INTERACTION BETWEEN VERTEBRATE SKELETAL AND UTERINE MUSCLE MYOSINS AND LIGHT MEROMYOSINS

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ABSTRACT

The specific contributions of this work may be summarized as follows: (a) No hybridization of uterine and skeletal myosin occurs at pH 6.0 although previous studies have shown that hybridization does occur at pH 6.5 (B. Kaminer et al. 1976. *J. Mol. Biol.* **100**:379–386.) or 7.0 (T. Pollard. 1975. *J. Cell Biol.* **67**:93–104.) (b) Hybridization of uterine and skeletal light meromyosins (LMM) occurs at pH 7.0 but not at pH 6.0, which is analogous to the hybridization of myosins. (c) In hybridized paracrystals there is a uniform distribution of both uterine and skeletal LMM molecules because all the paracrystals have only one axial repeat pattern. This makes it highly likely that in hybridized filaments the two myosins are also uniformly distributed throughout the filaments. (d) The 14-nm repeat of white bands observed in paracrystals of uterine LMM formed at pH 6.0, compared with the 14-nm repeat of dark bands observed with skeletal LMM under the same conditions, probably reflects differences in surface charge density along the different LMM molecules.

It has been shown that mixtures of skeletal and smooth muscle myosins (or nonmuscle myosin) in solution will form hybrid filaments, when the ionic strength of the solution is lowered, at pH 6.5 (9) and at pH 7.0 (13). The lengths of the filaments formed by the mixtures are intermediate between those formed by skeletal or smooth muscle myosins alone under the same conditions. This suggests that there are similarities in the intermolecular interactions involved in the assembly of smooth and skeletal muscle myosin filaments.

Uterine muscle myosin will form short (0.3–0.7-μm) synthetic myosin filaments that are structurally similar to the longer skeletal muscle myosin filaments formed under similar conditions (7). In both of these cases, the filaments are characterized by a bare zone in the middle and by tapered ends. We have observed the growth of longer (up to 1.2 μm[1]) synthetic uterine myosin filaments in the presence of 10 mM $MgCl_2$ (19). These longer filaments do not have a bare zone, and, from the observations, it was postulated that the increase in length resulted from "linear overlap of the tapered bipolar filaments and obliteration of the bare zone regions" (19). The resulting filament is made up of oppositely oriented myosin molecules along the entire length of the filament. Long filaments without bare zones have also been observed when other smooth muscle myosins have been used (3, 5, 16, 18). These differences in structure between synthetic skeletal and uterine myosin filaments

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[1] More recently, under these conditions, filaments as long as 2.3 μm, a length comparable to native smooth muscle filaments (1), have been observed (P. R. Wachsberger and F. A. Pepe, unpublished observation).

indicate that there are also differences in the intermolecular interactions involved in the assembly of the two types of filaments.

Huxley (7) observed clear, bare zones in short, synthetic, skeletal muscle myosin filaments as well as in longer filaments. However, clear, bare zones have not generally been observed in synthetic skeletal muscle myosin filaments (5, 8). Kaminer and Bell (8) concluded that the underlying filament was bipolar and that the absence of a bare zone was the result of additional myosin molecules aggregating onto the surface of the filament. Hinssen et al. (5) suggested that synthetic skeletal myosin filaments aggregate in a manner different from that of natural filaments.

The major purpose of this work is to study the coaggregation properties of skeletal and uterine myosin and skeletal and uterine light meromyosin (LMM) at pH 6.0 and pH 7.0. It was found that, in both cases, coaggregation or hybridization occurs at pH 7.0 but not at pH 6.0.

MATERIALS AND METHODS

Preparation of Myosins and LMM

The uterine myosin used for the filament studies was prepared from gravid rabbit uterus and was column purified as previously described (19). The skeletal myosin used for the filament studies was prepared from either rabbit or chicken muscle and was column purified as described by Pepe and Drucker (12). Uterine LMM was obtained by a 12-min trypsin digestion (trypsin:uterine myosin ratio of 1:50) of the 55% ammonium sulfate fraction of the crude myosin extract. The ethanol-resistant fraction of the LMM was used. Rabbit and chicken skeletal LMM were prepared by a 12-min trypsin digestion of myosin (trypsin:myosin ratio of 1:300) as described by Lowey and Cohen (10). The skeletal myosin used for digestion was prepared as described by Mommaerts and Parrish (11). The ethanol-resistant fraction of the LMM was obtained and then purified by column chromatography on diethylaminoethyl-Sephadex A 50 as described by Richards et al. (14).

