MUSCLE ACTIN FILAMENTS BIND PITUITARY SECRETORY GRANULES IN VITRO

RICHARD E. OSTLUND, JOYCE T. LEUNG, and DAVID M. KIPNIS

From the Metabolism Division, the Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT

Hog anterior pituitary secretory granules sediment at 3,000 *g*. When rat or rabbit skeletal muscle actin filaments are present with the granules, the sedimentation decreases markedly. Depolymerized actin or viscous solutions of Ficoll and collagen have no effect on granule sedimentation. With this assay, actin filaments bind secretory granules (consisting of the proteinaceous core plus limiting membrane), secretory granule membranes, mitochondria, artificial lecithin liposomes, and styrene-butadiene microspheres, but have little or no interaction with membrane-free secretory granule cores and albumin microspheres. A secretory granule-actin complex sedimentable between 3,000 *g* and 25,000 *g* can be isolated. Metal ions, nucleotides, salts, dithiothreitol, or pretreatment of the granules with trypsin do not destroy the binding, which appears to be a lipophilic interaction.

Microfilaments, which resemble skeletal muscle actin filaments in many characteristics, have been found in most nonmuscle mammalian cells (23, 2, 6). The potential importance of microfilaments is underscored by the fact that actinlike material constitutes up to 20% of total protein in several nonmuscle cell types (23). As a consequence, it has been postulated that actin filaments play a role in many functions, such as transport of secretory granules (10) and neurosecretory vesicles (1), cell movement (27), maintenance of cell shape (27), cell differentiation (30), and fast axoplasmic transport (15). The exact nature of the presumptive interaction between actinlike filaments and cell organelles has not been elucidated. However, Burridge and Phillips (3) have recently reported that both skeletal muscle actin and myosin can be found in association with adrenal medullary secretory granule membranes after mixing, and a definite morphological association has been established between microfilaments and the plasma membrane (2, 4, 22).

In this paper a simple system for examining the binding of large particles by microfilaments is detailed, and evidence is presented demonstrating that skeletal muscle actin binds hog pituitary secretory granules and granule membranes in vitro.

MATERIALS AND METHODS

Twice-polymerized actin was prepared from rat or rabbit back and hind limb muscle (24) without chromatographic purification and stored as a 6-mg/ml solution in 0.1 M KCl containing 2 mM Tris pH 7.5, 0.25 mM ATP, and 1.0 mM dithiothreitol at 4°C. Depolymerized actin was prepared by dialysis of this stock against storage buffer without 0.1 M KCl and sedimentation of any remaining filamentous actin by centrifugation at 105,000 *g* for 90 min. Secretory granules and granule membranes were prepared from fresh hog anterior pituitary glands (25). All procedures were carried out at 4°C. Pituitaries were mixed with 2 vol of 0.3 M sucrose containing 50 mM Tris-Cl pH 7.4 (Tris-sucrose buffer) and subjected to eight strokes of a motor-driven, loose-fitting Teflon-glass homogenizer. The homogenate was centrifuged at 5,000 rpm in a Sorvall SS-34 rotor (3,000 *g*, tip of the tube; DuPont Instruments, Sorvall Operations, Newtown, Conn.) for 10 min and washed with 2 vol of Tris-sucrose buffer. The supernatant fractions were combined

and centrifuged at 19,000 rpm for 30 min. The 19,000-rpm pellet was taken up in aqueous sucrose of density 1.20 to yield a final density of 1.18, and secretory granules were prepared by the discontinuous sucrose gradient procedure described by Poirier et al. (21). Granule membranes were prepared by hypotonic lysis. Purified granules (30 mg/ml) were suspended in 10 vol of distilled water, and after 60 min of stirring the suspension was centrifuged for 1.5 h at 25,000 rpm in an SW-27 rotor, using a discontinuous sucrose gradient consisting of 6 ml of 1.18 density sucrose, 6 ml of 1.14 density sucrose, and approx. 26 ml of hypotonic lysate. Granule membranes concentrated at the water-1.14 density sucrose and 1.14–1.18 density sucrose interfaces. The first interface was removed and dialyzed against 0.15 M NaCl containing 10 mM Tris-Cl pH 7.4. Secretory granule cores, which still contained some granule membranes, were collected from the pellet beneath the 1.18 layer. Granule cores free of granule membranes were prepared by homogenizing secretory granules (30 mg/ml) in 9 vol of 1.3% sodium desoxycholate containing 0.3 M sucrose followed by one wash in the same volume of desoxycholate buffer and two washes in Tris-sucrose. The granule cores were sedimented each time at 19,000 rpm (43,500 *g*) for 20 min in a Sorvall SS-34 rotor. Rat liver mitochondria were prepared by the method of Sjöstrand (26). Styrene-butadiene microspheres were purchased from Dow Chemical Co. (Midland, Mich.). Human albumin microspheres prepared by heat coagulation of an aqueous mist (31) were a gift of Dr. Norman Aspin of the Hospital for Sick Children, Toronto. Artificial liposomes were prepared by vigorously vortexing chromatographically purified lecithin (1 mg/ml) in 0.15 M NaCl containing 1 mM EDTA (19).

