X-RAY DIFFRACTION EVIDENCE FOR CROSS-BRIDGE FORMATION IN RELAXED MUSCLE FIBERS AT VARIOUS IONIC STRENGTHS

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ABSTRACT Equatorial x-ray diffraction patterns from single skinned rabbit psoas fibers were studied at various ionic strengths to obtain structural information regarding cross-bridge formation in relaxed muscle fibers. At ionic strengths between 20 and 50 mM, the intensity of the *11* reflection, I_{11}, of the relaxed state was close to that of the rigor state, whereas the intensity of the *10* reflection, I_{10}, was approximately twice that of the rigor reflection. Calculations by two-dimensional Fourier synthesis indicated that substantial extra mass was associated with the thin filaments under these conditions. With increasing ionic strength between 20 and 100 mM, I_{10} increased and I_{11} decreased in an approximately linear way, indicating net transfer of mass away from the thin filaments towards the thick filaments. These results provided evidence that cross-bridges were formed in a relaxed fiber at low ionic strengths, and that the number of cross-bridges decreased as ionic strength was raised. Above μ = 100 mM, I_{10} and I_{11} both decreased, indicating the onset of increasing disorder within the filament lattice.

INTRODUCTION

In vitro binding of myosin subfragment 1 (S-1) to regulated actin in the presence of ATP has been shown to occur to about the same extent in the presence and absence of Ca^{++} when the ionic strength is low (μ = 20–50 mM) (Chalovich et al., 1981; Chalovich and Eisenberg, 1982). The insensitivity of this binding to calcium concentration suggests that actomyosin cross-bridges, i.e., myosin heads attached to actin filaments, might be present in relaxed muscle fibers under similar conditions. Recently, it was found that relaxed rabbit psoas fibers showed substantial stiffness when rapid stretches were applied at μ = 20 mM and 5°C (Brenner et al., 1982). This stiffness was shown to be approximately proportional to the extent of overlap between thick and thin filaments in the sarcomere, suggesting that the observed stiffness is due to cross-bridges. These cross-bridges were thought to be in a rapid equilibrium between attachment and detachment, since at μ = 20 mM stiffness was only observed with very rapid stretches. As to whether cross-bridges are also present at higher ionic strengths, evidence from mechanical measurement is so far inconclusive. At μ =170 mM, there was nearly no detectable stiffness. Since the measured stiffness depends on the speed of stretch applied, a decrease in stiffness could be explained by either an increase in the rates of cross-bridge attachment-detachment or a decrease in the number of cross-bridges.

In the present study, equatorial x-ray diffraction from single skinned rabbit psoas fibers was obtained to determine whether structural evidence could be found for the presence of cross-bridges at various ionic strengths in the relaxed state. X-ray diffraction is a time-averaged measurement and thus the results are independent of the rates of cross-bridge attachment-detachment. We studied the two innermost reflections, *10* and *11*, from single fibers both in the relaxed state and in the rigor state. Two-dimensional electron density maps were calculated showing the relative mass associated with a thick and a thin filament. The results support the idea that a significant number of cross-bridges are present at low ionic strength in the relaxed fiber. The results further suggest that this number decreases when the ionic strength is raised. In addition, the mode of attachment in relaxed fibers at low ionic strength is probably different from that of the rigor state. A preliminary account of this work has been reported briefly (Brenner et al., 1983).

METHODS

Fiber Preparation

Equatorial patterns were obtained from single rabbit psoas fibers, the same type of preparation used in the stiffness measurements (Brenner et

al., 1982). Single fibers, 8–10 mm long and 80–150 μm in diam, were prepared from rabbit psoas muscle according to Brenner (1983). Relaxing solution at μ = 20 mM contained (in millimoles per liter): 1 ATP, 1 EGTA, 3 $MgCl_2$, 1 dithiothreitol (DTT), 10 imidazole, pH 7.0; rigor solution, 2.5 EGTA, 2.5 EDTA, 1 DTT, 10 imidazole. Ionic strength was generally adjusted by adding KCl; in a few experiments K propionate was used instead of KCl. All experiments were performed at 5°C.

There is little sarcomere dispersion along the fiber (Fig. 1), and the uniformity showed remarkable stability during experiments. The striation pattern was practically unchanged after repeated changes in conditions and no perceptible internal sarcomere movement or change in shape took place when conditions were changed from relaxation to rigor (Fig. 1).