Synthetic Filament Formation

Filaments were formed from solutions of myosin in 0.6 M KCl and 0.01 M imidazole, pH 7.0, at a concentration of 2-3 mg/ml. The solutions were slowly diluted to a final concentration of either 0.15 M KCl and 0.01 M imidazole, pH 7.0, or 0.025 M KCl and 0.075 M imidazole, pH 6.0, by using an Autoburette (Radiometer, Copenhagen) to deliver 0.01 M imidazole at pH 7.0 or 0.075 M imidazole at pH 6.0. Solutions were diluted in two stages: first they were diluted to 0.3 M KCl over a 1-min period and allowed to stand for 30 min in an ice bucket, and then they were diluted to the desired final concentration over a 5-min period. Filaments were formed from the individual myosins and mixtures of uterine and skeletal muscle myosin as indicated in Results.

Paracrystal Formation

Paracrystals were formed from solutions of the individual LMM and mixtures of uterine and skeletal LMM by dialysis against either 0.15 M KCl-0.01 M imidazole, pH 7.0, or 0.025 M KCl-0.075 M imidazole, pH 6.0, at a protein concentration of 0.5 mg/ml.

Electron Microscopy

Suspensions of either filaments or paracrystals were diluted to ~0.25 mg/ml with the appropriate buffer and placed on carbon-coated grids. Excess suspension was washed off with the appropriate buffer, and the grids were negatively or positively stained with 1% uranyl acetate solution in water. Electron micrographs were obtained at magnifications of 10,000 and 40,000 on a Siemens Elmiskop I.
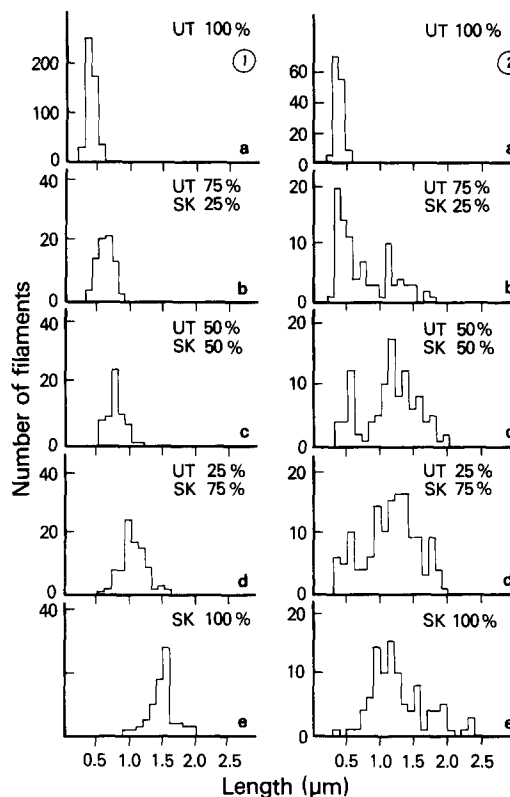

FIGURE 1 Length distributions of synthetic filaments formed from mixtures of rabbit uterine (UT) and skeletal (SK) myosins at pH 7.0. (a) Uterine myosin alone. (b–d) Coaggregation of uterine and skeletal myosin (see text). (e) Skeletal myosin alone.

FIGURE 2 Length distribution of synthetic filaments formed from mixtures of rabbit uterine (UT) and skeletal (SK) myosin at pH 6.0. (a) Uterine myosin alone. (b–d) Separate aggregation of uterine and skeletal myosins (see text). (e) Skeletal myosin alone.

SDS Polyacrylamide Gel Electrophoresis

SDS gel electrophoresis was carried out in 6% polyacrylamide gels as described previously (19).

RESULTS

Filament Hybridization

Synthetic filaments were formed from mixtures of rabbit uterine and skeletal myosins under conditions under which they individually would form filaments of different sizes. Filaments formed from rabbit uterine myosin alone, upon dilution to 0.15 M KCl in 0.01 M imidazole, pH 7.0, have a mean length of 0.44 ± 0.07 μm (Fig. 1 a). Under the same conditions, rabbit skeletal myosin alone formed filaments with a mean length of 1.53 ± 0.20 μm (Fig. 1 e). The mean length of filaments formed from mixtures of the two types of myosin under these same conditions is between 0.44 μm and 1.53 μm (Fig. 1 b–d). In each of the distribution curves in Fig. 1 a–e, the data were fit significantly better by a single normal distribution than by any two normal distributions, indicating that the distribution obtained with the mixtures is not the sum of the individual distributions of the uterine and the skeletal myosins. The change in

mean length as a function of the relative amount of uterine and skeletal myosin in the mixture is shown in Fig. 3 a. These results are in agreement with the work of Pollard (13) and Kaminer et al. (9) and are evidence for copolymerization of the two types of myosin to form hybrid filaments.

