THE MYOFILAMENT LATTICE: STUDIES ON ISOLATED FIBERS

II. The Effects of Osmotic Strength, Ionic Concentration, and pH upon the Unit-Cell Volume

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ABSTRACT

The effects of osmotic concentration, ionic strength, and pH on the myofilament lattice spacing of intact and skinned single fibers from the walking leg of crayfish (*Orconectes*) were determined by electron microscopy and low-angle X-ray diffraction. Sarcomere lengths were determined by light diffraction. It is demonstrated that the interfilament spacing in the intact fiber is a function of the volume of the fiber. It is also shown that the interfilament spacing of the skinned (but not of the intact) fiber is affected in a predictable manner by ionic strength and pH insofar as these parameters affect the electrostatic repulsive forces between the filaments. From these combined observations it is demonstrated that the unit-cell volume of the in vivo myofilament lattice behaves in a manner similar to that described for liquid-crystalline solutions.

INTRODUCTION

Loeb (32) noted that even in extremely hyperosmotic solutions muscles lost a maximum of 20% of the initial weight. Later, Overton (36) postulated that muscles behave as osmometers if allowances are made for dry weight and extracellular space. This osmotic behavior has been extensively studied in frog whole muscle (11, 22, 25, 44) and in frog single fibers (9, 16, 38, 41). Worthington (45) showed by X-ray diffraction that the separation between the myofilaments of insect muscle fibers increased upon exposure to hyposmotic media. It was suggested by Harris (24), from electron microscope observations, that osmotic swelling in the muscle occurred primarily between myofibrils and within cytoplasmic organelles; however, no reference to lattice volume was included. H. E. Huxley et al. (30) observed that frog fibers fixed for electron microscopy after exposure to hypertonic solutions appeared to have less space between the thick myofilaments than control fibers. Brandt et al. (12), also from an electron microscope study, reported that the spacing between the thick myofilaments in crayfish muscle fibers was doubled when the fibers were placed in a hypotonic medium before fixation whereas the myofilaments became more closely packed in a

hypertonic medium. Rome (40) demonstrated by X-ray diffraction that the myofilament lattice of frog muscle behaves as an osmometer with 38% of the lattice volume osmotically inactive.

This report demonstrates, using electron microscope and X-ray diffraction procedures, that the myofilament lattice of intact muscle fibers from the walking legs of crayfish (*Orconectes*) reflects the osmotic behavior of the whole muscle fiber. Experiments demonstrating that the interfilament spacing of skinned fibers is dependent upon the ionic composition, and the pH of the internal medium are also described. Brief accounts of these results have already appeared (1, 3, 6, 7).

MATERIALS AND METHODS

Most of the experiments described herein were carried out in conjunction with or subsequent to those reported by April et al. (4) in which the apparatus and basic experimental procedure are described.

For fixation in NaCl or urea media, either the control saline solution was used throughout (a modified van Harreveld's crustacean saline solution) or it was replaced for an equilibration period of 30 min by a solution made hypo- or hypertonic by adjustment of the NaCl concentration or of the urea concentration. Subsequently, to prevent contraction, an identical solution containing 1 mg/ml procaine hydrochloride was substituted before fixation with 1% OsO_4 as described by April et al. (4). For fixation in KCl media the control saline solution was replaced for 3 min by an isosmotic 30 mM/liter KCl saline solution to lower the membrane potential so that no large contraction occurred when isosmotic 200 mM/liter KCl solution was subsequently perfused through the chamber. The muscle fibers were fixed 30 min later. Procaine pretreatment was unnecessary in this instance, for the membrane is depolarized below the contraction threshold by the KCl.

The preparative technique and electron microscopy as well as the techniques utilized to determine the unit-cell volumes of the myofilament lattice of the fixed and living fibers are described in a previous paper (4).

In the experiments undertaken to determine the effects of pH upon the in vivo myofilament lattice of the intact and skinned fibers, the pH of the medium was adjusted to within 0.02 of a pH unit with propionic acid or KOH. The medium for some of the intact fibers was 200 mM/liter potassium propionate, 13.5 mM/liter $CaCl_2$, and 5 mM/liter Tris hydroxide. The medium for the remaining intact fibers and for all the skinned fibers was 200 mM/liter potassium propionate, 5 mM/liter $MgCl_2$, 10 mM/liter K_2-EGTA, 1 mM/liter adenosine triphosphate (ATP), and 20 mM/liter Tris hydroxide.

TABLE I

Lattice Parameters Determined by Electron Microscopy as a Function of Osmolarity

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69114E	95 mM/liter NaCl (0.2555 Osm)	8.90	556	3.56	31.8	618
68116E	" " " "	9.30	465	2.49	23.2	528
68075F	" " " "	8.45	496	2.84	24.0	537
69114A	130 mM/liter NaCl (0.3255 Osm)	9.70	482	2.68	26.0	559
69114D	" " " "	9.50	483	2.69	25.6	554
[7]	190 mM/liter NaCl (0.4455 Osm)	9.60	440 (±12)	2.23	21.5 (+1.1)	507 (±13)
69114B	240 mM/liter NaCl (0.5455 Osm)	9.70	407	1.91	18 6	472
69114C	280 mM/liter NaCl (0.6255 Osm)	9.50	386	1.72	16.3	443
69115C	380 mM/liter NaCl (0.8255 Osm)	8.75	362	1.51	13.2	399
69115D	" " " "	8.80	364	1.52	13.5	402
68075E	95 NaCl + 95 KCl (0.4455 Osm)	8.40	542	3.39	28.5	585
68074E	190 NaCl + 190 KCl (0.8255 Osm) 4 min	8.75	341	1.34	11.7	375
68075D	" " " " " " 30 min	8.70	442	2.25	19.6	485
68077H	190 NaCl + 400 Urea (0.8255 Osm) 4 min	8.65	380	1.66	14.4	416
68077C	190 NaCl + 400 Urea (0.8255 Osm) 30 min	8.85	414	1.97	17.5	459