Sedimentation of secretory granules (0.25–0.5 mg/ml) was examined in 0.5 or 1.0 ml vol of 0.15 M NaCl containing 10 mM Tris-Cl pH 7.4 (Tris-saline buffer) in the presence of actin (0.25–0.5 mg/ml). After the tubes were gently vortexed at 4°C, the mixture was heated to 37°C for 15 min with agitation and then centrifuged at 3,000 *g* (5,000 rpm in a Sorvall SS-34 rotor using clear 16 × 100-mm tubes) for 10 min at 4°C. The supernate was removed, and the amount of granule protein sedimenting was determined by the difference in turbidity (OD 660) between the supernate and the remixed supernate and pellet. Actin has negligible turbidity under these conditions. No significant differences in the turbidity were noted between freshly prepared granule suspensions and granule pellets remixed with the supernate after incubation and centrifugation, as shown in the following experiment with secretory granules and rabbit skeletal muscle actin, 0.25 mg/ml each:

	OD 660	
	Fresh	Remixed
Secretory granules	0.310	0.336
Secretory granules + actin	0.334	0.312

In some studies, the pellet and supernatant protein content were measured directly by the method of Lowry after trichloroacetic acid precipitation (12). In all experiments, simultaneous controls containing identical buffers and secretory granules but no actin were carried out. The effect of actin was calculated as sedimentation (percent of buffer control) equals micrograms of secretory granule pellet in the presence of actin per micrograms of secretory granule pellet in the absence of actin.

Type I phospholipase C (7.6 U/mg), type III trypsin (11,000 U/mg), and type IV acid-soluble calf skin collagen were purchased from Sigma Chemical Co. (St. Louis, Mo.). Collagen was dialyzed against 0.5 M acetic acid for 4 days and then against several exchanges of Tris-saline.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was done with the neutral phosphate system of Maizel (13). Viscosity measurements were made in a Cannon-Fenske viscometer (Cannon Instrument Co., State College, Pa.) with a water outflow time of 460 s. Relative viscosity was computed as the ratio of outflow time of protein in Tris-saline to outflow time of Tris-saline alone.

RESULTS

Secretory Granule Sedimentation

Porcine anterior pituitary secretory granules prepared as described are almost exclusively growth hormone- and prolactin-containing particles 0.3–0.7 μm in diameter (8). The dense hormone material of the granule core is surrounded by a limiting membrane (8). Between 50 and 90% of the granules sediment when suspended in Tris-saline buffer and centrifuged at 3,000 *g* for 10 min (Fig. 1, tubes *B* and *B'*). However, if skeletal muscle actin is included with the secretory granules (Fig. 1, tubes *C* and *C'*), the pellet formed is hardly visible, and the supernate is turbid. Under these mild conditions of centrifugation, actin alone does not sediment but forms a clear solution (Fig. 1, tubes *A* and *A'*).

The amount of sedimentation was quantitated by turbidimetric measurement at OD 660 nm or by determination of protein content in the supernatant fraction and pellet. Under the experimental conditions shown in Fig. 1, sedimentation of secretory granules was only 25.8 ± 2.4% of the sedimentation seen in the absence of actin.

The dose-response curve of the inhibition by actin of secretory granule sedimentation, i.e., actin binding, is shown in Fig. 2. A significant effect can be seen with as little as 25 μg/ml actin, an actin solution which has a relative viscosity of 1.004 compared to buffer control. Hence, in-

