HUMAN PLATELET MYOSIN

II. In Vitro Assembly and Structure of Myosin Filaments

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ABSTRACT

We have used electron microscopy and solubility measurements to investigate the assembly and structure of purified human platelet myosin and myosin rod into filaments. In buffers with ionic strengths of less than 0.3 M, platelet myosin forms filaments which are remarkable for their small size, being only 320 nm long and 10–11 nm wide in the center of the bare zone. The dimensions of these filaments are not affected greatly by variation of the pH between 7 and 8, variation of the ionic strength between 0.05 and 0.2 M, the presence or absence of 1 mM Mg^{++} or ATP, or variation of the myosin concentration between 0.05 and 0.7 mg/ml. In 1 mM Ca^{++} and at pH 6.5 the filaments grow slightly larger. More than 90% of purified platelet myosin molecules assemble into filaments in 0.1 M KCl at pH 7. Purified preparations of the tail fragment of platelet myosin also form filaments. These filaments are slightly larger than myosin filaments formed under the same conditions, indicating that the size of the myosin filaments may be influenced by some interaction between the head and tail portions of myosin molecules. Calculations based on the size and shape of the myosin filaments, the dimensions of the myosin molecule and analysis of the bare zone reveal that the synthetic platelet myosin filaments consists of 28 myosin molecules arranged in a bipolar array with the heads of two myosin molecules projecting from the backbone of the filament at 14–15 nm intervals. The heads appear to be loosely attached to the backbone by a flexible portion of the myosin tail. Given the concentration of myosin in platelets and the number of myosin molecules per filament, very few of these thin myosin filaments should be present in a thin section of a platelet, even if all of the myosin molecules are aggregated into filaments.

Many motile cells which lack the highly organized myofibrils found in striated muscle have been shown to contain significant quantities of actin and myosin (for review see reference 30). Circumstantial evidence supports the attractive idea that these contractile proteins interact to generate the force for cell movement. Direct proof that these cytoplasmic contractile proteins are involved with cell movement will depend, at least in part, upon knowledge of their localization within moving cells and the structural relationship of the contractile proteins to each other. The ultrastructural localization of cytoplasmic actin has been relatively straightforward due to (*a*) its formation of 6-nm wide filaments which are preserved in routine thin sections prepared for electron microscopy, and (*b*) the availability of a method for labeling actin filaments *in situ* with the head portion of the

muscle myosin molecule (14). On the other hand, there is considerable uncertainty about both the form and the localization of myosin in all nonmuscle cells.

As one approach to evaluate the form which myosin might take in vertebrate nonmuscle cells, we have studied in vitro the assembly of filaments from purified human platelet myosin. Since it is impossible to duplicate intracellular conditions precisely in vitro, we varied protein concentration and ionic conditions to determine their influence on the assembly process. The synthetic filaments formed were remarkable for their small size, being about 320 nm long and 10–11 nm wide. Neither the size nor the shape was of these filaments was influenced strongly by the conditions of their assembly. We believe that the low concentration of myosin in platelets and the small size of platelet myosin filaments can account for the apparent absence of myosin filaments in thin sections of resting platelets and nonmuscle cells.[1]

MATERIALS AND METHODS

Protein Purification

Human platelet myosin and human uterus myosin were prepared by the KI-gel filtration method of Pollard et al. (29). Platelet myosin rod, the tail portion of the myosin molecule, was obtained as a by-product of the myosin preparation and was purified by gel filtration (29). Myosin from rabbit back muscles was purified using ammonium sulfate fractionation according to Kielley and Harrington (17).

Determination of Platelet Myosin Solubility

1-ml samples with platelet myosin at a concentration of 0.7 mg/ml in 0.6 M KCl were dialyzed in narrow dialysis bags for 4 h at 4°C vs. various concentrations of KCl between 0.05 and 0.6 M, buffered with 10 mM imidazole chloride, pH 7.0. After dialysis, samples were taken for electron microscopy and K^+EDTA ATPase assay, which established that the myosin ATPase activity was stable in the various buffers. The remaining part of each sample was centrifuged sequentially in the same tubes in a Spinco Ti-50 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 17,000 rpm (26,000 *g*) for 20 min and 45,000 rpm (183,000 *g*) for 30 min. After each spin, the amount of myosin remaining in the supernates was determined by assay of K^+EDTA ATPase activity (28) and protein (20). The amount of myosin sedimenting during each spin was calculated by subtraction.

[1] Preliminary accounts of some of this work have been presented (23, 24, 26).

Formation of Platelet Myosin and Myosin Rod Filaments

Stock solutions of myosin or myosin rod in column buffer (0.6 M KCl, 10 mM imidazole chloride pH 7.0, 1mM dithiothreitol, 0.5 mM ATP, 0.1 mM $MgCl_2$) were dialyzed at 4°C against buffers in which the pH and the concentrations of monovalent and divalent cations were varied as described in the Results Section. Routinely, the dialysis lasted 4 h; on occasion, dialysis was terminated after 1 or 2 h to avoid the time-dependent clumping of filaments into large aggregates which formed in some buffers. Narrow dialysis tubing (9 mm flat width) allowed for equilibration of pH and conductivity within the tubing in less than 4 h. In some experiments, the stock solutions of proteins were rapidly diluted into various low ionic strength buffers.

Negative Staining

Protein solutions were applied to Formvar- and carbon-coated grids rendered hydrophilic by glow discharge in a partial vacuum. After 30 s, excess solution was withdrawn by contact with filter paper and the grid stained with 1% aqueous uranyl acetate for about 15 s.

Embedded Specimens

Intact resting platelets in citrated platelet-rich plasma were "lightly" fixed for 2 min at 37°C with an equal part of 0.1% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.2. After pelleting at 20,000 *g* for 10 min they were further fixed with 3% glutaradehyde in 0.05 M cacodylate buffer for 60 min. After washing in buffer, the pellet was treated with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at room temperature, followed by treatment with 1% aqueous uranyl acetate for 1 h at room temperature, dehydration, and embedding in Epon-Araldite.

Pellets of crude platelet actomyosin (29) were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.5 and then processed like the intact platelets.

Electron Microscopy

Electron micrographs were taken on Siemens Elmiskop 1A and 101 electron microscopes. The magnification of the specimens was determined at frequent intervals using, as a standard, negatively stained paracrystals of muscle tropomyosin having a periodicity of 39.5 nm.

Measurements

Sharply focused micrographs with well stained, well separated filaments were chosen for measuring three dimensions: length of the whole filament, length of the bare zone, and width in the middle of the bare zone. These features of the filaments are defined in Figs. 1 and 11. The filaments in every micrograph of a given specimen were remarkably similar to each other, so that

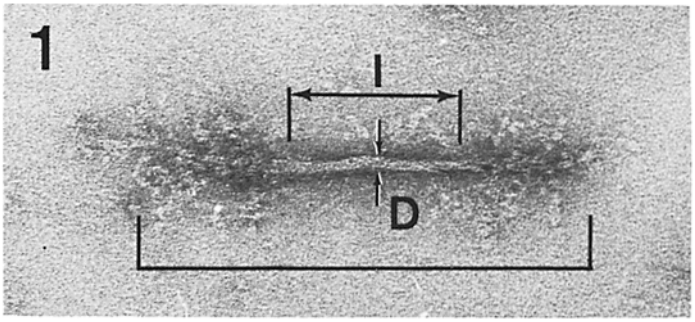


FIGURE 1 Electron micrograph of a platelet myosin filament negatively stained with 1% uranyl acetate. This filament was formed by dialysis against 0.1 M KCl, 1 mM $MgCl_2$ at pH 7. The length of the filament is marked with brackets. The length of the bare zone (*l*) and the diameter of the filament in the center of the bare zone (*D*) are indicated with arrows. The globular heads of the myosin molecules project from the shaft of the filament except in the bare zone. The filament is 400 nm long. × 150,000.

we believe that our selection of micrographs on the basis of their clarity did not introduce any bias into the measurements. The electron micrograph negatives were enlarged to about 700,000 × by projection, and the filament dimensions measured to the nearest millimeter with a ruler. The dimensions of *all* of the filaments on each selected micrograph were used to calculate the mean and standard deviation of each of three dimensions.

RESULTS

Platelet Myosin Filament Formation

Like muscle myosin (19), platelet myosin is a large asymmetric molecule with a molecular weight of about 500,000 (7,29), consisting of globular heads and a fibrous tail (3). Previous work established that platelet myosin forms filamentous aggregates at low ionic strength (3, 5, 6, 43). A typical filament is shown in Fig. 1. All of the platelet myosin filaments observed in this study have a bare central shaft, referred to as the bare zone, which is composed of fine parallel filaments. At both ends, a number of projections are attached to the shaft, giving the filament a bipolar symmetry. Earlier work by Adelstein et al. (3) showed that the projections are the heads of the platelet myosin molecules, containing the ATPase activity and actin-binding sites, while the shaft is formed by the lateral aggregation of the fibrous tail portion of the molecule. Negatively stained platelet myosin filaments are found in two configurations: in some cases the myosin heads clumped together on the surface of the filament backbone (Figs. 2 and 4 *c*), but frequently the heads are spread away from the filament backbone (Figs. 1 and 4). This indicates that the heads are loosely attached to the filament backbone by a flexible part of the myosin tail, which allows them to swing out up to 50 nm from the shaft of the filament. Other types of myosin filaments also exhibit both compact and spread configurations (27).

In the sections which follow, we consider the influence of the concentrations of monovalent and divalent cations, H^+, ATP, and myosin on the formation and structure of platelet myosin filaments.

INFLUENCE OF IONIC STRENGTH: Ionic strength has little influence on the size and shape of platelet myosin filaments (Fig. 2 and Table I), but it is a major determinant of the number of filaments formed from a given concentration of myosin (Fig. 3). In KCl concentrations of 0.05 to 0.2 M, platelet myosin forms short thin bipolar filaments (Fig. 2) with mean lengths of 300–350 nm and mean widths of about 10–11 nm (Table I). By eye, none of these filament suspensions were turbid, except the sample in 0.05 M KCl, which contained a precipitate consisting of the usual small myosin filaments aggregated end to end into nets.