In this and all following tables L_s means sarcomere length, and d_{m-m}, distance between myosin filaments. All numbers in brackets refer to the number of experiments.

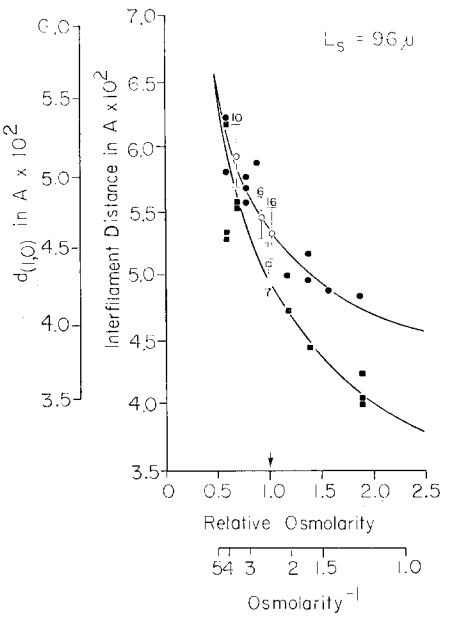

FIGURE 1 Interfilament distance as a function of osmolarity. The lattice spacing and interfilament distance obtained from fixed single crayfish fibers by electron microscopy (■) and from living single fibers by X-ray diffraction (●) are plotted against the relative osmolarity and reciprocal of the osmolarity of the medium. The sarcomere lengths of the fibers studied by electron microscopy were adjusted to 9.6 μ through the isovolumic relation, and those fibers from which the X-ray data were obtained were at 9.6 μ. The curves are calculated from the Boyle–van't Hoff equation. The open symbols (□, ○) are mean values, with the number of experiments and the standard deviation from the mean indicated.

RESULTS

Osmolarity and Ionic Strength

FIXED FIBERS: The interfilament distances of single fibers fixed in solutions made hypo- and hyperosmotic by adjustment of the NaCl concentration of the medium were determined by optical transform analysis of the electron micrograph plates. The sarcomere lengths were determined before fixation by light diffraction. The resultant unit-cell volumes varied as a function of the osmolarity as shown in Table I. The interfilament distances (adjusted to a sarcomere length of 9.6 μ through the isovolumic equation) are plotted against the osmolarity of the medium in Fig. 1 (solid squares).

Algebraic rearrangement of the Boyle–van't Hoff equation gives a linear form:

$$V/V_o = (1 - b/V_o)(P_o/P) + b/V_o \qquad (1)$$

where P_o and V_o are, respectively, the original osmolarity and volume, P the experimental osmolarity, V the equilibrium volume, and b is the minimal unit-cell volume. The relative unit-cell volume (V/V_o) is plotted against the reciprocal of the relative osmolarity (P_o/P) in Fig. 2 (solid squares), and a line is fitted to the experimental points by the method of least squares. The ordinate intercept (b/V_o) of this plot is 29% (±2% SE of estimate). This represents the fraction of the unit-cell volume which acts as an osmotic dead space within the fixed lattice.

From the volume term in the modified Boyle–van't Hoff equation, the interfilament spacing of the fiber lattice can be calculated over a wide

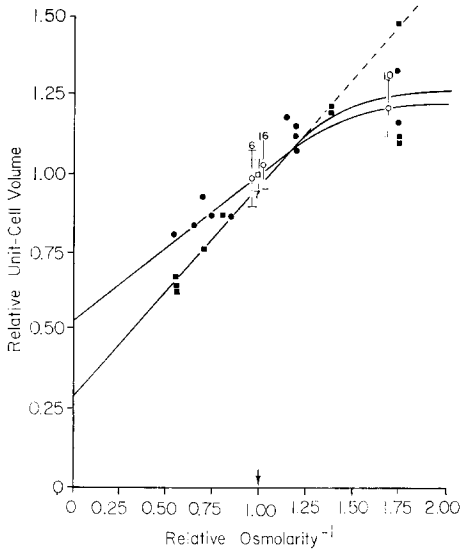

FIGURE 2 Lattice volume as a function of osmolarity. The ratio of the experimental unit cell volume to that of the control is plotted against the reciprocal of the relative osmolarity. The squares (■) are individual results obtained from electron microscopy while the circles (●) are the results from X-ray diffraction. The solid lines (——) are the least squares fits below 3.5 × 10^{-1} osmoles, above which the curves are fitted by eye. The ordinate intercepts indicate the myofilament lattice equivalent of the nonosmotic volumes for the living (53%) and the fixed (29%) fibers. The linear fit of the data indicates compliance with the Boyle–van't Hoff relation. The open symbols (□, ○) are mean values, with the number of experiments and the standard deviation from the mean indicated.

