ELECTRON MICROSCOPIC INVESTIGATIONS OF ACTOMYOSIN AS A FUNCTION OF IONIC STRENGTH

N. IKEMOTO, S. KITAGAWA, A. NAKAMURA, and J. GERGELY

From the Department of Muscle Research, Institute of Biological and Medical Sciences, Retina Foundation, and the Kennedy Memorial Laboratories, Massachusetts General Hospital, and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02114. Dr. Ikemoto's present address is the Department of Biology, Faculty of Science, Okayama University, Okayama, Japan. Dr. Kitagawa's present address is the Research Institute of Catalysis, Hokkaido University, Sapporo, Japan

ABSTRACT

Natural actomyosin at $\mu = 0.6$ appears in various forms, including the regular arrowhead structures originally reported by Huxley (1), when it has been stained negatively with 1% uranyl acetate. In addition to the arrowheads, thin whiskers, 700–1200 A in length and 20 A in width, attached to the arm of the arrowheads have been demonstrated. The dimensions of the whiskers and arms of the arrowheads are practically the same as those of the light meromyosin (LMM) and the heavy meromyosin (HMM) moieties of the single myosin molecule, respectively. Changes in the electron microscopically distinguishable elements during aggregation of natural actomyosin on reduction of the ionic strength have been observed. At $\mu = 0.4$, partial aggregation of the LMM whiskers begins to result in some parallel alignment of the arrowhead-bearing filaments (acto-HMM). In the range of $\mu = 0.3$–0.1, the LMM whiskers merge into smooth filaments which are arranged alternatingly with arrowhead-bearing filaments. Thus, lateral aggregation of composite actomyosin filaments (acto-HMM + LMM whiskers) results with the LMM moieties as links. This view is supported by the following facts: (*a*) acto-HMM is devoid of whiskers and does not show lateral aggregation at $\mu = 0.1$; (*b*) natural actomyosin digested with trypsin at $\mu = 0.6$, which was followed by removal of LMM aggregates at low ionic strength, is essentially the same as acto-HMM at $\mu = 0.1$; and (*c*) digestion with trypsin of natural actomyosin at $\mu = 0.2$ for varying periods of time leads to a separation of arrowhead-bearing filaments from LMM aggregates.

INTRODUCTION

H. E. Huxley's elegant electron microscopic demonstration of periodically repeated structures on actomyosin and acto-HMM filaments (the so-called arrowhead structures) opened up new possibilities in the study of the interaction of actin and myosin (1). Our previous electron microscopic studies on the interaction of actin and myosin (2) on specimens prepared from low ionic strength solution ($\mu = 0.1$) have demonstrated three major structural patterns, depending upon the concentrations of ATP and ionic milieu, which seemed to correspond to three states of

the intact system, rigor, relaxation, and contraction.

Under the conditions of rigor, viz. in the absence of ATP at $\mu = 0.1$, actin and myosin are combined into composite actomyosin filaments which are further aggregated into larger bundles, whereas at higher ionic strengths the majority of the arrowhead-bearing filaments are separated from each other.

This paper deals with an electron microscopic investigation of actomyosin, natural and reconstituted, in various ionic milieus. At $\mu = 0.6$ several arrowhead patterns appear, including the typical one described by Huxley (1). One of these is characterized by thin whiskers attributable to the LMM portion of a myosin extending from arrowheads. At lower ionic strength a new type of filament appears, owing to the aggregation of the whiskers. Additional information regarding the formation of the new type of filament has been obtained by studies on trypsin-treated preparations.

MATERIALS AND METHODS

Natural actomyosin was prepared by extracting ground muscle with Weber-Edsall solution for 24 hr and was purified by repeated precipitation and redissolution (3). Myosin was extracted and purified as described previously (4). H-meromyosin (HMM) was prepared by digestion of myosin (10 mg/ml) with trypsin (1:200 wt. ratio) in 0.6 M KCl, 50 mM Tris, pH 7.5, at 25°; the digestion was stopped after 10 min by a twofold excess of soybean trypsin inhibitor. After dialysis against 25 mM phosphate, pH 6.5, and removal of light meromyosin and undigested myosin by centrifugation for 1 hr at 20,000 *g*, HMM was precipitated with ammonium sulfate between 45 and 60% saturation at 0°. A final dialysis against 0.1 M KCl and 25 mM Tris, pH 7.5, followed. Actin was extracted at 0°C from acetone-dried muscle residue and was purified by repeated cycles of polymerization, centrifugation, and depolymerization (5). Reconstituted actomyosin was prepared by mixing myosin and actin in a 4:1 weight ratio, usually at $\mu = 0.6$.

The ionic strength of the suspension of the natural and reconstituted actomyosins was subsequently reduced to appropriate KCl concentrations by dilution with the medium containing 10 mM histidine (pH 7.0). Protein concentration was in a range of 0.3–1 mg/ml.

Negative staining was carried out on collodion-carbon-filmed specimen grids, with or without perforation, with the use of 1% uranyl acetate according to Huxley and Zubay (6) with slight modifications; a drop of water was placed on the grid and blotted prior to the addition of an actomyosin suspension, and the negative stain was applied after the suspension had been placed on the grid without having been washed with the solvent.

A Philips 200 electron microscope was employed with double condenser illumination and the use of 300 μ Pt condenser aperture, 20 or 50 μ objective aperture, 60 kv accelerating voltage, and an emission current of 36 μA.

