decrease of fiber width. In three fibers studied we found that, in 3% PVP solution, fixation with 1% glutaraldehyde leads to a decrease in width of 10%, with no change in sarcomere spacing. This could be modelled by Eqs. (3) and (4) by assuming that the concentration of cross-linkages increased by 10%.

Passive Tension

Flory (1953) utilizes the statistical thermodynamic theory of elasticity to characterize the relationship between extension and tension in a network polymer. His approach derives from the relation:

$$\left(\frac{\partial \Delta F}{\partial L}\right)_{T,P} = \tau\,,$$

where L is the length of the network and τ is tension. Flory's equation (Flory 1953, p. 492) for the one-dimensional distension of a swollen network whose volume is constant is:

$$\tau = RT(\nu_e/V_0)\nu_2^{1/3}\,(\alpha - 1/\alpha^2)\,, \tag{5}$$

where $\alpha = L/L_0$ with L_0 the length of the undistended network. Can this relation describe the passive length-tension relation of skinned fibers given values for ν_e/V_0 and ν_2 determined from the previous extension and compression studies?

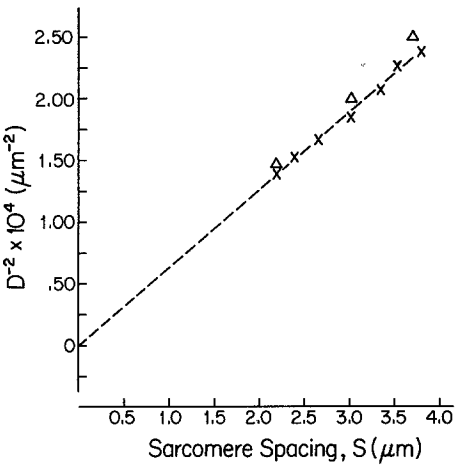

Fig. 8. Plot of the square of the reciprocal of the fiber width D vs. sarcomere spacings, as obtained from stretch experiments (×), and compression experiments (△) for fiber (10/11/78). Experiments carried out using relaxing solution containing 3% PVP. Dashed line, $D^{-2} = a_0 + a_1 s$, where $a_0 = -0.009$ and $a_1 = 0.548$, determined by linear regression fit of data ($r = 0.985$). Note, if $a_0 = 0$ the fiber obeys a constant volume relation, as appears to be nearly the case here

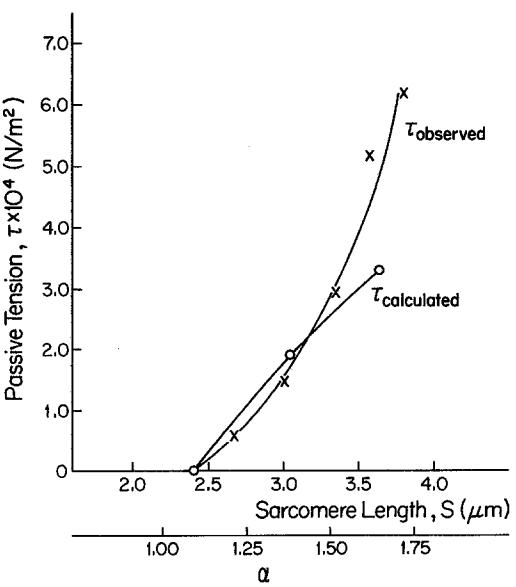

Fig. 9. The dependency of passive tension τ on sarcomere spacing s. Crosses (×) refer to data obtained from stretching fiber 10/11/78 (cf. Figs. 1–3 and Fig. 10) in relaxing solution containing 3% PVP (near in situ size). The parameter $\alpha = s/s_0$, where s_0 is the slack length of the sarcomere which in this case, was taken to be 2.4 μm (i.e., a spacing at which the fiber was taut but at which no detectable passive tension was observed)

Focussing on the fiber described previously in Figs. 1–3, we have determined the passive length-tension relation for this fiber in 3% PVP, since at this concentration, the volume remained approximately constant during stretch (cf. Fig. 8). The observed passive length-tension curve is compared in Fig. 9 with the theoretical relation obtained using the derived values for ν_e/V_0 for this fiber. We have calculated values of α using an L_0 of 2.4 μm, the slack length of the sarcomeres in this fiber following a pre-stretch.