TABLE II

Lattice Parameters Determined by X-Ray Diffraction as a Function of Osmolarity

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
68224B1	95 mM/liter NaCl (0.2555 Osm)	9.65	502	2.90	28.1	581
68225A1	" "	9.65	535	3.30	31.9	619
[10]	100 mM/liter NaCl (0.2655 Osm)	9.60	514 (±23)	3.05	29.3 (±2.5)	593 (±26)
68346A3	150 mM/liter NaCl (0.3655 Osm)	9.60	500	2.88	27.7	577
68346B2	" "	9.60	482	2.68	25.8	556
68347A4	" "	9.60	492	2.79	26.8	568
68348A2	160 mM/liter NaCl (0.3855 Osm)	9.60	505	2.94	28.3	583
[6]	190 mM/liter NaCl (0.4455 Osm)	9.60	472 (±17)	2.57	24.7 (±1.9)	545 (±20)
[16]	200 mM/liter NaCl (0.4655 Osm)	9.60	462 (±19)	2.46	23.7 (±2.0)	533 (±22)
68347A3	240 mM/liter NaCl (0.5455 Osm)	9.60	435	2.18	21.0	502
68347A2	280 mM/liter NaCl (0.6255 Osm)	9.60	432	2.15	20.7	498
68235B1	285 mM/liter NaCl (0.6355 Osm)	9.65	446	2.29	22.2	516
68347A5	320 mM/liter NaCl (0.7055 Osm)	9.60	425	2.08	20.0	490
68231A1	380 mM/liter NaCl (0.8255 Osm)	9.65	421	2.04	19.8	487
[8]	95 NaCl + 95 KCl (0.4455 Osm)	9.60	568 (±16)	3.72	35.8 (±2.0)	655 (±19)
[3]	90 NaCl + 200 Urea (0.4455 Osm)	9.60	517 (±2)	3.08	29.6 (±1.5)	596 (±2)
[9]	200 mM/liter K propionate (0.4455 Osm)	9.60	515 (±16)	3.06	29.4 (±1.4)	594 (±18)

range of osmotic pressures at any given sarcomere length:

$$P(V - b) = \phi nRT \qquad (2)$$

where P is the osmolarity of the medium, V is the volume of the unit cell, b is the minimal unit-cell volume, ϕ is the osmotic coefficient, and nRT has the usual significance. From the calculated volume of the unit cell of the fixed fibers and the assumption that the lattice equivalent to the osmotic dead space is 29% of the unit-cell volume, a curve can be generated which relates the reciprocal of the osmolarity to the interfilament distance. The experimental points closely approximate this theoretical curve (Fig. 1, lower curve). At the minimal unit-cell volume (6.2×10^{-3} μ^3) the thick filaments would be spaced 273 A apart (center-to-center) at the standard sarcomere length of 9.6 μ. The control unit-cell volume is 21.5 (±1.1) $\times$ 10^{-3} μ^3, representing an interfilament separation of 507 (±13) A at the standard sarcomere length used.

The interfilament distances of single intact fibers also vary with additions or isosmotic substitutions of urea and KCl to the bathing medium. The unit-cell volumes of fibers after 4 min in a solution made hyperosmotic by addition of 400 mM/liter urea average 14.4 $\times$ 10^{-3} μ^3, 67% of the control unit-cell volume. The lattice volume is an average 17.5 $\times$ 10^{-3} μ^3 when the fibers are fixed 30 min after the introduction of the urea solution. Although the 30 min value of the unit-cell volume is

still less than that of the control volume, the lattice tends to return to the control level with time. The unit-cell volumes average 28.5 $\times$ 10^{-3} μ^3 in cells fixed after 30 min exposure to a hyposmotic (less 95 mM/liter NaCl plus 95 mM/liter KCl) saline solution, 133% of the control volume.

LIVING FIBERS: The interfilament distances of living single fibers were determined by low-angle X-ray diffraction in bathing media of different osmolarities and tonicities. The results are listed in Table II. The equilibrium unit-cell volumes decrease when fibers are bathed in solutions made hyperosmotic and increased in solutions made hyposmotic by adjusting the NaCl concentration. The interfilament spacing is plotted against the osmolarity of the medium in Fig. 1 (solid circles) at a sarcomere length of 9.6 μ. The mean interfilament spacings ($\pm$ the standard deviation from the mean indicated by brackets) are indicated by open circles.

The relative unit-cell volume (V/V_o) is plotted against the reciprocal of the relative osmolarity (P_o/P) in Fig. 2 (solid circles). The line is the least squares regression fit for the X-ray data below P_o/P = 1.3. The ordinate intercept (b/V_o) is 53% ($\pm$ 3% SE of estimate).

A curve relating the reciprocal of the osmolarity to the distance between the thick myofilaments can be calculated as before from the Boyle–van't Hoff equation, using the computed unit-cell volume of the living fiber at a sarcomere length of 9.6 μ, 24.1 ($\pm$1.7) $\times$ 10^{-3} μ^3, and an assumed lattice equivalent to an osmotic dead space of 53%. The experimental results at the osmolarities studied approximate the theoretical curve (Fig. 1, upper curve) within 1 SD from the mean of the control points. Thus, the equivalent nonosmotic volume of the myofilament lattice corresponds to an in vivo minimal unit-cell volume of 12.9 $\times$ 10^{-3} μ^3 and represents a minimal in vivo interfilament spacing of 393 A at the standard sarcomere length of 9.6 μ. The control unit-cell volume is 24.1 ($\pm$1.7) $\times$ 10^{-3} μ^3 which represents an interfilament spacing of 538 ($\pm$19) A at the standard sarcomere length used.