Trypsin (2 × crystallized) and soybean trypsin inhibitor were purchased from the Worthington Biochemical Corp., Freehold, N J.

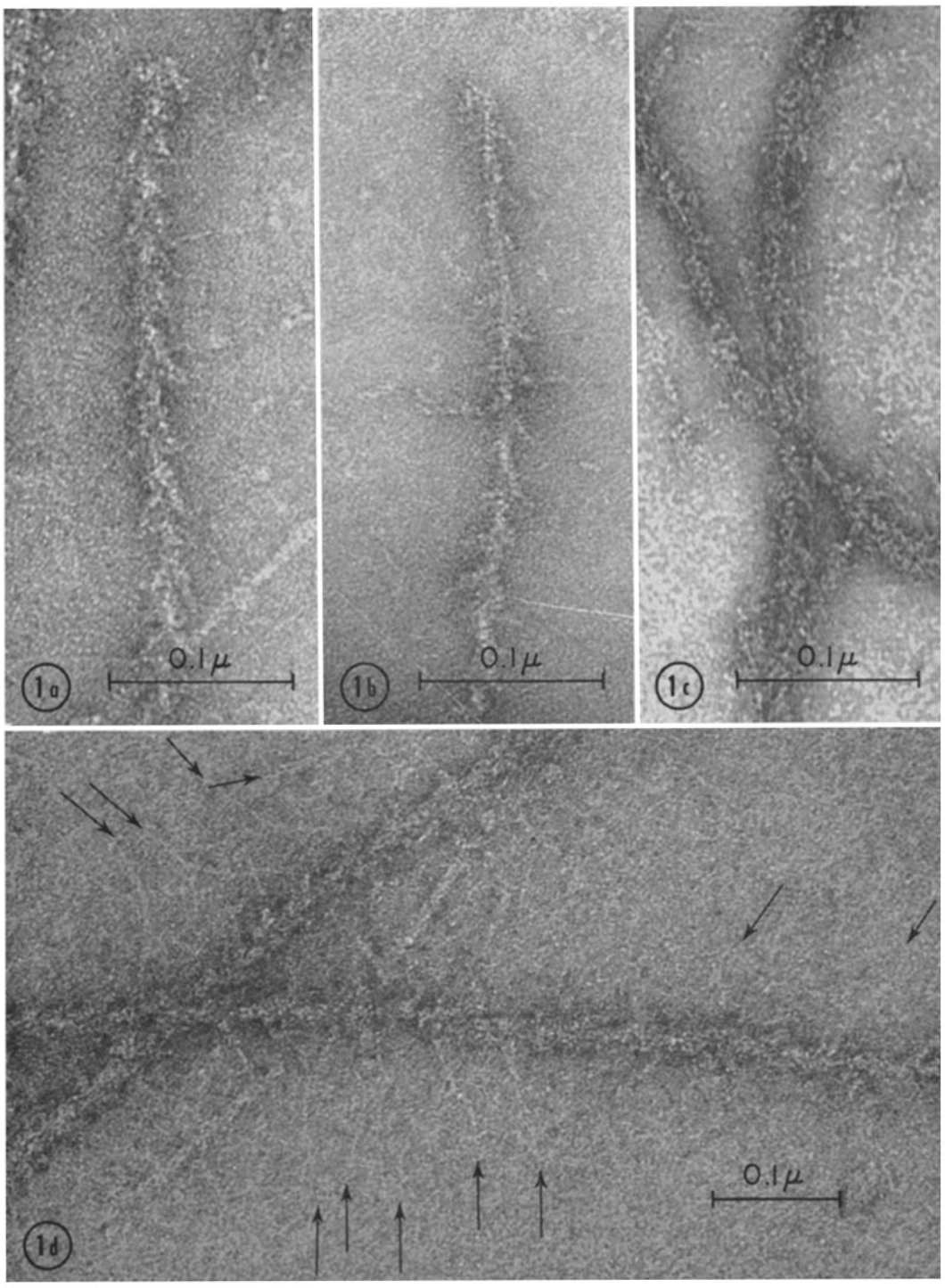
RESULTS

Natural Actomyosin at $\mu = 0.6$

Natural actomyosin appears in the form of filaments whose lengths are distributed about 1 μ and vary in a range of 0.3–2 μ. Numerous similar structures, Huxley's arrowheads (1), are seen on the filaments.

The arrowheads appear in various forms (Figs. 1 *b* and *c*) in addition to the typical one described originally by Huxley (Fig. 1 *a*) in which sets of paired "arms," 30–60 A and 100–300 A in width and length, respectively, are attached to the axis of the composite filaments at a constant oblique angle (30°–40°) and at roughly constant intervals of approximately 360 A. An equally or even more frequent appearance is that shown in Fig. 1 *b* where the regularity of the attachment of the arms is seen only on one side of the axis of the filament; on the other side shorter arms appear attached in an irregular fashion at intervals varying from 120 to 250 A and at a larger angle to the axis (60°-80°). There are instances when the irregularity in the attachment of the arms is seen on both sides of a filament, although the over-all thickness of the filament (320–360 A) is essentially the same as that of those in Figs. 1 *a* and *b*.