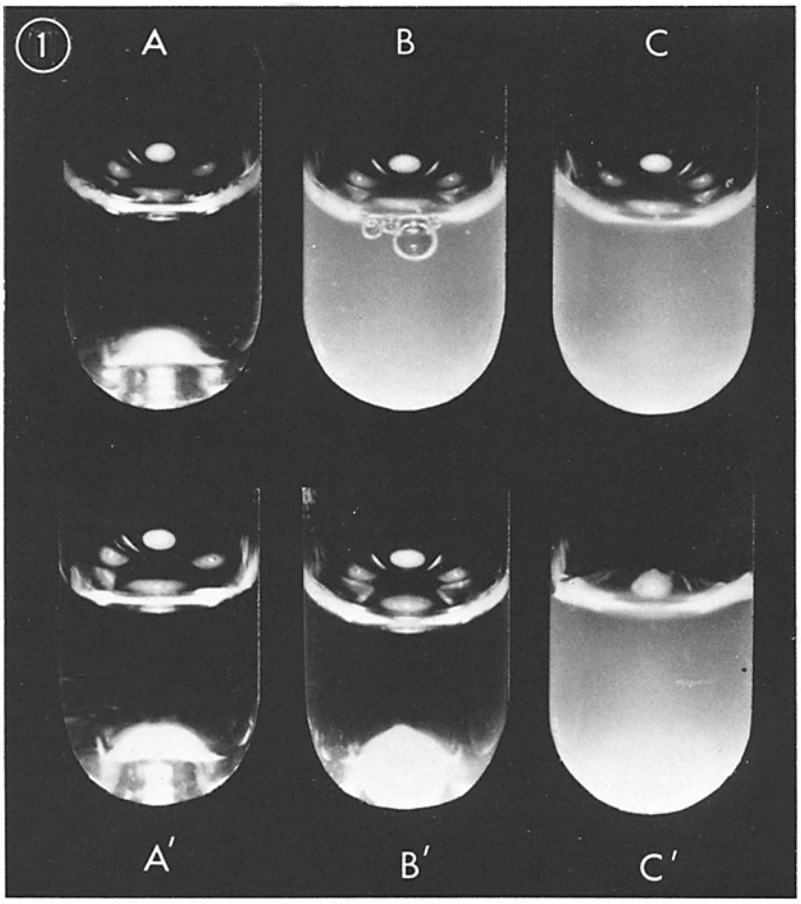

FIGURE 1 The effect of actin on secretory granule sedimentation. (*A*) Rabbit skeletal muscle actin 0.27 mg/ml. (*B*) Hog anterior pituitary secretory granules, 0.27 mg/ml. (*C*) Both actin and granules, 0.27 mg/ml each. Tube *A* shows a highlight (but no precipitate) at the bottom. The mixtures were incubated at 37°C for 15 min and then centrifuged at 3,000 *g* for 10 min at 4°C. The same tubes after centrifugation are labeled *A'*, *B'*, *C'*. Tube *A'* demonstrates only a highlight while tube *B'* contains a definite precipitate. Tube *C'* shows a very light precipitate with little sedimentation of the secretory granules.

creased viscosity is not necessary for actin-granule interaction.

Effect of Other Viscous Solutions on Secretory Granule Sedimentation

It might be expected that any viscous solution could produce similar inhibition of secretory granule sedimentation. The results with several such viscous solutions are shown in Table I. Both rat and rabbit skeletal muscle F-actin, i.e., polymerized fibrous actin, inhibited secretory granule sedimentation. G-actin (nonpolymerized globular actin), a nonviscous solution kept from polymerizing in Tris-saline buffer by its low concentration, was ineffective. The sediment obtained from the centrifugation of a mixture of G-actin and secretory granules (Table I) contained no extra protein (G-actin bound to and sedimenting with granules). Thus, the filamentous but not the monomeric form of actin-bound secretory granules. Increased viscosity by itself was not sufficient to alter granule sedimentation, since a very viscous solution of soluble collagen did not inhibit granule sedimentation. Collagen (tropocollagen form) is a filamentous protein 15 Å by 0.3 μm (29). Rabbit muscle actin polymerized at a concentration of about 6 mg/ml forms filaments approx. 80 Å by 0.5 μm (9). The viscous spherical polymer Ficoll (5) also had no effect on granule sedimentation. Thus, filamentous structure alone does not appear suffi-

cient to account for the granule-actin interaction, although the filamentous form of actin is necessary.

Effect of Actin on Sedimentation of Other Particles

The inhibitory effect of actin on sedimentation of particles other than secretory granules is shown in Table II. The particles were selected to be approximately the same size and shape as secretory granules. Mitochondria interacted with actin as did lecithin artificial liposomes and styrene-butadiene microspheres. However, the sedimentation of human albumin microspheres was not affected by actin.