The extent of filament formation was measured biochemically by centrifuging samples sequentially at 26,000 *g* and 183,000 *g* in a preparative ultracentrifuge (Fig. 3). Little myosin pelleted during the low speed spin, except for the nets of small filaments in 0.05 M KCl. The high speed spin sedimented over 90% of the myosin in the 0.05 and 0.1 M KCl samples, indicating that most of the myosin molecules were assembled into filaments under these conditions. The fraction of myosin pelleting from higher concentrations of KCl correlated well with the presence or absence of filaments detectable by electron microscopy. In

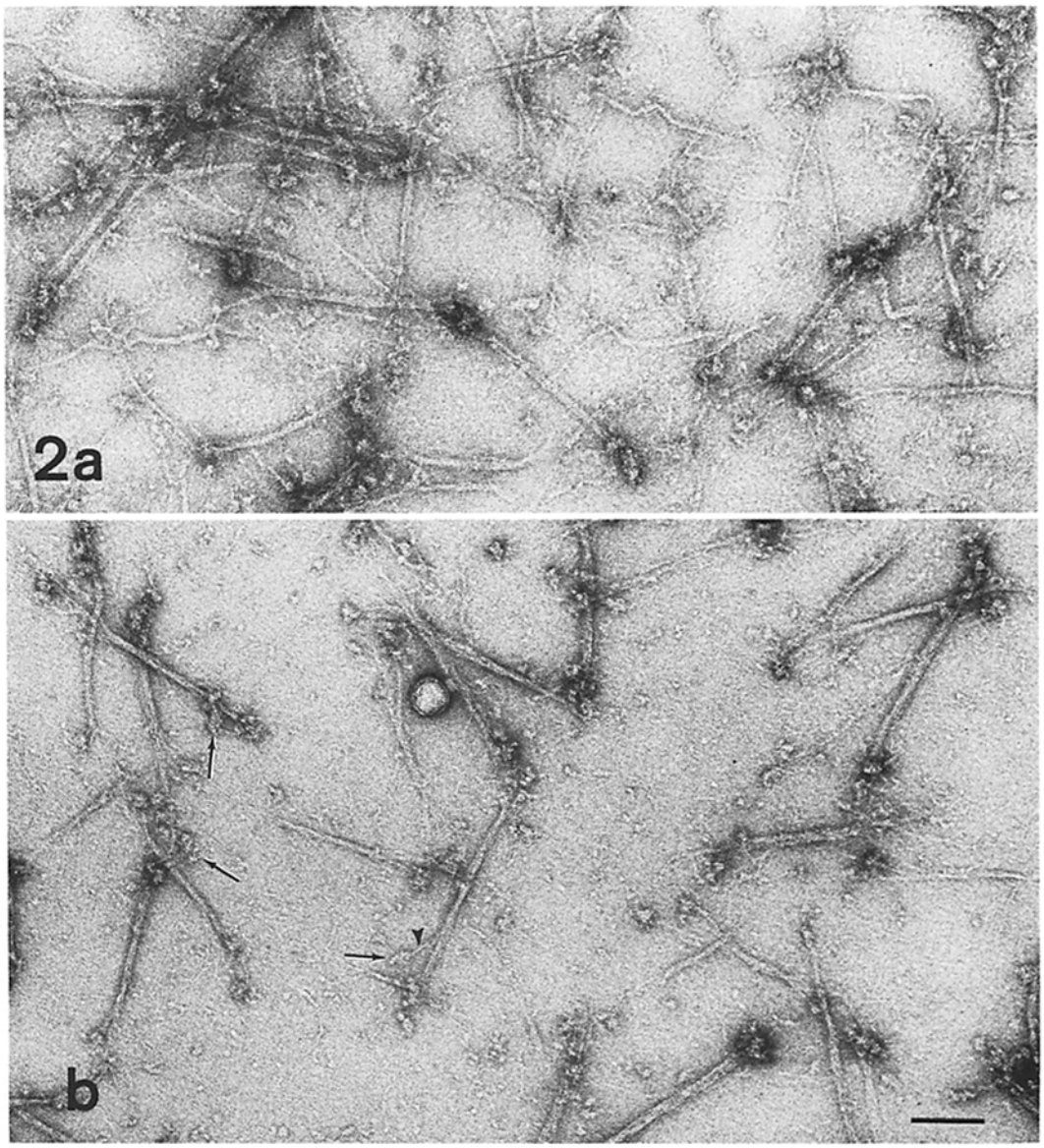


FIGURE 2 Influence of ionic strength on platelet myosin filament formation. Electron micrographs of negatively stained filaments formed from purified platelet myosin dialyzed against 0.05 M KCl (*a*) or 0.1 M KCl (*b*) buffered with 10 mM imidazole-chloride, pH 7. Most of these filaments have their projections clustered together near the shaft of the filament, but a few have their projections spread away from the shaft (arrows) tethered to the backbone by a thin fiber (arrowhead). Bar is 0.1 μm. × 100,000.

0.3 M KCl a few small filaments were observed by electron microscopy and 16% of the myosin sedimented, while in 0.5 M KCl no filaments were seen by electron microscopy and less than 8% of the myosin pelleted.

The low speed spin alone might have led to the erroneous conclusion that platelet myosin forms filaments in 0.05 M KCl but that it is "soluble" in KCl concentrations of 0.1 M or greater. The combination of the two spins and the electron microscopy show that (*a*) platelet myosin forms filaments which are too small to pellet in 20 min at 26,000 *g* (this point is discussed further in the Calculation Section), and (*b*) ionic strength influ-

TABLE I

*Influence of Ionic Strength on Platelet Myosin Filament Size**

Concentration of KCl‡	Total length	Bare zone length, *l*	Diameter, *D*	*n*§
M	*nm*	*nm*	*nm*	
0.05	309 ± 37 (249–368)	165 ± 23 (130–216)	9.9 ± 1.3 (7.8–12.5)	1.9
0.10	351 ± 25 (292–400)	173 ± 22 (130–216)	10.5 ± 1.8 (7.8–14.0)	2.3
0.15	349 ± 28 (303–411)	156 ± 21 (119–227)	11.2 ± 1.7 (7.8–17.2)	2.3
0.20	339 ± 5 (335–346)	Not measured	9.9 ± 1.0 (7.8–10.9)	
0.30‖				

* 40 filaments of each type were measured, except on the 0.2 M grid where an ordered array, similar to the Type I tactoids (26), was used to more accurately measure lengths. Figures given are the mean ± SD (range).

‡ All samples were dialyzed against a buffer which contained in addition to KCl, 10 mM imidazole chloride pH 7.

§ The number of myosin molecules in each axial repeat, *n*, is calculated using the equations developed in the Appendix.

‖ No filaments observed.

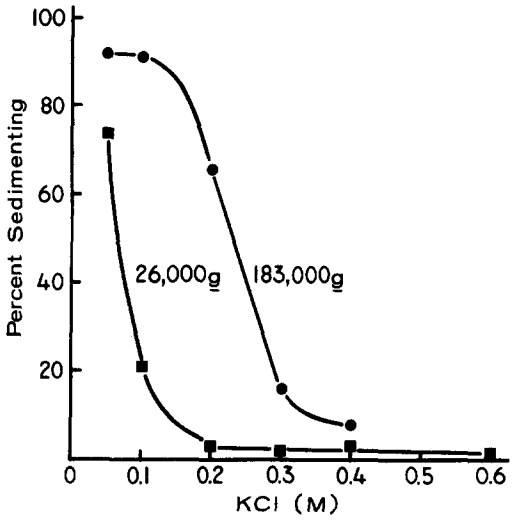


FIGURE 3 Platelet myosin solubility. Centrifugation of platelet myosin dialyzed against various concentrations of KCl buffered with 10 mM imidazole-chloride, pH 7, as described in the text. The fraction of myosin sedimenting after each spin was determined both ATPase assay and protein determination. The estimates of supernatant myosin by the two methods were similar, so the values were averaged.

ences the equilibrium between filaments and monomer, but not the size of the filaments.

INFLUENCE OF H^+ CONCENTRATION: Provided the KCl concentration is less than 0.2 M, H^+ concentration is the most significant factor we tested in determining the size of platelet myosin filaments (Fig. 4 and Table II). The pH influences both the length and width of the filaments. At each pH the variation in the dimensions is quite restricted, as indicated by the small standard deviations in Table II. The discrete size of the filaments at any pH suggests that each increment in the growth of the filaments has strict ionic determinants which are influenced by H^+ concentration. The filaments formed at pH 6.5 are somewhat longer and distinctly wider than the filaments formed at higher pH's. As discussed below in the Calculation Section, the greater width of the filaments at pH 6.5 may indicate that the myosin tails in the core of these filaments are packed differently than the myosin tails in the filaments formed at higher pH's.

INFLUENCE OF DIVALENT CATIONS: At a given pH and KCl concentration, 1 mM EDTA reduced and 1 mM $MgCl_2$ or $CaCl_2$ increased the size of platelet myosin filaments (Fig. 4 and Table III). Although EDTA blocks the formation of filaments by guinea pig granulocyte myosin (36), EDTA did not completely disrupt the platelet myosin filaments even at pH 8.2 where the platelet myosin filaments are relatively small. Thus, divalent cations facilitate the growth of platelet myosin filaments but are not absolutely required. Higher concentrations of $CaCl_2$ or $MgCl_2$ promote the formation of three different types of tactoids of platelet myosin (26) which will be described in detail in a subsequent paper.

INFLUENCE OF ATP: The presence of 1mM ATP has no apparent effect on filament assembly in 0.1 M KCl at pH 7 with 2 mM $MgCl_2$ and 0.2 mM EGTA (Fig. 5 and Table III). These conditions may resemble those within the platelet.

