

# Interaction of Rat Liver Lysosomal Membranes with Actin

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**ABSTRACT** Membranes were prepared from lysosomes purified 80-fold by centrifugation in a discontinuous metrizamide gradient. When salt-washed membranes were combined with rabbit muscle actin, an increase in viscosity could be measured using a falling ball viscometer. The lysosomal membrane-actin interaction was actin- and membrane-concentration dependent and appeared to be optimal under presumed physiological conditions (2 mM MgCl<sub>2</sub>, 1 mM MgATP, neutral pH, and free calcium concentration less than 10<sup>-8</sup> M). The actin cross-linking activity of the membrane was optimal at pH 6.4. The interaction was maximal between 10<sup>-7</sup> and 10<sup>-9</sup> M free calcium ions and inhibited by ~50% at concentrations of calcium greater than 0.5 × 10<sup>-7</sup> M. The actin-lysosomal membrane interaction was destroyed if the membranes were pretreated with Pronase, or if the membranes were purified in the absence of protease inhibitors. The interaction was not destroyed if membranes were washed with high salt or extracted with KCl and urea. In addition, a sedimentation assay for the actin-lysosomal membrane interaction was also performed to corroborate the viscometry data. The results suggest the existence of an integral lysosomal membrane actin-binding protein.

The cytoplasm of cells is dominated by a three-dimensional matrix of filamentous elements. Electron microscopy distinguishes three major classes of these elements based on their sizes; microtubules (~25-nm diam), intermediate filaments (10 nm), and microfilaments (6 nm). Subcellular organelles, including lysosomes, are embedded within and presumably attached to the cytoskeletal network. The network is not merely a passive scaffold since the various filaments presumably have several active properties and participate in localized events, such as movement of organelles, force generation, and cell division.

Targeting of extracellular macromolecules to lysosomes for digestion or processing begins by endocytosis, a complex process that has received considerable attention (for review see reference 1). Vesicles that are formed from coated pits on the cell surface must move into contact with lysosomes for fusion to occur. In some cell types coated pits appear to line up along actin stress fibers (2), suggesting an anchoring of these structures to the underlying cytoskeletal network. Whether this anchoring has anything to do with the endocytic process is not known.

Endosomes and lysosomes move through the cytosol in a characteristic fashion known as saltatory motion. Thin links can often be seen in electron micrographs connecting these organelles to cytoskeletal filaments. Microfilament bundles and short filaments have been observed to be associated with lysosomes in polymorphonuclear leukocytes (3), prompting

the suggestion that these filaments take part in moving lysosomes. Lysosomal movement may be similar to that of secretory vesicles during translocation to the plasma membrane, a process that is thought to involve microfilaments (4, 5).

In the present study we have examined the association of actin with purified lysosomal membranes using falling ball viscometry (6–8) and sedimentation to quantify the process. A preliminary report of this study has been presented (9).

## MATERIALS AND METHODS

**Lysosomal Membrane Purification:** Liver lysosomes from male Sprague-Dawley rats weighing 200–300 g were purified by isopycnic centrifugation of the light mitochondrial fraction (10) in a discontinuous metrizamide gradient (11). The buffer used to prepare the light mitochondrial fraction was 0.25 M sucrose, 1 mM EDTA, 40 mM HEPES, pH 7.6, with the following protease inhibitors: 5 μM leupeptin, 5 μM pepstatin, 5 μg/ml chymostatin, 5 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. The final pellet and the metrizamide were resuspended in 1 mM EDTA, 5 mM HEPES, pH 7.6 containing the same protease inhibitors and unless otherwise stated all solutions used contained 0.1 mM EDTA and the protease inhibitors described above. The gradients were centrifuged for 2 h at 24,000 rpm in a Beckman SW 28 rotor (Beckman Instruments, Inc.) and 1-ml fractions were collected from the top of the gradient by displacement with Fluorinert (ISCO, Inc., Lincoln, NE).

The gradient fractions were assayed for β-hexosaminidase, succinate dehydrogenase, and leucyl β-naphthylamidase activities to localize lysosomal, mitochondrial, and plasma membrane fractions, respectively (12). The lysosomal fractions lacking succinate dehydrogenase and leucyl β-naphthylamidase activity were pooled and diluted 10–20-fold in 0.1 mM EDTA, 5 mM HEPES, pH 7.6 (low-salt buffer), frozen, and thawed five times in a acetone/dry ice bath

and centrifuged at 80,000 *g* for 2 h. The resulting pellet (unwashed lysosomal membrane) was washed two times with 100–200 ml of low-salt buffer and the final membrane pellet (low-salt washed membrane) was resuspended in ~500  $\mu$ l of low-salt buffer and stored at  $-70^{\circ}\text{C}$ . Lysosome membranes were also isolated from rat liver by the procedure of Ohsumi et al. (30). These membranes, which were used for some of the sedimentation studies, had a similar protein composition to those prepared by the metrizamide gradient (data not shown).