Although the passive tension predicted by the theory is the same order of magnitude as that determined experimentally, the shape of the theoretical relation is not in congruence with experiment. While departures from theory are common with non-biological polymers (e.g., rubber) at large distensions, the departure at small extensions is unexplained.

Equation (5) demonstrates that passive tension should be a function of the concentration of chains ν_e/V_0. Thus one would predict that if changes in ionic strength have influence on ν_e/V_0 this should also influence passive tension. In stretched fibers we observed a slight increase in passive tension at 0.09 M ionic strength, and a slight decrease at 0.29 M compared to passive tension at 0.15 M ionic strength. The effect, however, was too small to quantify accurately. In experiments with skinned fibers, Gordon et al. (1973) also observed that increasing ionic strength above 0.17 M caused a decrease in passive tension, while decreasing ionic strength below 0.17 M caused an increase of passive tension. Both these sets of results are in qualitative agreement with those predicted from Eq. (5) and the postulated changes in ν_e/V_0 required from theory. Unfortunately, since Gordon et al. (1973) provided no data on the absolute magnitude of passive tension in their 0.17 M standard solution, it is not possible to determine the actual magnitude of passive tension changes with ionic strength in their experiments.

Discussion

Basis of Theory

Resting skeletal muscle behaves as an elastic body, in that it can be reversibly deformed by elongation and compression. Some of the elastic properties of intact muscle arise from the myofibrillar contents themselves and others arise from the structure and semi-permeable nature of the sarcolemma and possibly, the sarcoplasmic reticulum. We have chosen to examine the elastic behavior of the myofibrillar contents by simplifying the system through mechanical removal of the sarcolemma (i.e., by skinning the fiber) and by functional destruction of the sarcoplasmic reticulum with a non-ionic detergent. In previous papers, we have examined the elastic behavior of skinned fibers under a variety of conditions (Godt and Maughan 1977; Maughan and Godt 1979). In this paper, we attempt to unify our observations with a quantitative theory, based upon the physical chemistry of polymer networks.

The predominant theory of elasticity for polymer networks was developed by Flory (1953), using a statistical thermodynamic approach. Basically, the theory

presumes that the network is made up of long, randomly-coiled and inextensible polymer chains, consisting of identical monomers. The chains are assumed to be periodically cross-linked and to bear a net charge. The network is bathed in solvent containing ions. Under normal conditions, the distensibility properties of this network arise not from extending chains or breaking cross-links but from deforming the network (although the theory permits the number of cross-links to vary). Distensibility reflects entropic changes of the macroscopic network rather than changes in internal energy of the microscopic components. Both Flory (1953) and Hill (1960) have suggested that these conditions might well apply in skeletal muscle and that, therefore, the theory might help explain muscle properties.

Flory develops the theory by casting it in terms of the free energy changes associated with assembly of the network from the individual monomers, the interaction of solvent with the network, and the osmotic forces arising from the presence of co- and counter-ions within the charged network. When a skinned fiber is bathed in a solution containing large colloidal molecules, an additional free energy change occurs due to external osmotic compressive pressure. In the theoretical section, we have taken Flory's expression for free energy changes of a general charged polymer network and have added an additional term for osmotic compressive pressure (Eq. 2d). We have not included a term reflecting a Van der Waals attractive force since, as demonstrated in the Appendix, calculations indicate that the likely magnitude of such an attractive force is at least three orders of magnitude less than the compressive force due to PVP and is therefore negligible under our conditions.