Similarly, filaments were formed at pH 6.0 from mixtures of rabbit uterine and skeletal muscle myosins by dilution to 0.025 M KCl in 0.075 M imidazole, pH 6.0. Representative examples of the filaments formed under these conditions are shown in Fig. 4. In this case, the mean length of filaments from uterine myosin alone was 0.45 ± 0.07 μm (Fig. 2 a), and that from the skeletal myosin alone was 1.33 ± 0.41 μm (Fig. 2 e). Although the distribution of lengths for the uterine myosin alone was similar at pH 6.0 and at pH 7.0, that for the skeletal myosin showed a much more precise length determination at pH 7.0 than at pH 6.0. Whereas the distribution for rabbit uterine myosin filaments alone at pH 6.0 (Fig. 2 a) was best fit by a single normal distribution, that for skeletal myosin alone (Fig. 2 e) was fit significantly better ($P < 0.005$) by two normal distributions with peaks at 1.10 ± 0.16 μm and 1.51 ± 0.46 μm. It is quite clear that neither of the two peaks in Fig. 2 e significantly overlaps the narrow distribu-
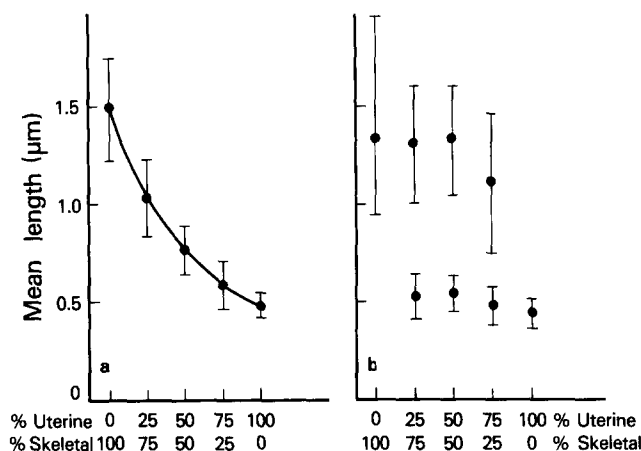