To prepare high-salt washed membranes, a portion of the unwashed lysosomal membrane was treated two times with 100–200 ml of 2 M KCl, 0.1 mM EDTA, 5 mM HEPES, pH 7.6 (high-salt buffer). The pellet was then resuspended in low-salt buffer and washed twice. The high-salt washed membrane preparation was stored in the low-salt buffer at  $-70^{\circ}\text{C}$ . Lysosome latency was measured as described (13). Protein was measured by the Bio-Rad microassay procedure (14) on membranes solubilized with 0.1 M NaOH. Samples were prepared for electron microscopy as described (15).

**Labeling and Electrophoresis of Membrane Protein:** Membrane samples were solubilized with 2% SDS in 100 ml of 10 mM Tris and iodinated with 500  $\mu\text{Ci}$  of carrier-free  $\text{Na}^{125}\text{I}$  (Amersham Corp., Arlington Heights, IL) using the iodogen procedure (16). Unreacted  $^{125}\text{I}$  was removed by passage of the sample over a 1-ml Sephadex G-50 column (17) (10 mM Tris, 1% SDS, pH 7.8). Aliquots of the sample were electrophoresed on 8% polyacrylamide gels in the presence of SDS and 2-mercaptoethanol (18), stained for protein with Coomassie Blue, and autoradiographed using Kodak XR-P-1 film and an intensifying screen. Myosin (200,000),  $\beta$ -galactosidase (116,250), phosphorylase B (92,500), BSA (66,200), and ovalbumin (45,000) were used as molecular weight standards.

**Preparation of Actin:** G-Actin was prepared from an acetone powder of rabbit skeletal muscle with a double cycle of polymerization and sedimentation as described by Spudich and Watt (19). The actin was resuspended in extraction buffer (2 mM Tris, 0.2 mM ATP, 0.5 mM 2-mercaptoethanol, 0.2 mM  $\text{CaCl}_2$ , pH 7.6), dialyzed against the same buffer for 36 h, and clarified by centrifugation at 100,000 *g* for 90 min. The actin preparation was stored on ice at  $4^{\circ}\text{C}$  in the presence of 0.01% wt/vol sodium azide and discarded after 2 d. The actin ran as a single 42,000-mol-wt band on SDS polyacrylamide gels (results not shown).

**Viscosity Measurements:** Viscosity was measured using a low-shear falling ball viscometer (6–8). Unless otherwise indicated the actin-lysosomal membrane interaction was assayed in 50 mM KCl, 20 mM HEPES, 1 mM MgATP, 2 mM  $\text{MgCl}_2$ , 5 mM EGTA ( $[\text{Ca}]_{\text{free}} = 10^{-8}$  M) at pH 6.8 (polymerization buffer). Membrane sample and actin were mixed with polymerization buffer at  $4^{\circ}\text{C}$  (final volume of 50  $\mu$ l), drawn into 100  $\mu$ l capillary tubes, and incubated in a horizontal position at room temperature for 60 min. The falling times of a 0.64 mm stainless steel ball (New Hampshire Ball Bearings, Inc., Petersborough, NH) were converted to apparent viscosities from standard curves obtained using glycerol solutions of known viscosities (8). The apparent viscosities shown are averages of a minimum of four measurements and in some cases five to eight measurements were averaged. Standard errors for all recorded values were  $\leq \pm 5\%$ . In addition, falling times were recorded over three separate 1-cm distances per tube. If inconsistencies in viscosity were noted within the same tube, that sample was not used. A gelled extract is defined as one through which a ball would not fall at a  $90^{\circ}$  angle.

**Sedimentation Assay:** Actin was iodinated with 500  $\mu\text{Ci}$  of carrier-free  $\text{Na}^{125}\text{I}$  (Amersham Corp.) using the iodogen procedure (16). Unreacted  $^{125}\text{I}$  was removed by passage of the sample over a 0.8-ml G-10 column (equilibrated with actin extraction buffer). Lysosome membranes (1.5 mg/ml) were incubated with  $^{125}\text{I}$ -labeled actin ( $\sim 9 \times 10^7$  cpm/mg) in polymerization buffer, pH 6.8. 50  $\mu$ l of the incubation mix was layered over 125  $\mu$ l of 20% sucrose in polymerization buffer, in Beckman Ultra-Clear microcentrifuge tubes (Beckman Instruments, Inc., Palo Alto, CA). After incubation at room temperature for the indicated times, the membranes were sedimented for 15 min at 30 psi in a Beckman airfuge (Beckman Instruments, Inc.). The supernatant was removed by aspiration and the pellets were washed gently with polymerization buffer (50  $\mu$ l). The washed pellets were counted for radioactivity in a Nuclear-Chicago (Tracor Analytic Co., Elk Grove, IL) gamma counter.

