A STRUCTURAL STUDY OF GELS, IN THE FORM OF THREADS, OF MYOSIN AND MYOSIN ROD

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INTRODUCTION

When a glycerinated, cross-striated muscle cell goes from relaxation to rigor at pH 7, with the removal of ATP, the fixed negative electrical charge in the A-bands increases by ~40% (Bartels and Elliott, 1980, 1981, 1982, 1985). The addition of ATP to the rigor muscle causes the A-band charge to decrease again; the charge change is reversible. The major A-band protein is myosin, and each head of the myosin molecule has a single nucleoside phosphate binding site (Young, 1967; Luck and Lowey, 1968). Possible sites for the initiation of the A-band charge change might therefore be the myosin heads or some other part of the myosin molecule under the influence of ATP binding to the heads.

To find out more about the site of the charge changes in the A-band, a gelled thread of purified myosin, containing oriented filaments, has been developed. In this preparation the protein concentration is comparable to the concentration of A-band myosin. A preparation of gelled purified myosin rod (the myosin molecule without the two globular heads) has also been prepared. Donnan potentials have been measured from these preparations in rigor and relaxing solutions at several ionic strengths (Bartels et al., 1985; and paper in preparation).

This paper and the one that follows (Poulsen et al., 1987) describe the preparation and structure of the threads, as investigated by electron microscopy and x-ray diffraction. The electrical measurements on the threads will be reported in full in a subsequent paper (Bartels, E. M., P. H. Cooke, G. F. Elliott, and R. A. Hughes, manuscript in preparation). Preliminary reports of some of these experiments have appeared previously (Cooke et al., 1984; Bartels et al., 1985, 1986).

METHODS

Preparation of Myosin and Myosin Rod

Back and leg muscles (400–500 g) were excised from rabbits that had been killed by cervical dislocation. Bulk quantities of myosin (2–7 g) were isolated and purified according to the method described by Starr and

Offer (1982). There was one modification of their method; the initial precipitation of protein from Guba-Straub solution was allowed to settle overnight at 4°C. About 50–80% of the crude myosin, subjected to anion-exchange (DEAE) chromatography, was eluted, after binding to the column, by the addition of 0.1–0.3 M KCl to the buffer. These KCl fractions contained the purified myosin from which the gelled threads of myosin were prepared.

Myosin rod was prepared from a papain digestion of purified myosin, using the procedures of Margossian and Lowey (1982) for producing heads and rod subfragment. The purified rod fraction was finally re-dissolved in a small volume of a solution containing 0.6 M KCl, 0.05 M potassium phosphate buffer (pH 7.0).

The purity of the myosin and rod subfragment preparations was checked by SDS gel electrophoresis, and no significant contamination by other proteins was observed.

The K^+ EDTA-activated ATPase activity of the purified myosin and myosin threads was estimated in a system containing 0.3 M KCl, 0.1 M Tris/HCl (pH 7.5) with 0.005 M K_2EDTA. The liberated inorganic phosphate was measured at 730 nm after the addition of ammonium molybdate and Fiske and Subbarrow (1925) reagent. The activity of myosin thread samples was also determined in the experimental solutions.

The Manufacture of the Protein Threads

To produce a fixed protein phase suitable for measuring Donnan potentials with microelectrodes, separate methods were devised for handling myosin and myosin rod. For myosin, 1–2 g of the protein at 5–10 mg/ml was induced to form bipolar filaments by dialysis to equilibrium in 0.1 M KCl, 0.005 M $MgCl_2$, 0.02 M potassium phosphate buffer (pH 7). This is the basic rigor solution, which we will call standard rigor solution (see Table I). After 24 h, the suspension of filaments was sedimented by centrifugation in 38-ml tubes at 65,000 g for 2 h. The translucent paste, containing sedimented filaments, was transferred to a 10-ml syringe equipped with a 4-cm long silicone rubber tubing with an inside diameter of 0.56 mm and a wall thickness of 1.57 mm. The paste of myosin filaments was extruded manually as threads up to 10-cm long into an ice-cold fivefold dilution of the standard rigor solution, at a rate of 1–2 cm s^{-1}. The extruded protein quickly gelled into an opaque thread and settled to the bottom of the container. It was allowed to stand undisturbed for several hours at 4°C, before further manipulation.

For myosin rod, 1–2 g of purified rod subfragment were dialyzed into filamentous tactoids according to the procedure of Margossian and Lowey (1982) and were then sedimented by centrifugation at 16,000 g for 40 min. The supernatant fluid was decanted and a small volume of 1.2 M KCl solution, equal to the volume of the precipitated rod, was added to the top of the pellet and mixed using a small magnetic stirring bar. This procedure slowly dissolved the precipitated rod tactoids into ~2 ml of concentrated solution containing 50–100 mg ml^{-1} protein in 0.6 M KCl. The solution was transferred by pipette to a syringe and extruded as a transparent thread, which quickly gelled, into 1–2 liters of an ice-cold solution of the fivefold diluted standard rigor solution. It was then handled in the same way as the threads of myosin.

COMPOSITIONS OF THE SOLUTIONS USED IN THIS STUDY

	KCl	MgCl$_2$	Phosphate buffer, pH 7	Na$_2$ATP	Na$_2$ADP	Ionic strength
						M
Standard rigor	100	5	20			0.141
½ Standard rigor	50	2.5	10			0.072
⅕ Standard rigor	20	1	4			0.030
Standard relax	100	5	20	2.5		0.149
½ Standard relax	50	2.5	10	2.5		0.081
⅕ Standard relax	20	1	4	2.5		0.044
Standard ADP	100	5	20		2.5	0.144
½ Standard ADP	50	2.5	10		2.5	0.080
⅕ Standard ADP	20	1	4		2.5	0.039

The amounts are given in millimoles per liter, apart from the ionic strength calculated by the Perrin programme, which is molar.

The myosin and the rod threads that were not used during the week of preparation were stored in a glycerinating solution at $-20°C$. The glycerinating solution contained 50% glycerol and 50% of a solution with a composition of 0.040 M KCl, 0.002 M MgCl$_2$, 0.008 M phosphate buffer, pH 7. The glycerinated threads were soaked for 1 h in fivefold diluted standard rigor solution before experiments were carried out. There were no differences in the measurements of charge-concentration obtained from experiments with fresh threads and with glycerinated threads.

Electron Microscopy

The threads were examined either as thin sections of embedded segments or as small fragments produced by mechanical homogenization. In embedding, oriented 3–4-mm long segments were treated with 1% glutaraldehyde in an appropriate rigor solution for at least 1 h and then immersed in a solution of 2% OsO$_4$ and 0.1 M s-collidine at pH 7.2. The threads were then dehydrated for 2 h by immersion in a graded series of ethanol solutions and finally embedded and cured in an epoxy resin mixture. Thin sections of oriented threads were stained with solutions of uranyl acetate and lead citrate.

Filaments were also isolated from segments of the threads by mechanical homogenization in a tissue grinder. The homogenate was adjusted to a protein concentration of 0.1–0.2 mg/ml by the addition of rigor solution and drops were applied to Formvar-carbon–coated specimen screens and stained with 2% uranyl acetate after removal of the excess solution by absorption into filter paper.