FIGURE 3 Mean lengths of synthetic myosin filaments represented in the length distribution curves obtained from mixtures of rabbit uterine and skeletal myosin in Figs. 1 and 2. (a) Hybrid filaments formed at pH 7.0. The mean and SD for the data in Fig. 1 are as follows: Fig. 1 a, 0.44 ± 0.07 μm; Fig. 1 b, 0.65 ± 0.12 μm; Fig. 1 c, 0.81 ± 0.13; Fig. 1 d, 1.08 ± 0.20; Fig. 1 e, 1.53 ± 0.20 μm. (b) Filaments formed at pH 6.0. For each mixture, two normal curves fit the data in Fig. 2 significantly better than a single normal ($P < 0.005$), and the two distributions correspond closely to those of uterine or skeletal myosin alone. The mean and SD for the data in Fig. 2 are as follows: Fig. 2 a, 0.45 ± 0.07 μm; Fig. 2 b, 0.48 ± 0.09 μm and 1.12 ± 0.36 μm; Fig. 2 c, 0.55 ± 0.09 μm and 1.33 ± 0.28 μm; Fig. 2 d, 0.53 ± 0.10 μm and 1.30 ± 0.31 μm; Fig. 2 e, 1.33 ± 0.41 μm.
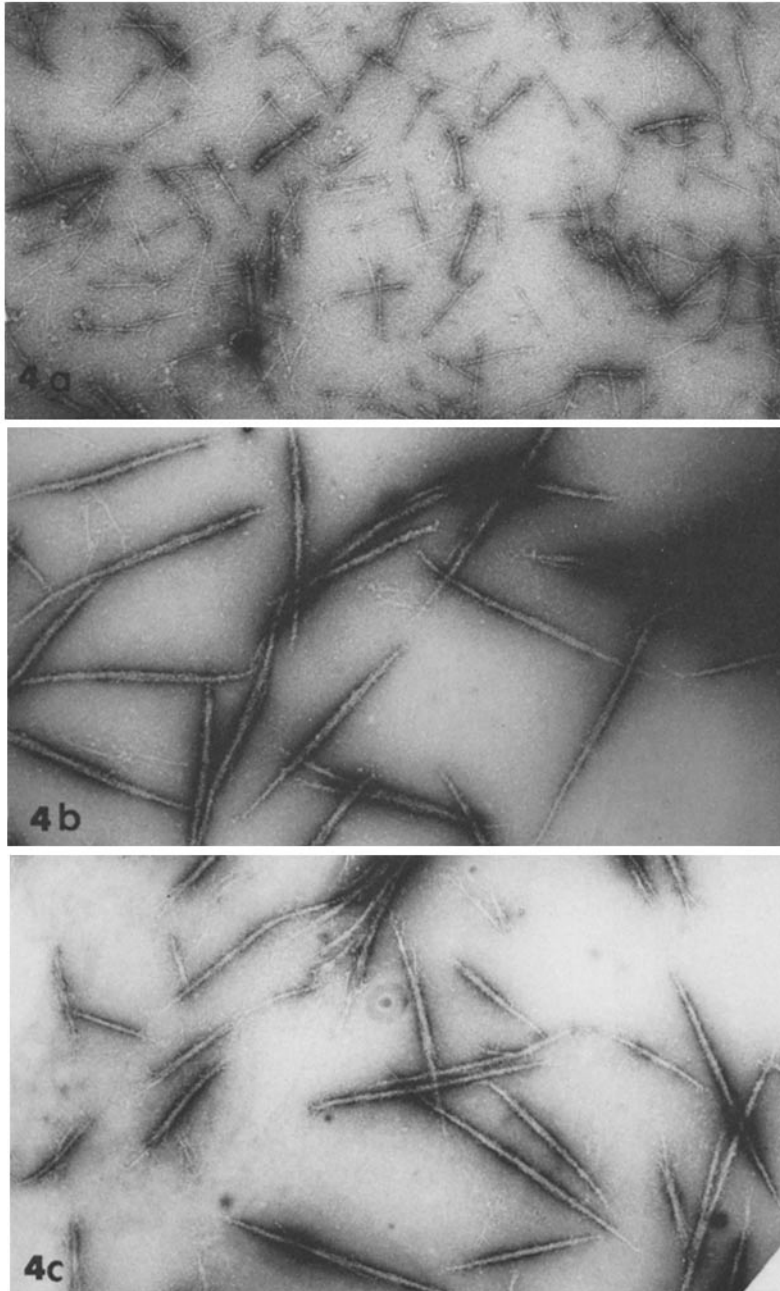
FIGURE 4 Synthetic filaments formed at pH 6.0. (*a*) Rabbit uterine myosin filaments. (*b*) Rabbit skeletal myosin filaments. (*c*) 1:1 mixture of rabbit uterine and rabbit skeletal myosin filaments. × 25,000.

tion corresponding to the lengths of the uterine myosin filaments (cf. Fig. 2*a* and *e*). The distribution of filament lengths obtained with mixtures of the rabbit uterine and skeletal myosin at pH 6.0 is shown in Fig. 2*b–d*. That there clearly are two populations in the length distribution of filaments obtained with the mixtures was verified by statistical analysis. Each distribution in Fig. 2*b–d* was fit significantly better ($P < 0.005$) by two normal distributions than by a single normal distribution.

In each case, one of the peaks corresponded closely to the length distribution for uterine myosin filaments alone, and the other corresponded closely to the length distribution for skeletal myosin filaments alone (Fig. 3 b). It is clear from these data that the two myosins do not form hybrid filaments at pH 6.0.

LMM Paracrystal Formation

The SDS gels for the uterine and skeletal LMM preparations are shown in Fig. 5. These gels were overloaded to detect the presence of minor components. The major component in the rabbit uterine LMM has a chain weight of 85,000 dalton (Fig. 5 c), which is in agreement with LMM obtained from chicken gizzard myosin (17). The higher chain weight component is a small amount of residual rod. The major component in both the rabbit and chicken skeletal LMM preparations has a chain weight of 73,000 dalton (Fig. 5 a and b). In contrast to rabbit and chicken skeletal LMM, uterine LMM did not form ordered paracrystals in 0.15 M KCl–0.01 M imidazole, pH 7.0. However, paracrystals were formed with uterine LMM in 0.025 M KCl–0.075 M imidazole, pH 6.0 (Fig. 6 e). The paracrystals formed from chicken and rabbit skeletal LMM preparations as well as from rabbit uterine LMM are shown in Fig. 6, and those
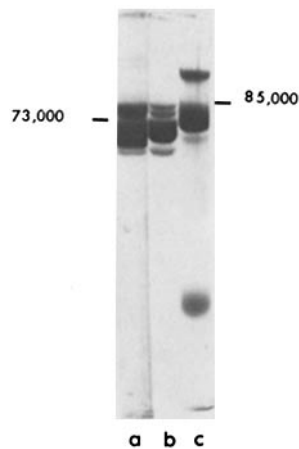