**pH Effect:** A different  $\text{Ca}^{++}/\text{EGTA}$  stock solution was prepared for each pH. The free calcium concentration was maintained at  $10^{-8}$  M using 5 mM EGTA and various concentrations of calcium determined from the  $K_{\text{app}}$  for calcium binding by EGTA at each pH as described by Amos (20): pH 6.0,  $1.58 \times 10^{-5}$ ; pH 6.4,  $2.82 \times 10^{-6}$ ; pH 6.8,  $4.73 \times 10^{-7}$ ; pH 7.2,  $8.0 \times 10^{-8}$ ; pH 7.6,  $1.4 \times 10^{-8}$ ; pH 8.0,  $2.24 \times 10^{-9}$ . The pH for each sample was determined prior to the addition of actin and adjusted to the desired pH with NaOH or HCl. The addition of actin had no further effect on the pH.

**Pronase Treatment of Membranes:** High-salt washed lysosomal membranes (260  $\mu\text{g}$ ) were pelleted (100,000 *g*, 45 min) and resuspended in 100  $\mu$ l of 20 mM phosphate buffer, pH 7.2 without protease inhibitors and with 0.4 mg/ml Pronase (Sigma Chemical Co., St. Louis, MO) for 30 min at  $37^{\circ}\text{C}$ . The

incubation was continued for 90 min following the addition of 25  $\mu$ l of 100 mM EDTA, pH 7.5. The proteolytic digestion was further quenched by the addition of diisopropylfluorophosphate (1.33 mg/ml), phenylmethylsulfonyl fluoride (0.3 mg/ml), and the mixture of protease inhibitors described previously. After 15 min at  $37^{\circ}\text{C}$ , the digested membrane sample was centrifuged at 100,000 *g* for 45 min, resuspended in 150  $\mu$ l of the quench solution, and incubated for 15 min at  $37^{\circ}\text{C}$ . This was repeated one additional time. The final pellet was resuspended in 50  $\mu$ l of 5 mM HEPES, pH 7.6, containing diisopropylfluorophosphate and protease inhibitors. This was used directly for actin viscometry assays. Protein determinations using the Bio-Rad microassay indicated an 85% loss of membrane protein.

**Extracted Membranes:** Rat liver lysosome membranes were extracted with 20 mM HEPES, pH 7.6, 2 M KCl, 7 M urea, 0.1 M glycine, 20 mM EDTA, and the protease inhibitors described above following the procedures of Luna et al. (25). The extracted membranes were washed with 5 mM HEPES, pH 7.6, 0.1 mM EDTA, and protease inhibitors. Protein recovery following the KCl-urea-extraction was 88%. NaOH-extracted membranes were prepared as described (32). The membranes were collected in a Beckman SW 56 rotor (Beckman Instruments, Inc.) at 100,000 *g* for 1 h. 3% of the starting membrane protein was recovered in the NaOH-extracted membrane pellet.

## RESULTS

### Purification and Characterization of Lysosomal Membrane

Metrizamide gradient fractionation of rat liver light mitochondrial fraction (10, 11) yielded a lysosomal preparation that was enriched at least 80-fold in specific activity of  $\beta$ -hexosaminidase. Leucyl  $\beta$ -naphthylamidase and succinate dehydrogenase, enzyme markers for plasma membrane and mitochondria, respectively, were not associated with the purified lysosomal fraction. The pooled lysosomal fractions displayed 60–70% latency for  $\beta$ -hexosaminidase activity and morphological examination showed intact organelles that had the appearance of lysosomes (Fig. 1). The electron dense bodies were rounded or elongated with diameters of 250–600