Fitting of Theory and Experiment

How well does this relatively simple model fit experimental data from skinned fibers? With certain key assumptions, we obtain a satisfactory fit of the observed relation between fiber width and osmotic compressive pressure (Figs. 3–4) over a wide range of pressures and at several sarcomere lengths. To fit this relation we needed to specify a number of parameters. We calculate ionic strength (I) from the known composition of the bath. We estimate the osmotic pressure (Π) from osmometric data, assuming the PVP colloid was completely excluded from the fiber. We estimate charge concentration (i/V_u) from the known concentration of myofilaments with estimates of their net charge from amino acid analysis and assuming that myofilament charge density is typical of all myofibrillar proteins. We estimate the volume fraction of polymer (v_2) from the size of the dehydrated fiber relative to its hydrated size, assuming that essentially all the solvent had evaporated and that the network was completely collapsed, with no internal spaces unoccupied by polymer. We could not, however, measure the interaction parameter, χ_1, or the concentration of chains (ν_e/V_0).

The major untestable assumption is that the interaction parameter, χ_1, is a linear function of v_2, i.e., $\chi_1 = a_0 + a_1 v_2$, as it appears to be for other polymer-solvent systems (e.g., polydimethylsiloxane in benzene, polystyrene in methyl ethyl ketone or toluene, and rubber in benzene (Flory 1953, Fig. 111).

We also had no direct way of determining or even estimating the number of chains (ν_e/V_0); however, we knew that ν_e/V_0 and χ_1 could be related through the general expression 3. Thus we determined ν_e/V_0 in each fiber by finding that value of ν_e/V_0 which gave a linear fit of the χ_1 versus ν_2 expression with a correlation coefficient greater than 0.99 and which best fit the D versus ln Π/Π_0 at any given sarcomere length. If the assumption of a linear relation between χ_1 and ν_2 is valid, our model (expressions 3 and 4) has only *one* arbitrary parameter (ν_e/V_0) for fitting a wide range of conditions.

While we had no physical way of determining the likely magnitude of ν_e/V_0, we had some expectations how it might change under different experimental conditions. Since $\nu_e/2\,V_0$ is approximately the number of cross-links, we expected that $\nu_e/2\,V_0$ should increase when the fiber was put into fixative, in so far as the value of chemical fixation of cells depends upon establishment of extra cross-linkages. We expected that $\nu_e/2\,V_0$ should be reasonably constant with changes in sarcomere length, which was fairly well borne out in most cases, although to fit the data satisfactorily, we were required to increase in $\nu_e/2\,V_0$ somewhat as the fiber was stretched. We expected that $\nu_e/2\,V_0$ might change with ionic strength since we know that muscle proteins salt in and out. Finally, we had no way of predicting how $\nu_e/2\,V_0$ might change with changes in pH so we assumed that it remained constant.

Changes with Fixation

The most straightforward, although the least quantitative test of the theory is the effect of fixation upon fiber width. We expected that when a fiber was fixed in glutaraldehyde that the number of cross-linkages should increase. The theory predicts that, in the absence of other changes, an increase of cross-linkages leads to a decrease of fiber width. We observed that in 3% PVP fiber width decreased by 10% with fixation in 1% glutaraldehyde. This is in accord with Eisenberg and Mobley (1975) who observed that the width of single fibers from semitendinosus muscle of the frog shrank an average of 10% with fixation in 5% glutaraldehyde. The theory predicts that a decrease of diameter of 10% can be produced by a 10% increase in the number of cross-linkages. Although there is no way to test this value experimentally, it seems reasonable in light of the similarity of appearance between frozen, unfixed fibers and glutaraldehyde-fixed fibers. One might expect that any large increases in cross-linking would be reflected in gross ultrastructural changes with fixation.

Changes with Sarcomere Length

Table 1 shows that in some fibers the theory can predict the change in width with sarcomere length with ν_e/V_0 invariant. However, in most cases, it was necessary to increase ν_e/V_0 by 5–50%, in one case, with sarcomere length to adequately fit the data (cf. Fig. 3). This necessity was unexpected and points up an inadequacy of the theory.

