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X-ray Measurements of the Bulk Modulus of the Myofilament Liquid Crystal in Striated Muscle

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Low-angle X-ray diffraction measurements of the distance between myosin filaments comprising the smectic B_1 liquid crystal found in skinned crayfish striated muscle were made. The interfilament distance as a function of applied compressive osmotic pressure was determined and the bulk modulus of this myofilament liquid crystal ascertained. Under compression the behavior of this myosin filament lattice is shown to be predicted well by the Murnaghan equation of state. The isothermal bulk modulus, B_0, of this myosin filament liquid crystal is 1.51×10^5 dynes/cm^2 at 2°C and at 46% actin overlap.

INTRODUCTION

Observations of a structural isomorphism between the spatial orientation of striated muscle myosin filaments and smectic liquid crystals have been reported.[1–5] The A or anisotropic band of myosin filaments in striated muscle fulfills the orientational requirements[6,7] of a smectic B liquid crystal. According to the nomenclature of De Vries,[8] the myosin filament lattice belongs to subgroup B_1. Observational deductions concerning the liquid-crystalline nature of the myosin filament lattice were tested by determining whether or not the behavior of the myosin filament lattice could be described by solid state physics equations. We have investigated the behavior of the myosin filament lattice with respect to compression. Previous work by other investigators (see discussion) used an indirect method and their results were based on empirical equations invalid for the range of pressures we investigated. The compression (as explained below) is applied in a uniform manner radially perpendicular to the *c* axis of the myosin filament lattice. Thus a uniform two-dimensional compression of the myosin filament lattice is achieved. Hence the stress tensor in these experiments is scalar.

The crayfish myofilament liquid crystal utilized in this study is composed of myosin filaments approximately 4.5 micrometers long and 18 nanometers in

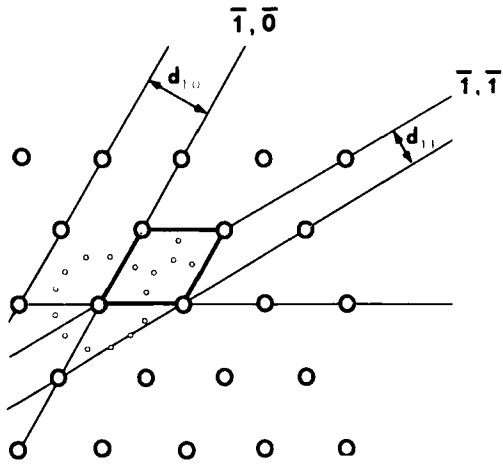

FIGURE 1 Schematic of the lattice of the long tonic fibers of the crayfish leg muscle. The large circles represent the myosin filaments which are arranged in a hexagonal array, forming lattice planes [1,0] with the distance between the lattice planes indicated ($d_{1,0}$). The small circles represent the actin filaments, twelve of which are arranged equidistant around each myosin filament, providing a 6:1 unit cell. There appear to be no structural connections in this muscle to which this arrangement may be attributed.

diameter. The filaments have a net negative charge with the isoelectric point at approximately pH 4.4. These filaments, suspended in a dielectric medium, are organized in the usual B_1 hexagonal array. *In vivo* a second type of polymer, composed primarily of actin, interdigitates the myosin filament lattice from both ends in a 6:1 unit-cell ratio. The actin polymers are approximately 3.6 micrometers long and 8 nanometers in diameter. The degree of interdigitation can be altered by the mechanical application of tension parallel to the *c* axis.

The spatial orientation of the myofilament lattice is illustrated in Figure 1.

THEORY

Following the presentation of Anderson[9] the isothermal bulk modulus is

$$B = -V(P)\left\{\frac{\partial P}{\partial V(P)}\right\}_T \quad \text{and} \quad B_0 = B_{P\to 0}. \tag{1}$$

We define

$$B_0' \equiv \left\{\left(\frac{\partial B}{\partial P}\right)_T\right\}_{P=0} = (B')_{P\to 0}. \tag{2}$$

Assuming the bulk modulus to be linear with pressure we may then write

$$B(P) = -V\left(\frac{\partial P}{\partial V}\right)_T = B_0 + B_0'P. \tag{3}$$

Anderson has verified the success of the approximation that the instantaneous bulk modulus is linear with pressure, and stated that this relation is generally applicable to solids of all types.