X-ray Diffraction

The x-ray data were obtained using the synchrotron radiation source at Daresbury. All recordings were taken on the low-angle scattering camera with a specimen-to-film distance of ~2 m; the exposure time on film was 30 min for a meridional pattern and 5 min for an equatorial pattern. Some x-ray diffraction patterns were taken on Caeverken AB Reflex 25 film, and some were recorded using the linear proportional counter. This depended on the configuration that was in use for the parallel x-ray studies on corneal collagen from this laboratory (e.g., Sayers et al., 1982).

The specimens consisted of straight parallel bundles of 5–10 threads. These were fixed in a circular muscle cell, similar to the perspex cell described in Naylor et al. (1985). The distance between the mylar windows in the cell was 1–2 mm. To use the beam dimensions for maximum resolution, the thread preparations were placed vertically for meridional diffraction and horizontally for equatorial diffraction.

Because the specimen-to-film distance varied from run to run at the synchrotron, the x-ray spacings were calibrated against the collagen period, assuming that the spacing in wet, freshly dissected, rat-tail tendon in (0.15 M) sodium chloride was 67.0 nm.

Measurement of the Volume and Volume Changes of the Protein Threads

The threads were normally taken to be cylindrical (constant diameter) and their volume was calculated as $\pi l r^2$, where l is the length of the thread and r is the radius. A few rod threads were clearly more ellipsoidal in shape and in those cases the volume was calculated as $\pi l ab$, where l is the length of the thread and a and b are the two half diameters of the ellipse. The diameter and length of the thread were measured under the microscope using a micrometer scale; 10 measurements were taken over a 1-cm long thread. These measurements were taken on a Zeiss Ultraphot microscope at a final magnification of 68 or on a Zeiss 405 inverted microscope with a final magnification of 25. In no case did we observe any length changes when the experimental solutions were changed.

A swelling factor, S_1, was defined as

$$S_1 = d_2^2/d_1^2,$$

where d_1 is the diameter in the equilibration solution and d_2 is the diameter in a particular experimental solution. This factor was then used to correct for volume changes between the different experimental solutions. The equilibration solution was the fivefold diluted standard rigor solution, at pH 7. The opacity of the threads was also noted during these experiments; some interesting transparency changes were observed.

Determination of the Protein Concentration in the Threads

Protein concentration was determined by the microbiuret methods (Leggett-Bailey, 1967). Threads of known volume were completely digested in a known volume of 3% NaOH before assay. The measurements gave the total amount of protein in a given sample, and the protein concentration in the thread was then calculated in milligrams of protein per milliliter.

Most protein determinations were carried out on samples in the equilibration solution (fivefold diluted standard rigor solution) since all threads are produced and are easy to handle in this solution. When the threads were transferred to the other experimental solutions, it was assumed that there was no diffusion of protein out of the sample, so the protein concentration of a thread in the new solution was P/S_1, where P is the protein concentration in the fivefold diluted rigor solution, and S_1 is the swelling factor, remembering that the threads did not change their length during swelling.
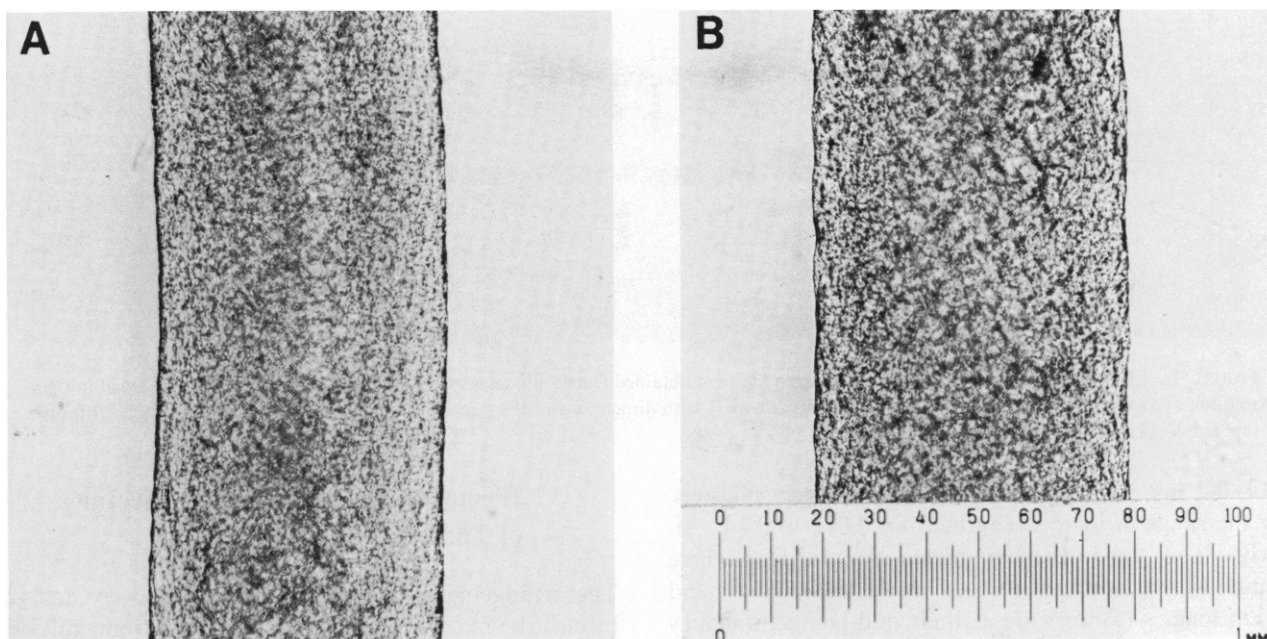
FIGURE 1 Two optical micrographs illustrating changes in diameter of segments of the same gelled thread of myosin in solutions of different ionic strength (the effect of the ligand ATP is similar). In *A*, the thread is condensed at low ionic strength (the fivefold diluted rigor solution, 0.030 M) after extrusion. In *B*, an increase in ionic strength to standard rigor solution (0.141 M) results in a 10% increase in diameter. Scale is 1 mm. 68×.

RESULTS

Morphology and Structure of the Protein Threads

The structure of the threads was designed empirically to provide solid, cylindrical gels of nearly microscopic dimensions at protein concentrations that compare with the density of myosin in the A-bands of muscle.

Light Microscopy

Fig. 1 shows a gelled thread of myosin at equilibrium with two solutions at different ionic strengths. At low ionic strength ($\mu = 0.030$ M) in dilute rigor solution excluding ATP, the diameter of the thread with a protein concentration of 125 mg ml^{-1} was uniformly ~550 μm (Fig. 1 *A*).

After (*a*) the addition of 0.0025 M ATP and adjusting the pH back to 7, (computed $\mu = 0.044$ M) or (*b*) increasing the ionic strength by substitution with standard rigor solution ($\mu = 0.141$ M), the diameter of the threads increase to 580–600 μm, which is an increase of ~10% (Fig. 1 *B* shows rigor, $\mu = 0.141$ M). A further addition of 0.0025 M ATP to the standard rigor solution at physiological ionic strength ($\mu = 0.149$ M) resulted in a >30% increase in diameter of the thread, to over 700 μm. These changes in diameter were essentially complete in 10–15 min and were reversible for up to 5 h of exposure to the solutions at ionic strengths <0.15 M. Table II gives the average changes in the swelling factor calculated from the diameter changes.