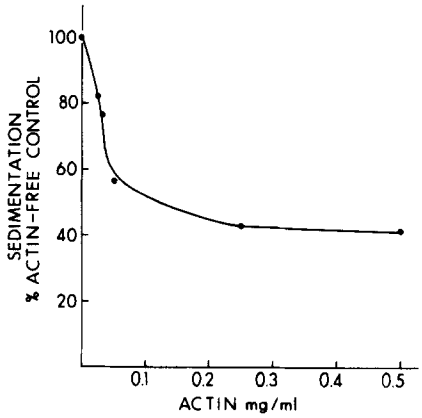

FIGURE 2 Effect of actin concentration on secretory granule sedimentation. Rat muscle actin and secretory granules, 0.5 mg/ml, were incubated and centrifuged as described in Materials and Methods. Sedimentation of granule protein when actin is present is plotted as a percent of sedimentation in the absence of actin.

Fractionation of Secretory Granules

Since secretory granules are surrounded by a limiting membrane, the interaction of actin with isolated secretory granule membranes and with membrane-free granule cores was examined. Secretory granule membranes were prepared by hypotonic lysis and granule cores by either hypotonic lysis or desoxycholate treatment. Cores prepared by hypotonic lysis alone were incompletely stripped of membranes, as judged by a persistence of approx. 50% of the magnesium-ATPase activity of the granule membrane (7). Granule cores prepared by desoxycholate treatment were found to be free of membranes when examined by electron microscopy. Granule cores prepared by hypotonic lysis interacted with both rat and rabbit skeletal muscle actin as well as did intact granules (Table II). However, sedimentation of cores prepared by the more vigorous desoxycholate method was not significantly different whether in the presence or the absence of rat muscle actin. When rabbit muscle actin was employed, a small inhibition of sedimentation was noted (74.5% of control sedimentation), but this was significantly less than the actin-induced inhibition of sedimentation of both secretory granules (25.8%) and hypotonically lysed granule cores (19.3%), $P < 0.001$. Other experiments also suggested that the small

TABLE I

Effect of Viscous Solutions on Secretory Granule Sedimentation

Solution	Granule sedimentation			Relative viscosity
	Buffer control	Viscous solution		
	μg	μg	% of buffer control	
Filamentous actin				
Rabbit muscle 0.25 mg/ml	197 ± 8.6	50.8 ± 4.7	25.8 ± 2.4*	1.184
Rat muscle 0.5 mg/ml	122 ± 4.9	48.3 ± 4.5	39.6 ± 3.7*	1.236
Depolymerized actin				
Rat muscle 0.5 mg/ml	376 ± 31	382 ± 18	102 ± 4.8	–
Ficoll, 20 mg/ml	185 ± 5.8	196 ± 13	106 ± 7.1	1.473
Soluble collagen 0.25 mg/ml	108 ± 0.6	114 ± 6.5	106 ± 5.2	1.975

Porcine anterior pituitary secretory granules, 0.25–0.5 mg/ml in 0.5–1.0 ml Tris-saline buffer with or without the viscous material, were incubated at 37°C for 15 min and centrifuged for 10 min at 3,000 *g*. Granule sedimentation was determined by either turbidimetry or protein assay. The amount of granule protein sedimenting in a viscous solution was compared to that sedimenting in buffer alone (buffer control). Each experimental condition was done in triplicate in the same assay as the control. Means ± standard error are given.

* $P < 0.001$ compared to control.

TABLE II

Sedimentation of Particles in the Presence of Actin

Particle	Sedimentation	Skeletal muscle actin	Particle diameter
	% ± SE	mg/ml	μm
Secretory granules	25.8 ± 2.4*	rabbit – 0.25	0.3–0.7
	39.6 ± 3.7*	rat – 0.50	
Secretory granule fractions			
Granule cores (hypotonic lysis)	17.3 ± 3.6*	rabbit – 0.25	
	43.7 ± 1.6*	rat – 0.50	
Granule cores (desoxycholate)	112.8 ± 6.9	rat – 0.50	
	74.6 ± 4.4*	rabbit – 0.25	
Granule membranes	80.9 ± 3.2*	rabbit – 0.20	
	73.1 ± 0.8*‡	rabbit – 0.43	
Other organelles and particles			
Rat liver mitochondria	42.3 ± 3.7*	rabbit – 0.25	0.5–1.0
Artificial lecithin liposomes	29.9 ± 2.0*	rabbit – 0.50	0.5–1.0
Styrene-butadiene microspheres	21.5 ± 2.9*	rabbit – 0.25	0.53
Human albumin microspheres	96.8 ± 8.8	rabbit – 0.25	0.50

Particles were suspended in 0.5–1.0 ml Tris-saline. Actin was added in the final concentration indicated. The mixtures were incubated and centrifuged as described in Materials and Methods. Particle sedimentation is expressed as a percent of that sedimenting in the absence of actin.