In some actomyosin filaments, as indicated in Fig. 1 *d*, a thin whisker originates at the end of the arms of the arrowheads and extends over a considerable distance. Such thin and long "whiskers" are particularly well demonstrable when the actomyosin filaments are treated with 25% glycerol prior to negative staining (Fig. 1 *d*). If several (~7) whiskers, together with corresponding arms, appear in a regular fashion (Fig. 1 *d*), they show the periodicity characteristic of the arrowheads (360 A). Sometimes the whiskers appear on both sides of the axis; in other instances,



FIGURES 1 *a–d* Various appearances of arrowhead structures of natural actomyosin at $\mu = 0.6$. Fig. 1 *a*, Arms of the arrowheads appear in a regular fashion on both sides of the filament (typical arrowhead pattern). Fig. 1 *b*, Arms appear regularly only on the right side of the filament. Fig. 1 *c*, Arrangement of arms is irregular on both sides. Fig. 1 *d*, Appearance of thin whiskers (arrows) extending from the arms of the arrowheads. Conditions: 0.6 M KCl, 10 mM histidine, pH 7.0. Scales show 0.1 μ. Figs. 1 *a–c*, × 273,000; Fig. 1 *d*, × 188,000.

they are seen only on one side (see Fig. 1 *d*). On some occasions, the unilateral location shifts from one side to the other side of the axis along the same actomyosin filament.

The whiskers measure approximately 20 A in width and 700–1200 A in length (from the attachment to the arm to the tip of the whisker). These dimensions correspond roughly to those of light meromyosin, and the whisker plus the arm would correspond to a single myosin molecule.

In preparations of natural actomyosin the predominant orientation of the whiskers is in a roughly perpendicular direction to the axis of the actomyosin filament (Fig. 1 *d*). Since, as mentioned above, the arms of the arrowheads are oriented obliquely, a bending takes place at the junction of the arm and the whiskers.

Actomyosin filaments reconstituted at $\mu = 0.6$ from purified actin and myosin are significantly longer than those of natural actomyosin. At the same time, the former are more irregular in their course (Fig. 2) than the typical arrowhead structures which are fewer and less clearly discernible (inset to Fig. 2). The roughly parallel orientation along the filament axis of the thin whiskers that results in their tangling and which is rather unusual in the case of natural actomyosin (see Fig. 1 *c*), is more common with reconstituted actomyosin.

Aggregation of Actomyosin Filaments at Lower Ionic Strengths

When the ionic strength of a suspension of actomyosin is lowered from $\mu = 0.6$ to $\mu = 0.4$, partial aggregation of the whiskers occurs, and a new type of thin filament appears between two parallel arrowhead-bearing filaments (Fig. 3 *a*).