Electron Microscopy of Whole Filaments

The microscopic structure of the threads made from myosin were based on typical reconstituted filaments (Huxley, 1963). Fig. 2 shows an electron micrograph of a single, isolated filament from the homogenate produced by liquid shearing of a gelled thread. For dilute suspensions

TABLE II

Solution	N. of experiments	S_1	$\pm\sigma$
Myosin			
Standard rigor	30	1.64	0.18
½ Standard rigor	66	1.27	0.16
⅕ Standard rigor	58	1	—
Standard relax	28	2.06	0.39
½ Standard relax	46	1.50	0.24
⅕ Standard relax	40	1.33	0.19
Standard ADP	14	1.73	0.22
½ Standard ADP	22	1.33	0.20
⅕ Standard ADP	20	1.15	0.20
Myosin rod			
Standard rigor	26	1.02	0.06
½ Standard rigor	26	1.02	0.06
⅕ Standard rigor	26	1	—
Standard relax	24	1.06	0.06
½ Standard relax	22	1.05	0.04
⅕ Standard relax	20	1.08	0.06

The number of experiments, and the swelling factors with their standard deviations in the various solutions, for threads of myosin and of myosin rod. With myosin rod the swelling factor is not significantly different from unity in any solution.
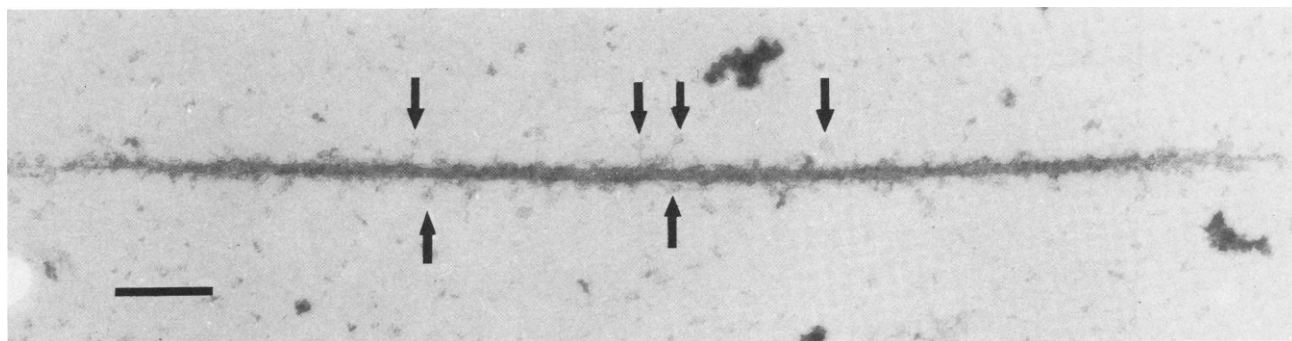
FIGURE 2 Electron micrograph of an isolated myosin filament obtained from a dilute suspension, made by homogenizing a thread in rigor solution at an ionic strength of 0.072 M. Projections (*arrows*) with dimensions of the size and shape of myosin molecules extend from the tapered, bipolar shape. Bar, 100 nm. 128,000×.

($\sim$0.1–0.2 mg ml^{-1} protein) in standard rigor solutions, over the range of ionic strengths from 0.072 to 0.141 M, nearly all of the isolated filaments examined as whole mounts by electron microscopy were between 1- and 1.5-μm long, $\sim$15-nm wide at their mid-points, and very gradually tapered along both ends. A central bare zone was not always observed; instead, irregular projections extended from the filament shafts along the entire length (see Fig. 2).

Electron Microscopy of Thin Sections of Threads

The arrangements of filaments in threads were analyzed by electron microscopy of thin sections cut from embedded segments. A set of results, corresponding to the series of swollen threads studied by light microscopy, is illustrated in Fig. 3.

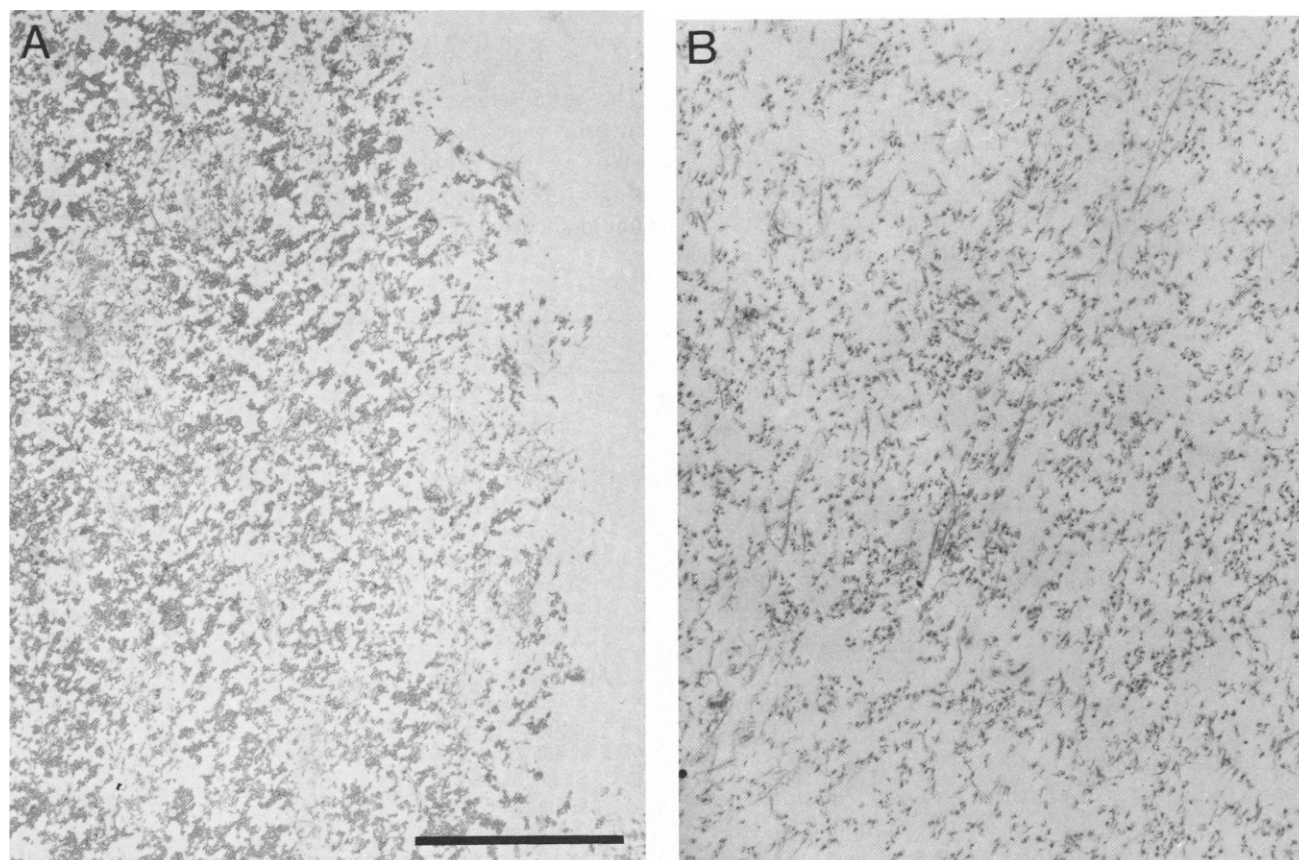In cross-sections of threads that were fixed at low ionic

FIGURE 3 Electron micrographs illustrating thin sections of a fixed embedded series of swollen threads. *A* indicates an area of the edge and a cortical area (25-μm deep) of a thread. Irregular bundles of close-packed filaments in nearly exact cross-section and resembling muscle A-bands in size are seen. The filament bundles are separated by clear regions of comparable areas. *B* demonstrates the effect of 0.0025 M ATP in a relaxing solution of low ionic strength (0.044 M). Bar, 1 μm. 28,000×.

strength (0.030 M) and viewed at low magnifications, nearly all the filaments appeared in cross-sectioned profile, indicating mainly axial orientations, and were tightly packed into irregular groups, with intervening clear areas of comparable and irregular size (Fig. 3 *A*). This pattern of arrangement and orientation among the filaments was uniform over large areas in cross-sections, primarily in its cortical 50 or 60 μm, or 25% of the thread diameter.

