EFFECTS OF MYOSIN AND HEAVY MEROMYOSIN ON ACTIN-RELATED GELATION OF HELA CELL EXTRACTS

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ABSTRACT

The gelation induced by warming (to 25°C) the 100,000 *g* supernatant fraction (extract) of HeLa cells lysed in a buffer containing sucrose, ATP, DTE, EGTA, imidazole, and Triton X-100 was studied in the presence of myosin and heavy meromyosin (HMM). Myosin mixed with extract induces shrinkage of the gel, but jelled extract or myosin alone does not shrink. In the concentration range, 0.14–1.04 mg/ml of myosin, the degree of shrinkage is roughly proportional to the concentration of myosin. Supplemental $MgCl_2$ also promotes shrinkage. HMM (0.4–0.8 mg/ml) can inhibit gel formation by extract in tubes or floated on a sucrose cushion. Gel electrophoresis of gels shrunken by added myosin or electrophoresis of the proteins which can be sedimented from extract after incubation in the presence of HMM indicate that both myosin and HMM interfere with the changes in sedimentability of the high molecular weight protein (HMWP) thought to participate (together with actin) in gel formation in HeLa cell extracts (R. R. Weihing, 1976. *J. Cell Biol.* **71**:303–307). These results, together with previous results showing that actin is present and that HMWP is enriched in the plasma membrane fraction of HeLa cells (R. R. Weihing, 1976. *Cold Spring Harbor Conf. Cell Proliferation.* **3**:671–684), point to the possibility of dynamic changes in the interactions of HMWP or myosin with actin in processes of movement occurring at the cell surface.

It has been recognized since 1960 that the cytoplasm of giant amebas can carry out lifelike movements after release from the cell (2). Since that time, studies on fractionation and ultrastructure of the cytoplasm and on the Ca^{++} and ATP requirements for movement and consistency changes (24, 30, 31, 32) have shown that rapid changes in the organization and interactions of actin and myosin most likely provide the molecular machinery for these changes. Recently, the studies on control of movement and consistency change by Ca^{++} and ATP have been extended to cultured mammalian cells (14).

The ability of cytoplasmic extracts to jell was recognized more recently, and it has been related to actin (22), actin and a high molecular weight actin-binding protein (4, 29, 34, 35), actin and two other proteins (15, 16), or actin and several low molecular weight proteins (20). The gels formed by three of these cytoplasmic extracts can shrink spontaneously (4, 22, 29), and the incorporation of myosin into the shrunken gels has suggested that interactions of actin and myosin induce gel shrinkage. These newer observations on gelation and shrinkage seem to be related to the older observations on consistency changes and movement of ameba extracts by the observation of a gel-like consistency for the ameba cytoplasm un-

der certain conditions (31) and the apparent involvement of actin and myosin in both sorts of systems.

I previously reported that suitable extracts of HeLa cells undergo a gelation reaction, which can be related to actin and a high molecular weight protein (HMWP) (34, 35). Gelation could be inhibited by submicromolar concentrations of cytochalasin B which also prevented the changes in sedimentability of actin and HMWP which are always found on gelation (34; cf. reference 13). In this paper, I report that exogenous myosin is required to induce shrinkage of jelled HeLa cell extract, and that gelation is inhibited by heavy meromyosin (HMM).

MATERIALS AND METHODS

HeLa cells were grown and harvested as described (34, 35), and the 100,000 *g* supernatant fraction of cells lysed in 1.8 vol of 0.34 M sucrose, 0.001 M ethyleneglycol bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), 0.001 M ATP, 0.001 M dithioerythritol (DTE), 0.01 M imidazole-Cl, pH 7 and 0.5% Triton X-100 was prepared as described (34, 35) and designated extract.

One milliliter of extract incubated for 1 h at 25°C in 10-ml Beckman tubes (Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) solidified to a jelly-like solid which was then isolated by centrifugation at 35,000 rpm (100,000 *g*) and 22°–24°C for 30 min with a Beckman FA-40 rotor. Such pellets were designated pelleted gel. Gels were also isolated by centrifugation from sucrose cushions. For these experiments, 3 ml of extract (prepared without sucrose and without ATP) was layered over 9 ml of 5% sucrose containing EGTA, DTE, and imidazole, pH 7, at the concentrations used in the buffer described above. The floating extract was incubated at 25°C for 1 h, and gel was then collected by centrifugation in an SW-41 rotor at 12,000 rpm (25,000 *g*) for 2 h at 22°–24°C.