INFLUENCE OF MYOSIN CONCENTRATION: Myosin concentration has little influence on the structure of synthetic platelet myosin filaments. Most of our experiments were carried out

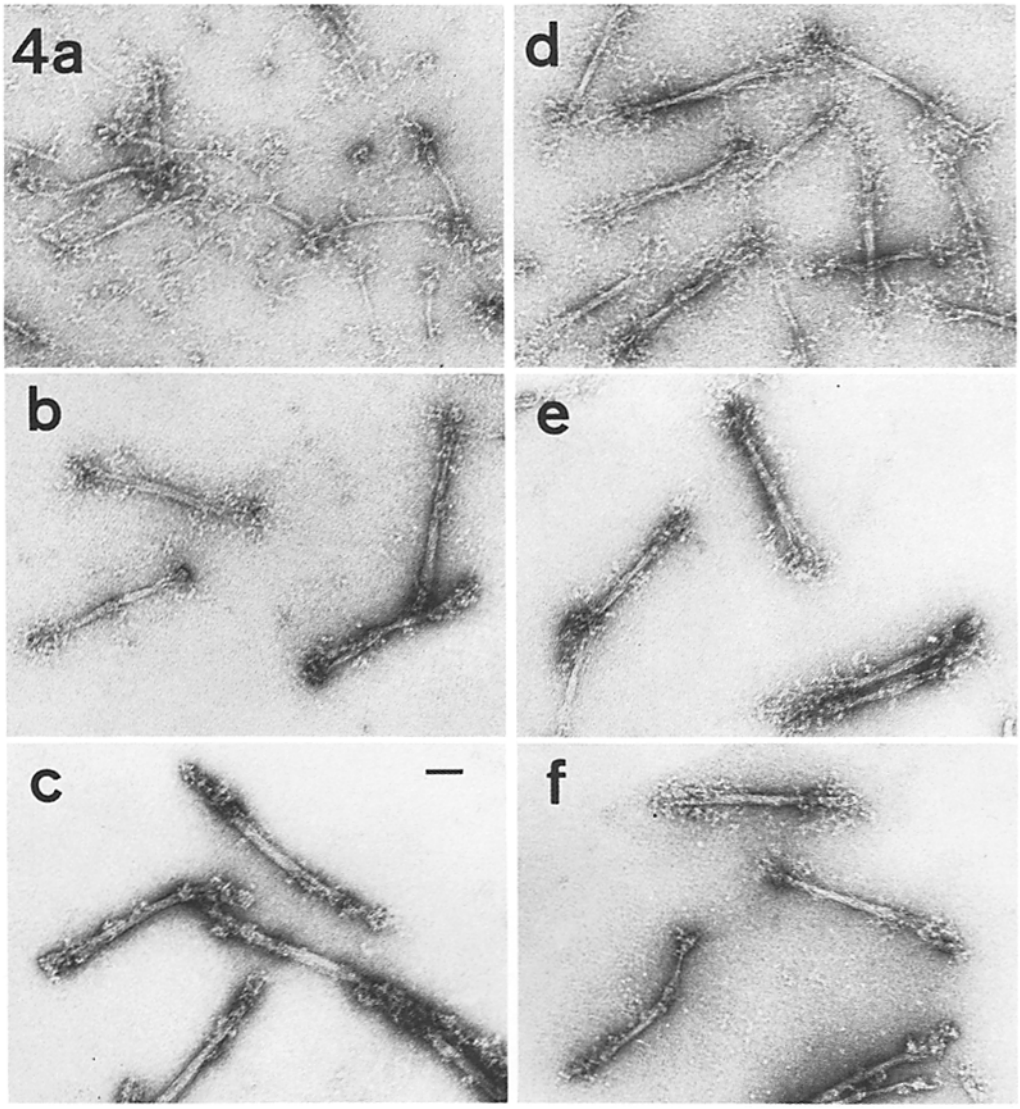


FIGURE 4 Influence of pH and divalent cations on platelet myosin filament formation. Electron micrographs of negatively stained filaments formed from purified platelet myosin dialyzed against 0.1 M KCl, 10 mM imidazole-chloride at (*a*) pH 8.2, (*b*) pH 7.2, (*c*) pH 6.5, or against (*d*) 1 mM EDTA, pH 7.0, (*e*) 1 mM $MgCl_2$, pH 7.0, or (*f*) 1 mM $CaCl_2$, pH 7.0. Bar is 0.1 μm. × 50,000.

with myosin at concentrations of 0.2–0.3 mg/ml, but electron microscopy established that variation of the myosin concentration between 0.05 and 0.7 mg/ml did not alter the size or shape of filaments formed (see also Fig. 8 in reference 27). The effect of myosin concentration on the extent of filament formation is less well understood. Two experiments with a single preparation of myosin gave identical puzzling results. The methods described in Fig. 3 were used to measure the number of myosin molecules incorporated into filaments in 0.1 M KCl at pH 7 as a function of myosin concentration between 0.05 and 0.7 mg/ml. At each concentration below 0.3 mg/ml, about two-thirds of the myosin pelleted. At higher concentrations of myosin, over 90% of the myosin pelleted as in the experiment in Fig. 3, so that less myosin remained in the supernate at 0.4 mg/ml than at 0.3

TABLE II

*Influence of pH on Platelet Myosin Filament Size**

pH‡	Total length	Bare zone length, *l*	Diameter, *D*	*n*
	nm	*nm*	*nm*	
8.2	298 ± 34 (230–370)	138 ± 18 (100–180)	10.5 ± 1.8 (8.8–13.2)	1.8
7.2	300 ± 37 (244–449)	170 ± 23 (112–245)	11.2 ± 2.0 (6.1 16)	2.5
6.5	391 ± 43 (264–481)	184 ± 24 (107–245)	16.7 ± 2.3 (12–21)	6.1

* 50 filaments of each size were measured.

‡ Each sample was dialyzed against a buffer containing 0.1 M KCl and 10 mM imidazole chloride with the pH adjusted to the value given.

TABLE III

Influence of Divalent Cations and ATP on Platelet Myosin Filament Size

Conditions*	Total length	Bare zone length, *l*	Diameter, *D*	*n*
	nm	*nm*	*nm*	
1 mM EDTA‡	271 ± 26 (216–324)	132 ± 17 (87–162)	8.6 ± 1.9 (5.9–11.7)	1.2
1 mM $CaCl_2$	359 ± 35 (292–433)	145 ± 15 (108–173)	13.0 ± 1.7 (10.2–17.6)	3.0
1 mM $MgCl_2$‡	350 ± 39 (270–455)	146 ± 19 (108–206)	11.6 ± 2.1 (8.0–17.6)	2.4
2 mM $MgCl_2$, 0.1 mM EGTA‡§	306 ± 26 (257–349)	148 ± 24 (113–216)	9.5 ± 1.5 (6.8–12.8)	1.6
2 mM $MgCl_2$, 0.1 mM EGTA, 1 mM ATP‡‖	320 ± 23 (267–369)	156 ± 19 (123–185)	10.5 ± 1.4 (6.8–12.8)	2.0

* All samples were dialyzed against a buffer containing 0.1 M KCl, 10 mM imidazole chloride, pH 7, in addition to the ions listed.

‡ 40 measured.

§ 25 filaments measured.

‖ The grid was washed very briefly, about 2 s, with this buffer but without ATP to avoid precipitates during staining.

mg/ml. Thus, there is no evidence for a critical concentration for filament assembly in the range examined. A more detailed interpretation of these experiments will require additional information.

INFLUENCE OF PURIFICATION METHOD AND THE PRESENCE OF PLATELET MYOSIN ROD: In earlier work (3) it was reported that platelet myosin formed bipolar filaments up to 700 nm long in 0.1 M KCl at pH 7. The platelet myosin used in the current work differs from that in the 1971 study in two important ways: (*a*) the present platelet myosin contains less than 5% platelet myosin rod, while the 1971 platelet myosin was contaminated with up to equal portions of platelet myosin rod; and (*b*) the present platelet myosin is exposed to 0.6 M KI during its purification, while the 1971 platelet myosin was not. Can either of these differences account for the smaller size of the myosin filaments in this study? Filaments formed from mixtures platelet myosin with an equal amount of rod were larger than the pure myosin filaments, confirming the earlier observations. In addition, an effect of KI on filament size can be ruled out by two control experiments: (*a*) platelet myosin purified with KCl (and consequently contaminated with some actin) assembled into small filaments similar to the KI myosin filaments described above; and (*b*) rabbit skeletal muscle myosin, purified by the platelet myosin method using KI, formed thick filaments of normal size in 0.1 M KCl at pH 7. These observations lead us to conclude that the larger size of the myosin filaments observed in 1971 was a consequence of platelet myosin rod being incorporated into the filaments and promoting their growth.

COMPARISON OF VERTEBRATE CYTOPLASMIC MYOSIN FILAMENTS: The myosins from mouse fibroblasts (2), guinea pig granulocytes (36), rabbit macrophages (35), and rabbit hepatocytes (8) all form filaments similar in size and form to platelet myosin filaments (Fig. 6). Granulocyte myosin filaments differ slightly from the others shown in Fig. 6 in requiring divalent cations for their assembly (36).

COMPARISON WITH MUSCLE MYOSIN FILAMENTS: To compare filaments formed under identical conditions, equal concentrations of platelet myosin, human uterine myosin, and rabbit

skeletal muscle myosin in 0.5 M KCl were dialyzed against 0.1 M KCl at pH 7. Electron micrographs of the resulting filaments reveal both important similarities and differences (Fig. 6). All three types of myosin form filaments under the same ionic conditions, and all of the resulting filaments have bipolar symmetry, but there are striking differences in their dimensions. Skeletal muscle myosin filaments are much longer and somewhat wider than the platelet myosin filaments, while the uterine myosin filaments are intermediate in size. In spite of these differences, there is some evidence that skeletal muscle myosin will form hybrid filaments with both platelet myosin (26, 27) and smooth muscle myosin (15, 26, 27).