When the cross-sectional profiles of the filaments were clearly resolved, differences in the packing arrangements in swelled threads were evident. At low ionic strength, bundles contain irregular groups of 50 to over 100 compact filament-profiles with an average near-neighbor spacing of ~34 nm, as determined by optical diffraction (Fig. 3 *A*). The morphological effects resulting from addition of the ligand ATP, causing a relatively minor increase in ionic strength from 0.030 to 0.044 M, are shown in Fig. 3 *B*.

In the presence of 0.0025 M ATP, the profiles of filaments appeared to be widely dispersed and retained no evidence of a uniform interfilament spacing or close packing into bundles separated by clear spaces. Similarly, a significant increase in total ionic strength of the solution phase (a shift from 0.030 to 0.141 M) appeared to disperse the bundles of filaments, and a broad distribution of distances between filament profiles were seen.

High-power transverse sections of the threads (Fig. 4) show that the myosin filaments are quasi-regularly packed,

that they have a solid back bone, and are surrounded by a continuous halo, which probably consists of disordered heads. The interstitial spaces are clearly seen in this micrograph, which is of a thread in the fivefold diluted rigor solution. It is, however, difficult to judge the degree of regularity in the filament packing from such micrographs; there are some regions where the packing appears roughly hexagonal but on the whole the order appears liquid-like, with perhaps a nearest neighbor spacing (see the x-ray results, below).

The shape of the filaments seen in cross-section is very variable, probably because the range of alignments means that the cross-section is rarely precise. Sometimes the cross-sectioned filament appears more circular, with a hint of fine subfilaments (or projection origins).

In longitudinal sections of plastic-embedded threads at low ionic strengths, the filaments were grouped into an axially oriented trabecular network of bundles. Each bundle contained a staggered arrangement of filaments, numbering from 5 to 10 filaments in a section of the small bundles to upwards of 50 filaments in large bundles. The bundles were roughly aligned with the fiber axis. This alignment varied from bundle to bundle, however, with most filaments in most bundles within ~ ±15° of the fiber axis. Occasional bundles, or single filaments, were at greater angles to the axis, and there are also electron-dense dots, some of which are probably cross-sections of such
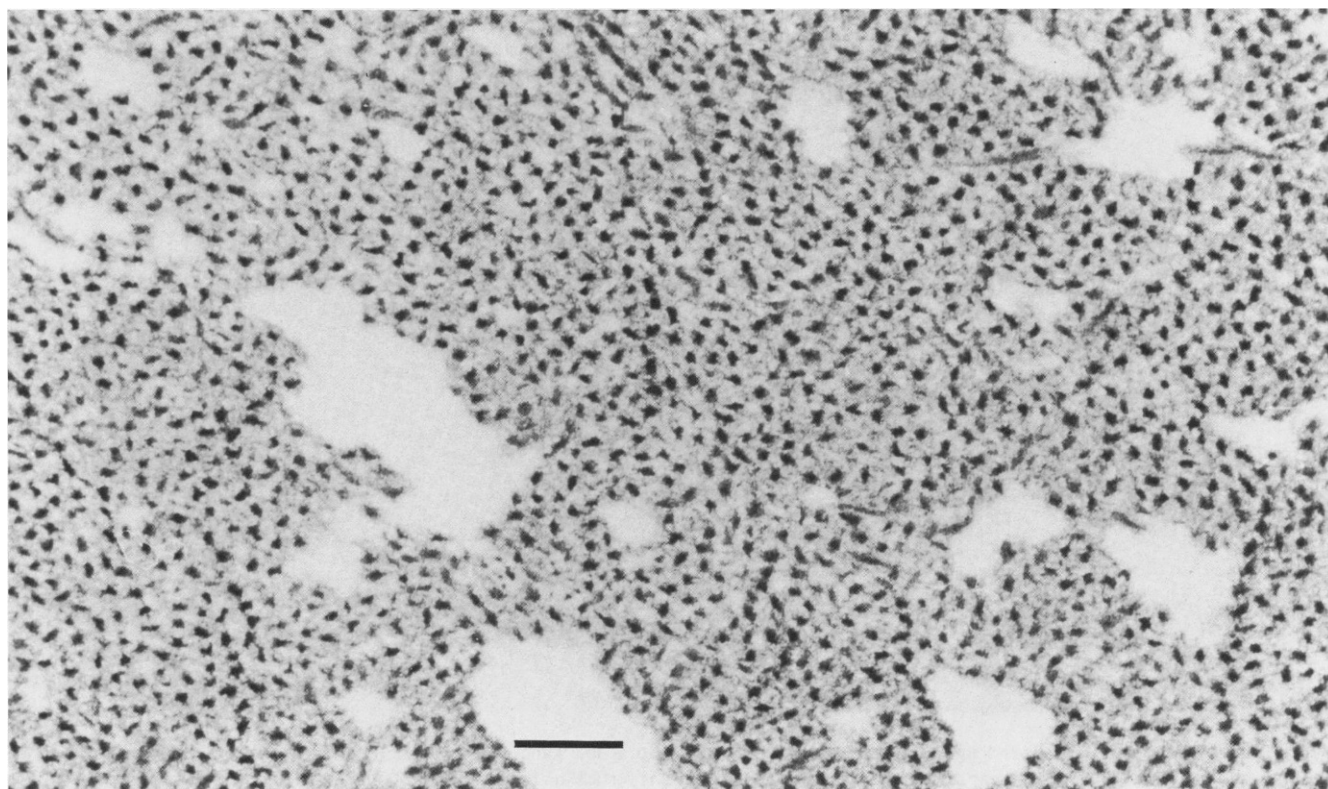