Protein in cell fractions was measured by the method of Lowry et al. (18), and gel electrophoresis, staining and destaining, and densitometry were done as previously described (34, 35). The back and hind leg muscles of New Zealand white rabbits were used to prepare myosin (17). HMM was prepared according to Woodrum et al. (36), except that the dialysis after digestion was done against 20 mM PIPES, pH 6.6, instead of 20 mM imidazole, pH 6.6. The high ratio of trypsin to myosin (36) was used for these experiments, and gel electrophoresis of the HMM produced a polypeptide pattern (Figs. 3*c* and 4*h*) which was quite similar to that originally reported (36). The concentrations of myosin and of HMM were calculated from the absorbance at 280 nm, using an extinction coefficient of 0.56 liters/g-cm for myosin (7) and 0.647 liters/g-cm for HMM (37).

RESULTS

Gelation of Extract

As previously reported (34, 35), when the cytoplasmic extract is incubated at 25°C for 1 h, it solidifies into a jelly-like solid. Provided the total protein concentration is high enough (undiluted extract is about 12 mg/ml), the gel may remain in the bottom of the container in which it is formed when the container is tilted (Fig. 1*a*) (designated 4+ gelation), or it may slide as a single mass from the bottom to the side of the container when it is tilted (3+ gelation). Extract diluted to about 8 mg/ml with the buffer used to extract cells forms a few large pieces (2+) or several smaller ones (1+). Dilution to 3–4 mg/ml prevents gelation. Strong gelation (3–4+) is usually evident within 5 min, but the lower degrees of gelation may take longer to become noticeable.

Gel electrophoresis was used to study the composition of the gel formed by HeLa cell extract. Centrifugation at 100,000 *g* produced compact pellets consisting either of a narrow, clear rim

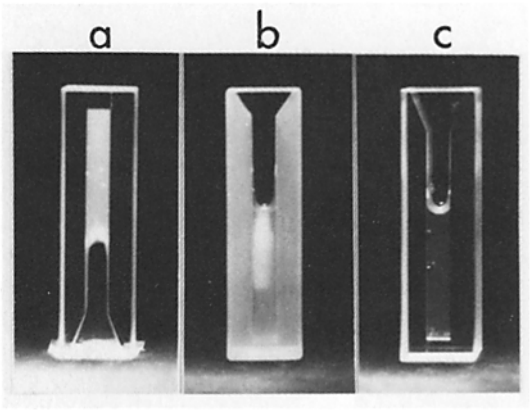

FIGURE 1 Effect of added myosin on gelation of cytoplasmic extract of HeLa cells. The contents of the cuvettes were as follows: (*a*) 1 ml of cytoplasmic extract (14.4 mg/ml total protein), 0.1 ml of 0.5 M KCl and 0.05 ml of 0.1 M $MgCl_2$; (*b*) 1 ml of cytoplasmic extract (14.4 mg/ml total protein), 0.075 ml of myosin (15.9 mg/ml) in 0.5 M KCl, 0.025 ml of 0.5 M KCl, and 0.05 ml of 0.1 M $MgCl_2$; and (*c*) 1 ml of complete buffer, 0.075 ml of myosin (15.9 mg/ml) in 0.5 M KCl, 0.025 ml of 0.5 M KCl, and 0.05 ml of 0.1 M $MgCl_2$. Final concentrations were as follows: total extract protein, 12.5 mg/ml; myosin, 1.04 mg/ml; $MgCl_2$, 4.3 mM; and KCl, 43 mM. The mixtures were incubated at room temperature for 3 h, and then photographed. However, the shrinkage was nearly complete after 30 min.

around a light tan, opaque center containing small holes in a "Swiss cheese" pattern or of a tan, veil-like material lying over a compact clear pellet. (A similar procedure only compressed comparable gels from *Acanthamoeba* [22].) Analysis of over 100 such experiments has always shown that the concentrations of a 42,000-dalton component, which comigrates with rabbit striated muscle actin, and of HMWP, which comigrates with the heaviest component of a partially purified preparation of actin-binding protein from macrophages (author's unpublished observations), are progressively enriched in extract (35) and pelleted gel (34, 35) (Fig. 2*a* and *b*). Centrifugation of gels formed after incubation of extract first diluted to 8 mg/ml produced pellets with similar enrichment for actin and HMWP (Fig. 2*c*). However, when gelation is inhibited by dilution to 3–4 mg/ml, then the pellets formed after incubation at 25°C and centrifugation at 100,000 *g* are no longer enriched for actin and HMWP (Fig. 2*d*). Thus, gelation is associated with the conversion of actin and HMWP to a more highly sedimentable form, and inhibition of gelation is accompanied by inhibition of these changes in sedimentation.