73,000 —

— 85,000

a   b   c

FIGURE 5   Polyacrylamide SDS gel electrophoresis of rabbit skeletal and uterine myosin LMM preparations. (a) Rabbit skeletal LMM. The major component has a chain weight of 73,000 dalton. (b) Chicken skeletal LMM. The major component has a chain weight of 73,000 dalton. (c) Rabbit uterine LMM. The major component has a chain weight of 85,000 dalton. The low chain weight component is a hemoglobin standard.

formed from mixtures of these LMM preparations are shown in Fig. 7.

At pH 7.0, chicken skeletal LMM formed paracrystals with a 14-nm axial repeat of narrow dark bands (Fig. 6 a), whereas rabbit skeletal LMM formed paracrystals with a 43-nm axial repeat of wide light bands (Fig. 6 b). Rabbit uterine LMM at pH 7.0 formed only small, nonperiodic aggregates. Therefore, at pH 7.0, the three LMM preparations clearly show different aggregation properties.

At pH 6.0, all three LMM preparations formed paracrystals with a 14-nm axial repeat. However, the skeletal muscle LMM preparations gave paracrystals with narrow dark bands repeating at 14 nm (Fig. 6 c and d), and the uterine LMM preparation gave narrow light bands repeating at 14-nm intervals (Fig. 6 e). Therefore, at pH 6.0, the rabbit and chicken skeletal LMM preparations have similar aggregation properties. The different pattern observed with the uterine LMM preparation may reflect a difference in aggregation properties, a difference in charge distribution (and, therefore, uranyl acetate binding along the myosin rod), or both.

Mixtures of chicken and rabbit skeletal LMM at pH 7.0 gave paracrystals with a 43-nm axial repeat (Fig. 7 a), which is characteristic of the pattern obtained with rabbit skeletal LMM alone (Fig. 6 b). None of the paracrystals obtained showed the 14-nm axial repeat characteristic of chicken skeletal LMM alone (Fig. 6 a), indicating that chicken and rabbit LMM hybridize. Similarly, rabbit uterine LMM hybridizes with chicken skeletal LMM at pH 7.0 inasmuch as the 14-nm axial repeat obtained with chicken skeletal LMM alone (Fig. 6 a) is replaced completely by a 43-nm axial repeat of wide light bands in the presence of uterine LMM (Fig. 7 b). Whether hybridization of uterine LMM and rabbit skeletal LMM occurs at pH 7.0 is not certain because the 43-nm axial repeat observed with the mixture (Fig. 7 c) is the same as that seen with rabbit skeletal LMM alone (Fig. 6 b).