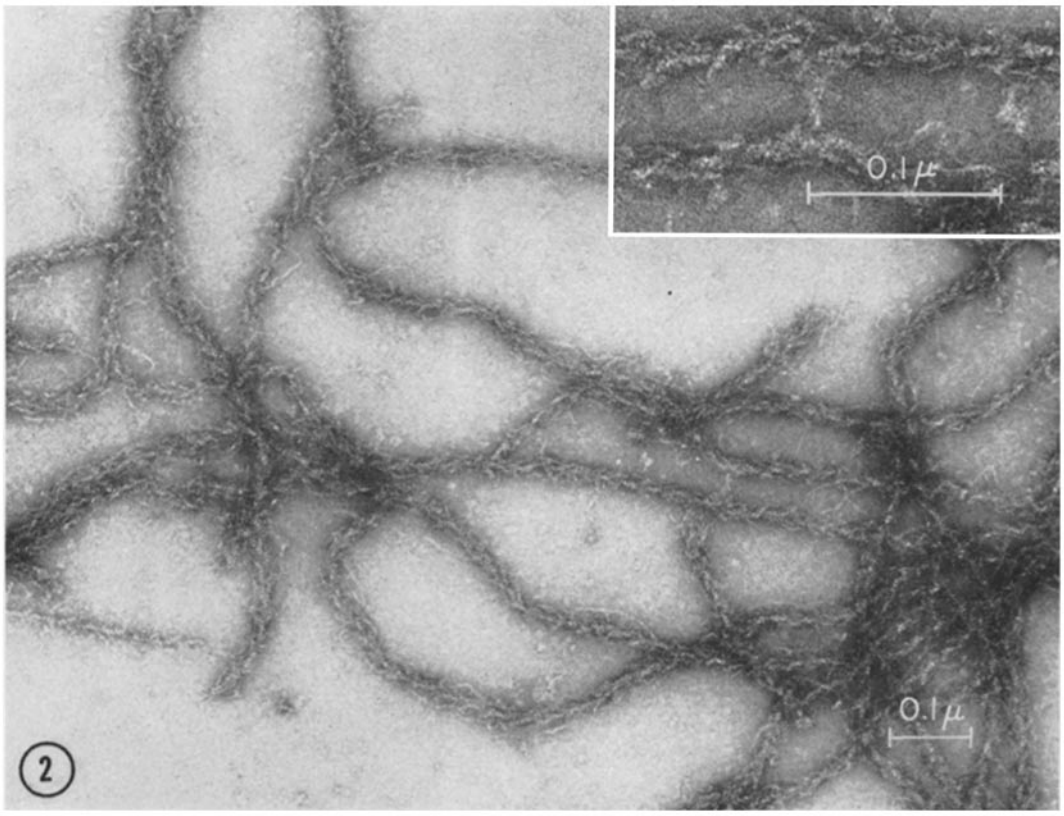
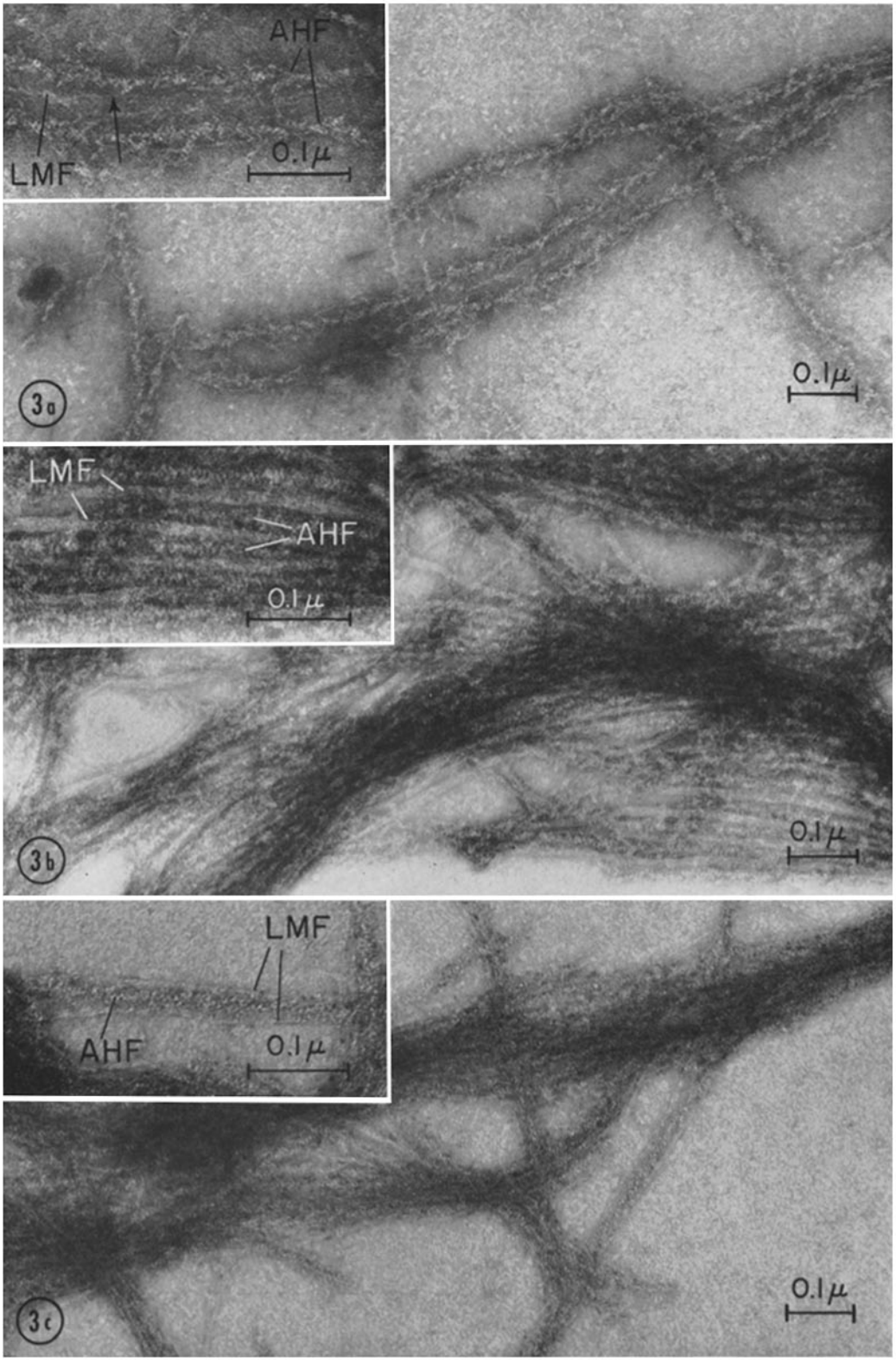


FIGURE 2 Reconstituted actomyosin at $\mu = 0.6$. Myosin and actin were mixed in a 4:1 weight ratio. Conditions: 0.6 M KCl and 10 mM histidine, pH 7.0; protein, 1 mg/ml. Scales indicate 0.1 μ. × 105,500; inset, × 252,000.



FIGURES 3 *a–c* Formation of natural actomyosin aggregates on lowering the ionic strength. Fig. 3 *a*, Appearance of a new type of filament (*LMF*) between two arrowhead-bearing filaments (*AHF*), resulting in the lateral aggregation of the latter filaments (μ = 0.4). Arrow in inset points to whisker merging into LMF. Fig. 3 *b*, More extensive appearance of the new type of filament (*LMF*) and of parallel arrangement of arrowhead-bearing filaments (*AHF*) (μ = 0.2); note the alternating arrangement of the two kinds of filaments. Fig. 3 *c*, Actomyosin aggregates at μ = 0.1. Scales indicate 0.1 μ. Figs. 3 *a–c*, × 100,000; insets, × 150,000.

Whiskers starting on the arrowheads can be traced to these thin filaments (LMF in Fig. 3 *a*, inset) into which they merge. At even lower ionic strengths, below 0.3, arrowhead-bearing filaments (AHF in Fig. 3) aggregate into large bundles, and the new type of filament resulting from the merging of the whiskers is more clearly discernible. Within the aggregate (Figs. 3 *b* and *c*), two kinds of filaments (AHF and LMF) are arranged alternatingly in parallel fashion; one still shows the presence of arrowheads, although not so clearly as at $\mu = 0.6$, whereas the other shows a smooth surface without periodic features.