Effect of Myosin

Unlike jelled extracts of other cells (4, 22, 29), jelled HeLa cell extract does not shrink to opaque masses upon prolonged incubation at 25°C. Instead, in two experiments in which the gel was inspected at 1, 2, 4, 12, and 24 h after beginning incubation at 25°C, 3+ gelation persisted unchanged for 4 h, and the gel disappeared after 24 h. Because contracted gels from other cells contain myosin (4, 22, 29), it was of interest to determine the effect of exogenous myosin on gel from HeLa cells. In several experiments, myosin purified from rabbit striated muscle was mixed with cell extract and incubated at 25°C for up to 4 h. Myosin had no immediate effect on gel formation because extract alone or extract mixed with myosin jelled in 5 min. However, 1.04 mg/ml of myosin caused noticeable shrinkage of the gel within 20 min of incubation at 25°C, and after 40 min the fraction of the original gel volume occupied by the shrunken gel could be as small as one-fourth of the original volume of gel (Table I-A). If lower concentrations of myosin were used, then shrinkage first became noticeable after longer times, and the degree of shrinkage was usually less (Table I-A). Shrinkage was also promoted by addition of $MgCl_2$ (Table I-B), an effect which resembles the acceleration of gel contraction by $MgCl_2$ previously reported for jelled macrophage extracts (29). Fresh myosin induced the most rapid shrinkage because, when the effects of fresh myosin (final concentration 1.04 mg/ml) were compared with the effects of myosin stored for 8 and 12 days in the cold room in 0.5 M KCl (final concentration 1.18 mg/ml), the fresh myosin in-

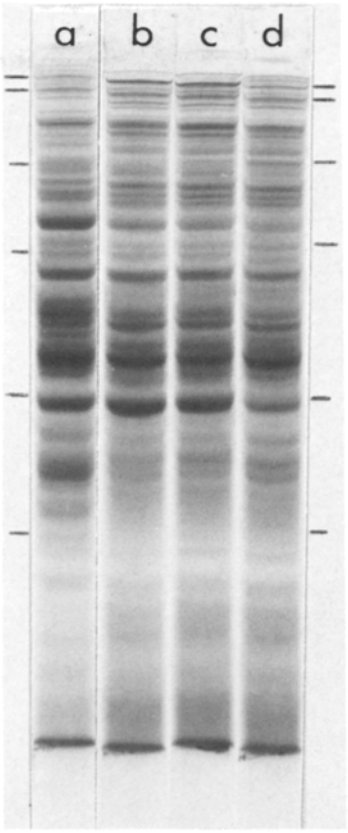

FIGURE 2 Gel electrophoretic patterns of HeLa cell extract and proteins derived from extract. (*a*) Cytoplasmic extract; (*b–d*) 100,000 *g* pellet fraction of extract incubated 1 h at 25°C after; (*b*), no dilution, total protein concentration of 13.2 mg/ml; (*c*) dilution of 0.67 ml of same extract as (*b*) with 0.33 ml of complete buffer; and (*d*) dilution of 0.33 ml of same extract as (*b*) with 0.67 ml of complete buffer. The lines to the left and right of the gels denote the positions of (from top to bottom) HMWP, myosin heavy chain (200,000 daltons), β-galactosidase (130,000 daltons), bovine serum albumin (69,000 daltons), rabbit skeletal muscle actin (42,000 daltons) and α-chymotrypsinogen (25,700 daltons).

TABLE I

Shrinkage of Gels Induced by Myosin

A. Effect of Concentration of Myosin

Myosin concn	Time when shrinkage first noted		Degree of shrinkage after 40 min	
	Exp 1	Exp 2	Exp 1	Exp 2
mg/ml	*min*			
1.04	20	20	3/4	1/2
0.69	40	20	2/3	2/3
0.35	40	40	1/4	3/8
0.14	40	50	1/8	0
0	None after 60 min		0	0

B. Effect of Concentration of $MgCl_2$

Concentration of $MgCl_2$	Time when shrinkage first noted		Degree of shrinkage after 40 min	
	Exp 1	Exp 2	Exp 1	Exp 2
mM	*min*			
4.3	40	20	2/3	1/2
0.86	None after 60 min	30	0	1/4