Platelet Myosin Rod Assembly

Platelet myosin rod is thought to be the tail portion of the myosin molecule, from which the heads, containing actin-binding sites and ATPase activity, have been cleaved (3). It is thought (1) that this cleavage occurs by proteolytic digestion of the intact platelet myosin molecule during the storage of platelets in the blood bank or during the isolation of the myosin. The platelet myosin rod is obtained in a highly purified form as a by product of the myosin purification, and has a subunit molecular weight of about 140,000 daltons. On the basis of its large Stokes radius of about 17.0 nm (29), it is thought to be a fibrous molecule like the corresponding fragment of muscle myosin. The assembly of the platelet myosin rod into filaments is reported here for comparison with the assembly properties of the intact myosin. In general, the effects of various conditions on the formation of filaments from isolated myosin rod parallels the effect of these conditions on the formation of filaments of intact myosin described above.

INFLUENCE OF IONIC STRENGTH: At KCl concentrations of 0.3 M or less, platelet myosin rod forms short aggregates with filamentous substructure (Fig. 7 and Table IV). These filaments are similar to myosin filaments, except that the rod filaments are wider and lack the bipolar projections which are characteristic of myosin filaments.

INFLUENCE OF DIVALENT CATIONS: Low concentrations of $CaCl_2$ cause rod filaments to grow wider and more heterogeneous than in the absence of Ca^{++} (Fig. 7). As in the case of the myosin, $MgCl_2$ has a less pronounced effect on filament size. For reasons which are not currently understood, the combination of 1 mM EDTA with 2 mM $CaCl_2$ largely blocks the effect of $CaCl_2$. Higher concentrations of $CaCl_2$ or $MgCl_2$ promote the formation of large paracrystals (23) which will be described in a subsequent paper.

INFLUENCE OF ATP: When filaments of platelet myosin rod in 0.6 M KCl were formed by dilution to 0.1 M KCl at pH 7 with 1 mM ATP, they were smaller than those formed without ATP (Table IV).

Structure of Platelet Actomyosin

The structure of platelet actomyosin has been examined by electron microscopy of thin sections (44) and negatively stained specimens (3, 5, 6, 43).

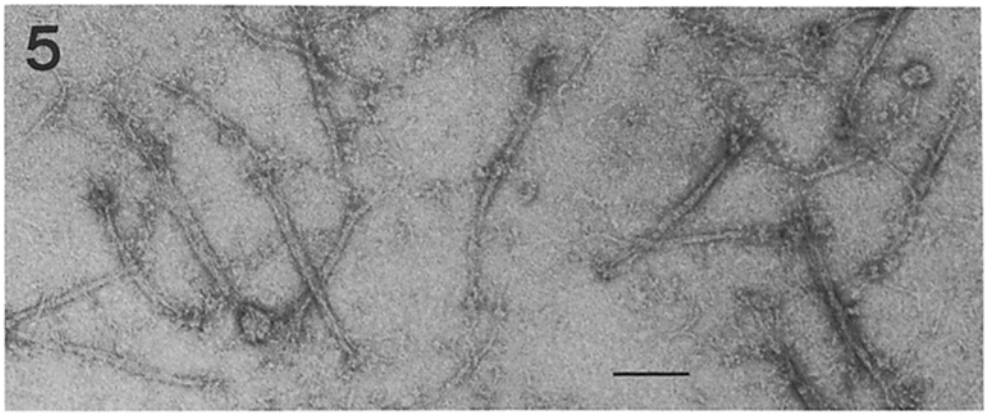


FIGURE 5 Influence of ATP on platelet myosin filament formation. Electron micrograph of negatively stained filaments formed from purified platelet myosin dialyzed against 0.1 M KCl, 10 mM imidazole-chloride pH 7, 2 mM $MgCl_2$, 1 mM ATP and 0.2 mM EGTA. Bar is 0.1 μm. × 100,000.

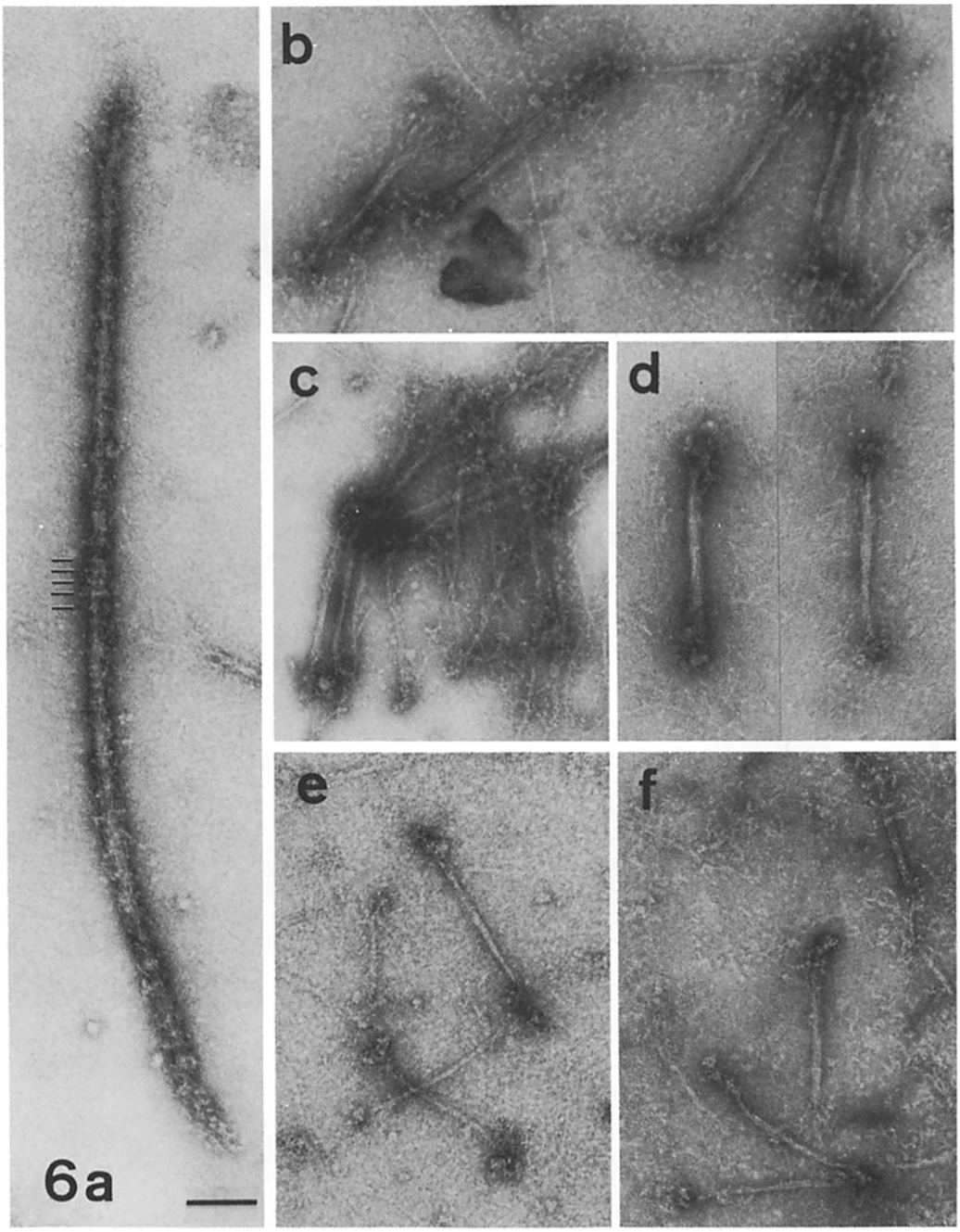


FIGURE 6 Comparison of various myosin filaments. Electron micrographs of negatively stained myosin filaments from various sources formed by dialysis against 0.1 M KCl, 10 mM imidazole-chloride pH 7. The buffer for the granulocyte myosin also contained 1 mM $MgCl_2$. (*a*) Rabbit skeletal muscle myosin with a distinct 14.5 nm axial periodicity visible in some regions marked with bars; (*b*) human uterus smooth muscle myosin purified by S. Thomas using the method in reference 29; (*c*) mouse fibroblast myosin purified by R. S. Adelstein, reference 2; (*d*) rabbit hepatocyte myosin purified by D. Brandon, reference 8; (*e*) guinea pig granulocyte myosin purified by T. P. Stossel, reference 36; (*f*) human platelet myosin. Bar is 0.1 μm. × 100,000.

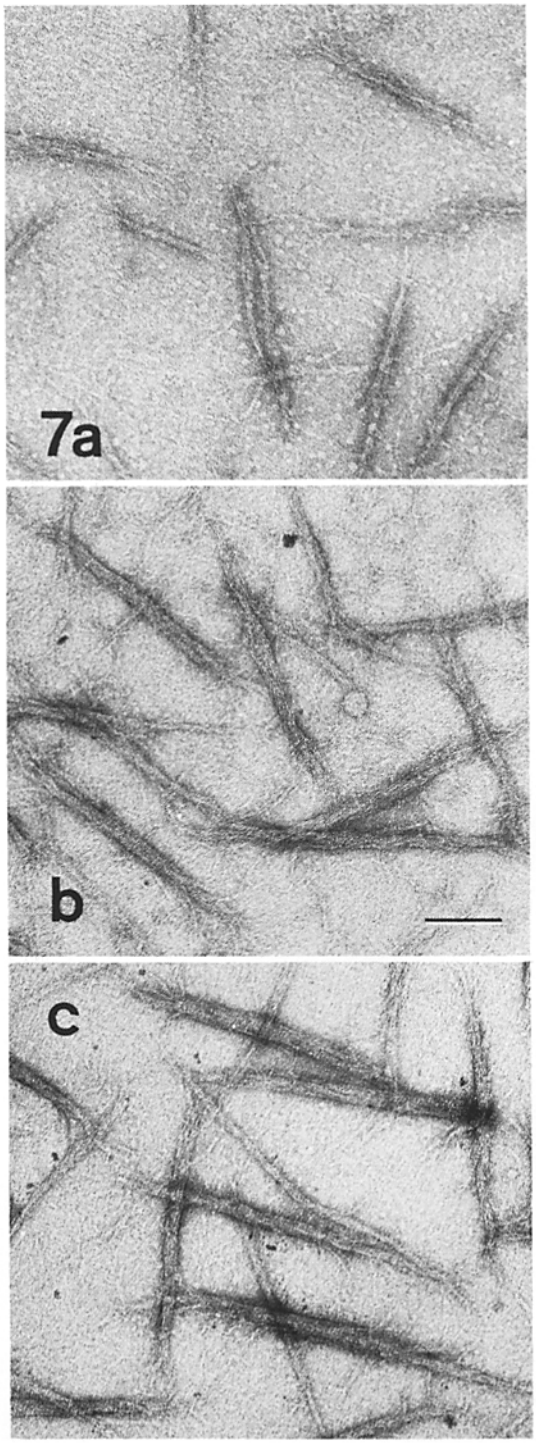


FIGURE 7 Filaments of platelet myosin rod. Electron micrographs of negatively stained filaments formed from purified platelet myosin rod by dilution into 0.1 M KCl, 10 mM imidazole-chloride pH 7, with (*a*) 1 mM EDTA, (*b*) 2 mM $MgCl_2$, or (*c*) 2 mM $CaCl_2$. Bar is 0.1 μm. × 100,000.