The detailed relationship between the two kinds of filaments is particularly clear where a thin bundle branches out from the thicker bundle (inset in Fig. 3 *c*). A filament (AHF) still carrying a few arrowheads is running between two parallel smooth filaments (LMF) that are 300–350 A apart. This dimension is comparable to the width of the actomyosin filaments with arrowheads at $\mu = 0.6$ (see Fig. 1).

Digestion of Actomyosin with Trypsin

Digestion of natural actomyosin aggregates with trypsid at 100:1 actomyosin-trypsin ratio by weight gives further insight into the genesis of the new type of filament described above. After a 2 min digestion in 0.2 M KCl (Fig. 4 *a*), the arrowhead pattern of one type (AHF) of filament becomes clearer, and it appears that these filaments are flanked by filaments of the other type (LMF); it is clear that the ends of the arms of the arrowheads merge into the latter at several places. These filaments are usually spindle-shaped, vary in thickness, and show a very close resemblance to LMM aggregates (1). After longer digestion (20 min) (Fig. 4 *b*), filaments that bear arrowheads become completely separated from the spindle-shaped aggregates which, at this stage, have grown to a larger size and often form an angle with the former.

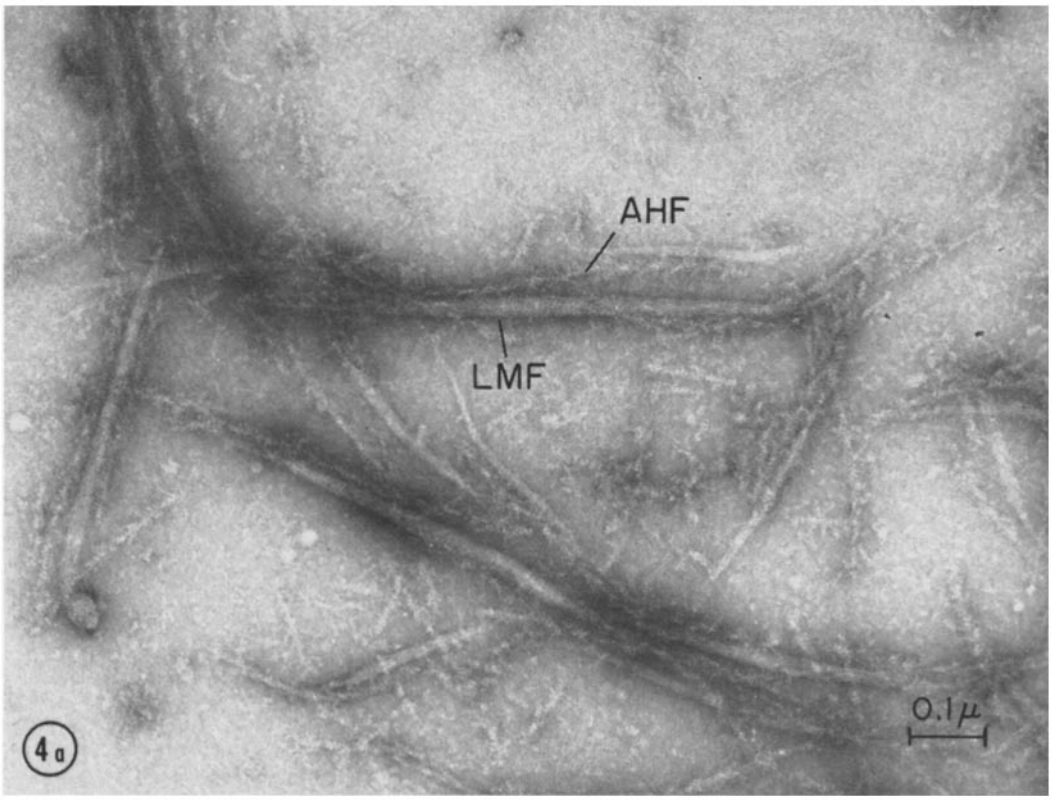
The whiskers found in natural and reconstituted actomyosin (see Figs. 1 and 2) cannot be seen with acto-heavy meromyosin filaments (1:2 actin-HMM ratio by weight, $\mu = 0.1$) (Fig. 5 *a*) and no spindle-shaped structures occur. Digestion of natural actomyosin at 0.6 M KCl (pH = 7.0, 22°C, and 1:100 trypsin-actomyosin ratio), followed by removal of LMM aggregates by centrifugation (20,000 *g*, 60 min) at 0.05 M KCl, leads to an electron microscopic pattern similar to that seen with acto-HMM (Fig. 5 *b*) with only a few remaining whiskers. An arrowhead pattern can be clearly seen, but there is no lateral aggregation of the filaments.