(A) One milliliter of extract made with complete buffer was mixed with 0.05 ml of 0.1 M $MgCl_2$, up to 0.075 ml myosin in 0.5 M KCl, and enough 0.5 M KCl to bring the final volume to 1.15 ml, and incubated at 25°C for 1 h. Degree of gelation and shrinkage was determined every 10 min for 60 min after beginning the incubation. The parameter, degree of shrinkage, was estimated by subtracting the fraction of the original gel volume occupied by the shrunken gel from 1; thus, when myosin was absent, the degree of shrinkage was 0 (= 1-1), and at the highest concentration of myosin in exp A-1 the degree of shrinkage was 3/4 (1 = 1-1/4).

(B) One milliliter of extract made with complete buffer was mixed with 0.05 or 0.01 ml of 0.1 M $MgCl_2$, 0.075 ml of myosin in 0.5 M KCl, 0.025 ml of 0.5 M KCl, and sufficient water to bring the volume to 1.15 ml. The tubes were incubated and assayed as described above. The final concentration of myosin was 1.04 mg/ml.

A single myosin preparation was used for exp A-1 and B-1; a single, different preparation was used for the other experiments.

duced shrinkage after 20 min, but the older preparations induced shrinkage only after 2 and 4 h of incubation at 25°C. The effects of ATP were not investigated because it was found that gels formed from extracts prepared with a buffer lacking sucrose, ATP, and Triton also underwent a myosin-induced shrinkage. Gel shrinkage required the addition of myosin to extract because myosin or extract alone did not shrink (Fig. 1). Electrophoresis of shrunken gels prepared by carefully aspirating the fluid from around a shrunken gel (Fig. 3*c*) or of shrunken gels collected by pelleting at 100,000 *g* (Fig. 3*b*) showed that actin and myosin heavy chain were major components. The concentration of HMWP appeared decreased, relative to nearby high molecular weight bands (cf. Fig. 3*a* with 3*b* and *c*); a similar effect has been reported by Boxer and Stossel (4).

Effect of HMM

These results made it of interest to determine the effects of HMM on gelation. For these experiments, extract was prepared using buffer without ATP and sucrose. Extract alone jelled normally (Table II), but the lowest concentration of HMM used caused formation of a gel which was looser (2+) than the control gel. The highest concentration completely inhibited gelation.

HMM also affected the sedimentation of actin

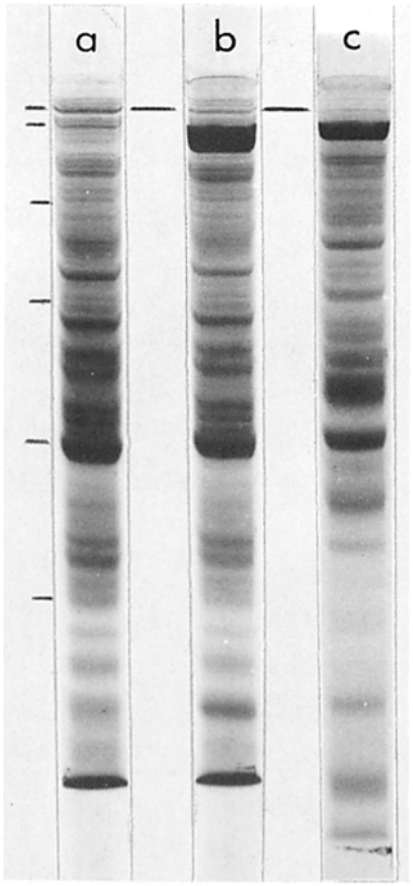

FIGURE 3 Effect of myosin on electrophoretic patterns of gel. Fig. 3*a–b* are 100,000 *g* pellet fractions collected after gelation of: (*a*) mixture of 1 ml of extract (13.9 mg/ml total protein) with 0.05 ml 0.1 M $MgCl_2$, and 0.1 ml of 0.5 M KCl; (*b*) mixture of 1 ml of extract used in (*a*) with 0.05 ml of 0.1 M $MgCl_2$, 0.025 ml 0.5 M KCl; and 0.075 ml of myosin (15.9 mg/ml) in 0.5 M KCl. For Fig. 3*c* the mixture shown in Fig. 1*b* was used. The liquid was carefully aspirated from the shrunken gel with a Pasteur pipette, and the gel was macerated in water for protein and electrophoretic analysis. The lines to the left of (*a*) mean the same as in Fig. 2. The enlargement of (*c*) has been adjusted to align actin and myosin heavy chain in Fig. 3*a–c*. The position of HMWP has been indicated with the lines connecting the gels.