X-ray Diffraction

The x-ray source was a rotating anode generator (model GX-6; Marconi-Elliott, Borehamwood, England) using the line focus configuration. Diffraction patterns were recorded by a high resistance wire detector (Podolsky et al., 1976). Typical exposure time was 1,000 s. The original data were smoothed once by a three-point averaging routine with weights (1,2,1) and integrated intensities were obtained by means of a cursor stripping method (Yu et al., 1979). The intensities were taken as the mean values of the two sides of the patterns. The cursor-stripping method assumes that the background under each peak is linear and that the peaks do not have long tails. Preliminary attempts have been made to obtain intensities by a curve-fitting method. The intensity ratio, I_{11}/I_{10}, obtained by the curve fitting is ~10% smaller than that obtained by the cursor-stripping method.

The muscle fiber was placed between two Mylar windows of a thermoelectrically cooled chamber. The striation pattern and the position of the fiber within the specimen chamber were monitored continuously by an inverted microscope through a window at the bottom of the chamber. Sarcomere length was first adjusted at the set-up bench to 2.2 to 2.4 μm using the light microscope and later monitored by laser diffraction during the experiments.

To compare intensities, I_{10} and I_{11}, from different fibers under different x-ray camera conditions, the following normalization procedure was adopted. The intensity of the *10* reflection at μ = 20 mM ($I_{10}^{\mu=20\,mM}$) was recorded and normalized with respect to I_c, the intensity of the x-ray beam through the backstop, several times during the course of the experiment and then averaged. I_{10}/I_c and I_{11}/I_c obtained under other conditions but from the same fiber were then normalized with respect to this averaged value of $(I_{10}^{\mu=20\,mM})/I_c$. Care was taken such that the same area of the fiber was sampled throughout the experiment.

The order of changing salt concentration did not follow any fixed routine. Patterns from μ > 170 mM were, in general, obtained near the end of the experiments, since high salt concentrations seemed to cause some irreversible disorder within the filament lattice. Experiments on relaxed and rigor patterns were generally performed on different fibers except that relaxed patterns at μ = 20 and 170 mM were always obtained as controls. However, during one experiment, cycling between relaxed and rigor states at various salt concentrations was performed, and the results were not distinguishable from others.

Control Experiments

(*a*) To see whether the relaxed fiber was partially depleted of ATP due to ATPase activity in the absence of calcium, a backup system (10 mM phosphocreatine and creatine kinase at 300 units/ml; Sigma Chemical Co., St. Louis, MO) was added to the relaxing solution. The lowest ionic strength in this case was 50 mM.

(*b*) To ensure that all the ATP binding sites of myosin were saturated in the presence of 1 mM MgATP, the concentration of MgATP was lowered to 50 μM in the presence of the backup system.

(*c*) To determine whether changes in intensities as a function of ionic strength were related to interaction between the thick and thin filaments, a few experiments were performed at sarcomere lengths of 4.2–4.6 μm, where there was no overlap between the thick and the thin filaments.

(*d*) Possible effects of lattice spacing on reflection intensities were investigated by adding various amounts of dextran T500 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) (1–5% wt/vol) to the relaxing solution at μ = 170 mM. In addition, in a few experiments K propionate was used

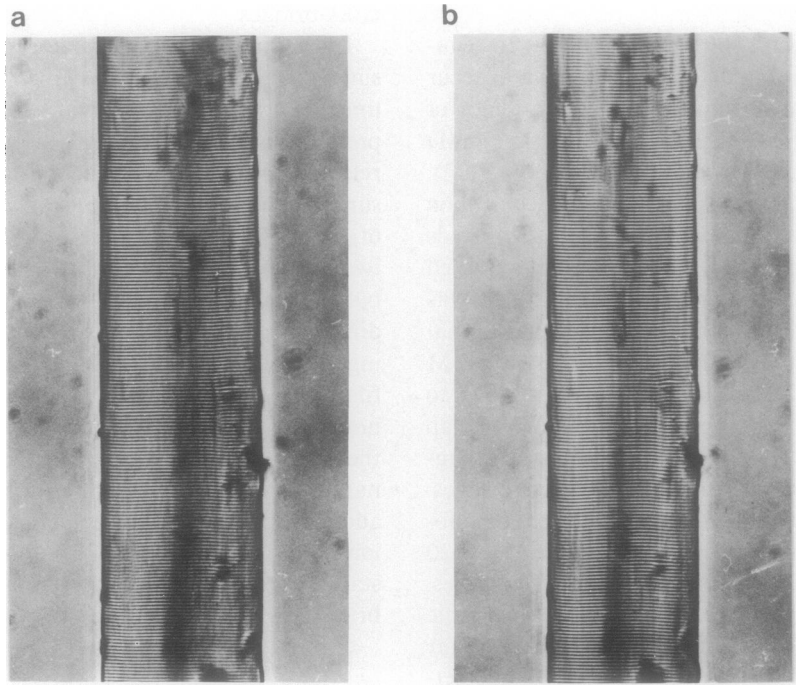

FIGURE 1 Micrographs of single skinned rabbit psoas fibers, in (*a*) the relaxed state and (*b*) the rigor state, both at μ = 170 mM, 5°C. The sarcomere length is 2.35 μm. In the present study, a total of 16 fibers were used, with 5 fibers mostly in the relaxed state, 6 fibers mostly in the rigor state, and 5 fibers in the dextran experiments.

instead of KCl, since in this case the lattice spacing was found to be constant between μ = 20 and 80 mM.