* $P < 0.01$ compared to control.

‡ Sedimentation performed at 10,000 *g*.

effect of rabbit actin on desoxycholate-prepared granule cores was qualitatively different from the effect on secretory granules (Fig. 3). Progressively increasing quantities of secretory granules were centrifuged with a fixed amount of actin, 0.25 mg/ml. A dose-response curve of granule-actin interaction was observed. When granule cores prepared with desoxycholate were used, a constant minimal degree of binding was found, suggesting that it was probably nonspecific in nature. The possibility that traces of desoxycholate might remain on the cores and reduce actin binding was considered. Styrene-butadiene particles were treated with desoxycholate and washed in the same fashion as granules. The binding of these particles and untreated styrene-butadiene microspheres to actin was tested. Particle sedimentation in the presence of actin compared to sedimentation in the absence of actin was 46 ± 7.4% (SE) for desoxycholate-treated and 45 ± 0.9% for control styrene-butadiene microspheres. This indicates that treatment of a stable particle with desoxycholate need not disrupt binding to actin.

Secretory granule membranes also interacted with rabbit actin (Table II). Because membranes could not be prepared in quantities sufficient to develop a dose-response curve as shown in Fig. 3, their interaction with actin was studied in discontinuous sucrose gradients (Fig. 4). The location at

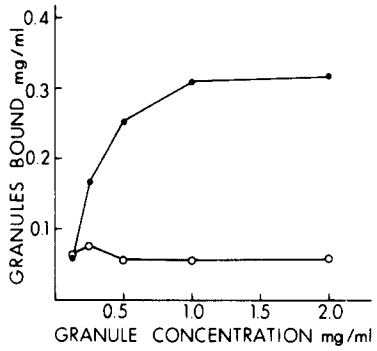

FIGURE 3 Effect of increasing granule concentration on granule-actin binding. Either secretory granules (●——●) or desoxycholate-prepared granule cores (○——○) were suspended in Tris-saline containing actin 0.25 mg/ml or no addition in the concentrations plotted. After incubation and centrifugation as described in Materials and Methods, the supernate was removed and analyzed for granule protein. Granule binding was computed as the difference in granule sedimentation between tubes without and with actin.

which secretory granule membranes accumulated was changed from the water-1.14 density sucrose interface to just below the 1.14–1.18 density sucrose interface by the addition of actin. Furthermore, the gross appearance of the membranes was also altered, in that they were more "granular" in the presence of actin.