We have rexamined platelet actomyosin using both of these methods to determine whether the presence of actin filaments influences the formation of myosin filaments and how the myosin filaments interact with actin filaments.

Mixtures of actin and myosin in 0.5 M KCl were dialyzed or diluted to lower the KCl concentration to 0.1 M to form actomyosin precipitates. Negatively stained preparations of actomyosin (Fig. 8 *a*) clearly demonstrate that short thin filaments of platelet myosin form in the presence of actin and that these myosin filaments can link adjacent actin filaments. The cross-links are formed by the bare central shaft of the myosin filaments, while the myosin heads at both ends of the bipolar filaments bind to actin filaments forming an arrowhead pattern. In many cases it is clear that the myosin molecules decorating the actin filaments are part of myosin filaments, but in other cases myosin molecules attached to the actin filaments are not clearly associated with a myosin filament.

The identification of myosin filaments and their relation to actin filaments are much less clear in thin sections of crude actomyosin (Fig. 8 *b*). The filaments in these preparations fall into two sizes classes; there are thin filaments which undoubtedly are actin and fewer thicker filaments which may be composed of myosin. The presumed myosin filaments are about 11.2 nm wide in these preparations. Under similar conditions, purified platelet myosin forms filaments which are 16 nm wide in negatively stained preparations, so there may be some lateral shrinkage of these filaments during fixation, dehydration, or embedding. This type of lateral shrinkage of the myosin filament has been noted also in embedded platelet myosin paracrystals (26).

It is worth noting here that platelet actomyosin fractions contain large numbers of membrane fragments. Judging from their size, they could only be derived from the plasma membrane. These plasma membrane fragments are not entirely separated from the myosin until the final step in the myosin purification, gel filtration on 4% agarose.

Filaments in Intact Platelets

Judging from the size of the platelet myosin filaments in the studies described above and from

TABLE IV

Platelet Myosin Rod Summary

Conditions	Length	Width
	nm	*nm*
Influence of divalent cations*		
0.1 M KCl, 1 mM EDTA	335.0 ± 55.2 (236–480)	11.68 ± 2.60 (6.3–18.9)
0.1 M KCl, 2 mM Mg	365.7 ± 67.3 (220–511)	13.00 ± 2.80 (7.9–18.9)
0.1 M KCl, 2 mM Ca	382.1 ± 88.7 (228–613)	19.80 ± 7.0 (7.9–40.9)
0.1 M KCl, 1 mM EDTA, 2 mM $MgCl_2$	Not measured	10.93 ± 2.14 (6.3–14.2)
0.1 M KCl, 1 mM EDTA, 2 mM $CaCl_2$	Not measured	12.27 ± 3.78 (6.3–23.6)
Influences of ATP‡		
0.1 M KCl, 10 mM imidazole	261.05 ± 39.49 (165–329)	11.00 ± 2.84 (7 48–17.95)
0.1 M KCl, 10 mM imidazole + 1 mM ATP	200.90 ± 45.58 (127–269)	6.66 ± 2.41 (3.0–10.5)

* Experiment 8.

‡ Experiment 7.

our observation that fluorescein-labeled antibodies to platelet myosin stain the cytoplasm of platelets leukocytes, and other cells[2] one would expect to observe myosin filaments in thin sections of intact platelets. Instead, high resolution images of resting platelets (Fig. 9) reveal that the cytoplasm is filled with reticular material but no clear 10–11-nm myosin filaments, confirming similar observations by others (5, 39, 41, 42). Some but not all platelets in retracted clots (Fig. 9; references 5, 39, 41 and 42) and platelets treated with trypsin (31) have a population of thick filaments which are considerably wider than the synthetic myosin filaments described above. These large filaments may be composed of myosin, because purified platelet myosin forms large tactoids with a similar appearance in high concentrations of divalent cations and at low pH (26).

The reasons for the absence of visible myosin filaments in resting platelets (and other vertebrate nonmuscle cells) remain a mystery, although the calculations which follow shed some light on the question.

Calculations

STRUCTURE OF THE PLATELET MYOSIN FILAMENT: Using the size and shape of negatively stained platelet myosin filaments, the dimensions of the platelet myosin molecule, the assumptions given below, and the equations from the Appendix of this paper, we have calculated the value of n, the number of myosin molecules whose heads project from the filament in each axial repeat. These values of n for platelet myosin filaments formed under various conditions are given in Tables I–III. In most cases n is approximately 2, although there are interesting exceptions discussed below. The calculated value of n and other dimensions of these filaments suggest a model for the platelet myosin filaments which consists of 28 myosin molecules (Fig. 10).

For the calculation of n, we made a number of assumptions:

(*a*) There is no significant shrinkage or flattening of the negatively stained filaments so that the dimensions measured from the micrographs are close to the true hydrated dimensions of the filaments. Glutaraldehyde fixation before negative staining does not alter the appearance of these negatively stained filaments, supporting the validity of this assumption.

(*b*) The tail of the platelet myosin molecule is a completely alpha-helical coiled-coil like the corresponding region of the muscle myosin molecule (19). The large Stokes radius (29), the low content of proline and the high content of acidic residues of isolated platelet myosin rod[3] are consistent with this assumption.

(*c*) The tail of platelet myosin is 160 nm long. This is the length expected for an alpha-helical

[2] Fujiwara, K., and Pollard, T. D. 1974. Unpublished experiments.

[3] There are 8 mol of proline, 115 mol of aspartic acid and 353 mol of glutamic acid per mol of electrophoretically purified platelet myosin rod. (T. D. Pollard. 1974. Unpublished observation.)

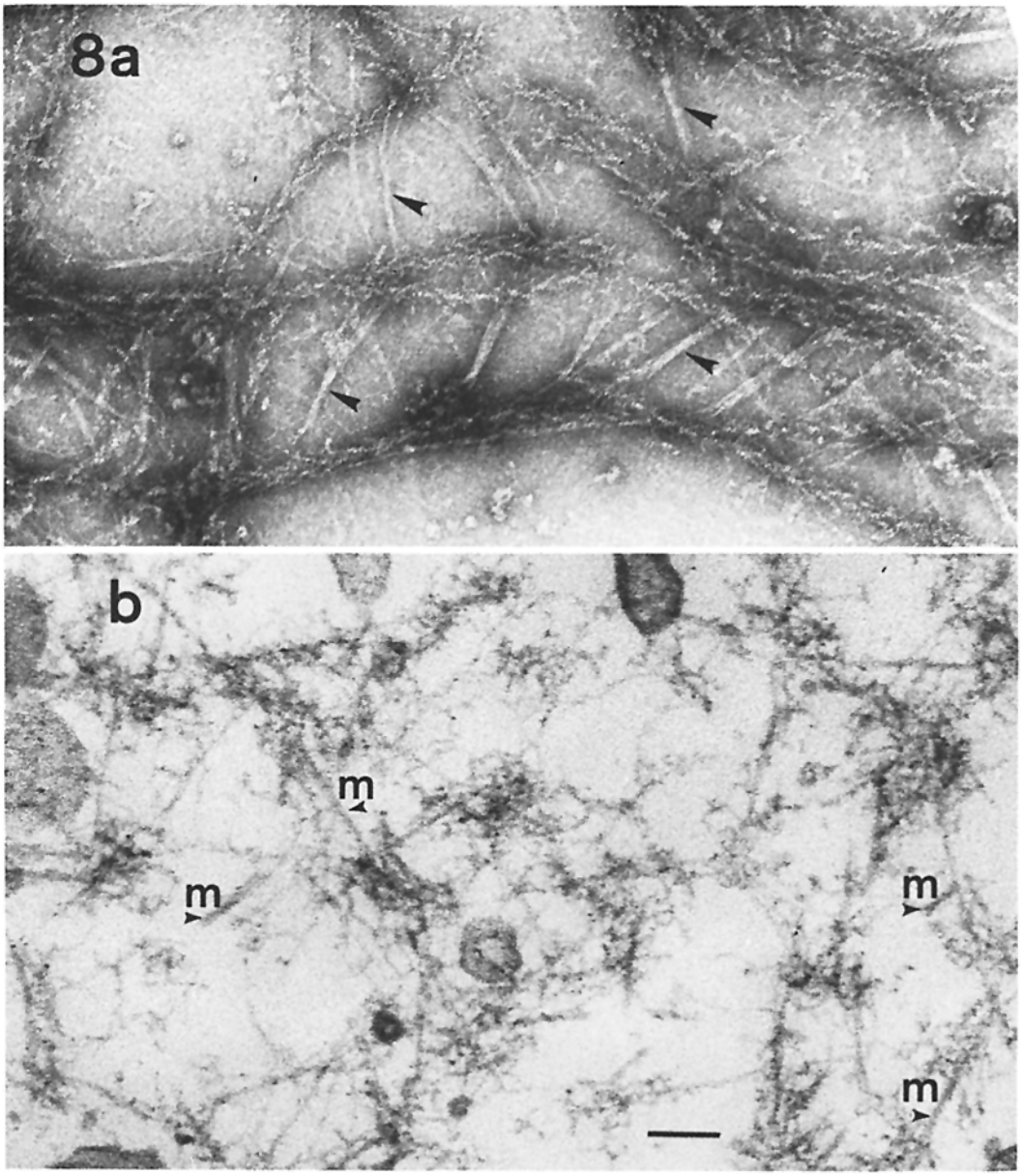


FIGURE 8 Electron micrographs of mixtures of platelet actin and myosin. (*a*) Sample is negatively stained. Several myosin filaments cross-linking decorated actin filaments are marked with arrowheads. (*b*) Sample is a thin section of a pellet of crude actomyosin prepared according to reference 29 by precipitation in 0.15 M KCl at pH 6.5 and fixed at the same pH. Several presumptive myosin filaments are marked (*m*). Bar is 0.1 μm. × 100,000.

coiled-coil consisting of two polypeptides with molecular weights of 140,000 daltons (our estimate of the molecular weight of the isolated platelet myosin rod). This is approximately the length of the tail of smooth muscle myosin (16) and is somewhat longer than the tail of skeletal muscle myosin, 145 nm (11, 19).