When fibers are equilibrated in solutions made hypotonic either by deletion of 95 mM/liter NaCl or by substitutions of 190 mM/liter urea for 95 mM/liter NaCl, the unit-cell volumes are 29.3 ($\pm$2.5) $\times$ 10^{-3} μ^3 and 29.6 ($\pm$1.5) $\times$ 10^{-3} μ^3, respectively, which is about 124% of the control unit-cell volume. When fibers are equilibrated for 30 min in a solution made hypotonic, but kept isosmotic, by substitution of 95 mM/liter KCl for an equivalent amount of NaCl, the average unit-cell volume is 35.8 ($\pm$2.0) $\times$ 10^{-3} μ^3, representing about 144% of the volume of the control (Table II).

SKINNED FIBERS: The interfilamen distances of skinned fibers bathed in media of various osmolarities were determined by low-angle X-ray diffraction and the data are presented in Tables III A and B. In fibers bathed in the potassium propionate medium there is an approximate 22% increase in the unit-cell volume (29.8 $\times$ 10^{-3} μ^3), which is followed by an additional 38% increase in the unit-cell volume (39.4 $\times$ 10^{-3} μ^3) upon removal of the sarcolemma.

TABLE III A

Lattice Parameters Determined by X-Ray Diffraction as a Function of Ionic Strength in Intact Fibers

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69210B5	50 mM/liter K propionate (i = 0.0955)	10.60	569	3.73	39.6	690
69212B3	" " " " "	9.60	567	3.71	35.6	654
69214A2	" " " " "	10.10	567	3.71	37.5	671
69219A2	75 mM/liter K propionate (i = 0.1205)	12.60	548	3.46	43.7	724
69210B4	100 mM/liter K propionate (i = 0.1455)	10.60	572	3.77	40.0	694
69212B2	" " " " "	9.60	567	3.71	35.6	654
69210B3	150 mM/liter K propionate (i = 0.1955)	10.60	552	3.51	37.3	669
[11]	200 mM/liter K propionate (i = 0.2455)	9.60	518	3.09	29.7	598 ($\pm$21)
69210B2	300 mM/liter K propionate (i = 0.3455)	10.60	481	2.67	28.3	583
69216A2	" " " " "	10.40	456	2.40	25.0	548
69216A3	400 mM/liter K propionate (i = 0.4455)	10.40	434	2.17	22.6	521

TABLE III B

Lattice Parameters Determined by X-Ray Diffraction as a Function of Ionic Strength in Skinned Fibers

Experiment	Condition	Ls	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69219B3	0 mM/liter K propionate (i = 0.0455)	9.80	535	3.30	32.4	624
69210A5	50 mM/liter K propionate (i = 0.0955)	11.20	552	3.51	39.4	688
69214B2	" " " " "	11.90	521	3.13	37.3	669
69221A5	" " " " "	10.30	547	3.45	35.6	654
69219B2	75 mM/liter K propionate (i = 0.1205)	9.80	567	3.71	36.4	661
69210A4	100 mM/liter K propionate (i = 0.1455)	11.20	557	3.58	40.1	694
69221A4	" " " " "	10.30	553	3.53	36.4	661
69210A3	150 mM/liter K propionate (i = 0.1955)	11.00	557	3.58	39.4	688
69221A3	" " " " "	10.30	570	3.75	38.6	681
69225A2	" " " " "	9.60	622	4.46	42.9	718
[18]	200 mM/liter K propionate (i = 0.2455)	9.60	595	4.08	39.2	687 (±21)
69210A2	300 mM/liter K propionate (i = 0.3455)	11.20	569	3.73	41.9	709
69216B3	" " " " "	10.90	594	4.07	44.4	730
69221A2	" " " " "	10.30	594	4.07	42.0	710
69225A3	" " " " "	9.60	630	4.58	44.0	727
69216B3	400 mM/liter K propionate (i = 0.4455)	10.90	594	4.07	44.4	730
69225A4	" " " " "	9.60	658	4.99	48.0	759
69225A6	" " " " "	9.60	642	4.75	45.7	741
69225A5	500 mM/liter K propionate (i = 0.5455)	9.60	658	4.99	48.0	759

In one series of experiments the effects of ionic strength upon the myofilament lattice were investigated. A pair of single fibers isolated from the same bundle and attached to the same tendon were mounted together in the chamber, and one fiber was skinned. The interfilament distances of the intact and skinned fibers were determined sequentially by X-ray diffraction, and the results are listed, respectively, in Tables III A and B. The interfilament distances are plotted against the relative ionic strength of the medium in Fig. 3. Since the propionate is nonpermeant, ionic strength with respect to the intact fibers is practically equivalent to osmolarity, and indeed the behavior of the lattice of the intact fiber is accordant with the Boyle–van't Hoff relation. The curve for the intact fibers is that calculated from the pressure-volume equation, using the volume of the unit-cell in 200 mM/liter potassium propionate solution (29.8×10^{-3} μ^3) and the in vivo nonosmotic volume of 53%.