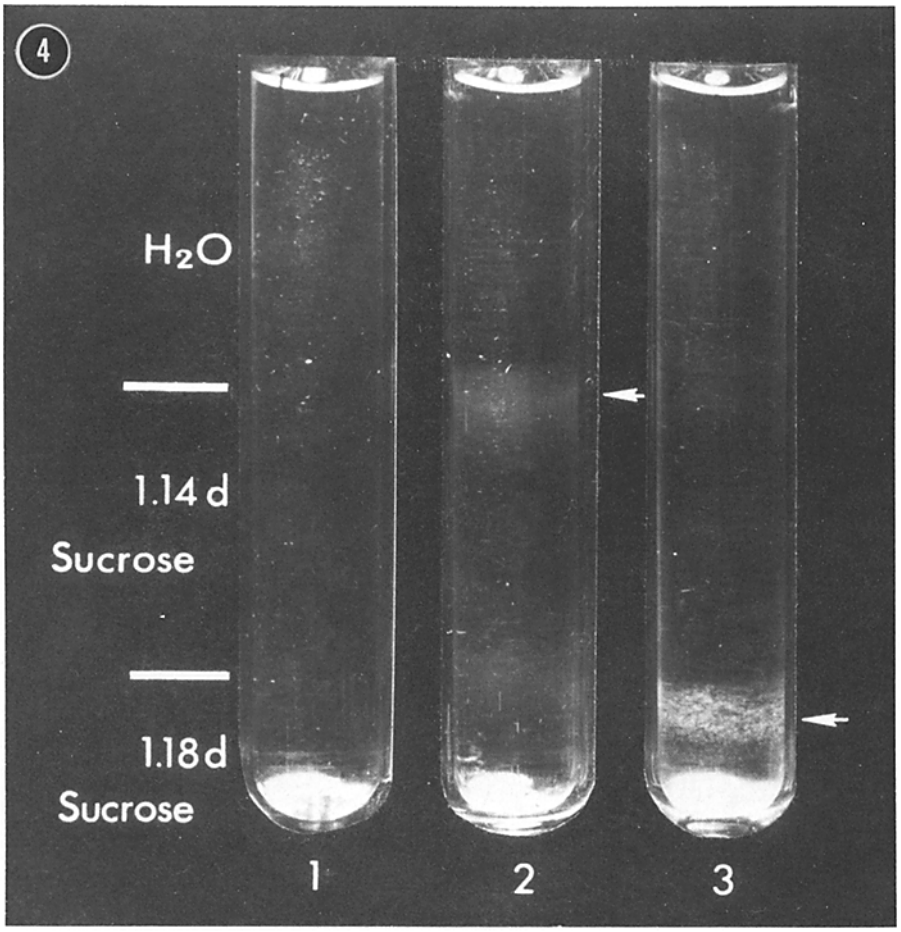

FIGURE 4 Discontinuous sucrose gradient centrifugation of secretory granule membranes in the presence and absence of actin. Secretory granule membranes, 420 μg in sucrose of density 1.06, were mixed with 245 μg rabbit skeletal muscle actin in a final volume of 1.1 ml. After incubation at 37°C for 15 min, the suspension was mixed with 0.99 ml of 1.32 density sucrose to give a final density of 1.18. This was then placed in the bottom of an SW-41 centrifuge tube and overlayered with 5.0 ml of 1.14 density sucrose and 5.5 ml of water. The tubes were centrifuged at 41,000 rpm for 3 h and photographed. Tube *1* contained only actin, tube *2* contained only secretory granule membranes, and tube *3* contained both actin and secretory granule membranes. No visible material appeared in tube *1* (actin), whereas a milky layer of membrane was seen at the H_2O-1.14 density sucrose interface in tube *2* (arrow). In tube *3*, the membranes are found near the 1.14–1.18 density sucrose interface and appear granular.

Isolation of the Secretory Granule-Actin Complex

Filamentous muscle actin does not sediment significantly at 25,000 *g* over 30 min. About 50% of the secretory granules (heavy secretory granules) can be sedimented at 3,000 *g* for 10 min. Consequently, actin and heavy secretory granules should be easily separated if no binding occurs. However, when actin and heavy secretory granules were mixed and centrifuged after incubation at 37°C for 15 min, a significant amount of material sedimented between 3,000 and 25,000 *g* (Table III). Heavy secretory granules were prepared by centrifugation of secretory granules at 3,000 *g* for 10 min and resuspension of the pellet in Tris-saline. Actin was clarified by centrifugation at 25,000 *g* for 30 min on the day of the experiment. Granules and actin were then mixed at final concentrations of 0.5 mg/ml and 0.25 mg/ml, respectively, and incubated at 37°C for 15 min. The tubes were centrifuged at 3,000 *g* for 10 min and the super-

TABLE III

Isolation of Secretory Granule-Actin Complex

	Undiluted			10 times dilution	
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2
		μg/ml		μg/ml	
Actin	46	48	20	40	29
Heavy secretory granules	9	24	40	25	11
Heavy secretory granules + actin	406	288	375	412	186

Isolation of the secretory granule-actin complex. Heavy secretory granules and actin were prepared as described in Results. Secretory granules and actin were mixed in final concentrations of 0.5 and 0.25 mg/ml, respectively, and incubated at 37°C for 15 min. The tubes were centrifuged at 3,000 *g* for 10 min and the pellets discarded. The supernates were centrifuged at 25,000 *g* for 30 min. In exp 1 and 2, an aliquot of the 3,000 *g* supernate was diluted 10-fold with Tris-saline and then centrifuged in tubes identical in geometry to those containing the undiluted specimens. Results are expressed as μg 3,000–25,000 *g* pellet/ml of original incubation mixture.

nates removed. Supernates from tubes containing both granules and actin were quite turbid while those containing granules or actin alone were clear. The supernates were then centrifuged at 25,000 *g* for 30 min and the pellets taken for protein determination and polyacrylamide gel electrophoresis. These 3,000–25,000 *g* pellets (Table III) contained a protein complex (387 ± 40 μg/ml original incubation mixture) when actin and granules were mixed, but little protein was found when either actin (51 ± 14 μg) or secretory granules alone (31 ± 9 μg) were used. To determine whether this complex was stable on dilution, an aliquot of the 3,000 *g* supernate of exp 1 and 2 was diluted 10-fold in Tris-saline and centrifuged at 25,000 *g*, using centrifuge tubes identical in geometry to those used for the undiluted samples. As shown in Table III, this complex did not readily dissociate on dilution. By SDS-polyacrylamide gel electrophoresis, both actin and secretory granule proteins were demonstrated in the granule-actin complex (Fig. 5). The actin content was 28.1 ± 3.5% (SD) of the total protein content (four determinations) and was not significantly affected (24.2%) by dilution (Fig. 5).