and HMWP as illustrated in Fig. 4. The 100,000 *g* supernate from extract incubated with the highest concentration of HMM used in exp 1 of Table II (Fig. 4, scan *a*) contained actin at a lower concentration relative to the other proteins than the supernate from the control extract incubated without HMM (Fig. 4, scan *b*). HMWP showed reciprocal changes, its concentration in the supernate being raised by incubation with the high concentration of HMM in comparison with the supernate incubated without HMM. Because the total amount of protein recovered in the respective supernates is not very different (4.5 and 4.75 mg) and because other gross changes in band intensity are not visible, the result implies that HMM concomitantly increased the sedimentability of actin and lowered the sedimentability of the HMWP. If this is true, then one would expect to find the reciprocal changes in the pellet fraction from extract incubated with HMM, i.e., decreased HMWP and increased actin concentration. This seems to be the case for HMWP and may also be so for actin (Fig. 4*c* and *d*), but for actin the increase expected at the high concentration of HMM is difficult to see. Presumably, this is because the increase in intensity of the actin is superimposed upon an intensity which is already high, and because the changes in the total pattern which arise from the presence of the bands due to HMM make it more difficult to compare the relative intensities of bands in this pair of gels.

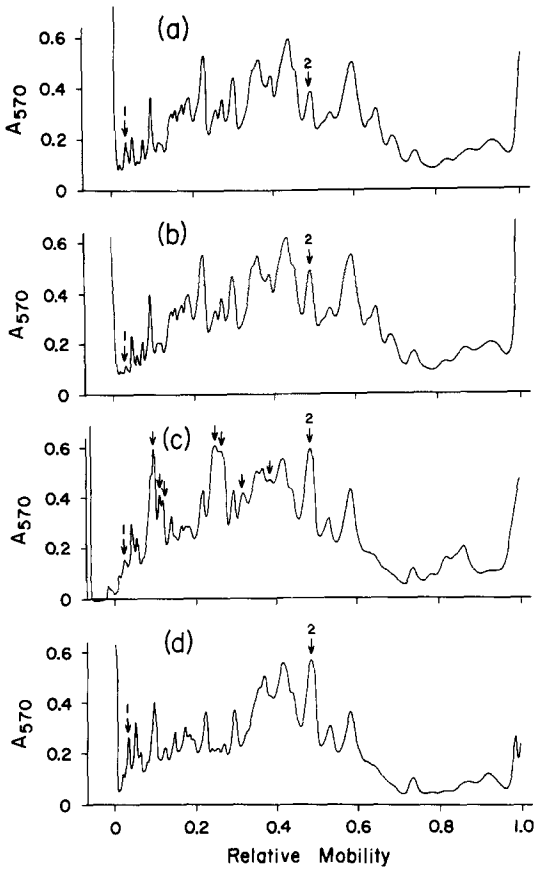

FIGURE 4 Spectrophotometric scans of the gel electrophoretic patterns of fractions produced by centrifugation of extract with and without heavy meromyosin at 100,000 *g*. The fractions were prepared as described in the text. Scan *a* is for the supernatant fraction of a mixture of extract and heavy meromyosin (Table II, Exp. 1, column 5). Scan *b* is for a control supernatant fraction from extract without added HMM (Table II, Exp. 1, column 1). Scans *c* and *d* are for the pellet fractions corresponding to scans *a* and *b*, respectively. Arrow *1* indicates the position of HMWP; arrow *2* indicates the position of actin. The unnumbered arrows in scan *c* indicate the positions of the polypeptides of HMM. The presence of these bands in the pellet fraction (scan *c*), but not in the corresponding supernatant fraction (scan *a*), indicates that all the HMM was sedimented.

TABLE II

Inhibition of Gelation by HMM in Tubes

A. Concentration of HMM (mg/ml)	0	0.2	0.41	0.61	0.82	1.9
B. Degree of Gelation						
Exp 1	3+	2+	1+	±	0	–
Exp 2	3+	–	–	1+	–	–
Exp 3	3+	–	–	–	–	0

10-ml Beckman centrifuge tubes were loaded as follows: 0.5 ml of cytoplasmic extract, amounts (up to 0.1 ml) of HMM in 0.02 M PIPES, pH 6.6 to give the final concentration shown, enough 0.02 M PIPES, pH 6.6 to bring the total final vol to 0.6 ml. The tubes were then incubated for 1 h at 25°C when the degree of gelation was determined. The gels or other proteins sedimenting under the conditions used to collect pelleted gel were then collected, and the fractions were subjected to protein and electrophoretic analysis.