FIGURE 4 Higher power transverse section at 0.030 M ionic strength, showing details of the cross-sectional profiles of the filaments, and the surrounding halo, which represents the myosin head region. Bar, 250 nm. 59,000×.
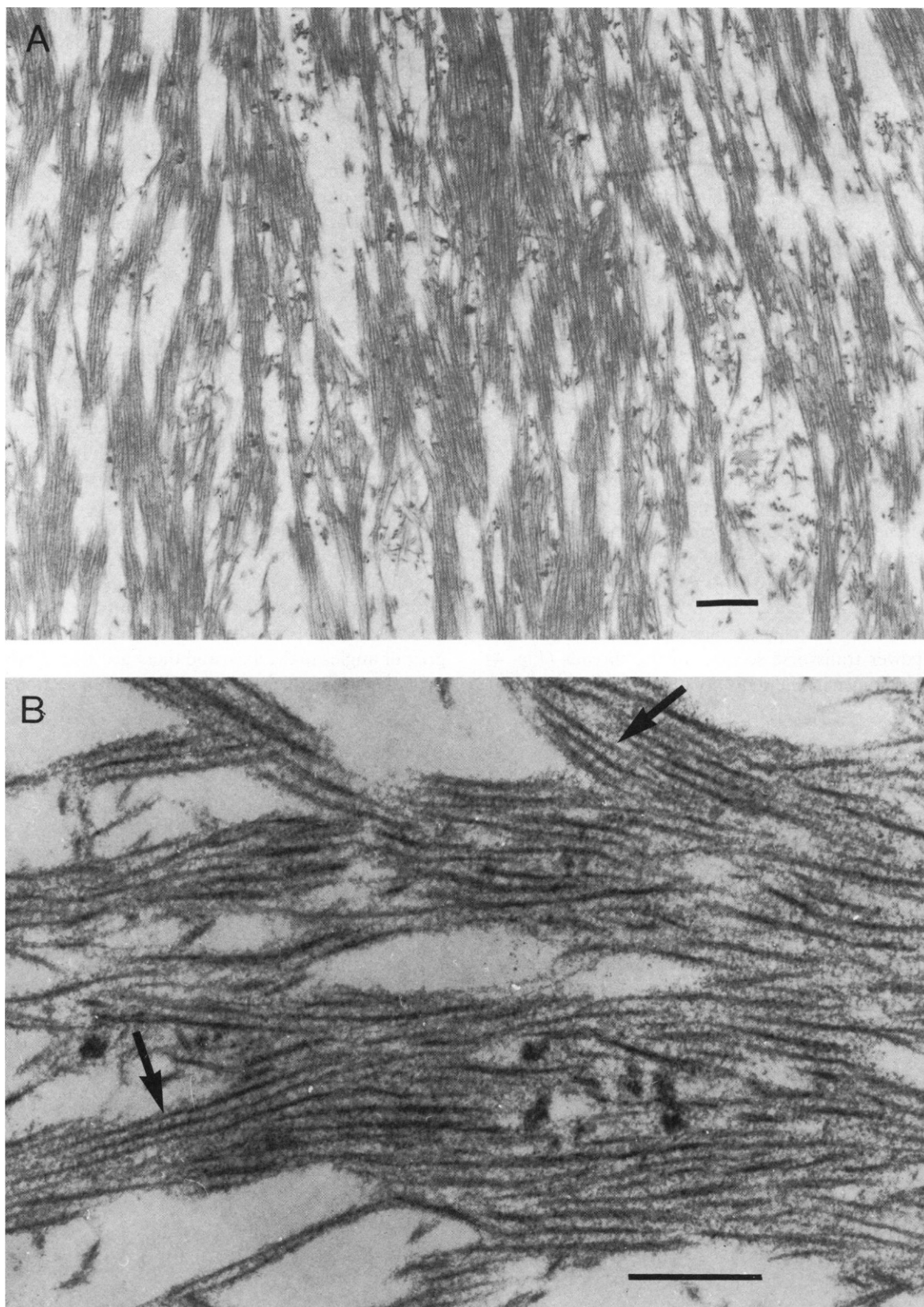
FIGURE 5 *A* shows low-power longitudinal section of a myosin thread at low ionic strength (0.030 M). The orientation of the bundle directions, and the intervening clear regions, can be seen. The fiber axis is vertical on the page. Bar, 500 nm. 20,000×. *B* shows higher power longitudinal section showing a single myosin filament bundle. The arrows show the cross connections, arranged like the rungs of a ladder. The fiber axis is horizontal on the page. Bar, 250 nm. 88,000×.

misaligned filaments. The interstices of the filamentous network were clear and comparable in size to the filament bundles. These interstitial spaces appeared to contain little or no electron dense materials. All these features can be seen in low power micrographs of longitudinal sections (Fig. 5 A).

In higher magnification longitudinal sections (Fig. 5 B), the filaments appear to have a dense shaft, ~10–12 nm in diameter, and wispy projections can be seen from these shafts. In adjacent filaments these projections often give a ladder-like effect (see the arrowed regions in Fig. 5 B). The spacing of the rungs on these ladders could not be measured with any precision, but it seemed to be in the range 13–20 nm.

Electron Microscopy of Myosin Rod

When aliquots of concentrated tactoids made from aggregated myosin rod subfragments in the standard rigor solution were extruded over the same ionic strength gradient that was used for myosin filaments, stable gelled threads did not form. Instead, the threads dissociated into a suspension of rod filaments or tactoids within a few hours (Fig. 6 A), presumably because the aggregates of rod were unable to cross-link into a network of bundles.

To induce soluble rod subfragments to gel in extruded threads, very concentrated solutions of rod were extruded into a large volume of low ionic strength salt solution (see Manufacture of Threads). The structure of the resulting gels in thin section was unusual (Fig. 6 B): one component was identical to the filamentous tactoids, but another major component was unique. It consisted of stellate or elongated clusters of fine radially oriented strands, possibly molecules or small aggregates of rods, ~200-nm long, mutually linked into a geodesic network extending throughout the longitudinal and transverse axes of the gelled threads. This geodesic network encloses rod filaments or tactoids, which look very much like those in the suspension seen in Fig. 6 A, taking account of the different staining conditions of the two micrographs. Probably these tactoids are identical in the two preparations.