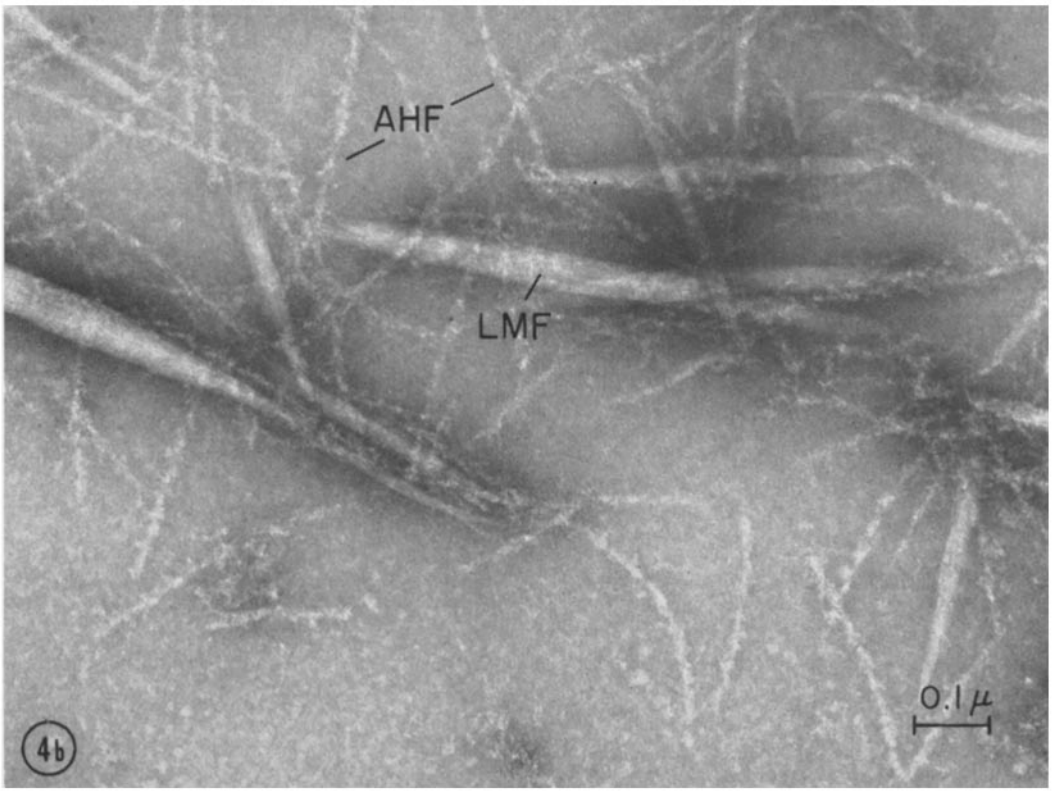
DISCUSSION

Reducing the ionic strength from $\mu = 0.6$ to $\mu = 0.1$ permits the tracing of the formation of actomyosin aggregates from the filaments characterized by the presence of arrowheads seen in negatively stained preparations.

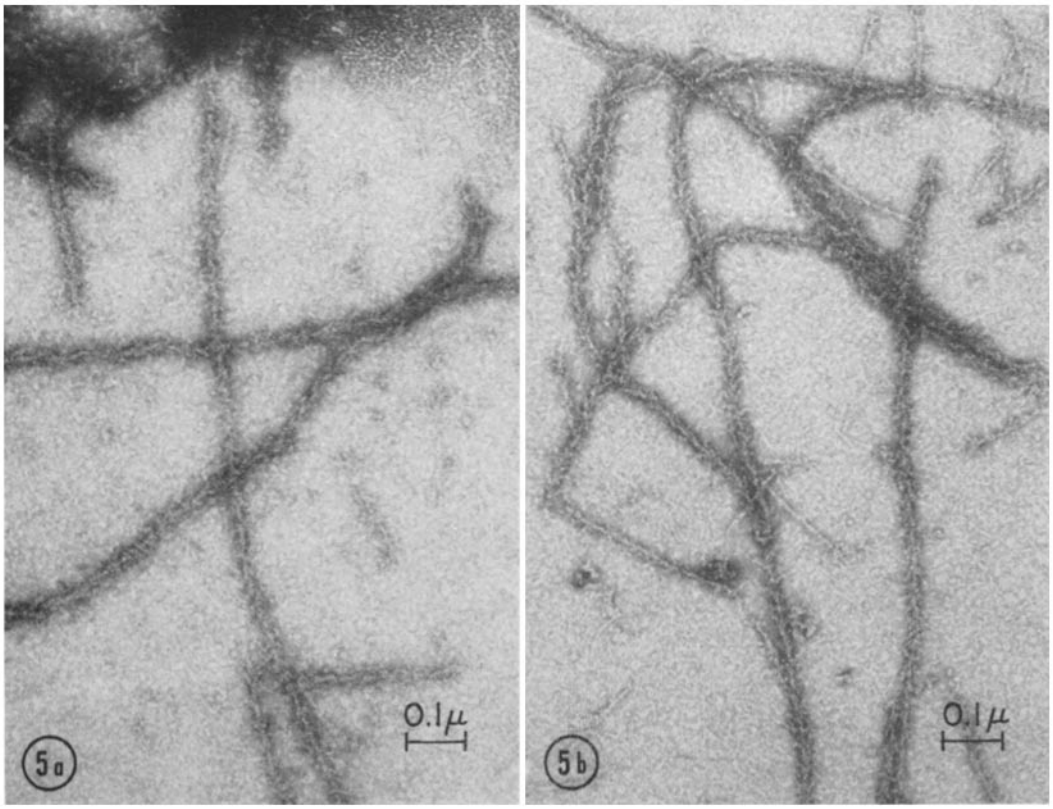
It seems that the key elements in the formation of actomyosin patterns in the absence of ATP (2) are the thin whiskers described above that extend from the arrowheads. These thin whiskers, often 700–1200 A long and 20 A in diameter, are well demonstrable after pretreatment of actomyosin with 25% glycerol, particularly in the case of natural actomyosin. Since the arrowhead structures are attributable to the HMM part of myosin attached to F-actin (as seen in Fig. 5), it seems reasonable to consider the whisker as corresponding to the LMM part of the myosin molecule. It may well be that the helical part of HMM (7) is also included in the proximal part of the whisker. The maximum over-all length of the attachments to the actin filament, taken as the sum of the thin distal whisker and the thicker proximal arm portion, comes rather close to the generally accepted length of the individual myosin molecules, viz. 1600 A. The diameters of both portions, viz. that of the arms of the arrowheads (30–60 A) and that of the whiskers (20 A), are in the range of that of HMM (30–50 A) and LMM (15–20 A) (1, 8–12), respectively.