Changes in Ionic Strength

The ionic strength, I, appears explicitly in Eq. (3), reflecting the tendency of ions in solution to shield fixed charges on the network. Increasing ionic strength should decrease fiber width and vice versa. In fact, the experimental observations are quite the opposite to that which we initially expected. However, we can bring theory into correspondence with data if we assume that increasing ionic strength not only increases the electrostatic shielding, but also decreases the number of cross-links $\nu_e/2\,V_0$. Conversely, decreasing ionic strength decreases shielding and increases the number of links. It seems to us that this dual action of ionic strength is not unreasonable, especially since the change in $\nu_e/2\,V_0$ required to fit the data is relatively small (4–16%).

It is well-known that ionic strength affects the structural integrity of muscle proteins. Conventional biochemical techniques for purification of myofibrillar proteins utilize this structure-disrupting property of altered ionic strength. For instance, myosin is extracted (i.e., salts in) at high ionic strengths, whereas actin is extracted at low ionic strength (Ebashi and Nonomura 1973). Conversely, solubilized myosin forms filaments (i.e., salts out) as ionic strength is decreased from, e.g., around 0.5 M, and monomeric actin at low ionic strength forms filaments as ionic strength is increased above, e.g., 0.01 M (Ebashi and Nonomura 1973). Thus it seems reasonable to expect that, over the smaller range of ionic strength we examined (0.09–0.29 M), an increase of ionic strength might weaken structural integrity somewhat. This weakening would be reflected qualitatively in our theory as a decrease in the concentration of cross-links.

Which of the known elements in relaxed skinned muscle fibers are important for structural integrity? While crossbridges probably play no significant role in relaxed fibers, possible candidate structures linking myofilaments are M-lines and Z-disks. In addition Maruyama and colleagues have identified webs of a protein they term connectin which appears to permeate both skeletal and cardiac muscle cells (Maruyama et al. 1977). One way to determine the relative role played by each of these structures might be to observe the mechanical consequences of selective extraction of these structures. For example, M-lines have been preferentially extracted from chicken breast muscle myofibrils (Eaton and Pepe 1972). Attempts to destroy the connectin network in skinned fibers with elastase, however, lead to irreversible dissolution of the fiber (Maughan and Godt 1979).

Changes in pH

We attempted to test the model by altering the charge density on the network, i/V_u, by changing solution pH. We expected that, as i/V_u decreased, fiber width should decrease and that the maximum decrease in width should occur when $i/V_u = 0$. In other words, from width measurements at neutral pH and at the isoelectric point of the network one would expect that the fit between theory and experiment would yield the actual i/V_u, assuming that $\nu_e/2\,V_0$ was not affected by

pH. Furthermore, the i/V_u so determined should be near that obtained from amino acid analysis of myofibrillar proteins and the concentration of these proteins. We find that fiber width does decrease with decreasing pH, as was reported previously (Godt and Maughan 1977), and the width decrease appeared to be maximal around pH 4.0. The isoelectric point of myosin is reported to be 5.4 (Erdös and Snellman 1948); that of actin is 5.2 (Szent-Gyorgyi 1947); and that of tropomyosin is 5.1 (Young 1963). If these isolated proteins are characteristic of the polymeric network of the skinned fiber we would expect a minimum fiber width at pH 5 or above, rather than 4.5 or below. However, measurements of the electric charge on the proteins in glycerinated psoas muscle as determined from microelectrode measurements of Donnan potentials (Elliott et al. 1978) indicate that the isoelectric point of the myofibrillar proteins is between 4 and 4.5.

If the isoelectric point of relaxed skinned fibers is between 4 and 4.5 the experimental data in 3% PVP (cf. Figs. 5 and 6) could be fitted with an i/V_u at pH 7 of 0.34 M. This corresponds to a concentration of fixed charges in intact muscle of $(i/V_u)D_0^2/D_{3\%}^2 = 57$ meq/l. This is well within the range of other estimates. Elliott (1973) gives a value of 40 meq/l on the myofilaments alone, derived from amino acid analysis and protein concentrations of myofilaments. Conway (1957) estimates that the concentration of total fixed charges in rat leg muscle is 100 meq/l. Collins and Edwards (1971) estimate a charge density around 56–58 meq/l at pH 7.5 in glycerinated frog ventricle as determined from microelectrode measurements of Donnan potential. While the agreement of our data with others seems excellent, it must be treated with reserve in view of the uncertainties involved in the diameter measurements at low pH mentioned previously. Furthermore this value of i/V_u does not predict the full magnitude of the diameter change with pH in 13% PVP solutions.