X-ray Diffraction Patterns

Myosin. The electron micrographs of the myosin gels showed regular, roughly parallel, myosin filaments. The x-ray pattern, as would be expected, shows a merid-
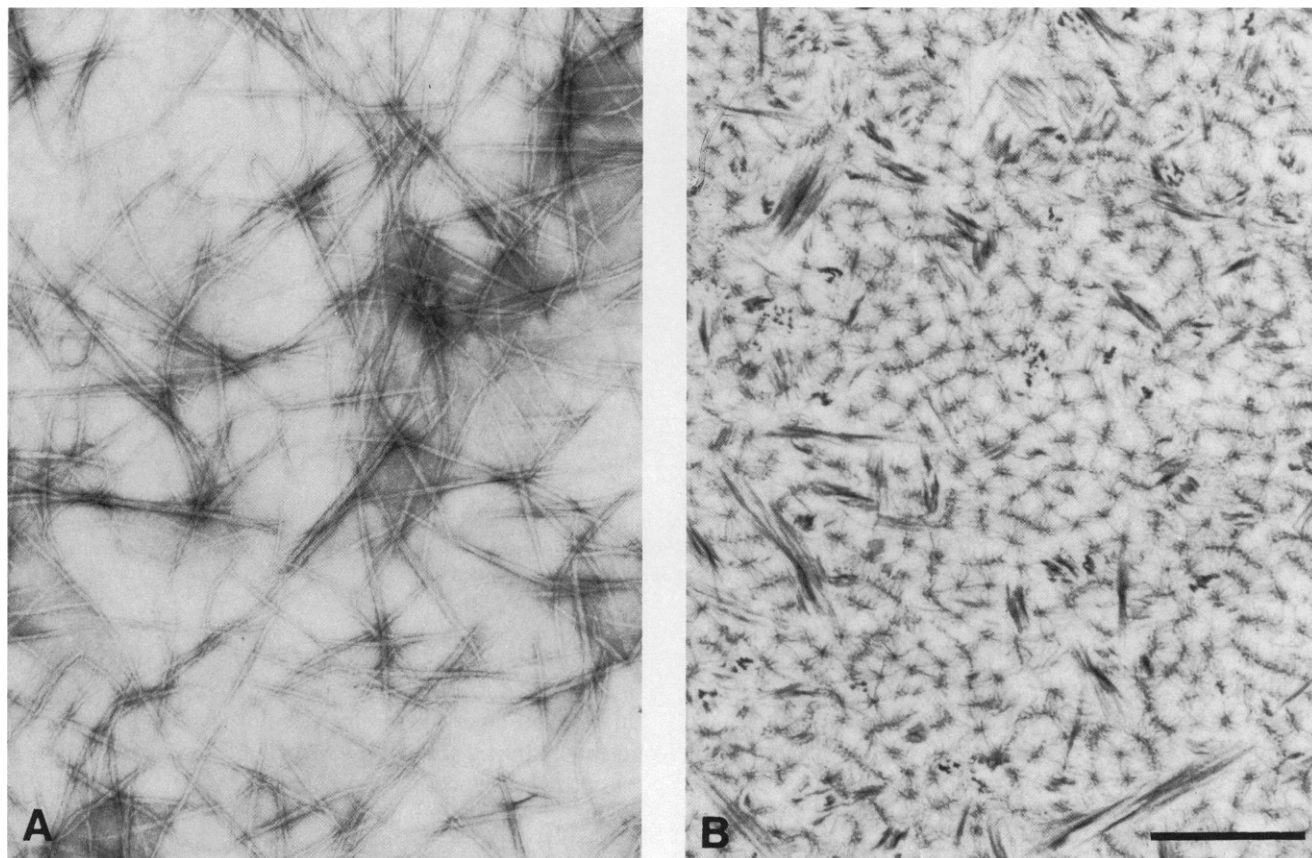

FIGURE 6 In *A*, typical tactoidal filaments of purified myosin rod subfragment are shown in a negatively stained preparation. These filaments did not form a solid gelled thread. In *B*, rod subfragments in a gelled thread are shown, organized as a mixture of filamentous tactoids and cross-linked stellate clusters, in a thin-sectioned, embedded preparation. Bar, 1 μm. 20,000×.
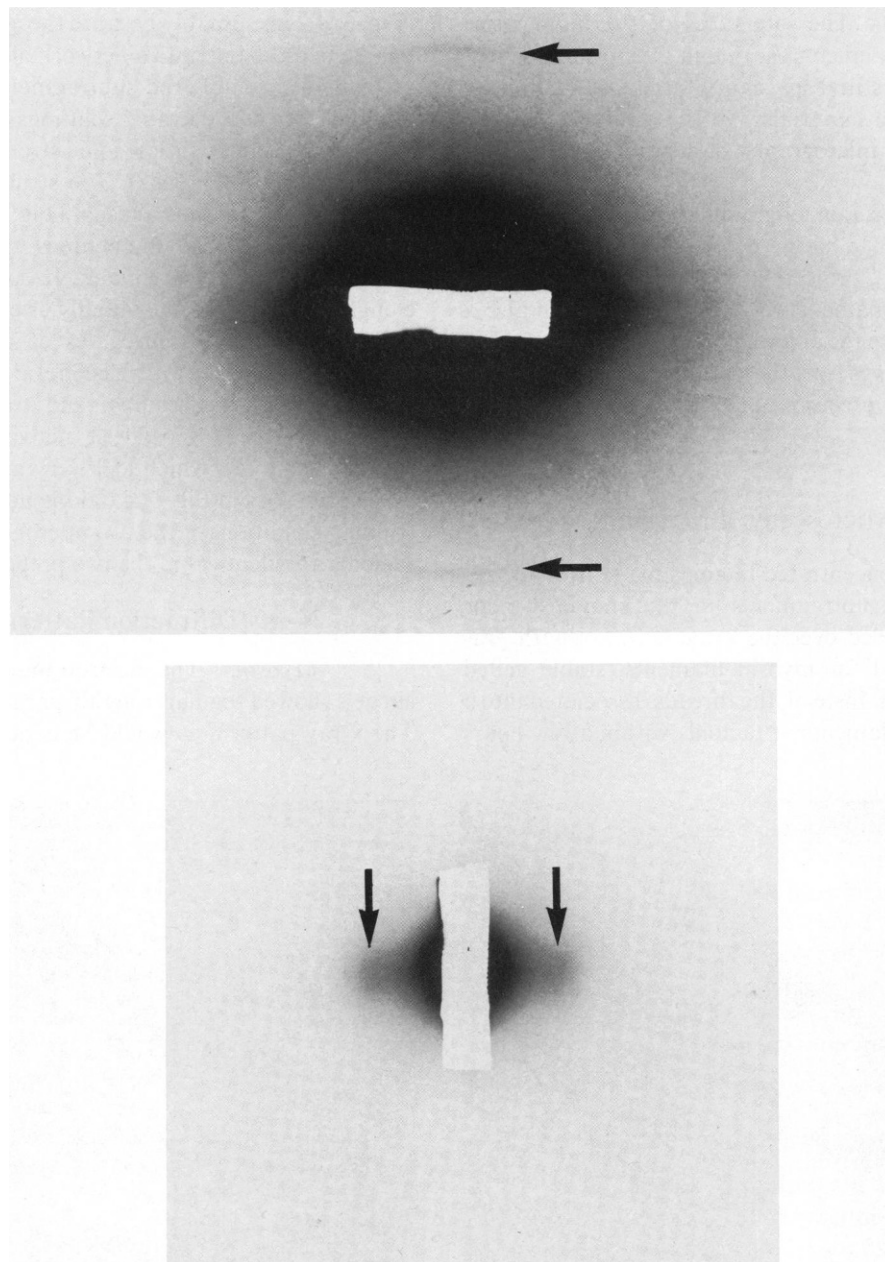
FIGURE 7 X-ray diagrams from myosin threads, fiber axis vertical, in the fivefold diluted (0.030 M) rigor buffer. (*Top*) The meridional pattern, showing the clear, arced, 14.4-nm meridional reflection (marked by an arrow) and the continuous low-angle scattering. (*Bottom*) The equatorial pattern, showing the single diffuse equatorial reflection at 38.0 nm, marked by arrows.

ional spacing of 14.4 ± 0.7 nm (SD, 12 observations, four on film and eight using the proportional counter). Fig. 7 *A* shows a meridional x-ray pattern of a myosin thread taken on film. The 14.4-nm meridional reflection is seen clearly, but compared with an intact muscle it shows considerable arcing across the meridian. The 14.4-nm meridional reflection must come from a relatively well-ordered component and this is likely to be the filament backbone, probably consisting of the light meromyosin subfragment of the myosin molecules. Occasionally there were traces of other meridional reflections, particularly on the counter spectra,

but these could not be measured with any precision, and might have been artifacts of the apparatus. No off-meridional layer lines, which could have been interpreted as an organized state of the myosin heads, were seen in any of our patterns.