Murnaghan[10,11] analyzed (3) and found, assuming the strain and P_0 to be constant over a homogeneous medium, that

$$P = \frac{B_0}{B_0'}\left\{\left(\frac{V_0}{V}\right)^{B_0'} - 1\right\} \tag{4}$$

This equation, generally referred to as "the Murnaghan equation", is an equation of state for an isotropic deformable medium. Anderson considered both polynomial equations of state as well as the Murnaghan equation of state and found the Murnaghan equation to be generally superior to the polynomials. Indeed, Anderson demonstrated the success of the Murnaghan equation in predicting the compression of a variety of substances up to very high pressures.

Drickerman *et al.*[12] used the Murnaghan Eq. (4) to describe their results of lattice parameters under very high pressure. Among the substances tested were molecular crystals. The success of the Murnaghan equation in describing their data for molecular crystals over a large pressure range stimulated our interest in determining whether or not the behavior of the myosin filament liquid crystal could be described by the Murnaghan equation.

In our experiments pressure is the controlled variable and V_0 is determined from low-angle X-ray measurements of the unit cell taken when $P_{(\text{applied})} = 0$. In general the unit-cell volume is determined from the relation

$$V = 2Ld_{1,0}^2/\sqrt{3} \tag{5}$$

where L represents the length (parallel to the c axis) of the myofilament lattice (assumed to be invariant in these experiments) and $d_{1,0}$ the lattice planes illustrated in Figure 1.

MATERIALS AND METHODS

Single long tonic muscle fibers were dissected from the meropodite of the walking leg of the crayfish (*Orconectes*) and were stripped of the sarcolemma (plasma membrane) as described by April *et al.*[13-15] These single living skinned fibers in bathing solutions were mounted in a low-angle X-ray camera of a modified Franks design and, using an Elliott GX-6 rotating anode generator, X-ray diffraction patterns from the 1,0 lattice planes (see Figure 1) were recorded on Kodak NS film with exposures of approximately 2 hours.[13-15] The diffraction of light by the regular sarcomere repeat of muscle was utilized to determine accurately the sarcomere length and thus the degree of actin interdigitation. A 0.3 mW He-Ne laser (λ = 6328 Å; Optics Technology Inc., Los

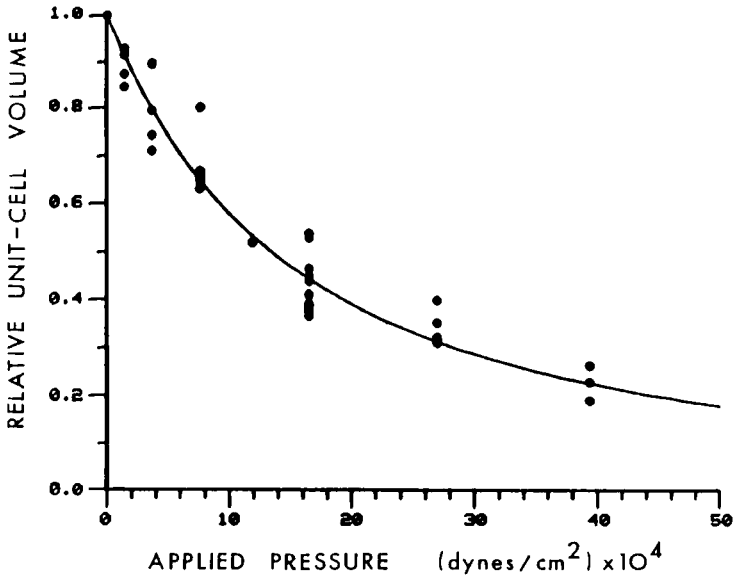

FIGURE 2 The effect of pressure on unit-cell volume. Relative unit-cell volume (determined by X-ray measurements of skinned striated muscle fibers) plotted against applied pressure. The applied (osmotic) pressure was adjusted by varying the PVP-10 (polyvinylpyrrolidone, molecular weight = 10,000) concentration of the medium. PVP-10 was the sole osmotically active agent with respect to the myosin filament lattice of skinned striated muscle. The curve demonstrates the behavior predicted by the Murnaghan equation.