The effect of the ionic strength upon the lattice of the skinned fiber is not only markedly less than that upon the intact fiber, but it is in the opposite direction. The data approximate a straight line fitted by the method of least squares. The lattice spacing of the skinned fiber is therefore directly proportional to the ionic strength which is quite unlike the greater inverse function in the intact fiber and which is accountable to the osmolar concentration of the external medium. It is thus apparent that the myofilament lattice of the skinned fiber no longer behaves as an osmometer.

The relative unit-cell volumes are plotted against the reciprocal of the relative ionic strength in Fig. 4. The lattice of the intact fiber behaves as an osmometer whereas that of the skinned fiber is independent of the osmolarity. At low osmolarities a limit to the capacity of the lattice of the intact fiber to expand is reached and the interfilament spacing becomes similar to that of the skinned fiber.

pH

INTACT FIBERS: The effects of the pH of the medium upon the myofilament lattice of the intact fiber were studied in another series of experiments. The results are listed in Table IV A, and the interfilament spacing is plotted against the pH of the external medium in Fig. 5. There is little change in the interfilament spacing of the intact fibers as the

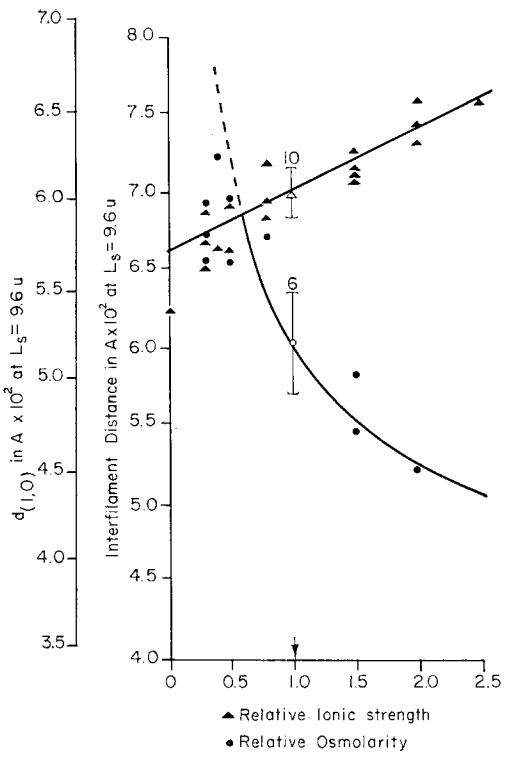

FIGURE 3 Interfilament spacing in crayfish as a function of osmolarity and of ionic strength. The lattice and the interfilament spacing determined by X-ray diffraction for living single fibers (●) and living fibers from which the sarcolemma has been removed (▲) are plotted against the same conditions expressed, respectively, as the relative osmolarity and the relative ionic strength of the medium. The curve for the intact fibers is calculated from the Boyle–van't Hoff equation, while that for the skinned fibers is fitted by the method of least squares. The open symbols (○, △) represent mean values, with the number of experiments and the standard deviation from the mean indicated.

pH of the external medium is changed between 6.4 and 9.4. Over this range the interfilament distance averages 601 (±17) A which is very close to the mean of the control values, 598 (±21) A, and the value, 595 (±18) A, reported previously (4). Below pH 6.4, however, the lattice spacing becomes progressively less, reaching 488 A at pH 4.4. In the intact fibers these effects are not reversible below pH 6.4. The pH effects were indistinguishable in both of the potassium propionate solutions used.

SKINNED FIBERS: In the skinned fibers the myofilament lattice spacing follows a direct nonlinear relation with the pH of the medium. These results are listed in Table IV B and are plotted in Fig. 5. The mean of the control values is 687 (±21) A which is considerably larger than the value, 608 (±26) A; reported previously (4) where the pH was not so closely controlled. There is a distinct effect of pH upon the interfilament distance of the skinned fiber at all of the pH values studied. At pH 4.4, the spacing in the skinned fiber averages 445 A whereas at pH 9.4 the interfilament spacing averages 722 A. At pH values below 6.4, the effect of pH upon lattice spacing is not reversible, and in this range the curves of the intact and skinned fiber appear as if they might overlap.

DISCUSSION

Osmotic Pressure Effects

Numerous studies of the osmotic properties of the single muscle fiber of the frog (9, 16, 38, 41) have produced values for the apparent osmotic dead space of from 33 to 35% of the total volume of the fiber. This apparent osmotic dead space is considerably greater than the reported 26% (16), later recalculated to 20% (9) for the nonaqueous volume. Impossibly low values would have to be assigned to the densities of the solid matter of the sarcoplasm if this were to be the whole source of the observed dead space. The apparent dead space exceeds the nonaqueous volume by a factor of 1.7. Dick (15) indicated that such discrepancies may be attributable to (*a*) the presence of bound water, (*b*) possible variations of osmotic coefficients of the cytoplasmic proteins with changes in the concentration, and (*c*) the presence of mechanical rigidity opposing the osmotically induced volume changes.