In the equatorial direction, a single reflection is seen (Fig. 7 *B*). This looks too diffuse to be the (1, 0) spacing of a hexogonal lattice, and by analogy with the cornea (Sayers et al., 1982), it is probably the nearest neighbor distance in a packing that is essentially like a two-dimensional liquid. The spacing of this reflection is ~39

nm both in the fivefold and the twofold diluted rigor solution.

A strong diffuse scatter is seen in the central region, similar to the scatter reported in intact muscles (Poulsen and Lowy, 1983) as arising from the myosin head subfragments, scattering in a disordered mode. A full analysis of this diffuse scatter is described in the paper that follows (Poulsen et al., 1987).

Myosin Rod. Only a few x-ray patterns were recorded from myosin rod threads, and these were taken with the camera operating with the counter, because this was the mode that was operative when rod specimens were available. As might be expected from the electron micrographs, the spacings are different from those of the myosin threads and spacings recorded from intact muscles. In both the meridional and equatorial directions these preparations gave a diffuse spacing at ~19.0 nm. There is, then, some regularity in the structure of these threads, and this is probably related to the cluster and sheet appearance seen in Fig. 6. We intend to follow up on these observations in future experiments at the Daresbury synchrotron.

ATPase Activity of Myosin

With myosin threads solubilized in the assay reagents, typical K^+ ATPase values were 0.1–0.2 μM mg protein^{-1} min^{-1}. This is similar to classical values under these conditions. In the fivefold diluted rigor solution used in the thread experiments, these values were reduced to 6 nM mg^{-1} min^{-1}, showing that the ATPase is largely inhibited under our experimental conditions.

Protein Concentration

The protein concentration in the threads of whole myosin ranged from 60 to 170 mg ml^{-1}, and in the threads of the rod subfragment the concentration ranged from 60 to 120 mg ml^{-1}, all measured in the fivefold diluted standard solution. Most myosin threads in our experiments had a concentration of ~120 mg ml^{-1}, and most rod threads had a concentration of ~90 mg ml^{-1}.

Volume Changes
between Different Conditions

The myosin threads swell with increasing ionic strength, with ATP, and to some extent with ADP, as seen in Table II where the swelling factors are given. In contrast, myosin rod preparations show negligible swelling.

DISCUSSION

Morphology of the Threads

Myosin. The electron micrographs show that the myosin threads are composed of typical reconstituted myosin filaments (Huxley, 1963), which are packed roughly parallel to the axis of the thread and are suffi-

ciently well aligned to give both equatorial and meridional x-ray diffraction. The meridional 14.4-nm x-ray reflection arises at least in part from the backbone of the myosin filaments (Elliott and Worthington, 1959; Worthington, 1960; Elliott, 1964), since it is also seen from oriented preparation of light meromyosin (Szent-Györgyi et al., 1960). This reflection is not accompanied by the myosin layer lines, which have been attributed to the regular helical arrangement of the myosin heads or cross-bridges on the surface of the myosin filaments (Elliott, 1964; Huxley and Brown, 1967). This must mean that the myosin heads are not regularly arranged in these myosin threads but extend out randomly to form the halos that are apparent in the cross-sections. The appearance of these halos seems to depend on ionic strength and the presence of nucleotides. The halos are most extended and evident at low ionic strength. At higher ionic strength the halos are not so diffuse or so extended. Possibly this is related to the low ionic strength "cross-bridges" observed by Brenner et al. (1982), in mechanical experiments on single rabbit fibers, and also characterized structurally by Brenner et al. (1984).

A further point may be noted. The myosin threads do not disappear into solution on gentle agitation, as do the rod threads made by a similar technique. This suggests that there must be some mechanical interaction between neighboring filaments, since it is now generally agreed that the Van der Waals attractive forces between myosin filaments are insufficient to account for the cohesion of the myosin filament lattice against the disruptive effect of electrostatic repulsion between the filaments (see, for example, Millman and Nickel, 1980) so that extra elastic forces must exist. In our myosin threads, which contain only purified myosin, these elastic forces certainly involve the myosin heads. It may be sufficient to involve a "tangling" of the heads between neighboring myosin filaments. In contrast, there may perhaps be more specific head–head interactions, as has been suggested from electron micrographs of smooth muscle myosin by Sobieszek (1972) and Cooke (1975) and of striated myosin by Suzuki and Pollack (1986). Furthermore, in the low-resolution x-ray analysis of myosin subfragment 1 in tannic acid–embedded crystals (Winkelmann et al., 1985), there appears to be head–head subfragment 1 dimers, with a close interaction between the two larger regions of the tadpole-shaped myosin molecules, at the crystallographic twofold axes in the [100] planes (see Fig. 6 of Winkelmann et al.). Such dimers might also occur in our gels. In solution studies, Morel and Garrigos (1982) observed that dimers of (skeletal muscle) S-1 were predominant in the analytical ultracentrifuge at low ionic strengths and in the presence of Mg-(nucleotide). Under the conditions of their experiments, however, the EDTA light chain was missing, so that a different interaction might have been predominant (see, for example, Pastra-Landis and Lowey, 1986). The continuous low-angle scattering from these threads, arising from

the myosin heads, is discussed in the paper that follows (Poulsen et al., 1987).

Myosin Rod. Threads made from myosin rod did not cohere except under conditions where the three-dimensional geodesic network was formed (see Methods). This network has not been reported before for rod preparations, though a similar open-mesh packing was sometimes seen in negatively stained preparations of light meromyosin by Huxley (1963), Phillpott and Szent-Gyorgyi (1954), and King and Young (1970). Yagi et al. (1981) have shown that light meromyosin (LMM), which undergoes slow dialysis from 0.35 to 0.1 M KCl, forms an extensive three-dimensional network of strands, crossing at 64.4-nm intervals. They suggest that in these nets the LMM molecules pack antiparallel, with the NH_2-terminal end of the subfragment molecule starting at a node in the lattice and the COOH-terminal end finishing in an overlap region midway between nodes. If a similar packing occurs in the rod nets seen in our threads, the extra length introduced by two S-2 subfragments, each ~70-nm long, would simply account for the difference between the figures of 64.5 nm in the LMM nets and the ~200 nm, which we observe in the rod nets (see Fig. 6 *B*).

The Swelling of Myosin and Myosin Rod

Inspection of the swelling coefficients of the myosin rod gels (Table II) show that these swell very little, <10% by volume, under any of the conditions examined. This probably reflects the three-dimensional nature of the geodesic network, which is likely to constrain, with strong elastic forces, any volume changes that might otherwise be generated by charge changes. The myosin gels are a different matter; in the phosphate buffer the volume in the standard rigor solution is 64% greater than in the fivefold diluted equilibration solution. The addition of ATP to the fivefold diluted equilibration solution, to give the equivalent relaxing solution, causes a 33% volume increase, and standard relaxing solution causes the largest increase, a 100% increase by volume over the equilibration solution. The rigor and relaxed series, and indeed the ADP series as well, all swell with increasing ionic strength, but at a given ionic strength the relaxed series is more swollen than either the rigor or the ADP series, by about an extra 10% (volume) at low ionic strengths and by as much as an extra 30% (volume) at the highest ionic strengths. It is thus clear the volume of these gels is not simply related to the charge. In general the charge increases with ionic strength (Bartels, E. M., P. H. Cooke, G. F. Elliott, and R. A. Hughes, manuscript in preparation; see also Bartels and Elliott, 1985) and the volume increases with ionic strength in both the absence and the presence of ATP, so the increased swelling could be a function of the extra charge. However, in the presence of ATP the charge is lower than in its absence (Bartels et al., 1985), so the increased volume in the presence of ATP must be due to other effects of the

nucleotide. Perhaps ATP decreases the head–head interaction between different filaments (see Myosin in Discussion), which would constrain the gel from swelling. This conflicts with the observations of Morel and Garrigos (1982), who observe extra dimerization of myosin heads in the presence of Mg ATP. It does, however, agree with our own electron microscope observations (Fig. 3, *A* and *B*), where the addition of ATP at constant salt concentration appears to decrease the clumping of the myosin filaments and to make them distribute more evenly in the available space. This phenomenon is also in accord with the qualitative interpretation of the transparency changes. These interesting effects warrant further study.