Fourier Transforms

Two-dimensional electron density maps of the axially projected filament lattice based on the *10* and *11* reflections were represented as Fourier transforms by means of the PIC system on a PDP 11/70 computer (Digital Equipment Corp., Marlboro, MA) (Trus and Steven, 1981). The amplitudes of both reflections were assigned phases of 0° (Huxley, 1968).

For the diffraction pattern of the relaxed muscle at μ = 20 mM, the zeroth-order Fourier component, F_{00}, was assigned to be 1,000 (arbitrary units) and the amplitude, F_{10}, to be 100. All other amplitudes derived from diffraction patterns under various conditions, including those of the rigor state, were normalized with respect to these two assigned amplitudes. The F_{00} term was not constant, since d_{10}, the lattice spacing of the *10* plane, expanded almost linearly as a function of increasing ionic strength when chloride was the predominant anion (see Results). The x-ray beam was shown to be ~50 μm in height, which was smaller than the fiber diameters, and great care was taken so that the beam passed through the middle portion of the fiber. The total amount of mass in the x-ray beam path consequently varied in inverse proportion to the diameter of the fiber, or d_{10} of the lattice. Since the term F_{00} is proportional to the total mass, it was normalized to be 1,000 × $(d_{10}^{\mu=20\,mM}/d_{10})$, where $d_{10}^{\mu=20\,mM}$ is the measured lattice constant of the relaxed state at μ = 20 mM and d_{10} is the lattice constant obtained under other conditions.

The relative mass, (A/M), defined as the ratio of mass associated with a thin filament to that with a thick filament of the unit cell, was calculated with the assumption that the background level was the lowest density level of each individual map. The filament regions were taken to be circular (disk) areas, whose diameters extended to the minimum of the line joining the thick and thin filaments. The diameters thus defined increased with ionic strength at μ = 20 mM, the diameter of the thick filament area (d_M) was 290 Å, the diameter of the thin filament area (d_A) was 215 Å; at μ = 170 mM d_M increased to 350 Å, d_A to 225 Å. Visible mass is defined as the total density of the ordered structure above the background level.

RESULTS

The single-skinned rabbit psoas fiber preparation was found to be well suited for obtaining equatorial patterns. Strong and sharp *10* and *11* reflections appeared above background in ~50 s under low salt conditions. Longer exposure time was used to improve the counting statistics. Fig. 2 shows typical patterns obtained from a single fiber cycled between the relaxed state at 20, 100, and 170 mM