If a portion of this discrepancy is due to some unspecified physical consequence of the highly organized lattice structure, then presumably, since there is a greater thin-to-thick myofilament ratio in the crayfish (6:1) than in the frog (2:1), the ratio of the nonaqueous volume to the apparent osmotic dead space might be higher in the crayfish. Single muscle fibers of the crayfish have an apparent osmotic dead space of 40% (37) while the nonaqueous volume is 18% (M. Berman and J. P. Reuben, unpublished results). The apparent osmotic dead space thus exceeds the nonaqueous volume by a factor of 2.2.

Our observation in the crayfish that the minimal in vivo lattice volume (53%) is larger than the apparent osmotic dead space of the whole fiber

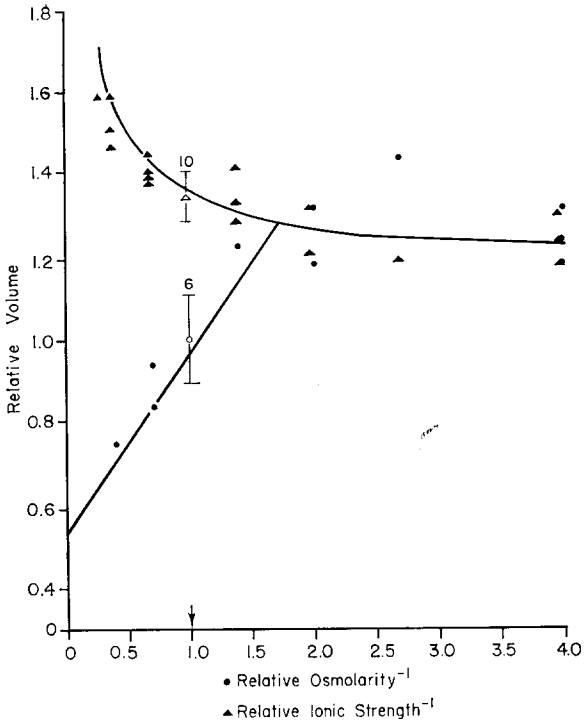

FIGURE 4 Unit-cell volume as a function of osmolarity and ionic strength. The relative unit cell volumes of intact fibers (●) and skinned fibers (▲) as determined by X-ray diffraction are plotted against the same conditions expressed, respectively, as relative osmolarity and relative ionic strength. The disparity in the behavior as a function of osmolarity and ionic strength is evident. The curve for the intact fibers is fitted by the method of least squares, while that for the skinned fibers is fitted by eye. It is also evident that there is a restriction upon the amount to which the lattice can swell. The open symbols (○, △) represent mean values, with the number of experiments and the standard deviation from the mean indicated.

TABLE IV A

Lattice Parameters Determined by X-Ray Diffraction as a Function of pH in the Intact Fiber

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69207A4	pH 4.4	9.60	423	2.06	19.8	488
69194A4	pH 5.4	9.10	484	2.70	24.6	544
69207A3	"	9.60	448	2.31	22.2	517
69194A3	pH 6.4	9.10	513	3.03	27.6	576
69198A2	"	9.60	514	3.05	29.3	593
69207A4	"	9.60	511	3.01	28.9	590
[11]	pH 7.4	9.60	518	3.09	29.7	598 (±21)
69194A2	pH 8.4	9.10	539	3.35	30.5	605
69198A3	"	9.60	525	3.18	30.6	606
69204A2	"	9.60	512	3.02	29.0	591
69198A4	pH 9.4	9.60	527	3.20	30.8	608
69204A3	"	9.60	528	3.21	30.9	609
69205A2	"	9.10	546	3.44	31.3	613

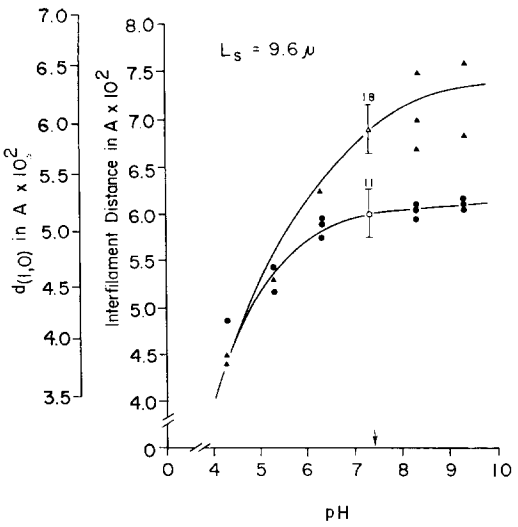

FIGURE 5 Interfilament spacing as a function of pH. The lattice spacing and interfilament spacing of living single muscle fibers (●) and skinned fibers (▲) is plotted against the pH of the medium. The curves are fitted by eye. The open symbols (○, △) represent mean values, with the number of experiments and the standard deviation from the mean indicated.

(40%) is similar to the results obtained for the frog lattice in which the minimal in vivo lattice volume (38%, 40) is larger than the apparent dead space of the whole fiber (33%, 38). Dick (15) discusses evidence suggesting that various constituent organelles of the cell have different non-osmotic volumes. The observed dead space of the whole muscle fiber may represent the weighted average of the various minimal volumes of the sarcoplasmic constituents.