Relative Magnitude of Chemical Potentials Within Relaxed Skinned Fibers

The relative absolute magnitudes of the four types of chemical potentials proposed in the theory (electrostatic, configuration elastic, mixing entropy and osmotic) are compared in Fig. 10. Here is plotted each of the four terms of Eq. (3) as a function of PVP concentration for the fiber (10/11/78) shown in Figs. 1–4. In the fully swollen fiber (0% PVP) the mechanical behavior is determined mainly by a balance between the elastic and the mixing entropy chemical potentials, with electrostatic potential playing an important though subsidiary role. In 3% PVP, when the fiber returned to near its in vivo size (cf. Maughan and Godt 1980) the mixing entropy and the elastic potentials still predominate over the osmotic and electrostatic potentials, which are roughly in balance. Note, however, that as PVP concentration increases, the osmotic force rises rapidly so that above about 10% PVP the mixing entropy and osmotic chemical potentials predominate over the electrostatic and elastic potentials.

In the intact fiber, which corresponds to the 2–3% PVP case, (cf. Maughan and Godt 1980) the fiber is in mechanical equilibrium (i.e., terms in Eq. (3) sum to zero) since the semi-permeable membrane provides an osmotic compressive

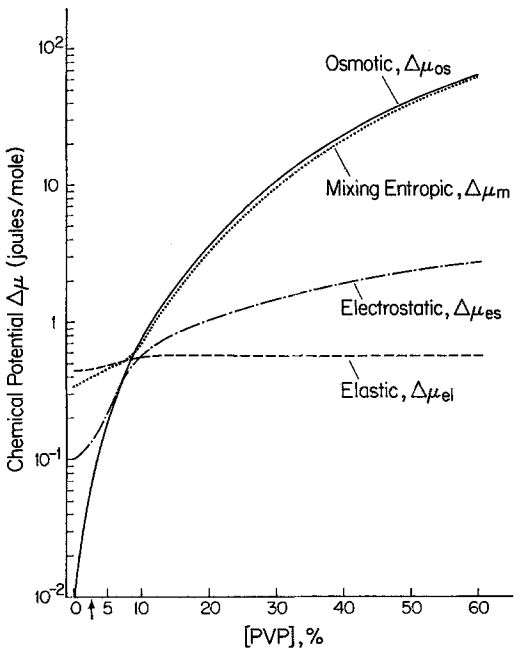

Fig. 10. Comparison of chemical potentials within a skinned fiber bathed in aqueous relaxing solution containing 0–60% PVP. The absolute values of the mixing entropic, configuration elastic, electrostaticand osmotic chemical potentials of Eq. (1) are plotted as a function of PVP concentration, where $\Delta\mu_{os} = V_1\pi$, $\Delta\mu_m = RT \cdot \ln [(1-v_2) + v_2 + \chi_1 v_2^2]$, $\Delta\mu_{es} = RTV_1 \cdot (i/V_u)^2 \cdot V_2^2/4I$ and $\Delta\mu_{el} = RTV_1 \cdot v_e/V_0 (v_2^{1/3} - v_2/2)$. Note that in PVP-free relaxing solution, the elastic term dominates, followed by the mixing entropy and electrostatic terms, whereas at PVP concentrations above 10%, the order reverses. To convert to units of pressure (force per unit fiber surface) divide each term by RTV_1. The arrow between 2 and 3% PVP indicates the concentration of PVP necessary to compress this particular fiber (10/11/78) back to its initial width in oil, as corrected for dehydration; i.e., back to its in situ width (cf. Maughan and Godt 1979)

force to constrain the network. When the fiber is skinned in 0% PVP relaxing medium the polymeric network is no longer in equilibrium since Eq. (3) sums to a positive value. To reach a new equilibrium, the positive terms (electrostatic and mixing entropy) must decrease since the only remaining negative term (elastic) increases only slightly with fiber volume. The new equilibrium state is reached by increasing fiber volume, since only in this way will the positive terms decrease until Eq. (3) sums to zero again, at which point the fiber is in chemical equilibrium.