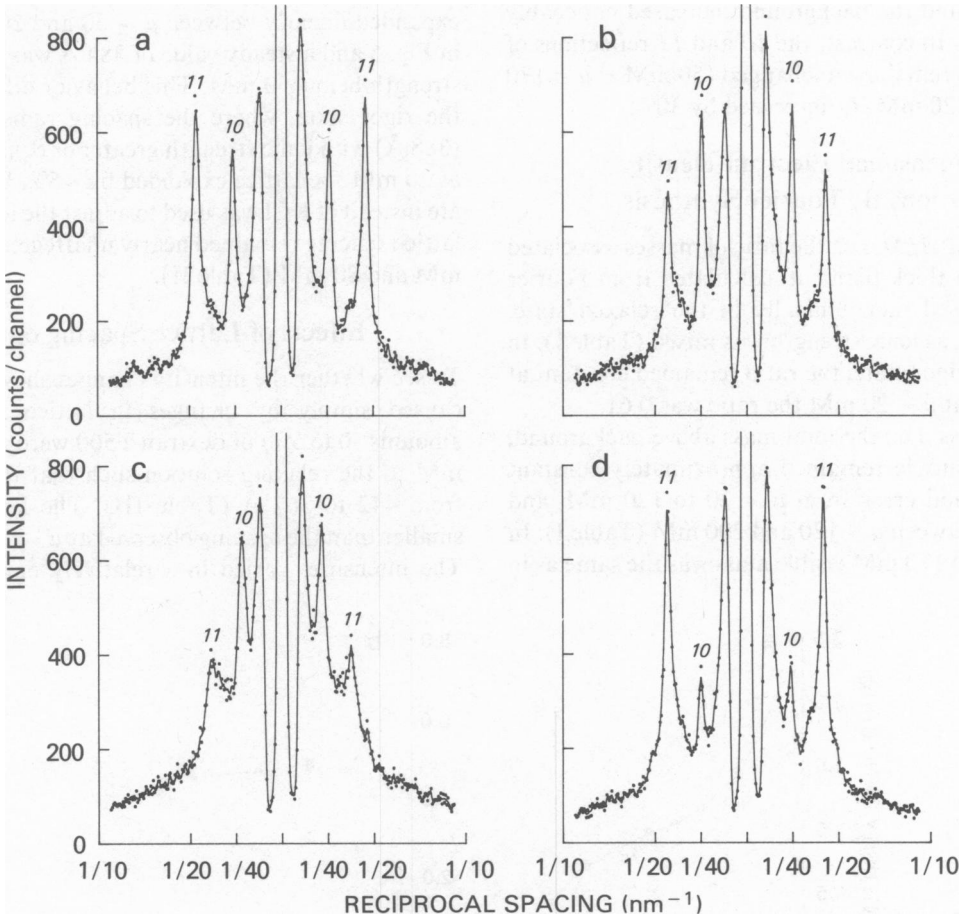

FIGURE 2 Equatorial diffraction patterns obtained from a single fiber in the relaxed state (*a*) at μ = 20 mM, (*b*) at 100 mM, and (*c*) at 170 mM; in the rigor state (*d*) at μ = 20 mM. Dots (·) correspond to original data; lines correspond to data smoothed by a three-point weighted average routine. The ease of changing states and the stability of the fiber are demonstrated by the fact that 20 solutions of various composition were applied to this fiber during this experiment without producing significant changes in the control patterns at μ = 20 mM (relaxed). Fig. 2 *a* was obtained with the first solution; *b*, the fourth; *c*, the fifteenth; and *d*, the nineteenth. KCl was used to change ionic strength.

and the rigor state at 20 mM, using KCl to adjust ionic strength.

Intensity Changes

Fig. 3 is the summary of changes in the intensity ratio, I_{11}/I_{10}, of the relaxed and rigor states associated with various ionic strengths. The ratio for the relaxed pattern decreased monotonically from 2.03 to 0.41 when the ionic strength was raised from 20 to 200 mM. The rigor patterns were virtually unaffected between 50 and 170 mM with the ratio remaining at ~5. Below 50 mM the rigor pattern showed a pronounced increase in the intensity ratio. Note that this change in intensity ratio was accompanied by a corresponding increase in d_{10}, the lattice constant (see Fig. 5).

Although the intensity ratio, I_{11}/I_{10}, changed monotonically, the behavior of the absolute intensities was more complicated (Fig. 4). Below μ = 100 mM, I_{10} and I_{11} changed in an approximately reciprocal manner: I_{10} increased and I_{11} decreased with increasing ionic strength. However, above μ = 100 mM both reflections decreased in intensity with the decrease in I_{11} being steeper. The widths of the reflection and the background increased noticeably for $\mu \geq 170$ mM. In contrast, the *10* and *11* reflections of the rigor patterns remained unchanged (50 mM $\leq \mu \leq$ 170 mM), but at μ = 20 mM, I_{11} increased by 30%.

Two-dimensional Electron-Density Distributions By Fourier Synthesis

The relative mass, A/M, i.e., the ratio of masses associated with a thin and a thick filament, calculated from Fourier synthesis, decreased monotonically in the relaxed state, from 0.43 to 0.20, as ionic strength was raised (Table I). In contrast, for the rigor state, the ratio remained constant at 0.56 except that at μ = 20 mM the ratio was 0.61.

The visible mass, i.e., the total mass above background, for the relaxed muscle remained approximately constant within experimental error from μ = 20 to 120 mM, and then decreased between μ = 120 and 200 mM (Table I). In rigor for μ = 50 to 170 mM visible mass was the same as in the relaxed fibers between μ = 20 to 120 mM. When ionic strength was 20 mM, visible mass in rigor increased by ~20%.

Saturation with ATP

We investigated the possibility of the fiber being in partial rigor at low ionic strength, which might be caused by (*a*) ATPase activity in the absence of Ca^{++}, depleting ATP in the center of the fiber; (*b*) insufficient concentration of ATP in the fiber to saturate all the myosin ATP binding sites.