Angeles, Calif., Model 170) was used to monitor the sarcomere length as previously reported by April *et al.*[14] Osmotic pressure was applied to the A-band lattice by introducing polyvinyl pyrrolidone (PVP-10, MW = 10,000) into the bathing solutions.[16–18] PVP-10 is the sole osmotically active particle with respect to the myosin filament lattice of the skinned muscle fiber. Since the concentrations of PVP-10 used were very small, the radial compressive osmotic pressures of the bathing solutions were determined using the virial expansion

$$P = \sum_{i=1}^{3} A_i (C\gamma)^i$$

where C denotes the prepared molar concentration of PVP-10, $A_i (i = 1,2,3)$ the virial coefficients of PVP-10, and γ the coefficient relating the number of osmotically active particles in a given PVP-10 sample to the calculated concentrations of the prepared solutions. The calculated pressures for low PVP-10 concentrations were very close to those obtained by using intact muscle cells as osmometers.[19] Data was collected for skinned muscle fibers in the relaxed state.[20]

TABLE I

Lattice parameters determined by X-ray diffraction as a function of applied (osmotic) pressure in the skinned striated muscle fiber with the corresponding relative unit-cell volume predicted by the Murnaghan equation

Number of observations	Applied pressure (dynes/cm^2)	Mean relative unit-cell volume	Std. dev.	Theoretical relative unit-cell volume
4	1.42×10^4	0.891	0.038	0.913
5	3.68×10^4	0.808	0.086	0.798
5	7.65×10^4	0.682	0.068	0.648
1	1.19×10^5	0.518	0	0.533
13	1.65×10^5	0.425	0.055	0.443
6	2.70×10^5	0.335	0.035	0.312
3	3.94×10^5	0.226	0.037	0.225

RESULTS AND ANALYSIS

The lattice parameters of the myosin filament liquid crystal were measured using the low-angle X-ray diffraction method described above at applied pressures ranging from 0 to 3.94×10^5 dynes/cm^2. The results of these measurements are given in Figure 2.

All of the data were utilized in "curve-fitting" the Murnaghan equation. The resultant coefficients of the Murnaghan equation were used to generate the curve demonstrated in Figure 2. At 2°C and 46% actin overlap the Murnaghan constants, B_0 and B_0', for the myosin filament liquid crystal in crayfish striated muscle are 1.51×10^5 dynes/cm^2 and 0.69 respectively. The predicted behavior shown in Table I and the fit of the curve to the data ($r = 0.977$) shown in Figure 2 demonstrate the applicability of the Murnaghan equation in describing the behavior of the myosin filament liquid crystal under compression.

DISCUSSION

Other laboratories have studied similar systems. Using optical techniques which provide an indication of the unit-cell dimensions on single fibers, Maughan and Godt[16,21] have reported values for the bulk modulus of a myofilament lattice obtained from the distal third of the semitendinosus muscle of the frog *Rana pipiens*. Their data analysis poses a few difficulties in terms of general application. They derive an empirical expression for the bulk modulus that is valid only for pressures ranging from 2.59×10^4 to 4.097×10^5 dynes/cm^2 and specific to their specimen. While their expression works well in the prescribed range for their specimen, it is essentially invalid at very low and

very high pressures. Consequently, calculation of the isothermal bulk modulus (1) using their equations is not possible.

Matsubara *et al.*[22] and April *et al.*[13] have shown the $d_{1,0}$ lattice spacing to be a linear function of sarcomere length, i.e. the degree of actin interdigitation. However, the relationship between the sarcomere length and the myosin filament lattice elastic constants has yet to be quantitated. In light of this we held the sarcomere length constant (9.61 ± 0.0079 micrometers). Also temperature was held constant at 2°C.

Use of the Murnaghan equation with X-ray diffraction data enables the determination of B_0 and B_0' in a non-empirical manner that is valid for large pressure ranges and many substances. We are presently using this methodology on striated muscle fibers in the rigor[20] state. The success of the Murnaghan equation in predicting the compression of the myosin filament lattice in crayfish striated muscle validates the approximation that the instantaneous bulk modulus is linear with pressure in this system. That this approximation holds for the myosin filament lattice further supports a solid state model for the striated muscle myosin filament lattice.

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