Time-Course of Actin-Secretory Granule Binding

Granule binding to actin was studied after incubation of the reaction mixtures at 37°C for 2–30 min, and binding at this temperature was found to be completed within 2 min. However, when samples incubated at 4°C for 15 min were compared to samples incubated at 37°C for the same period, binding was decreased at the lower temperature. After incubation at 4°C, granule sedimentation in the presence of actin was 69.3 ± 9.6% of the actin-free control, whereas at 37°C the sedimentation was 47.8 ± 9.8% of the actin-free control ($P < 0.05$).

Attempts to Disrupt Actin-Secretory Granule Binding

Metal ions (2 mM Mg, 2 mM Ca), 1 mM ethyleneglycolbis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetate (EGTA), 1 mM EDTA, 0.8 M KCl, 5 mM dithiothreitol, and MgATP up to 10 mM did not substantially disrupt actin-granule binding (Table IV). Likewise, the pretreatment of granules with either trypsin up to 1.0 mg/ml or phospholipase C up to 25 μg/ml did not disrupt actin-granule interaction (Table V). Larger concentrations of trypsin (1.0 mg/ml) appeared to enhance binding.

DISCUSSION

When centrifuged at 3,000 *g* for 10 min, most porcine pituitary granules sediment; however, skeletal muscle actin filaments under the same conditions remain in the supernate. When granules and actin filaments are mixed, incubated, and centrifuged together, granule sedimentation is much less than expected (Table I). The granules are bound by filamentous actin and remain in the supernate (Fig. 1). Whether the secretory granule-actin complex floats or sinks depends upon the balance of frictional resistance to sedimentation and the centrifugal field. A relatively low centrifugal force of 3,000 *g* was chosen to minimize the tendency toward sedimentation, and, as a consequence, the granule-actin complex is found in the supernate.

The binding is specific because other particles of similar size, e.g., albumin microspheres, granule

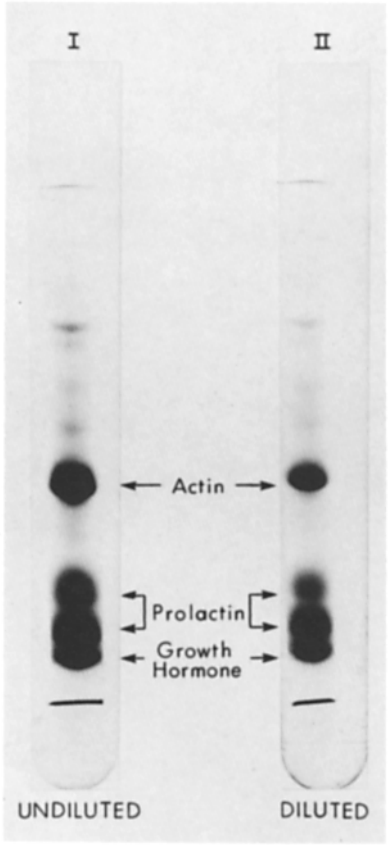

FIGURE 5 Secretory Granule-Actin Complex. (I) Electrophoresis in 0.1% SDS 6% polyacrylamide gels was performed using 100 μg of a 3,000–25,000 *g* pellet from exp 2, Table III. (II) Parallel electrophoresis of 95 μg of the same material collected after dilution of the 3,000 *g* supernate 10-fold in Tris-saline (see Table III, legend). The principal components of secretory granules, which contained negligible amounts of protein co-electrophoresing with actin, were growth hormone and prolactin (split band).

cores (Table II), are affected little or not at all, and because a new species complex composed of actin and secretory granule proteins can be isolated (Table III). If actin simply formed a viscous "net" in which secretory granules were trapped, then all particles of similar size would be prevented from sedimenting and other viscous fibrous proteins such as collagen could replace actin. Such was not the case. In fact, increased viscosity was neither a necessary nor sufficient condition for inhibition of secretory granule sedimentation. A corollary of the actin net hypothesis is that dilution of the actin-secretory granule complex would be expected to interfere markedly with complex formation. However, even after a 10-fold dilution it was still possible to recover the complex quantitatively by centrifugation (Table III).