Patterns obtained with and without the backup system in the presence of 1 mM MgATP at μ = 50 mM were indistinguishable from each other, thus ruling out MgATP depletion in the center of the fiber. At μ = 50 mM, with the backup system, no detectable changes in the diffraction patterns were observed by lowering the concentration of MgATP until it reached 50 μM, suggesting complete saturation in the presence of 1 mM MgATP.

Changes in Lattice Spacing

In a relaxed fiber, with KCl as the added salt, the lattice expanded linearly between μ = 50 and 200 mM as shown in Fig. 5 and a steady value of 384 Å was reached for ionic strength below 50 mM. This behavior differs from that of the rigor state, where the spacing remained unchanged (388 Å) with ionic strength greater or equal to 50 mM, and at 20 mM the lattice expanded by ~5%. When K propionate instead of KCl was used to adjust the ionic strength, the lattice spacing remained nearly unaffected between μ = 20 mM and 80 mM (Table II).

Effects of Lattice Spacing on I_{10} and I_{11}

To see whether the intensity changes shown in Fig. 4 were caused simply by changes in lattice spacing, various amounts (0 to 5%) of dextran T500 were added at μ = 170 mM to the relaxing solution such that the lattice shrank from 442 to 367 Å (Table III). The latter spacing was smaller than the spacing observed at μ = 20 mM (relaxed). The intensities varied in a relatively narrow range com-

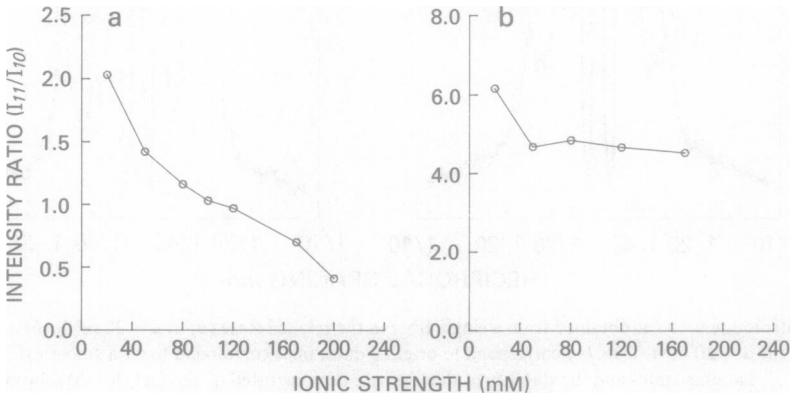

FIGURE 3 Intensity ratio, I_{11}/I_{10}, at various ionic strengths of the (*a*) relaxed and (*b*) rigor states. KCl was used to change ionic strength.

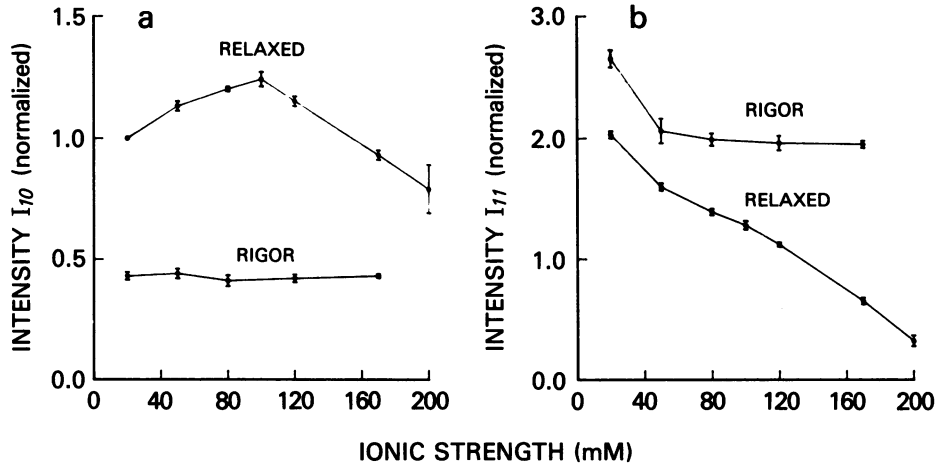

FIGURE 4 (*a*) I_{10} and (*b*) I_{11} of the relaxed fibers at various ionic strengths. The averaged, normalized intensity, $I_{10}^{\mu=20\text{ mM}}/I_c$ was used as normalizing factor for all intensities under various conditions obtained from the same fiber. The data shown here are averages from nine fibers. Error bars are standard error of the mean. KCl was used to change ionic strength.

pared with changes observed at various ionic strengths. In the presence of K propionate, where the lattice spacing remained unchanged between μ = 20 and 80 mM, the intensities (Table II) showed similar behavior as in Figs. 3 and 4, where KCl was used. Results shown in Tables II and III indicate that lattice spacing does not have significant effects on I_{10} and I_{11}.