REFERENCES

Bartels, E. M., and G. F. Elliott. 1980. Donnan potential measurements in the A- and I-bands of cross-striated muscles and calculation of the fixed charge on the contractile proteins. *J. Muscle Res. Cell Motil.* 1:452.

Bartels, E. M., and G. F. Elliott. 1981. Donnan potentials from the A- and I-bands of skeletal muscle, relaxed and in rigor. *J. Physiol. (Lond.).* 317:85–87P.

Bartels, E. M., and G. F. Elliott. 1982. Donnan potentials in rat muscle: difference between skinning and glycerination. *J. Physiol. (Lond.).* 327:72–73P.

Bartels, E. M., and G. F. Elliott. 1983. Donnan potentials in glycerinated rabbit skeletal muscle: the effect of nucleotides and of pyrophosphate. *J. Physiol. (Lond.).* 343:32–33P.

Bartels, E. M., and G. F. Elliott. 1985. Donnan potentials from the A- and I-bands of glycerinated and chemically skinned muscles, relaxed and in rigor. *Biophys. J.* 48:61–86.

Bartels, E. M., P. H. Cooke, G. F. Elliott, and R. A. Hughes. 1985. Donnan potential changes in rabbit muscle A-bands are associated with myosin. *J. Physiol. (Lond.).* 358:80P.

Bartels, E. M., P. H. Cooke, G. F. Elliott, and R. A. Hughes. 1986. Donnan potentials in muscle A-bands and in myosin gels. *J. Muscle Res. Cell Motil.* 7:74.

Brenner, B., M. Schoenberg, J. Chalovich, L. Greene, and E. Eisenberg. 1982. Evidence for cross-bridge attachment in relaxed muscle at low ionic strength. *Proc. Natl. Acad. Sci. USA.* 79:7288–7291.

Brenner, B., L. C. Yu, and R. J. Podolsky. 1984. X-ray diffraction evidence for cross-bridge formation in muscle fibers at various ionic strengths. *Biophys. J.* 46:299–306.

Cooke, P. H. 1975. Filamentous aggregates of purified myosin from smooth muscle. *Cytobiologie.* 11:346–357.

Cooke, P. H., E. M. Bartels, G. F. Elliott, and K. Jennison. 1984. Myosin threads. *Biophys. J.* 45:7a. (Abstr.)

Elliott, G. F. 1964. X-ray diffraction studies on striated and smooth muscles. *Proc. R. Soc. Lond. B Biol. Sci.* 160:467–472.

Elliott, G. F., and C. R. Worthington. 1959. Low angle X-ray diffraction patterns of smooth and striated muscle. *J. Physiol. (Lond.).* 149:32–33P.

Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorous. *J. Biol. Chem.* 66:375–400.

Huxley, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281–308.

Huxley, H. E., and W. Brown. 1967. The low-angle x-ray diagram of vertebrate striated muscle and its behaviour during contraction and rigor. *J. Mol. Biol.* 30:383–434.

King, M. V., and M. Young. 1970. Selective non-enzymatic cleavage of the myosin rod. Electron microscope studies on crystals and paracrystals of light meromyosin-C. *J. Mol. Biol.* 50:491–507.

Leggett-Bailey, J. 1967. Miscellaneous analytical methods: estimation of protein. *In* Techniques in Protein Chemistry. 2nd ed., Elsevier, New York. Chapter 1. 341.

Luck, S. M., and S. Lowey. 1968. Equilibrium binding of ADP to deaminase-free myosin. *Fed. Proc.* 27:519.

Margossian, S. S., and S. Lowey. 1982. Preparation of myosin and its subfragments from rabbit skeletal muscle. *Methods Enzymol.* 85(B):55–71.

Millman, B. M., and B. G. Nickel. 1980. Electrostatic forces in muscle and cylindrical gel systems. *Biophys. J.* 32:49–63.

Morel, J. E., and M. Garrigos. 1982. Dimerization of the myosin heads in solution. *Biochemistry.* 21:2679–2686.

Naylor, G. R. S., E. M. Bartels, T. D. Bridgman, and G. F. Elliott. 1985. Donnan potentials in rabbit psoas muscle in rigor. *Biophys. J.* 48:47–59.

Pastra-Landis, S. C., and S. Lowey. 1986. Myosin subunit interactions. *J. Biol. Chem.* 261:14811–14816.

Philpott, D. E., and A. G. Szent-Györgyi. 1954. The structure of light meromyosin, an electron microscope study. *Biochim. Biophys. Acta.* 15:165–173.

Poulsen, F. R., and J. Lowy. 1982. Small-angle x-ray scattering from myosin heads in relaxed and rigor frog skeletal muscles. *Nature (Lond.).* 5913:146–152.

Poulsen, F. R., J. Lowy, P. H. Cooke, E. M. Bartels, G. F. Elliott, and R. A. Hughes. 1987. Diffuse x-ray scatter from myosin heads in oriented synthetic fibers. *Biophys. J.* 51:959–967.

Sayers, Z., M. H. J. Koch, S. B. Whitburn, K. M. Meek, G. F. Elliott, and A. Harmsen. 1982. Synchrotron x-ray diffraction study of corneal stroma. *J. Mol. Biol.* 160:593–607.

Sobieszek, A. 1972. Cross-bridges on self-assembled smooth muscle myosin filaments. *J. Mol. Biol.* 70:741–744.

Starr, R., and G. Offer. 1982. Preparation of C-protein, H-protein, X-protein and phosphofruktokinase. *Methods Enzymol.* 85(B):130–138.

Suzuki, S., and G. H. Pollack. 1986. Bridgelike connections between thick filaments in stretched skeletal muscle fibers observed by the freeze fracture method. *J. Cell Biol.* 102:1093–1098.

Szent-Györgyi, A. G., C. Cohen, and D. E. Philpott. 1960. Light-meromyosin fraction 1: a helical molecule from myosin. *J. Mol. Biol.* 2:133–142.

Winkelmann, D. A., H. Meekel, and I. Rayment. 1985. Packing analysis of crystalline myosin sub-fragment 1. *J. Mol Biol.* 181:487–501.

Yagi, N., M. J. Dickens, P. M. Bennett, and G. Offer. 1981. Electron microscopy and X-ray diffraction of a hexagonal net of light meromyosin. *J. Mol. Biol.* 149:787–803.

Young, D. M. 1967. On the interaction of ADP with myosin and its enzymatically-active subfragments. *J. Biol. Chem.* 242:2790–2792.