(*d*) The tails of the platelet myosin molecules are packed tightly in a hexagonal array in the shaft of the filament with a center to center spacing of 2.0

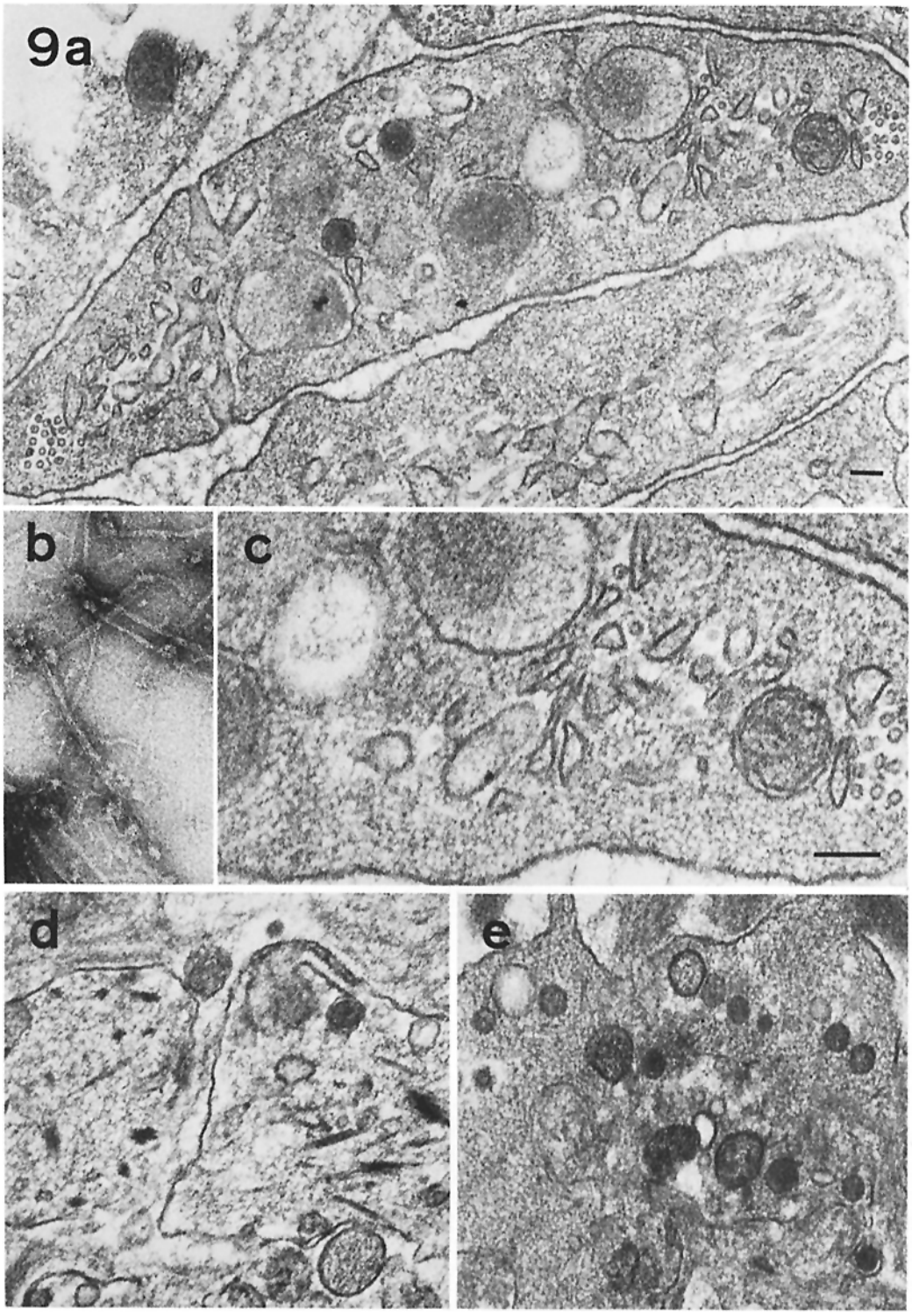


FIGURE 9 Electron micrographs of thin sections of platelets compared with negatively stained myosin filaments. (*a*) Micrograph shows a resting platelet cut in cross section. This is the platelet discussed in the text which has a surface area of about 1.4 μm^2 and which may contain about 170 myosin molecules. (*b*) Micrograph shows negatively stained platelet myosin filaments at high magnification for comparison with the detail of the platelet in (*c*) at the same magnification. (*d* and *e*) Micrographs are thin sections of platelets from retracted clots of platelet-rich citrated plasma recalcified with 20 mM $CaCl_2$. Some of the platelets (*d*) develop large tactoids throught by some to be myosin, while many platelets in the same clot have none of these tactoids (*e*). Bars are 0.1 μm. (*a*, *d*, and *e*) × 50,000; (*b* and *c*) × 100,000.

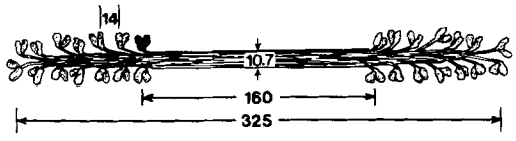


FIGURE 10 Line drawing of a two-dimensional model of a platelet myosin filament, derived as described in the text. The size of one myosin molecule is illustrated by the solid tail and heads at the upper left edge of the bare zone. All of the heads are shown closely associated with the filament backbone, although the heads on many negatively stained filaments are spread out from the backbone, obscuring their periodicity. The dimensions are in nanometers.

nm. This is thought to be the spacing of other ordered assemblies of alpha-helical coiled-coils including paramyosin and tropomyosin (9, 34).

(*e*) There is no hole in the core of the filaments.

(*f*) The 14–15 nm periodicity of surface projections on three types of platelet myosin paracrystals (26) represents the axial repeat of myosin heads (and therefore myosin molecules) in platelet myosin filaments. This assumption is supported by two facts: the heads in skeletal muscle myosin filaments also have a 14.3 nm axial periodicity (13) and platelet myosin appears to copolymerize with skeletal muscle myosin (27).

Platelet myosin filaments formed in vitro under conditions which may approximate the physiological state, such as 0.1 M KCl, 2 mM $MgCl_2$, 1 mM ATP, and 0.1 mM EGTA at pH 7, have two myosin molecules in each axial repeat (Tables I–III). From the dimensions of these synthetic myosin filaments, there are 14 axial repeats per filament and thus 28 myosin molecules per filament. Fig. 10 is a rough two-dimensional drawing of how the myosin molecules might be arranged in one of these synthetic myosin filaments. The model is shown in two dimensions, because there is no information available on the azimuthal arrangement of the projecting myosin heads on the surface of the filament. The drawing shows the heads close to the shaft of the filament, to emphasize their periodic arrangement, but it is clear from the micrographs of real filaments that the heads are usually spread away from the backbone of the filament.

The model depicted in Fig. 10 resembles in some ways Pepés 1967 (25) model for the bare zone of skeletal muscle thick filaments[4] and has several appealing features. The bare zone is the same length as a myosin tail, so that the tails of the myosin molecules bordering the bare zone overlap each other completely. The filament is twice as long as a single myosin molecule, so that the tails of the myosins at opposite ends of the filament just meet, but do not overlap in the center of the bare zone. Consequently, all of the molecules in these small filaments are arranged so that they might make extensive antiparallel overlaps with other myosins. It is possible that the assembly of these platelet myosin filaments requires bonds between antiparallel tails and that the observed limitation of filament elongation is related to the unavailability of these bonds for molecules whose heads are situated more than 160 nm from the center of the bare zone.

Under some conditions the mean diameter of synthetic platelet myosin filaments is not 10–11 nm, indicating that the structure of these filaments may differ from the model shown in Fig. 10. In the case of the small filaments formed in the presence of EDTA (Table III, Fig. 4), *n* is closer to one than two, so that these filaments may be partially disrupted. The diameter of the filaments which formed in the presence of 1 mM $CaCl_2$ (Table III, Fig. 4) is large enough to accommodate up to three myosin molecules per axial repeat, while the very thick filaments formed at pH 6.5 (Table II, Fig. 4) could have as many as six myosins in each axial repeat. The large number of surface projections on these thicker filaments also suggests that there are more than two myosins per axial repeat in these two cases. Alternatively, the greater width of these filaments may be due to either the myosin tails packing more loosely in the shaft or the presence of a hollow space within the shaft.

PREDICTION OF THE SEDIMENTATION COEFFICIENT OF PLATELET MYOSIN FILAMENTS: One way of confirming the model given above would be to measure the sedimentation coefficient and molecular weight of the filaments directly, using hydrodynamic methods. This work

[4] The model we propose for platelet myosin filaments complements both the model for striated muscle myosin filaments first proposed by Pepe in 1967 (25) and the general model for myosin filaments first proposed by Squire in 1971 (34). The measured dimensions of the platelet myosin filament fall within the range of values predicted by Pepe's model. The number of myosin molecules in the platelet myosin filament is the number which would be required to fill the missing rows of myosin heads adjacent to the bare zone in Squire's model (25).

has not yet been done, but we have calculated the sedimentation coefficient expected for a filament having the structure shown in Fig. 10 and find that the sedimentation behavior of the real myosin filaments (Fig. 3) is consistent with the model.