The unit-cell volume of the myofilament lattice of crayfish fibers fixed in media of various tonicities follows a Boyle–van't Hoff relation (cf. Figs. 1 and 2). In these electron microscope studies, therefore, the volume of the myofilament lattice is an inverse function of the osmolarity. Similar results are obtained by X-ray diffraction studies of living crayfish fibers (cf. Figs. 1 and 2), confirming the observations reported by Rome (40) for whole frog muscle. It is apparent that the unit-cell volume of the myofilament lattice is an index of the osmotic behavior of the whole muscle fiber.

Comparing the electron microscope and X-ray results, it is interesting to note that while the myofilament lattice volume shrinks during fixation by 11% in the control medium, the minimal unit-cell volume shrinks by 51% determined by extrapolation to infinite osmolarity. It seems that the minimal unit-cell volume shrinks more than the cytoplasm as a whole shrinks. This fixation shrinkage, which appears to be mainly located within the osmotic dead space, might be due to several possible effects of the fixative. First, the fixation shrinkage might involve a conformational change in the structure of the myofilaments so that the diameters decrease, but the surface-to-surface distance between the myofilaments remains unchanged. There is some evidence in support of this from the work of Elliott et al. (19), who found that they could best account for the equatorial distribution of X-ray intensity for living frog and rabbit muscle by taking the effective filament diameters in vivo to be about twice those inferred from measurements from fixed tissue, sectioned and examined in the electron microscope. A second

TABLE IV B

Lattice Parameters Determined by X-Ray Diffraction as a Function of pH in the Skinned Fiber

Experiment	Condition	L_s	$d_{1,0}$	Area	Volume	d_{m-m} at L_s = 9.6 μ
		μ	A	$10^{-3}\mu^2$	$10^{-3}\mu^3$	A
69188A4	pH 4.4	9.60	394	1.79	17.2	451
69188A5	"	9.60	379	1.65	15.9	437
69188A3	pH 5.4	9.60	460	2.44	23.4	531
69188A2	pH 6.4	9.60	540	3.36	32.3	623
[18]	pH 7.4	9.60	595	4.08	39.2	687 (±21)
69190A2	pH 8.4	10.20	631	4.59	46.9	751
69191A2	"	9.75	599	4.14	40.4	697
69192A2	"	11.60	529	3.23	37.5	671
69191A3	pH 9.4	9.75	651	4.89	47.7	757
69192A3	"	11.90	534	3.29	39.2	686

possibility involves a change in the minimum surface-to-surface distance because of a possible change in the forces acting between the myofilaments or a change in the amount of water bound to the myofilament surfaces. It is also quite possible that a combination of all of these effects might occur.

One general conclusion at least emerges from these experiments. In a fiber with the sarcolemma intact the lattice volume invariably follows the fiber volume, which in turn depends upon the osmotic constraints (Table III). When the sarcolemma is removed from the fiber there is usually a volume increase. This increase is least when the fiber is initially swollen and greatest when the fiber is shrunken (cf. Figs. 2 and 5). As a working hypothesis we suggest that the in vivo lattice is normally constrained from expansion by the sarcolemma, which is consistent with the observed expansion upon removal of that organelle. This implies that there may not be a true osmotic balance across the sarcolemma and that the relatively large minimal unit-cell volume of the in vivo lattice corresponds to the point at which the residual outwardly directed forces of the lattice are balanced by the inwardly directed osmotic forces and the intrinsic elasticity of the sarcolemma. This relationship would hold within the structural limits of the fiber lattice. It is plausible that the fiber volume is a primary factor in the interfilament spacing. At low osmolarities the maximum expansion of the lattice of the intact fiber appears to be similar to the limit in the skinned fiber (cf. Figs. 3 and 4). It is generally thought that the constraint to extreme muscle swelling is the elasticity of the sarcolemma (23). With respect to the myofilament lattice, however, the constraint in the extreme swollen state seems to be independent of the sarcolemma. The fiber can swell to 300% of the control volume (37) while the lattice only expands to about 175% (Fig. 4). This could be due to mechanical limitation such as Z line or M line structure; but, it could also represent the true balance between the van der Waal's and electrical double-layer forces which are thought to stabilize the hexogonal lattice (17, 18). Again, further experiments in this area are needed.

One further point worthy of note is that Rome (40) found that the lattice spacing in glycerinated rabbit psoas muscle was, over most of the sarcomere length range, greater than that in living whole muscle. Rome also found that in frog muscle something appeared to prevent the filament lattice from swelling maximally in hypotonic Ringer's solution (see Fig. 6 in reference 40). In the case of the skinned fibers, the sarcolemmal constraint is no longer operative and, since ample ATP is present, the filaments are free to separate. These observations support the role which the sarcolemma plays in our tentative hypothesis.

The observation and quantitation of the effects of osmotic pressure upon the myofilament lattice of the intact muscle fiber is of further interest, for not only can this behavior be explained in terms of the proposed (20, 21, 34, 42) liquid-crystalline nature of the lattice, but the limiting role of the sarcolemma is defined as it contributes to the phenomenon of interfilament spacing (2).

Ionic Strength Effects

In a number of our experiments the osmolarity of the medium was varied independently of the ionic strength, and the effects upon the myofilament spacing were studied in fixed fibers, in living fibers and in "living" fibers with the sarcolemma removed. The results from the fixed and in vivo studies indicate that changes in the lattice volume of fibers with intact membranes are not due to changes in the ionic strength of the internal medium since an isosmotic, but hypotonic, KCl solution causes the lattice to swell even though the internal ionic strength is thought to remain constant (5, 10, 31, 33). Similar volume changes induced with urea (14) show that, in all cases where the cell membrane is intact, the lattice volume follows the fiber volume (cf. Tables I and II).