Long Sarcomere Length

When fibers were stretched to 4.2–4.6 μm, where there was no overlap between the thick and thin filaments, the lattice spacings became smaller when ionic strength (using KCl) was lowered: 360 Å at 170 mM and 299 Å at 20 mM (Table IV).[1] At the same time I_{10} changed < 10% for 20 mM $\leq \mu \leq$ 170 mM, and decreased above 170 mM (I_{11} was not observable at this sarcomere length). This indicates again that the changes in I_{10} observed when ionic strength was varied at full overlap are not due to changes in lattice spacing, but rather are effects of ionic strength on interactions between the filaments.

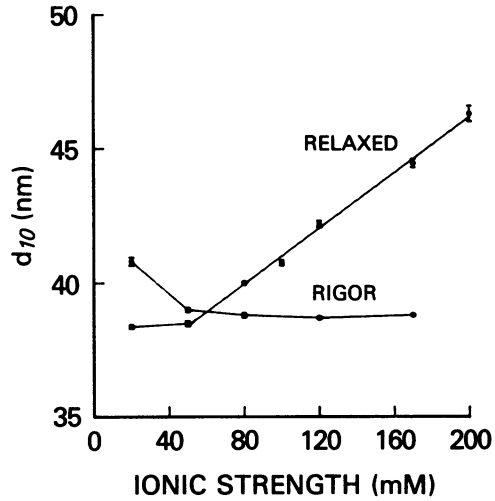

FIGURE 5 Lattice spacing, d_{10}, of relaxed and rigor states at various ionic strengths. Error bars are standard error of the mean. KCl was used to change ionic strength.

[1]This shrinkage behavior out of overlap indicates that the spacing change observed at full overlap was not simply caused by myosin heads interacting with the thin filaments.

DISCUSSION

Evidence for Attached Cross-Bridges in the Relaxed Fiber at μ = 20–50 mM

One of the striking features of the relaxed patterns at low ionic strengths is the strong I_{11}. At μ = 20 mM and 50 mM, I_{11} (relaxed) is only ~20% less than I_{11} (rigor). Since the fibers were saturated with ATP, the high intensity of *11* could not be caused by rigor cross-bridges. Based on the intensity data, calculations by Fourier synthesis showed that the relative mass, A/M, associated with the thin and thick filament region of the relaxed state at μ = 20 mM is

TABLE I
RELATIVE MASS (A/M) ASSOCIATED WITH A THICK AND THIN FILAMENT AND VISIBLE MASS

	μ (mM)						
	20	50	80	100	120	170	200
A. Relaxed state							
A/M*	0.43	0.39	0.35	0.33	0.32	0.26	0.20
Visible mass‡	1.00	0.95	1.00	1.02	1.00	0.90	0.79
B. Rigor state							
A/M	0.61	0.56	0.56	—	0.56	0.56	—
Visible mass	1.23	1.00	0.98	—	0.97	0.97	—

*Relative mass, A/M, is the ratio of the mass associated with one thin filament and one thick filament in the unit cell.
‡Visible mass equals the total densities of the unit cell above the minimum density level. Visible mass at μ = 20 mM of the relaxed state is set as 1.

TABLE II

EFFECT OF IONIC STRENGTH (ADJUSTED BY ADDING K PROPIONATE) ON EQUATORIAL X-RAY DIFFRACTION PATTERNS IN THE RELAXED STATE

μ (mM)	20	50	80	120	170
d_{10}(Å)	384 ± 1 (16)*	378 ± 1 (8)	381 ± 1 (10)	395 ± 1 (8)	414 ± 1 (10)
I_{10}/I_C‡	1.00	1.08 ± 0.04	1.12 ± 0.04	1.29 ± 0.04	1.18 ± 0.07
I_{11}/I_C‡	1.69 ± 0.10	1.41 ± 0.07	1.32 ± 0.04	1.22 ± 0.04	0.83 ± 0.07

*Numbers in parentheses denote the total number of data points used.

‡The normalized intensities, I_{10} and I_{11}, with respect to I_C were further normalized with respect to I_{10}/I_C at μ = 20 mM.