One of the fundamental properties of actin is its

TABLE IV

Effect of Buffer Conditions on Actin-Granule Binding

Experimental condition	Granule binding + agent / Granule binding − agent
Metal ions	
2 mM $MgCl_2$	0.89 ± 0.36
2 mM $CaCl_2$	1.19 ± 0.02
1 mM EDTA	1.01 ± 0.28
1 mM EGTA	1.09 ± 0.09
0.8 M KCl	0.68 ± 0.11
5 mM Dithiothreitol	0.93 ± 0.05
ATP + $MgCl_2$	
0.5 mM	0.94 ± 0.01
10.0 mM	0.98 ± 0.02

Granule binding to actin was quantitated as in Fig. 3. The effect of various agents studied is expressed as binding in the presence divided by binding in the absence of the specific agent. All values represent the mean ± SE of three or more determinations.

TABLE V

Effect of Granule Pretreatment on Granule-Actin Binding

Pretreatment	Granule binding + pretreatment / Granule binding − pretreatment
	% mean untreated controls
None	104
	87
	109
Phospholipase C (5 μg/ml)	89
Phospholipase C (25 μg/ml)	118
Phospholipase C (25 μg/ml) + lysolecithin (0.5 mg/ml)	112
Trypsin (0.1 mg/ml)	104
Trypsin (1.0 mg/ml)	230

Secretory granules, 2.5–7.5 mg/ml in Tris-saline, were incubated for 10 min at 37°C with either phospholipase C and a final concentration of 2 mM $CaCl_2$ and 0.2% bovine serum albumin or with trypsin. The granules were then washed twice with Tris-saline, sedimented at 41,000 *g* for 20 min, and resuspended in Tris-saline at a final concentration of 0.5–1.5 mg/ml. Actin was added to appropriate tubes at the same concentration. Granule-actin binding was determined for granules with and without pretreatment as described in Fig. 3.

ability to bind a wide variety of proteins, such as myosin, tropomyosin, actinin (a muscle Z-band protein), DNase (11), and the erythrocyte membrane protein spectrin (20). The association of actinlike filaments with lipid structures has also been shown in several ways. For example, actin has been identified in erythrocyte membranes (28), and actinlike filaments have been shown to be associated with mammalian (2, 4, 28, 16) and amoeba (22) plasma membranes as well as with intestinal brush border membranes (14).

The nature of the secretory granule-actin binding appears to be lipophilic. Since trypsin treatment may even enhance the ability of secretory granules to bind actin (Table V), it seems unlikely that a specific membrane protein is present which functions as an actin receptor. Dithiothreitol has no effect, and 0.8 M KCl only minimally decreases the ability of actin to bind secretory granules, suggesting that disulfide and ionic interactions are not major determinants for binding. Since artificial liposomes bind actin, it is apparent that lipid *per se* is sufficient for binding. In fact, all the species of particles which we have studied that bind actin contain membrane or are lipophilic, e.g., styrene-butadiene microspheres, whereas secretory granule cores and albumin microspheres exhibit little or no binding. It appears that phospholipids are not essential since phospholipase C does not affect binding of secretory granules to actin. In summary, our evidence suggests that actin-organelle binding can take place in the absence of protein-protein interaction.

Most cell organelles, e.g., mitochondria, nuclei, endoplasmic reticulum, secretory granules, and vesicles, are bounded by membranes. The ubiquity of actin in nonmuscle cells (23) and the demonstration of actin binding to membrane-bounded organelles offer an explanation for the manner by which organelle movement may be influenced by microfilaments. Our experiments demonstrate that a stationary bundle of actin filaments, such as those of the cell web, could inhibit secretory granule movement in vivo. In this context, it has recently been reported that disruption of the pancreatic B-cell web with cytochalasin B increases the number of secretory granules in apposition to the plasma membrane and increases insulin secretion (17). Another hypothesis is that microfilaments may cause vectorial movement of bound cell organelles by a sliding filament contraction mechanism involving nonmuscle myosin (23). Consistent with this thesis are the observations that myosin is found in secretory tissues such as anterior pituitary (18), and that unidirectional actin filaments have been described in intestinal brush border (14).

It is proposed that binding of membranous organelles to microfilaments may represent a fundamental requirement for many types of intracellular movement, and especially for the secretion of hormones stored in membrane-covered granules or vesicles.

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