It was not possible to determine whether the chicken and rabbit skeletal LMM preparations hybridize at pH 6.0 because paracrystals formed from the mixture have the same axial repeat pattern as the individual LMM preparations (Fig. 6 c and d). At pH 6.0, mixtures of uterine LMM and either chicken (Fig. 7 d) or rabbit (Fig. 7 e) LMM preparations formed mixtures of two types of paracrystals, namely, those with a narrow dark band
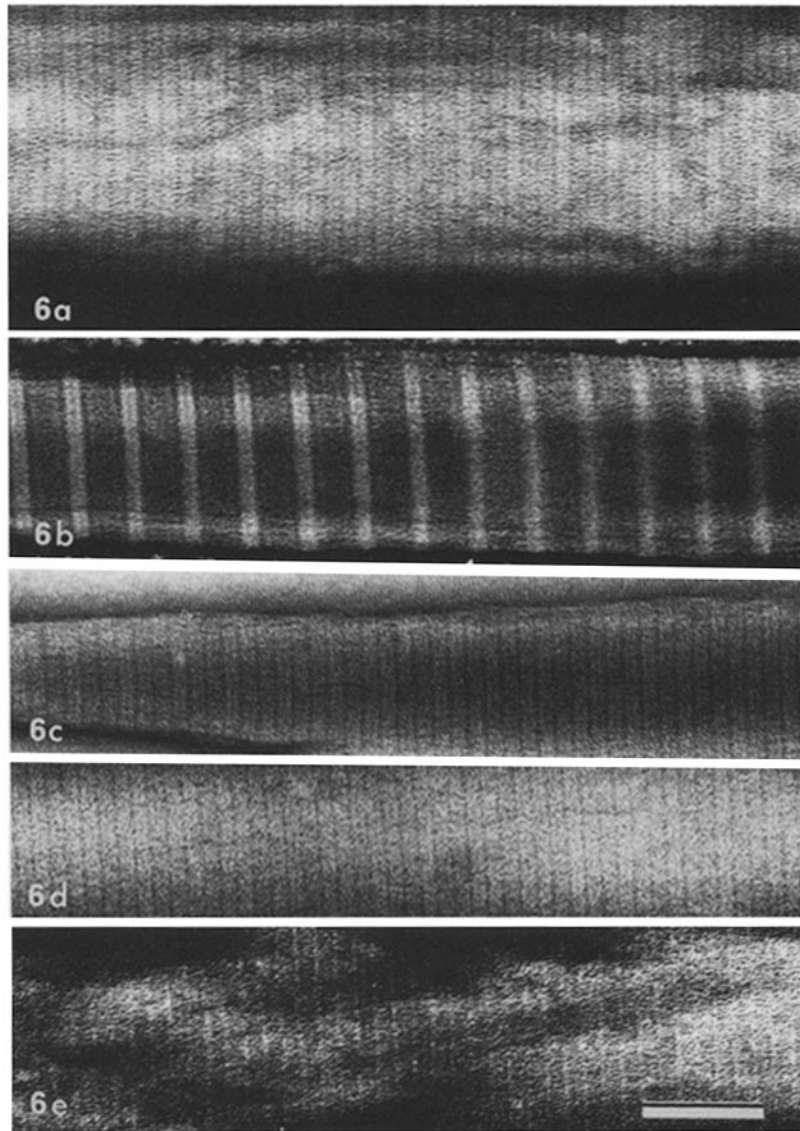
FIGURE 6   LMM paracrystals formed from the uterine and skeletal LMM preparations at pH 7.0 and pH 6.0. Bar, 0.1 μm. × 160,000. (a) Chicken skeletal LMM at 7.0 gives an axial repeat of narrow dark bands at 14 nm intervals. (b) Rabbit skeletal LMM at pH 7.0 gives an axial repeat of wide light bands at 43 nm intervals. (c) Chicken skeletal LMM at pH 6.0 gives an axial repeat similar to that in a. (d) Rabbit skeletal LMM at pH 6.0 gives an axial repeat similar to that in a and c. (e) Rabbit uterine LMM at pH 6.0 gives an axial repeat of narrow light bands at 14-nm intervals.

every 14 nm and those with a narrow light band every 14 nm. Therefore, it is clear that the uterine and skeletal LMM preparations do not hybridize at pH 6.0.

Chowrashi and Pepe (2) have shown that the 43-nm axial repeat observed with the ethanol-resistant fraction of chicken skeletal LMM can be converted to a 14-nm axial repeat by the removal of contaminating RNA by RNase treatment. Orcinol determination of RNA content (15) showed that the uterine LMM preparation contained 20 μg RNA/mg and that the chicken skeletal LMM preparation was free of RNA contamination. Therefore, we checked the possibility that the

RNA present in the uterine LMM may be responsible for the change from a 14-nm axial repeat to the 43-nm axial repeat observed when chicken skeletal LMM is mixed with uterine LMM (Fig. 7 b) at pH 7.0. To do this, we treated the uterine LMM preparation with RNase (RNase:uterine LMM ratio of 2:1) for 1 h at room temperature, decreasing the RNA content from 20 μg RNA/mg of protein to <3 μg/mg of protein. This had no effect on the paracrystals formed by the mixture of uterine and chicken skeletal LMM, inasmuch as the same 43-nm axial repeat (Fig. 7 b) was observed.

DISCUSSION

We have observed similarities and differences in aggregation of uterine and skeletal muscle myosins by comparing filament formation and LMM paracrystal formation, individually and as mixtures, at pH 6.0 and pH 7.0. Similarities in aggregation properties are most apparent at pH 7.0, where hybridization occurs, and differences in aggregation properties are most prominent at pH 6.0, where hybridization does not occur.