0.43, which is 60% of the rigor value (Table I) and practically the same as a fully activated psoas fiber at μ = 170 mM and 5°C (0.45) (Brenner and Yu, 1983). Furthermore, by theoretical calculation, if there were no myosin heads attached to the thin filaments, the relative mass should be only 0.20 for full overlap with the masses of the thin and thick filaments based on presently known biochemical data and filament structures. One might argue that the relatively high values of A/M of the relaxed fiber in the present study could be due to omitting higher order reflections beyond *11* on the equator, since the spatial resolution in the present study is ~230 Å and thereby the boundaries of the filaments are not accurately defined. However, neglecting the higher orders is unlikely to cause substantial error in estimating the values of A/M. Based on the two reflections, *10* and *11*, the value of A/M of the intact, relaxed frog sartorius was 0.23, whereas with three more reflections, 20, 21, and 30, (spatial resolution ~120 Å) (Yu et al., 1984; Yu, L. C., A. C. Steven, G. R. S. Naylor, R. C. Gamble, and R. J. Podolsky, manuscript accepted for publication, *Biophys. J.*), the corresponding ratio was 0.18. Thus, it is reasonable to say that there is substantial mass associated with the thin filaments. This is consistent with the idea, as suggested by the mechanical measurements (Brenner et al., 1982), that a significant number of cross-bridges is formed at low ionic strength even though there is no axial force.

Mass Transfer Away From the Thin Filament as Ionic Strength Is Raised Above 20 mM

The monotonic decrease in the relative mass, A/M, as ionic strength is raised (Table I) suggests graded transfer of mass away from the thin filament. The most likely interpretation is that the number of attached cross-bridges decreases. This interpretation is supported by the fact that the amounts of decrease in I_{11} and increase in I_{10} are similar to those observed at decreasing degrees of activation of the psoas fiber at μ = 170 mM and 5°C (Brenner and Yu, 1983). The changes also correspond closely with those of intact frog sartorious during graded activations (Yu et al., 1979). Furthermore, the constancy of I_{10} out of overlap at various ionic strengths indicates that the changes observed at full overlap are related to interaction between thick and thin filaments, most likely due to cross-bridges.

Changes in intensities might be caused by changes in lattice spacings. However, evidence from the three control

TABLE III

EFFECT OF DEXTRAN (T500) CONCENTRATIONS ON EQUATORIAL X-RAY DIFFRACTION PATTERNS OF SINGLE SKINNED RABBIT PSOAS FIBERS AT μ = 170 mM*

Percentage (wt/vol) of dextran T500	0	1	1.5	2	3	4	5	Relaxed at μ = 20 mM
d_{10} (Å)	442(19)‡	433 (1)	429 (4)	421 (1)	388 (7)	367 (5)	367 (1)	382 (28)
I_{11}/I_{10}	0.96 ± 0.02§	0.90	0.87 ± 0.11	1.26	1.00 ± 0.13	1.15 ± 0.32	1.40	2.20 ± 0.07
I_{10}/I_c‖	1	0.98	1.20 ± 0.18	1.04	0.95 ± 0.18	1.03 ± 0.13	0.56¶	1.20 ± 0.01
I_{11}/I_c‖	1	0.82	1.16 ± 0.12	1.20	1.02 ± 0.17	1.14 ± 0.10	0.78	2.76 ± 0.07

There have been studies on intensity changes accompanying shrinking or swelling of lattices (Perutz, 1942). Our preliminary modeling shows that I_{10} and I_{11} should increase with swelling. However, in the present study, the x-ray beam size was smaller than the fiber diameter, thereby swelling was accompanied by a decrease in the volume of irradiated material, proportional to the change in lattice constant. Modeling shows that intensity increases accompanying swelling could be roughly compensated for by decreases due to the decrease in the diffracting mass.

*The data in this table are averages of measurements from five fibers.

‡Numbers in the parentheses denote the total number of data points used.

§SEM.

‖The normalized intensities, I_{10} and I_{11}, with respect to I_c were further normalized with respect to I_{10} and I_{11} at μ = 170 mM, respectively.

¶At 5% dextran, disorder was clearly present in the fiber, causing both intensities to deteriorate considerably.

TABLE IV
INTENSITY OF THE *10* REFLECTION AT SARCOMERE LENGTHS OF 4.2–4.6 μm

μ (mM)	20	50	80	100	120	170
d_{10} (Å)	299 ± 2	326 ± 2	337 ± 6	344 ± 2	349 ± 2	360 ± 2
	(10)*	(8)	(6)	(10)	(6)	(10)
I_{10}/I_C‡	1.00 ± 0.03	1.08 ± 0.02	1.06 ± 0.04	1.09 ± 0.04	1.00 ± 0.09	1.02 ± 0.05

*Numbers in parentheses denote the total number of data points used.
‡The normalized intensity, I_{10}, with respect to I_C were further normalized with respect to I_{10}/I_C at μ = 20 mM.