The molecular weight of a myosin filament composed of 28 myosin molecules is 12.9×10^6 daltons. If the partial specific volume of the filament is taken to be 0.73 and the filament is 325 nm long, the axial ratio of the hydrodynamically equivalent prolate ellipsoid can be determined. The frictional ratio of this ellipsoid can be calculated from Perrin's equation (See reference 37, Equation 19–14). Using Stokes' law together with this information, one can calculate the frictional coefficient of the equivalent sphere. Finally, taking the density of the solution to be 1.0, the sedimentation coefficient at infinite dilution can be predicted to be about 80S.

The sedimentation properties of real synthetic platelet myosin filaments (Fig. 3) are consistent with their having a sedimentation coefficient of 80S. A front of 80S particles would travel about 2 mm in 20 min at 26,000 × g. Since the distance from the meniscus to the bottom of the rounded centrifuge tubes was about 7 mm, finding 80% of myosin in the supernate after the low speed spin agrees with the expected properties of the model filament. An 80S filament front would travel about 27 mm in 30 min at 183, 000 × g, and, accordingly, more than 90% of the myosin pelleted from 0.1 M KCl during the high speed spin.

PREDICTION OF THE NUMBER OF MYOSIN FILAMENTS IN A THIN SECTION OF A PLATELET: Knowing that the concentration of myosin in a platelet is about 1.5 mg/ml (29), one can calculate how many filaments with structures like that shown in Fig. 10 might be found in a thin section through an intact platelet. A weight concentration of 1.5 mg/ml of platelet myosin is equivalent to 3.3×10^{-6} M, or 2.0×10^{18} molecules per liter. A sagittal thin section through a platelet, like that shown in Fig. 9 *a*, has a surface area of about 1.4×10^{-8} cm^2 and a thickness of about 60 nm, so that the volume of platelet cytoplasm within the section is about 8.3×10^{-17} liters. If the platelet myosin molecules are distributed homogeneously throughout the cytoplasm, then there should be about 170 whole myosin molecules in the section shown in Fig. 9 *a*. If all of the myosin molecules were assembled into filaments, there would be enough myosin within the section to form six complete myosin filaments.

DISCUSSION

Our observations on human platelet myosin filaments provide some new information about their formation and structure, which may be helpful in understanding the physiological function of myosin in platelets and nonmuscle cells.

First, under in vitro conditions designed to approximate physiological conditions, platelet myosin forms short, relatively thin bipolar filaments consisting of about 28 myosin molecules. The information for the assembly of these filaments is contained in the tail portion of the molecule, while their length and width are apparently determined by some interaction between heads and tails. Cytoplasmic myosins from other vertebrate cells also form these small filaments and therefore may share this length-regulating mechanism. In contrast, skeletal muscle myosin forms large synthetic filaments which vary considerably in length. The small size of the platelet myosin filaments may have certain advantages of a nonmuscle cell whose contractile apparatus is not rigidly ordered like that in striated muscle. For example, small myosin filaments would diffuse through the cytoplasm more freely than large filaments, facilitaing the transfer of the myosin from inactive regions to sites of force generation.

Second, platelet myosin filaments are considerably thinner than skeletal muscle myosin filaments and consequently must have a different internal structure. The dimensions of the platelet myosin filaments are consistent with a model where the heads of two myosin molecules project from the surface of the filaments at intervals of 14–15 nm, while it has been suggested that three or four myosins are found in each 14.3-nm interval in skeletal muscle thick filaments (21, 34, 38).

Third, the head portions of the platelet myosin molecule appear to be loosely attached to the filament backbone, judging from their disordered appearance in electron micrographs of negatively stained specimens. This apparent disorder of the heads could result from disorder within the filament core, but this seems unlikely because the myosin heads are highly ordered in the platelet myosin paracrystals (26). Therefore, we believe that there is a flexible connection between the platelet myosin head and the filament backbone, like that thought to exist in skeletal muscle thick filaments (18, 27).

Fourth, the bipolar structure of platelet myosin filaments is likely to have the same physiological

significance that it has for the thick filaments in striated muscle, namely that a platelet myosin filament can cross-link two oppositely polarized actin filaments to form a contractile unit which might reasonably use a sliding mechanism to develop contractile force, as suggested previously (12, 22, 26, 33). If this model for the contractile event is correct, the small size of the platelet myosin filament would be the factor limiting the number of cross-bridges formed between a myosin filament and actin filaments. Since the force developed by muscle is proportional to the number of cross-bridges (10), the size of the myosin filament might be a factor limiting contractile force in platelets and other cells. Since the forces required to move nonmuscle cells are very small (see reference 40 for an interesting calculation), these small myosin filaments appear to be a reasonable compromise between making myosin aggregates large enough to develop the required force, but reasonably small to maximize the number of contractile units that might be formed from the myosin available within one cell.

Fifth, our data on the distribution of platelet myosin molecules between monomers and filaments, the number of myosin molecules per filament, and the concentration of myosin within platelets allow one to consider quantitatively the problems in localizing myosin in thin sections of intact platelets and nonmuscle cells. From the calculations described above, we know that there are about 170 platelet myosin molecules in the volume of cytoplasm in the section shown in Figure 9 *a*. Because physiological ionic strength, pH, and myosin concentration strongly favor filament formation, and because variations in ionic conditions within the physiological range have a relatively small influence on the size of platelet myosin filaments, one can predict with some confidence that the 170 myosin molecules in the section should form five or six myosin filaments containing 28 myosin molecules each. Since it is impossible to identify with certainty any of these postulated filaments in the micrograph, it is necessary to consider explanations for this enigma. Two general possibilities exist: either the filaments are present and invisible or they are truly absent.

A number of factors may contribute to the apparent absence of the filaments. (*a*) Geometry: because the filaments are 320 nm long and the sections are about 60 nm thick, less than 5% of randomly oriented filaments will be contained completely within the section. Consequently, there is a low probability that any complete filaments will be seen in a random thin section; rather, we expect that there are pieces of 15–20 different myosin filaments in such a section. Most of these pieces will be cut obliquely or in cross section, so that most of the myosin filaments will appear as circular or somewhat elongated profiles, not as obvious filaments. (*b*) Size of the filaments: the platelet myosin filaments are much thinner than the thick filaments found in muscle, being only 10–11 nm wide. When the myosin filament is fixed, dehydrated, and embedded, the diameter of its shaft shrinks about 15% (Fig. 8 and reference 26), so the filaments expected to form under physiological conditions will be about 8–9 nm wide in thin sections. Paracrystals of paramyosin, another assembly of alpha-helical coiled-coils, also shrink laterally about 15–20% when dehydrated (9). Myosin filaments 8–9 nm wide are likely to be confused with actin filaments which appear 6–8 nm wide in thin sections. (*c*) Quantity of the filaments: the molar ratio of actin to myosin in platelets is roughly 100 to 1. If all of the actin were polymerized within the cell, there would be about 7 μm of actin filament for each myosin filament 0.3 μm long. Even if only a fraction of the actin is polymerized, most of the filaments in the 6–9 nm size range will be actin, making identification of the myosin difficult. (*d*) Cytoplasmic density: to make visualization of the myosin filaments even more difficult, the cytoplasm of platelets and other cells is frequently rather dense. This grey amorphous material might obscure the filaments if present. Together these factors are sufficient to account for difficulty in visualizing the myosin filaments in platelets. The filaments might also be destroyed during fixation, although synthetic myosin filaments and myosin filaments in platelet actomyosin are adequately preserved by standard fixatives.

Alternatively, the myosin in platelets may not be assembled into filaments. The apparent absence of filaments in thin sections suggests that some caution might be necessary in applying the results of in vitro assembly studies to the situation in intact cells. For example, the ionic conditions in the cytoplasm are unknown and the real conditions may not favor myosin filament assembly, or the cell may contain proteins or other factors which inhibit filament formation.

Behnke and his colleagues (5) investigated the puzzle regarding the form of myosin in platelets and found in glycerinated platelets some thin filaments 2–10 nm wide which did not bind heavy

meromyosin like actin fialments. Because these thin filaments sometimes appeared to cross-link actin filaments, they suspected that they might be myosin. Allera and Wohlfarth-Bottermann (4) and Schroeder (32) found similar presumptive myosin filaments in glycerinated *Physarum* and contractile rings of glycerinated HeLa cells although positive identification was not made. We believe that the problem requires new approaches such as the use of tracers coupled to antimyosin for electron microscopic localization of myosin.

We are grateful to S.M. Thomas for help in the preparation of the uterine myosin, to D. A. Goodenough for his patient help with the electron microscopes, to R. S. Adelstein for preparing the fibroblast myosin, to D. Brandon for the hepatocyte myosin, to T. P. Stossel for the granulocyte myosin, and to W. Stafford for assistance with some of the calculations.

This work was supported by United States Public Health Service Research Grant GM 19654 and Career Development Award GM 5 KO4GM 70755-02.

Received for publication 5 August 1974, and in revised form 29 April 1975.

APPENDIX

Derivation of a General Equation Relating Some of the Structural Parameters of Myosin Filaments

In this section, we derive a general equation which relates several structural parameters of myosin filaments to the size of the myosin molecule and the dimensions of negatively stained myosin filaments. The equation is particularly useful for calculating the number of myosin molecules with heads in each axial repeat from measurements of the length of the bare zone, the diameter of the filament in the center of the bare zone, and the length of the axial repeat. The number of myosin molecules per filament is then simply calculated from the number of myosins per axial repeat and the length of the filament.