Filament Formation

It is clear from the length distributions of synthetic myosin filaments formed from mixtures of uterine and skeletal myosins at pH 7.0 (Figs. 1 and 3 a) that the myosins coaggregate. The hybrid filaments varied in length with the ratio of the two myosins (Fig. 3 a), as has previously been reported for pH 6.5 or pH 7.0 (9, 13). From observations of the hybrid filaments alone, it is not possible to learn much about the mechanism of hybridization. It is possible that the two myosins are uniformly distributed throughout the length of the filament. This would be a true copolymer, and the distribution of interacting sites on the two myosins probably would have to be quite similar. The length of the hybrid filament would presumably be determined by the different length-determining information provided by each of the two myosins. This, in turn, would depend upon the ratio of the two myosins. Alternatively, it is possible that the two myosins are segregated in the individual filaments. This could result if the uterine myosin inhibited the growth of skeletal myosin filaments by attaching to the ends of the filament. With increasing uterine myosin concentration, the inhibition might occur at shorter and shorter lengths, leading to a decrease in the length of the filaments

with increasing ratios of uterine to skeletal myosin. Both possibilities would produce the results observed, i.e., the formation of hybrid filaments.

In contrast to the observed hybridization at pH 7.0, the length distribution of synthetic filaments formed from mixtures of uterine and skeletal muscle myosins at pH 6.0 is clearly made up of two distributions, which correspond to the distributions obtained for uterine or skeletal myosins alone (Figs. 2 and 3 b). The most likely explanation for the difference in aggregation properties observed at pH 7.0 and pH 6.0 is that the concentration of charged groups at pH 6.0 and the distribution of interacting sites on the two myosins are significantly altered, thus eliminating the possibility of coaggregation.

Paracrystal Formation

The differences in aggregation properties between the LMM fragment of uterine and skeletal muscle myosins is accentuated upon adjustment from pH 7.0 to pH 6.0. At pH 7.0, coaggregation occurs, and, at pH 6.0, it does not, which is analogous to the behavior of the intact myosin molecules on formation of synthetic filaments. The axial repeat patterns of the paracrystals formed from uterine and skeletal LMM at pH 6.0 are different. At pH 6.0, with uterine LMM, the axial repeat of LMM paracrystals consists of a narrow light band at intervals of 14 nm (Fig. 6 e), whereas, with skeletal LMM, it consists of a narrow dark band at 14 nm intervals (Fig. 6 c and d). When paracrystals are formed from a mixture of the two LMM at pH 6.0, a mixture of the two types of paracrystals is obtained (Fig. 7 d and e). It is possible that these differences between uterine and skeletal LMM fragments result from differences in the distribution of charged groups on the two molecules and that these are accentuated by adjustment to pH 6.0. Comparisons of the electrophoretic mobility of smooth and skeletal muscle LMM have shown that smooth muscle LMM has a greater net negative charge than skeletal muscle LMM (6), which is consistent with the finding that smooth muscle myosins tend to have less histidine, lysine, proline, and glycine, while having more aspartic and glutamic acids than skeletal muscle (4).