experiments (dextran, K propionate, and out of overlap; Table II–IV) shows that lattice spacing does not have a significant effect on the intensities. In the dextran experiment (Table III), it was shown that compressing d_{10} by 15% at constant ionic strength caused variations in I_{11} that were small compared with the changes for the relaxed fibers in Fig. 4 *b*; the influence of dextran on I_{10} also appears to be less than the effect shown for the relaxed fibers in Fig. 4 *a*, although the uncertainties associated with the dextran data are not small enough to exclude completely some effects of lattice spacing on I_{10}/I_c. However, in the K propionate experiment (Table II), where d_{10} remained constant, the changes in intensities as a function of ionic strength were close to those found with KCl. In the out of overlap experiment (Table IV), where there was no interaction between myosin and actin, and spacing changed by ~20% when ionic strength was increased from 20 to 170 mM, I_{10} was nearly unaffected by ionic strength. We conclude from these three lines of evidence that, at most, only a small part of the changes in intensities as a function of ionic strength at full overlap may be attributed to an effect of lattice spacing.

Order-disorder transition in the filaments (Elliott et al., 1963) could theoretically cause qualitatively similar effects as those observed in the relaxed fiber. However, the reflections are very sharp up to $\mu \approx 120$ mM (the widths of *10* reflections are generally broadened by only ~2% compared with the direct beam), and then the pattern deteriorates at higher ionic strengths. Therefore it is unlikely that in the range of μ = 20 to 100 mM, order-disorder transition is the major cause in intensity changes.

The mass shift might also be interpreted as a configurational change of the attached myosin heads (Lymn, 1978). However, the more likely explanation is that the number of cross-bridges decreases as ionic strength is raised, since binding of S-1 to regulated actin in the presence of ATP is weakened by increasing ionic strength, and the low stiffness measured at at μ = 170 mM could be interpreted as a decrease in the number of cross-bridges. A possible way of resolving the uncertainty is to improve the spatial resolution of the diffraction pattern by obtaining additional reflections.

At μ = 100 mM, the relative mass, A/M, of the relaxed fiber is still significantly greater than the calculated value of 0.20 mentioned above. As was pointed out earlier, the difference is unlikely due to limited resolution. Therefore, some of the myosin heads may be attached to the thin filaments in the rabbit psoas fibers at μ = 100 mM. Incidentally, it is interesting to note that the intensity ratio at $\mu \approx$ 80–100 mM found in the present work is comparable with that of living rabbit psoas muscle (Huxley, 1968).

Disorder in the Lattice Predominates for $\mu \geq 100$ mM

For ionic strengths above 100 mM, both intensities decreased. The increase in the widths of the reflections as well as the increase in background indicate increasing disorder in the filament lattice. Some of the ordered structure appears to become part of the background, as shown by the decrease in the visible mass (Table I). It is very likely that there is further detachment of cross-bridges, since the relative mass, A/M, continues to decrease, but interpretation is complicated by the disordering effects. It is interesting to note that the onset of disorder in the thick filament array of Limulus muscle occurs at the same range of ionic strength (Wray et al., 1974).

Attachment of the Relaxed State May Be Different from that of the Rigor State

At 50 mM ionic strength, I_{11} of the relaxed patterns is ~80% of the rigor value, whereas I_{10} is about twice as strong. The difference in intensities could not simply be due to difference in number of cross-bridges formed when fibers are relaxed or in rigor. The reasoning is the following. Presumably, at any ionic strength, there are more cross-bridges formed in the rigor state. However, the present study suggests that if more cross-bridges were to be formed in the relaxed state at any given ionic strength, it will cause changes in both I_{11} and I_{10}, with much larger changes in I_{11} than I_{10}. Thus, it would be impossible to match the rigor values for both I_{10} and I_{11} by assuming additional formation of cross-bridges of the type observed in the relaxed state. Accordingly, the mode of attachment, and hence probably the configuration of the cross-bridges formed in the relaxed state, is different from that of rigor cross-bridges. This conclusion is consistent with the fact that the myosin-based layer line patterns are different in the two states (Matsuda and Podolsky, 1984).

CONCLUSION

The strong intensity of the *11* reflection of the relaxed psoas fiber at μ = 20 mM and 5°C indicates that there is substantial mass associated with the thin filaments under these conditions. As the ionic strength is raised, mass is transferred away from the thin filaments towards the thick filaments. These results support the finding by stiffness measurements that a significant number of cross-bridges is formed at low ionic strength (μ = 20 mM) (Brenner et al., 1982) and further suggest that the number of attached cross-bridges decreases as ionic strength is raised. Like the previous study, the present work also provides evidence that generation of net axial force is not a necessary consequence of cross-bridge formation. Rather, an activation process subsequent to myosin binding to actin is apparently required for force generation.

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