DEFINITIONS: We have used, where possible, the nomenclature suggested by Squire (34) to describe the parameters of myosin filaments. The meanings of some of the terms which follow are illustrated in Fig. 11: L, length of the myosin tail; r, radius of the myosin tail; a, effective cross-sectional area occupied by each myosin tail in the core of the filament. (This includes the cross-sectional area occupied by the tail itself, plus the fraction of the intermolecular space between the tails which is attributable to each tail); l, length of the bare zone; c, length of the axial repeat of the filament; D, diameter of the filament in the center of the bare zone; A, cross-sectional area of the myosin filament in the center of the bare zone; H, cross-sectional area in the center of the bare zone occupied by non-myosin, core protein or a hole; n, number of myosin molecules with heads projecting in each axial repeat; N, number of myosin tails which cross the center of the bare zone; R, number of rows of myosin heads whose tails cross the bare-zone center. R is related to n and N such that $R = N/n$.

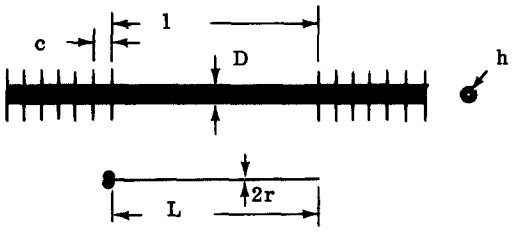


FIGURE 11 Line drawings illustrating the definitions used in the calculations in the Appendix.

DERIVATION: The cross-sectional area in the bare zone (A) is the sum of the area occupied by myosin tails and the area (H) occupied by hollow space or nonmyosin core protein. Since the area occupied by myosin tails is the product of the number of tails crossing the center (N) and the effective cross-sectional area of one myosin tail (a),

$$A = N a + H. \tag{1}$$

Substituting for N we get

$$A = Rna + H. \tag{2}$$

Solving for n, we obtain the general relationship:

$$n = \frac{A - H}{Ra} \tag{3}$$

To use this general equation, it is necessary to express the quantities A, R, and a in terms of dimensions which can be measured from electron micrographs or X-ray diffraction patterns: D, l, and c.

The term A is related to D by the equation

$$A = \pi(D/2)^2. \tag{4}$$

The term R (the number of rows of myosin heads whose tails cross the center of the bare zone) is related to the length of the bare zone (l), the length of the myosin tail (L), and the axial repeat of the filament (c) by the equation

$$\frac{N}{n} = R = \frac{2L - l}{c} + l \tag{5}$$

This equation is derived from the geometry of the bare zone as follows.

The length of the antiparallel overlap of the ends of the myosin tails bordering the bare zone is equal to $2L - l$ as

illustrated in Fig. 12. The maximum number of myosin tails crossing the center line when $n = 1$ is simply the number of axial repeats into which this maximum overlap can be divided, plus one for the myosin in the zero position.

Another way of looking at this relationship is shown in Fig. 13. Starting with the myosins bordering the bare zone, additional myosins are added at each end with one myosin at each axial repeat (in this example, n = 1). When the distance between the heads of symmetrically placed antiparallel myosins exceeds 2 L, the tails of these myosins will not overlap each other and will not contribute to the cross-sectional area of the bare zone. From the drawing, it can be seen that the number of myosins whose tails cross the center of the bare zone is determined by the length of that portion of the filament containing the heads of these myosins (that is 2 $L - l$) divided by the axial repeat, c, plus one for the myosin at the zero position (Equation 5). (The additional one arises from the fact that dividing the length 2 $L - l$ by the axial repeat gives the number of *intervals* into which that length can be divided, while there is a myosin head placed at each *interval boundary* and there is one more interval boundary than intervals.)

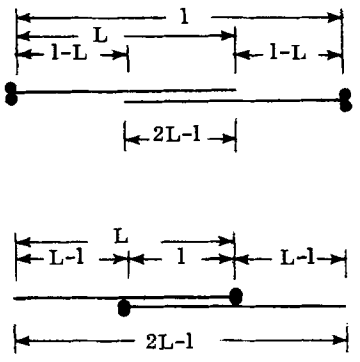


FIGURE 12 Line drawings illustrating the origin of the term $2L-l$ in Equation 5.

From this derivation of R, it is clear that Equation 5 is true only when the myosin filament is long enough that the number of axial repeats at each end of the filament is greater than or equal to $R/2$. In all cases examined, including the very short myosin filaments described in this report, the filament length is long enough for Equation 5 to be applicable.

The value of the term a, the effective cross-sectional area occupied by each myosin tail, will depend on the packing arrangement of the myosin tails in the core of the filament and the dimension of the tails. It is known that the usual center to center distance of hydrated assemblies of alpha-helical coiled-coils like myosin is 2.0 nm, so the radius of the myosin tail (r) is 1.0. If the

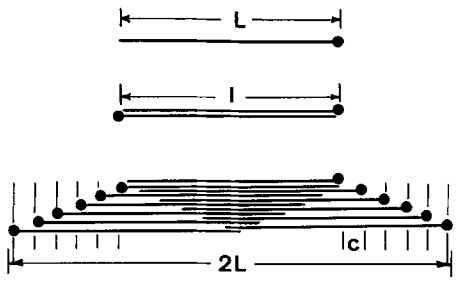


FIGURE 13 Line drawings illustrating myosin tail overlaps in the bare zone.

myosin tails are packed so tightly that there is no intermolecular space,

$$a = \pi r^2 = 3.14 \text{ nm}^2. \quad (6)$$

This is unlikely, but it represents the lower limit of the value for a. If the tails are packed in a hexagonal lattice[5]

$$a = (r)(r \tan 30)(6) = 3.44 r^2 = 3.44 \text{ nm}^2. \quad (7)$$

If the tails are packed in a square lattice

$$a = (2r)^2 = 4.0 \text{ nm}^2. \quad (8)$$

Thus, one can measure or make reasonable estimates for all of the dimensions necessary to calculate n, except for the value of H, the area occupied by nonmyosin core proteins or hollow space. In the case of the thin filaments formed from pure platelet myosin in this study, it is reasonable to assume that H is zero, at least for a first approximation. For thicker myosin filaments, like native skeletal muscle thick filaments, some estimate of H from chemical analysis or microscopical observation is necessary. If one has an independent value for n (20, 34), Equation 3 can be used to calculate H from dimensions measured in the electron microscope.

SAMPLE CALCULATION: Using the data in Table III and the value for c measured from paracrystals (22), we will calculate n for platelet myosin filaments formed in 0.1 M KCl, 20 mM imidazole pH 7, 2 mM $MgCl_2$, 1 mM ATP, and 0.1 mM EGTA. We will assume that H is zero, and that the myosin tails are 160 nm long and packed in a hexagonal array in the core of the filaments.

$$A = \pi(10.5 \text{ nm}/2)^2 = 86.6 \text{ nm}^2,$$

$$H = 0,$$

$$R = \frac{2(160 \text{ nm}) - 150 \text{ nm}}{14.5 \text{ nm}} + 1 = 12.3,$$

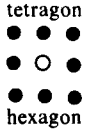
$$a = 3.44(1 \text{ nm})^2 = 3.44 \text{ nm}^2,$$

$$n = \frac{86.6 \text{ nm}^2}{(12.3)(3.44 \text{ nm}^2)} = 2.04 \text{ myosins per axial repeat.}$$

[5] The equation $a = (r)(r \tan 30)(6)$ is the area of a hexagon surrounding a circle of radius r.

TABLE V

Influence of Assumptions and Measurements on the Calculated Value of n

Filament diameter, D	Filament area, A^*	Hole area, H^*	Monomer packing	Effective area of tail, a^*	$\frac{R}{2L\text{-}l+}{c}$	$\frac{n}{\frac{A\text{-}H}{R\,a}}$
nm	nm^2	nm^2		nm^2		
I‡						
10.5	86.6	assume no hole	hexagonal 	3.44	12.3	2.0
II§						
10.5	86.6	no hole	total	3.14	12.3	2.2
10.5	86.6	no hole	tetragon 	4.0	12.3	1.8
10.5	86.6	9 (10% of 86.6)	hexagon	3.44	12.3	1.8
III‖						
7.5	44.2	no hole	hexagon	3.44	12.3	1
10.5	86.6	no hole	hexagon	3.44	12.3	2
13.5	143.1	no hole	hexagon	3.44	12.3	3

* Cross-sectional.

‡ (I) Data and assumptions used to determine actual value for n in platelet myosin filaments.

§ (II) Influence of varying *assumptions* on the calculated value for n. If there is total packing of the myosin monomers (i.e.: so that there are no intermolecular spaces), the effective cross-sectional area for each tail will decrease to 3.14 nm^2 and n will increase to 2.2. If the monomers are packed in a tetragonal array, the effective cross-sectional area for each tail will increase to 4.0 nm^2 (the area of a square surrounding a circle with a radius of 1 nm). If the filament contains a hole or central protein core which assumes 10% of the total cross-sectional area (34), n will decrease to 1.8.

‖ (III) Calculated values for n if D is 3 nm greater than or less than the actual measured diameter.

DISCUSSION: The calculated value for n will be influenced by errors in the assumptions and measurements used in the calculation.

In the calculations leading to the model proposed in this paper, we made two assumptions: (*a*) the hexagonal packing arrangement of the myosin tails within the filament back bone; and (*b*) the absence of a central hole or core protein. The influence of varying these assumptions is indicated in Table V. Extremes of packing indicate that n is not sensitive (in this model) to significant changes in packing or the presence or absence of a small hole.

We feel confident in the accuracy of our measurements because of the limited variability in the size of the filaments that we measure (D = 10.5 with a standard deviation of ± 1.4 nm). Therefore, a filament diameter large enough or small enough to change n by one integer is greater than two standard deviations from the diameter which we measure (Table V). In other words, there is less than a 3% chance that the filament has a value of n of 1 or 3. Consequently, we are reasonably confident that n = 2 for the platelet myosin filaments, but feel that some caution will be necessary in applying this method of analysis to thicker myosin filaments. For example, if n is large (>3), small errors in the measured D, the assumed H, or the assumed tail packing can change n by a full integer or more.

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