At pH 7.0, uterine and skeletal LMM coaggregated, as is evidenced by a change in the axial repeat pattern of the paracrystals. For instance, the 14-nm axial repeat pattern observed with
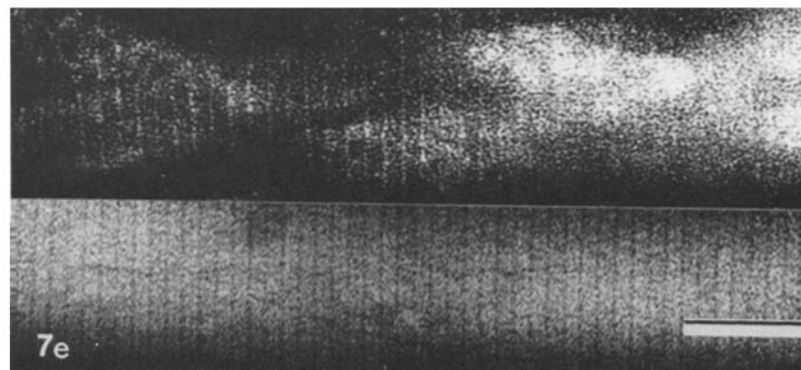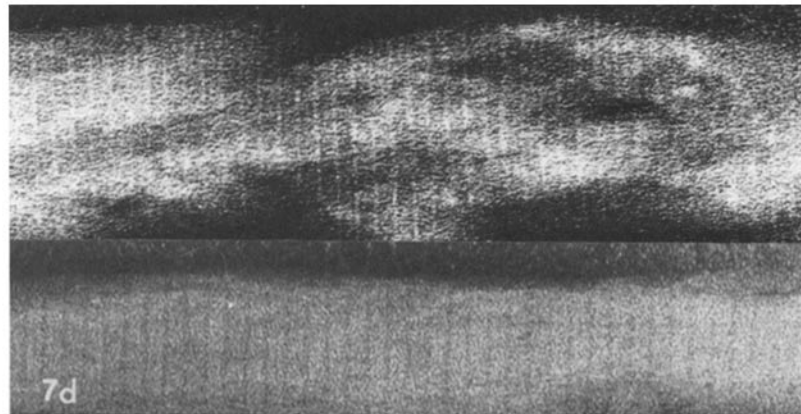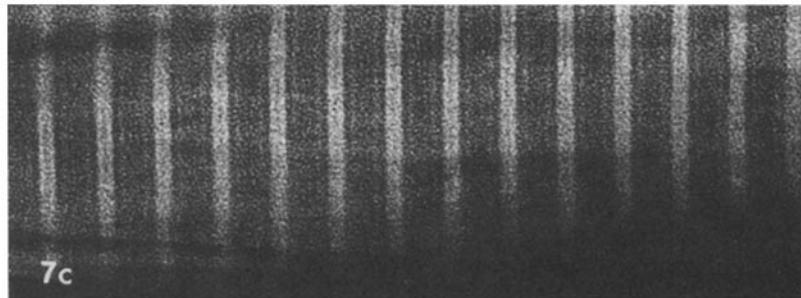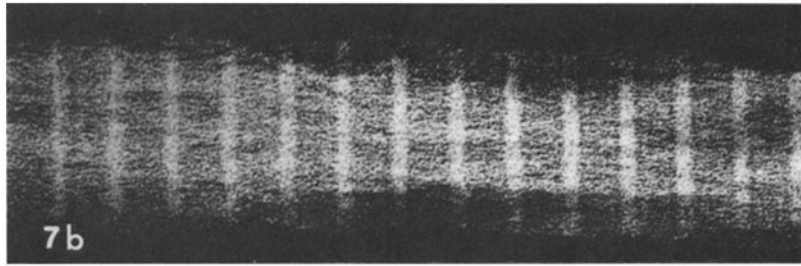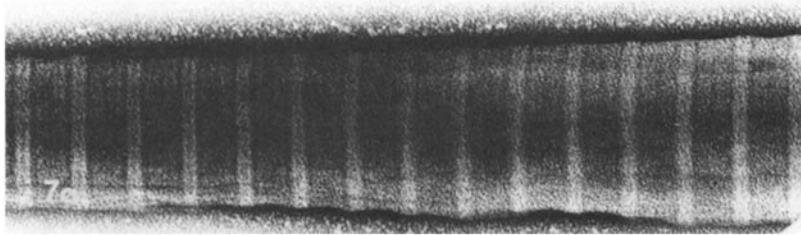
chicken skeletal LMM (Fig. 6a) was converted entirely to the 43-nm axial repeat pattern characteristic of rabbit skeletal LMM (Fig. 6b) when paracrystals were formed from a mixture of the two (Fig. 7a). The 14-nm axial repeat of chicken skeletal LMM (Fig. 6a) was also converted entirely to a 43-nm axial repeat when paracrystals were formed from a mixture of chicken skeletal and rabbit uterine LMM (Fig. 7b). The latter change is more dramatic because the uterine LMM alone, under these conditions, did not form paracrystals. By analogy, it is very likely that rabbit skeletal LMM and rabbit uterine LMM also coaggregate, even though the axial repeat pattern of paracrystals obtained with skeletal LMM alone (Fig. 6b) and when mixed with uterine LMM (Fig. 7c) are the same.

Because the hybridized paracrystals have uniform axial repeats over the entire paracrystal, it is most likely that the two types of LMM molecules are uniformly distributed throughout the paracrystal. It is, therefore, also likely that the different myosin molecules in hybrid myosin filaments are uniformly distributed along the length of the hybrid filament.

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FIGURE 7 LMM paracrystals formed from mixtures of the various LMM preparations. Bar, 0.1 µm. × 160,000. (a) Mixture of rabbit and chicken skeletal LMM at pH 7.0 gives an axial repeat of wide light bands at 43 nm intervals. (b) Mixture of rabbit uterine and chicken skeletal LMM at pH 7.0 gives an axial repeat similar to that in a. (c) Mixture of rabbit uterine and rabbit skeletal LMM at pH 7.0 gives an axial repeat similar to that in a and b. (d) Mixture of rabbit uterine and chicken skeletal LMM at pH 6.0 gives two types of paracrystals, one with a 14-nm axial repeat of narrow light bands (top) characteristic for uterine LMM and the other with a 14-nm axial repeat of narrow dark bands (bottom) characteristic for skeletal LMM. (e) Mixture of rabbit uterine and rabbit skeletal LMM at pH 6.0 gives the same two types of paracrystals shown in d.