The X-ray diffraction experiments in intact and skinned fibers also distinguished between the effects of osmolarity and ionic strength. After removal of the sarcolemma the myofilament lattice volume expands to a new volume and henceforth responds markedly less and in the opposite direction to the same osmolar changes, expressed as ionic strength, which greatly modify the volume of the intact fiber (cf. Fig. 3). It is evident that it is the osmolarity of the external medium, not the ionic strength of the internal medium, which has the major effect upon the in vivo interfilament spacing. It further seems likely that the effects upon the myofilament lattice spacing in the living frog muscle bundle attributed partly to ionic strength and to the special nature of potassium

ions (40) are in fact due to osmotic pressure changes across the sarcolemma.

The ionic strength effect is more equivocal. There is no doubt, however, that ionic strength does exert a distinct effect upon the myofilament spacing in the living fiber which can only be observed when the sarcolemma is removed. These results support to some degree the observations reported by Rome (40) in which changes in the ionic strength at sarcomere lengths longer than rest length result in similar variations in the lattice spacing of glycerol-extracted rabbit psoas muscle. Our results do not cover the lower range of ionic strengths as thoroughly as do Rome's, but over the normal to higher ionic strengths the trend appears to be similar. Rome (40) has ascribed the effects of ionic strength upon the filament separation of glycerinated muscle to the effect of the counterions on the electric-double-layer associated with the filaments. This explanation was initially proposed by Bernal and Fankuchen (8) for similar behavior observed in the liquid-crystalline system of tobacco mosaic virus (TMV). In the case of both TMV and the glycerinated muscle a distinct saturation effect is observed at higher ionic strengths. There is, in the skinned fibers as well as in the glycerinated muscle, a slight reversal of this saturation effect which in the latter case has been shown to be somewhat dependent upon sarcomere length. This effect of ionic strength in this range is in a direction that is opposite to that given by the common-sense argument, which would hold that an increased concentration of ions in solution would increase the electrostatic shielding and thus decrease the repulsive double-layer forces, and would lead to a *decrease* of the interfilament spacing. A point to consider, however, is the possibility that the ionic strength in the operative region of the electrical double-layer may not be related to the medium in a straightforward manner. Additionally, the theoretical work of Nineham and Parsegian (35) opens the possibility that the van der Waal's (dispersive) forces, to which the attraction between filaments has been ascribed (17, 18, 20, 39, 40, 43), may also be a function of the ionic composition of the interfilament medium. We intend to make further calculations with respect to these effects. Obviously, more experiments must also be undertaken with respect to the effects of sarcomere length in the skinned fiber.

Preliminary light microscope observations on skinned crayfish fibers (April, unpublished) suggest that, under some conditions, at low ionic strengths the whole fiber swells dramatically, as Rome (40) also observed with glycerinated muscles at short sarcomere lengths. These data on the effect of ionic strength upon the myofilament lattice of living fibers stripped of the sarcolemma appear to support some aspects of the electrostatic hypothesis for filament separation proposed by Elliott (17, 18) and Rome (40). This hypothesis has been modified and extended by April (2) to account for the differences between intact and skinned fibers.

pH Effects

Experiments on living fibers involving change in the pH of the bathing medium suggest that long-range electrostatic forces are involved in the maintenance of the myofilament separation. The filament lattice of the intact single fiber exhibited very little change, if any, when the pH was varied over one unit on either side of the physiological control point (cf. Fig. 5). It had been shown that the internal pH of living muscle is relatively independent of the pH of the external medium (13, 26), although Rome (40) did observe a definite decrease in lattice spacing when the pH of the medium was changed by bubbling CO_2 through it. In the living skinned fibers in our experiments, the pH of the bathing medium was found to have definite effects upon the myofilament separation at all values of pH tested (cf. Fig. 5). These results in living fibers support and confirm the observations of Rome (40) who found that the lattice dimensions of glycerinated rabbit psoas muscle were inversely proportional to the pH of the medium, with some indication that the same may be true in the living muscle.

The pH effects are not simple, and our results cannot be considered conclusive because they were generally nonreversible beyond a narrow range. The denaturing effect of low pH upon protein is well established, and it might be assumed that this is the cause of the nonreversibility of the lattice shrinkage at low pH values.

Rome (40) ascribed the lattice behavior in response to pH to alteration of the net amount of negative charge on the myofilaments. Again, this explanation was originally proposed by Bernal and Fankuchen (8) for similar behavior observed in liquid-crystalline solutions of TMV. In general, the pH data appear to support the

postulation that interfilament spacing is determined to some extent by electrostatic repulsive forces originating from the negative charge on the myofilaments.

The results of the experiments reported in this and a previous paper (4) appear to support a hypothesis for the liquid-crystalline nature of the resting myofilament lattice (as defined by Elliott and Rome, 20). Several papers by H. E. Huxley and his coworkers (27, 28, 29) have illustrated the role of "cross-bridge" interaction in the generation of muscle tension. While this paper has described the morphological behavior of the myofilament lattice under specific physical conditions, a subsequent paper will deal with the dynamics of the active myofilament lattice by correlating these morphological data with the generation of tension.

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