These difficulties were overcome by investigating the effects of HMM on gelation of extract floated on a sucrose cushion. For these experiments, it was first determined that extract floating on a cushion could jell, as shown by observation of entrapment of bubbles in floating, jelled extract. It was also observed that the protein sedimented from such floating gels (see Materials and Methods) contained a higher concentration of actin and HMWP than pelleted gel (cf. Figs. 2*b* with 5*a*), and that such pellets could be induced to form gels which easily collapsed to insoluble aggregates, if they were resuspended in the buffer from which they were derived, at a total protein concentration

of 0.7–0.9 mg/ml, supplemented with 0.1 M KCl or 5 mM $MgCl_2$, and incubated at 25°C. Thus, observations on these pellets were relevant to the previous observations on gelation in tubes.

In the first experiment (Table III), gelation was inhibited at about the same concentration of HMM which partly inhibited gel formation in tubes (cf. Tables II and III), but in exp 2 (Table III) about twice the concentration of HMM was required to observe an effect on gelation. The pellets formed after inhibition by HMM were always runny instead of being firm. In both experiments, the lowest concentration of HMM used (which did not inhibit gelation) inhibited the sedimentation of HMWP because its concentration was lower in this pellet relative to its concentration in the pellet formed in the absence of HMM (cf. Fig. 5*a* and *b*). At the higher concentrations of HMM, the sedimentation of HMWP appeared nearly completely inhibited (Fig. 5*c–e*), and reciprocal changes are observed in the supernatant fractions (Fig. 5*f* and *g*). Thus, HMM inhibits the changes in sedimentability of HMWP and concomitantly inhibits gelation. Failure of sedimentation of HMWP and inhibition of gelation cannot be simply the result of the dilution of the extract caused by adding HMM, because the degree of dilution of extract would be insufficient to inhibit gelation or inhibit the sedimentation of actin and HMWP.[1]

TABLE III

Inhibition of Gelation by HMM on Cushions

A. Concentration of HMM (mg/ml)					
	0	0.19	0.38	0.56	0.75
B. Gelation					
Exp 1	Yes	Yes	No	No	No
Exp 2	Yes	Yes	Yes	Yes	No
C. Character of Pellet					
Exp 1	Firm	Firm	Intermediate	Runny	Runny
Exp 2	Firm	Firm	Firm	Firm	Runny

12-ml SW41 rotor tubes were loaded as follows: 2.5 ml of extract made without sucrose and ATP plus amounts of HMM in 0.02 M PIPES, pH 6.6 to give the final concentration shown (up to 0.5 ml), and enough 0.02 M PIPES, pH 6.6 to bring the final volume to 3 ml were layered over 9-ml cushions as described in Materials and Methods. The tubes were incubated at room temperature for 1 h when the presence or absence of gelation was determined. The tubes were then centrifuged as described in Materials and Methods. The supernates were decanted, the character of the pellets was determined, and the fractions were subjected to protein and electrophoretic analysis. Separate, fresh preparations of HMM were used for exp 1 and 2.