One might also mention here the possibility of testing the model by means of thermal measurements. For example, one would expect on the basis of Fig. 10 that the mixing entropy would provide, as heat, approximately (63 joules/mole) $\times$ (0.239 cal/joule) = 15.1 cal/mole fiber water as the fully swollen fiber is shrunken with 60% PVP. Assuming that the fiber of Fig. 10 contains approximately a microliter of water, this thermal energy corresponds to (15.1

cal/mole) × (55.6 mole water/liter water) × (10^{-6} l water) = 8.4 × 10^{-4} cal, which may be measureable using modern calorimetry.

Passive Tension

Flory (1953) has demonstrated that his statistical thermodynamic model, which we have applied to skinned fibers, predicts the relative force developed upon deformation of the network. Thus, as adapted for relaxed skinned fibers, the model predicts a particular relation between passive tension and length. Furthermore, since a measurement of tension does not enter into the determination of any of the parameters used to calculate the relationship, the magnitude and form of the relationship provide an additional test of the theory. While the magnitude of the passive tension predicted by expression (5) differs by no more than a factor of 2 from the observed value, the shape of the length-tension relation predicted by theory is not in accord with experiment; at small extensions the theoretical value is too high, and at large extensions, too low. This lack of congruence is also a failure of the Flory theory applied to rubber (cf. Hill 1960, Fig. 3–4). Flory has suggested that in rubber the great increase in retractive force at long extensions is due to "crystallization" of the polymer with stretch. That is, stated simply, the entropy of the systems drops sharply due to increased alignment and ordering of the hitherto random polymer chains. Thus, our observation that the actual length-tension relation at long extensions is steeper than that expected by theory is consistent with morphological evidence from studies of muscle using X-ray diffraction and electron microcopy, which demonstrate a high degree of alignment and ordering with the myofibrillar structure. In this regard, our model of the skinned fiber as a random polymeric network is clearly an oversimplification.

Value of this Model

We feel that this theory, nevertheless, provides an adequate model of much of the mechanical behavior of relaxed skinned fibers. In the first place, it provides a plausible molecular explanation of the macroscopic properties of the muscle using a well-accepted statistical thermodynamic approach. It gives insight into the chemical potentials and resultant forces underlying the behavior and an indication of their relative magnitudes under different experimental conditions. The theory fits the experimental data rather well with one arbitrary parameter, the concentration of polymer chain units in the muscle, with other necessary quantities being either known, experimentally estimated or derived from the other variables.

This theory demonstrates that electrostatic forces play an important role in the elastic behavior of skinned fibers, as has been proposed by others (Elliott 1973). The theory also shows direct configurational elastic forces play an important role, as might have been obvious, but with the important addendum that this elasticity arises not from the elastic distension of individual elements,

but from the changes in configuration of a network of inextensible elements. Finally, the theory demonstrates that the entropic force due to mixing of polymer and water is very important, and that at high compressive pressures it predominantes over both the configurational elastic and the electrostatic forces.

Appendix

Relative Magnitude of Van der Waals Forces in Relaxed Skinned Fibers

Under the assumption that the major contribution of Van der Waals forces to the overall size of the fiber will be those forces between adjacent myofilaments, we can use relations developed by Parsegian (1973) for Van der Waals interaction between parallel rods of length L. The Gibbs free energy of interaction per unit length for two parallel rods of radius a, whose length is much longer than their center-to-center spacing, r, for the case of large separation ($r \gg a$) is given approximately by:

$$F^{vw} \simeq -\frac{9\pi kT}{16}\frac{a^4}{r^5}S,$$

where k is Boltzmann's constant and S is a function of the transverse and parallel rod polarizabilities and is of the order of 1 for nonpolar, nonconducting bodies in an aqueous environment. Setting $S = 1$, the attractive force per unit length will be given by:

$$f_{vw}(r) = -\frac{dF^{vw}}{dr} = -\frac{45\pi kT}{16}\frac{a^4}{r^6}.$$

Thus Van der Waals attractive force will tend to shrink the fiber in a manner similar to the osmotic compressive force due to excluded polymer molecules. Consider a myofilament in a hexagonal lattice surrounded by six nearest neighboring myofilaments at a distance r. Geometrically, this unit forms a cylinder of radius r and length L. The effective area over which the attractive force is applied is $2\pi rL/6$, that is, the area of the cylinder surface ascribed to each nearest neighbor. Thus the net compressive pressure on the unit cylinder arising from the Van der Waals attractive force will be:

$$\Pi^{vw} = \frac{3f_{vw}}{\pi r} = -\frac{135kT}{16}\frac{a^4}{r^7}.$$

In intact skeletal muscle at full overlap of thick and thin filaments, where Van der Waals forces will be greatest, the interfilament distance between thick and thin filaments is 24.8 nm (Elliott and Matsubara 1972). Thus, taking the filaments to be 6 nm in diameter, the Van der Waals compressive pressure on the unit cylinder will be 7.68 N/m^2 (7.6×10^{-5} atm) at room temperature. This is negligible compared to the osmotic compressive pressure necessary to return the

skinned fiber to near its in vitro size (i.e., 3% PVP) which is 4.64×10^3 N/m^2. As the fiber is compressed with higher concentrations of PVP, the interfilament spacing will decrease and Van der Waals attractive forces will increase markedly. Under the assumption that r decreases in proportion to fiber width, D, one can calculate Π_{vw} at other concentrations of PVP. Thus in 60% PVP, where width is only 0.56 of the width in 3% PVP, Π_{vw} is:

$$\Pi_{vw}^{x\% \text{ PVP}} = \Pi_{vw}^{3\% \text{ PVP}} \left(\frac{D_{3\%}}{D_{60\%}}\right)^7 = \frac{7.68 \text{ N/m}^2}{(0.56)^7} = 444.7 \text{ N/m}^2 .$$

This is again negligible compared to the osmotic compressive pressure of 3.41×10^6 N/m^2 exerted by a 60% PVP solution. In these very compressed fibers the condition $r \gg a$ no longer applies so one must use another approximation to obtain the Gibbs free energy due to Van der Waals forces and thus the Van der Waals compressive pressure (Parsegian 1973, Eq. 3.16a). The Π_{vw} calculated from this more appropriate equation is only five-fold larger than that given above and thus is still negligible compared to the osmotic compressive pressure.

Acknowledgements. We thank Elizabeth Low and Chris Recchia for their considerable help with the experiments. We also thank Ken Adler for carrying out scanning electron microscopy, Bill Boldosser for transmission electron microscopy, Jackie Fischl for writing the computer program, Nancy Duerinck for typing the manuscript, and Joseph Trono for constructing much of the experimental apparatus.

This work was supported by PHS grant HL 21312-01 and AHA grant 78-634 (DWM); and PHS grants AM 17828 and 25851 (REG). DWM is an Established Investigator of the American Heart Association.

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Received March 17, 1980/Accepted June 18, 1980

Note Added in Proof. Some of the anomalous behavior with changes in ionic strength might be explained by an effect upon the isoelectric point of the myofilament proteins. N. K. Sarkar (Enzymologia 14, 237–245, 1950) observed that increasing ionic strength with KCl over the range we studied decreased the isoelectric points of both myosin and actomyosin. A decrease in isoelectric point would tend to increase the net negativity of the proteins at pH 7 and thus to increase the electrostatic repulsive force between them. This increase would oppose and might predominate over the effects of increased shielding of charges with increasing ionic strength and thus could provide an alternate explanation for the swelling of skinned fibers with increasing ionic strength.