As the ionic strength is decreased to 0.4, lateral aggregation of the whiskers, belonging to two different actomyosin filaments, takes place which initiates the parallel aggregation of the two filaments. As the ionic strength is further lowered, the aggregation of the whiskers is more extensive, resulting in the emergence of a new type of filament and in the lateral aggregation of arrowhead-bearing filaments. Within these aggregates, the two kinds of filaments are arranged alternating in a parallel fashion. The wider ones still show the arrowhead patterns; the thinner smooth ones result from the merging of the whiskers. Thus, in view of the above identification of the whiskers with the light meromyosin portion of myosin, the smooth filaments visible in negatively stained actomyosin preparations at lower ionic strengths





FIGURES 4 *a* and *b* Natural actomyosin digested with trypsin. Weight ratio of trypsin to actomyosin 1:100, 0.2 M KCl, pH 7.0. Fig. 4 *a*, After a 2-min digestion the two types of filaments (one bearing arrowheads and the other smooth) show partial separation. Fig. 4 *b*, After digestion for 20 min the arrowhead-bearing filaments are almost totally separated from the other type of filament which shows a tendency to form bigger, spindle-shaped aggregates (*LMF*). *AHF*, arrowhead-bearing filaments. Scales indicated 0.1 μ. × 100,000.



FIGURES 5 *a* and *b* Lack of smooth filaments in acto-HMM and trypsin-treated natural actomyosin following removal of LMM. Fig. 5 *a*, Acto-HMM: F-actin mixed with HMM in a 1:2 weight ratio at $\mu = 0.1$. Note the arrowhead filaments devoid of whiskers and the absence of lateral aggregation of filaments. Fig. 5 *b*, Natural actomyosin: treated with trypsin in a 1:100 weight ratio, 0.6 M KCl, pH 7.0, followed by removal of LMM aggregates after dilution to 0.05 M KCl by centrifugation. Note essentially the same features as with acto-HMM. Scales indicate 0.1 μ. × 79,500.

($\mu = 0.1$–0.3) are essentially aggregates of the light meromyosin (LMM) moiety.

The identification of the various electron microscopically distinct elements in actomyosin aggregates with parts of the myosin molecule is further supported by observations on actomyosin following trypsin treatment or on reconstituted acto-HMM preparations.

The smooth filaments are never seen in acto-HMM or in actomyosin digested extensively with trypsin at $\mu = 0.6$ followed by removal of LMM at $\mu = 0.05$. On the other hand, after brief treatment of actomyosin by trypsin at low ionic strength, acto-HMM filaments still attached to filamentous aggregates of LMM are clearly seen.[1] Longer digestion leads to rather characteristic LMM aggregates separated from, and often forming an angle with, the arrowhead-bearing filaments.

The results with briefly digested actomyosin are of special interest in the light of its use for assaying the EGTA[2]-sensitizing factor (13). Fig. 4 shows the results of digestion carried out at $\mu = 0.2$, but a similar picture was obtained following digestion at $\mu = 0.1$ for 20–30 min, the conditions used for

[1] Dr. R. V. Rice has called our attention to the possibility that the rungs in the ladder-like structure described by Rice, Asai, and Morales (1963. *Proc. Natl. Acad. Sci. U.S.* **50**:549) may primarily consist of aggregates of the LMM portions of myosin molecules pulled out by the stretching process, the connections to the actin rails being formed through a few HMM regions.

[2] EGTA = ethylene glycol-bis-(β-aminoethyl-ether)N,N′-tetraacetic acid.

obtaining EGTA-insensitive actomyosin. In view of the changes occurring (even on mild tryptic digestion) in the myosin moiety of actomyosin, it may be an oversimplification to regard the effect of trypsin treatment as mainly resulting in the removal of the EGTA-sensitizing factor. Thus the similarities between the trypsin-treated actomyosin and the system reconstituted from purified actin and natural myosin, and the recently described desensitized natural actomyosin (14), may in some respect be fortuitous.

The present study permits some comment on the structure of the composite actomyosin filaments. It is apparent from Huxley's work and is also clear from our pictures that the arms of the arrowheads obliquely overlap with the actin filament over a distance of about 150 A. If each arm of an arrowhead corresponds to an HMM, it appears that an HMM attaches to a G (globular)-unit in one of the two strands making up the actin double helix and makes contact with one or more G-units in the other strand. The recent identification of the actin double helix as a right-handed one (15) made us look for the expression of the sense of rotation in the attachment of the myosin molecules. Indeed, in several cases (see Fig. 1) it appears that within the same arrowhead-bearing filament one arm passes under, the other over, the actin filament. If a strict sense of rotation persisted for the attachment, all arms on one side of the filament would have to be in the same relation to it. However, several instances occur in which some arms pass over the actin filament, others under it, on the same side of the filament.