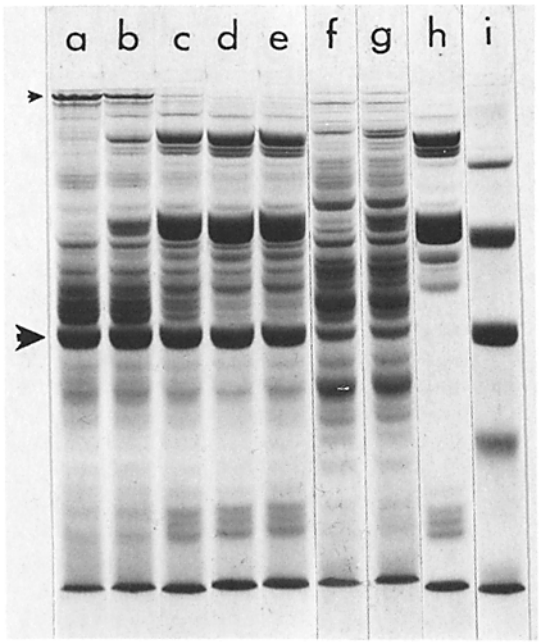

FIGURE 5 Gel electrophoresis patterns showing the effect of HMM on the sedimentation of proteins from extract layered on sucrose cushions. The results of Exp. 1 of Table III are shown. In Fig. 5*a–e* the pellets are shown: (*a*) no HMM, (*b*) 0.19 mg/ml HMM; (*c*) 0.38 mg/ml HMM; (*d*) 0.56 mg/ml HMM; (*e*) 0.75 mg/ml HMM; (*f* and *g*) are supernatant fractions corresponding to pellets (*a*) and (*e*), respectively; (*h*) is the pattern of HMM alone. The small arrowhead points to HMWP; the large one, to actin. Gel (*i*) is of standards; from top to bottom these are β-galactosidase, bovine serum albumin, rabbit skeletal muscle actin, and α-chymotrypsinogen.

DISCUSSION

In common with *Acanthamoeba* (22, 23), macrophages (28, 29), leukemic cells (4), sea urchin eggs (15, 16), and giant amebas (31), suitable extracts of HeLa cells can form a gel. In all cases, gelation can be related to a polymerization of actin induced by warming, but the generality of the role of the other proteins which have been isolated with gels is less well established. In HeLa cells, gelation is also associated with conversion of HMWP to a more easily sedimentable form, and inhibition of gelation by dilution (this report) or treatment of extracts with cytochalasin B (34) with inhibition of the changes. Thus, it is likely that

[1] This is because the total protein concentration was reduced by a factor of 0.83 in the experiments of Tables II and III, whereas gelation and the concomitant increase in sedimentation of actin and HMWP proceed perfectly well when the total protein concentration is reduced by a factor of 0.67.

both actin and HMWP participate in gelation of HeLa cell extracts. It should be pointed out, however, that the electrophoretic patterns of even the most highly purified gels (from cushions) are still somewhat complicated, so it is not yet possible to decide whether other proteins, which could resemble the intermediate molecular weight protein found in gels formed by sea urchin egg extracts (15, 16), or the highly potent, low molecular weight gelation factors reported for *Acanthamoeba* (20), also participate in gelation.

While jelled extracts of *Acanthamoeba* (22, 23), macrophages (28, 29), and leukemic cells (4) shrink spontaneously, neither jelled extracts of sea urchin eggs (15, 16) nor those of HeLa cells (this report) will do so. Why is a myosin supplement necessary for HeLa cell extracts to contract? Myosin is undoubtedly present in HeLa cells, having been identified by means of a highly purified antimyosin antibody (10). Furthermore, the 100,000 *g* supernatant fraction of HeLa cells lysed in 0.01 M NaCl, 0.0015 M $MgCl_2$, and 0.01 M Tris, pH 7.2, and then supplemented with 0.5 M KCl, contained a polypeptide which comigrated with myosin heavy chain, and which coprecipitated with myosin added to such extracts and precipitated by dialysis to 0.05 M KCl (author's unpublished observations). However, gel electrophoresis shows that the intensity of the bands in the myosin heavy chain region of HeLa cell extract (Fig. 3) is very low, while for cell extracts which undergo endogenous shrinkage (22) or lifelike movements (31) the intensity of the myosin heavy chain bands relative to the other proteins seems to be higher. Thus, the simplest explanation for the myosin requirement is that the myosin concentration of HeLa cell extract is too low to support shrinkage. (The myosin content of sea urchin egg extract cannot be estimated from available data [15, 16].) Although quantitative comparisons of the composition of these systems will be required to prove this explanation, it is of interest to note that the lowest concentration of myosin used in these experiments (0.14 mg/ml, Table I) did induce shrinkage and that it is close to the estimated concentration of myosin (0.13 mg/ml) in *Acanthamoeba* extracts, which shrink endogenously after gelation (22).

Inhibition of gelation by HMM is a new observation. Although the precise molecular mechanism of inhibition is not yet clear, the present data, which show that HMM prevents the change in sedimentability of HMWP which occurs during gelation, suggest that HMM inhibits the interactions of actin and HMWP believed to underlie formation of the gel network (8). On the other hand, while myosin has similar effects on sedimentation of HMWP (Fig. 3), gel formation proceeded normally in the presence of myosin. These results seem contradictory because one would expect that prevention of interaction of HMWP with actin should prevent gelation in both cases.

At least two explanations of this apparent contradiction are possible. First, the particular conditions used to study contraction might also promote gelation, either by direct promotion of the interactions underlying gelation, or by preventing a potential reversal of gelation by myosin. Here it is of interest to note the report that Mg^{++} supplements can promote gelation of macrophage extracts (29). In addition, when HeLa cell extract is diluted to 8 mg/ml from the usual 12 mg/ml, the gel formed in the presence of 5 mM $MgCl_2$ is as firm as the gel formed by undiluted extract, while diluted extract without $MgCl_2$ forms a looser gel (author's unpublished observations). This suggests that the $MgCl_2$ required for contraction could also overcome potential inhibition of gelation by myosin. If this is so, then $MgCl_2$ should also prevent inhibition of gelation by HMM, but this possibility has not yet been tested. Second, the mechanism of network formation might be different in the presence and in the absence of myosin. When myosin is absent, actin and HMWP presumably form the gel network. The myosin heads, which correspond to the activity of HMM that prevents interaction of HMWP, would no doubt be present on bipolar filaments which could not only inhibit change in sedimentability of HMWP but could also cross-link actin into a three-dimensional network. On the other hand, actin and myosin mixed under conditions similar to those of these experiments failed to jell (4, 29), which argues against this possibility.

Is there a physiological role for the effects of HMM and myosin on jelled extracts? While firm conclusions are not yet possible, certain speculations seem of interest as guides to future experiments. If we accept the view that actin and associated proteins form a contractile cytoskeleton (23), then the present in vitro results suggest that, in resting cells, actin and actin-binding protein (which resembles HMWP)[2] interact to form a noncontractile phase of the cytoskeleton. During movement, myosin would then displace actin-binding protein while undergoing the interactions

with actin which are believed to underlie cell movement (25). The observation that more actin-binding protein can be extracted from actively phagocytosing macrophages than from resting macrophages (29) is consistent with this suggestion. In addition, the reciprocal changes, i.e., displacement of myosin by actin-binding protein, may also be possible; this suggestion is based on two recent reports that filamin (which resembles actin-binding protein and HMWP)[2] can interfere with the Mg^{++} ATPase of acto-HMM (5, 20). Thus, it may be possible to distinguish contractile and structural phases of the contractile cytoskeleton, on the basis of whether actin is interacting preponderantly with myosin or with some other protein such as actin-binding protein.

The plasma membrane fraction of HeLa cells contains a concentrated form of HMWP (11, 35), and actin-binding protein has been reported to be present in macrophage plasma membranes (21) and the cortical blebs released from macrophages by sonication (6). The recent observation of actin-binding protein by immunofluorescence techniques on phagolysosomes of polymorphonuclear leukocytes (3) suggests that it (or HMWP) is located on the inner surface of the plasma membrane. Here, together with actin, it could form a gel (34) which could account for the high consistency commonly observed for cortical cytoplasm (19). This possibility implies that it will be of interest to determine the role, if any, of these in vitro effects in processes such as the cortical sol-gel transitions of giant amebas (1, 19), the formation of the contractile ring with its concomitant changes in cortical consistency and contractility (26), or phagocytosis where related processes involving actin, myosin, and actin-binding protein have already been postulated to occur (28).

[2] Protein with the electrophoretic mobility of actin-binding protein from macrophages (12, 18, 27) has been isolated from leukemic leukocytes (4), and has been identified in striated and smooth muscle (28). Contracted gels from *Acanthamoeba* also contain a protein of nearly identical mobility (22). HMWP has also been found to comigrate with the component of highest molecular weight in a partially purified preparation of actin-binding protein from macropahges (author's unpublished observations), suggesting that they are the same protein. Furthermore, while the molecular weights of actin-binding protein and filamin (a high molecular weight protein first isolated from gizzard and found by antibody staining to have a distribution similar to that of myosin cables in cultured cells) (33) do not appear to have been compared directly, the high molecular weight of this protein (5, 9, 33) and its reported ability to induce gelation of actin (9, 20) indicate a striking similarity between filamin, actin-binding protein, and HMWP.

I thank Mrs. Ann Elliott and Miss Pamela Langley for expert technical assistance and Drs. J. H. Hartwig and T. P. Stossel for a gift of partially purified actin-binding protein. This work was supported by United States Public Health Service research grant no. CA-15229 (to R. R. Weihing) and P30-12708 (to Mahlon B. Hoagland) from the National Cancer Institute.

Received for publication 22 November 1976, and in revised form 2 May 1977.

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