Huxley has argued, from the fact that the period of the arrowheads (350 A) roughly corresponds to the periodicity of the actin helix, that there must be a 1:1 particle ratio between myosin and G-actin units (1). In view of the uncertainty in the helix period of F-actin, as seen in negatively stained preparations (16, 17), this argument may be open to question. The relation of the HMM moiety to the actin units discussed above also renders a 1:1 molecular ratio (1) between myosin or HMM and G-actin units somewhat difficult to conceive, particularly if the published dimensions for the head of the myosin molecule, 200 A (18), or for the HMM-S1 particle, 100 A (18, 19), are taken into account. It should be noted that the discrepancy between dimensions obtained on shadow-cast and negatively stained preparations remains to be resolved. Although a 1:1 molar ratio for the combination of actin and HMM in solution has recently been reported (20), a 1:2 myosin-actin molar ratio, corresponding more closely to the weight ratio for the combination of myosin and actin, as determined from enzymatic studies (21), would appear more feasible for steric reasons.

These studies show that in actomyosin aggregates the relation of actin and myosin in some aspects resembles that obtained *in situ* in the sarcomere. The formation of LMM aggregates with HMM portions joined to actin filaments is reminiscent of the postulated linkage of the "cross-bridges" on the thick filaments to the actin filaments (22). Huxley has demonstrated polarity within actin filaments and the central symmetry in the myosin filament *in situ*. It seems that a different symmetry relation obtains in actomyosin aggregates. Since in this case the myosin moiety of the aggregates appears to be organized on long actin filaments which have the same polarity along a given filament, it would appear reasonable to assume that the myosin moiety of the aggregates does not change its polarity within the same aggregate as it does in the muscle or in myosin aggregated in the absence of actin. The relation of ATP-induced superprecipitation of actomyosin to the contraction of myofibrils has been a baffling one. These studies, although not having solved the problem, may shed some new light on it by the demonstration of some common features between the two systems.

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BIBLIOGRAPHY

1. HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**:281.
2. IKEMOTO, N., S. KITAGAWA, and J. GERGELY. 1966. Electron microscopic investigation of the interaction of actin and myosin. *Biochem. Z.* **345**:410.
3. SZENT-GYÖRGYI, A. 1951. Chemistry of Muscular Contraction. Academic Press Inc., New York. 2nd Edition.
4. GERGELY, J., A. MARTONOSI, and M. GOUVEA. 1959. The Role of SH groups in the interaction of myosin with phosphate compounds and with actin. *In* Sulfur in Proteins. R. Benesch et al., editors. Academic Press Inc., New York. 297.
5. DRABIKOWSKI, W., and J. GERGELY. 1962. The effect of temperature of extraction on the tropomyosin content in actin. *J. Biol. Chem.* **237**:3412.
6. HUXLEY, H. E., and G. ZUBAY. 1960. Electron microscope observations on the structure of microsomal particles from *Escherichia coli*. *J. Mol. Biol.* **2**:10.
7. LOWEY, S. 1964. Myosin substructure: isolation of a helical subunit from heavy meromyosin. *Science.* **145**:597.
8. RICE, R. V. 1961. Conformation of individual macromolecular particles from myosin solutions. *Biochim. Biophys. Acta.* **52**:602.
9. RICE, R. V. 1964. Electron microscopy of macromolecules from myosin solutions. *In* Biochemistry of Muscle Contraction. J. Gergely, editor. Little, Brown and Company, Boston.
10. ZOBEL, C. R., and F. D. CARLSON. 1963. An electron microscopic investigation of myosin and some of its aggregates. *J. Mol. Biol.* **7**:78.
11. ZOBEL, C. R. 1967. An electron microscopic investigation of heavy meromyosin. *Biochim. Biophys. Acta.* **140**:222.
12. CARNEY, J. A., and A. L. BROWN. 1966. An electron microscope study of canine cardiac myosin and some of its aggregates. *J. Cell Biol.* **28**: 375.
13. EBASHI, S., and F. EBASHI. 1964. A new protein component participating in the superprecipitation of myosin B. *J. Biochem. (Tokyo).* **55**:604.
14. PERRY, S. V., V. DAVIES, and D. HAYTER. 1966. "Natural" tropomyosin and the factor sensitizing actomyosin adenosine triphosphatase to ethyl enedioxybis (ethylene amino) tetraacetic acid. *Biochem. J.* **99**:1c.
15. DEPUE, R. H., and R. V. RICE. 1965. F-actin is a right handed helix. *J. Mol. Biol.* **12**:302.
16. HANSON, J. 1967. Axial period of actin filaments: Electron microscope studies. *Nature.* **213**:353.
17. HANSON, J. 1967. The structure of actin filaments. *Proc. Intern. Congr. Biochem.* 7th, Tokyo, 1966. **2**:327 (Abstr.)
18. SLAYTER, H. S., and S. LOWEY. 1967. Substructure of the myosin molecule as visualized by electron microscopy. *Proc. Natl. Acad. Sci. U.S.* **58**:1611.
19. RICE, R. V., A. C. BRADY, R. H. DEPUE, and R. E. KELLY. 1966. Morphology of individual macromolecules and their ordered aggregates by electron microscopy. *Biochem. Z.* **345**:370.
20. YOUNG, M. 1967. Studies on the structural basis of the interaction between myosin and actin. *Proc. Natl. Acad. Sci. U.S.* **58**:2393.
21. MARUYAMA, K., and J. GERGELY. 1962. Interaction of actomyosin with adenosine triphosphate at low ionic strength. I. Dissociation of actomyosin during the clear phase. *J. Biol. Chem.* **237**:1095.
22. HUXLEY, H. E. 1957. The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol.* **3**:631.