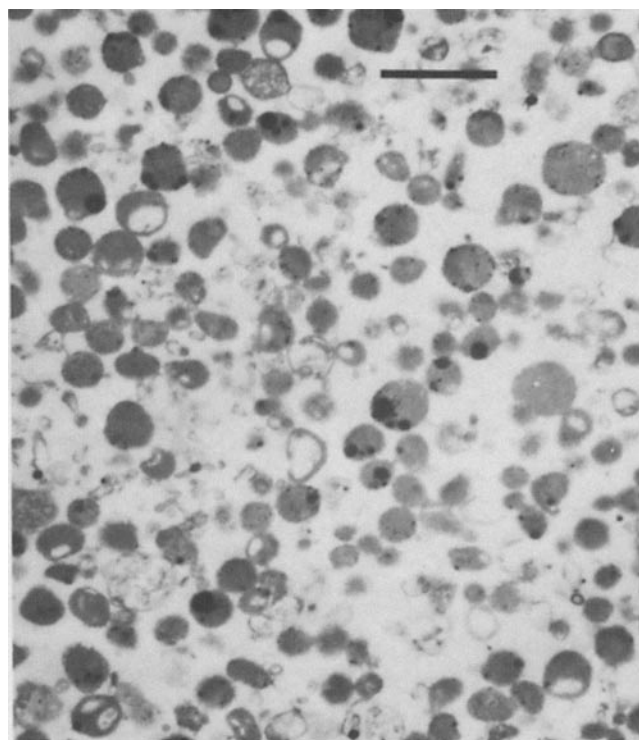


FIGURE 1 Electron micrograph of purified rat liver lysosomes. A sample of the metrizamide gradient purified lysosomal fraction used to prepare lysosomal membrane was centrifuged at 100,000 *g* for 45 min and fixed as described (4). Bar, 1,000 nm.  $\times 30,250$ .

nm, frequently containing vacuoles and vesicles. This fraction appeared to be free of other contaminating organelles. Occasionally, membrane fragments were visible which were probably due to breakage of the fragile lysosomes during purification.

Lysosomal membranes were prepared free of soluble hydrolases and other peripherally bound proteins by repeated washings of frozen and thawed organelles (see Materials and Methods). SDS gel electrophoresis of the low-salt washed  $^{125}\text{I}$ -labeled membranes showed a fairly equal distribution of Coomassie Blue-stained protein bands in the 200,000–45,000-mol-wt region (Fig. 2, lane A). The corresponding autoradiogram of the membrane proteins showed a very similar protein pattern (lane B), implying a general accessibility of lysosomal membrane proteins to iodination. When the electrophoretic pattern of the low-salt washed membrane was compared with high-salt (2 M KCl) washed membrane, staining with Coomassie Blue revealed very little difference in protein distribution between the two preparations (lanes C and D). The high-salt washed membranes showed a slight decrease in the staining of proteins in the 200,000–80,000-mol-wt range, but these proteins were not considerably depleted despite the large number of washes. Autoradiography of the high-salt washed membranes (not shown) revealed very little variability in the protein pattern when compared with the low-salt washed membrane autoradiogram. None of the lysosomal membrane preparations examined by electrophoresis contained a distinctive band around 42,000 mol wt indicating that they are substantially free of endogenous actin.

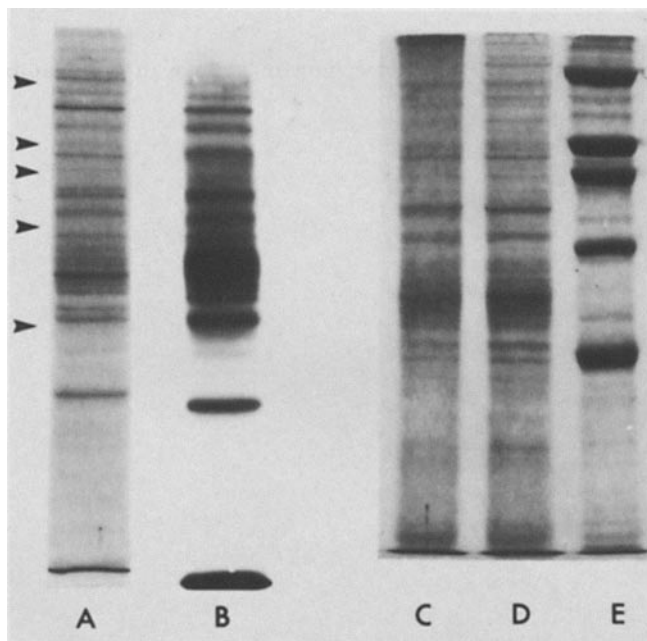


FIGURE 2 Polyacrylamide gel electrophoresis of purified lysosomal membrane. (A) Coomassie-Blue staining of  $^{125}\text{I}$ -labeled, low-salt washed lysosomal membrane proteins (17  $\mu\text{g}$ ). (B) Autoradiogram corresponding to A. (C) Coomassie-Blue staining pattern of high-salt washed lysosomal membrane proteins (18  $\mu\text{g}$ ). (D) Coomassie-Blue staining of the same lysosomal membrane preparation seen in C but treated with low salt only (18  $\mu\text{g}$ ). (E) Molecular weight standards: myosin (200,000),  $\beta$ -galactosidase (116,250), phosphorylase B (92,500), BSA (66,200), and ovalbumin (45,000). Arrows correspond to the position of the molecular weight standards shown in E.

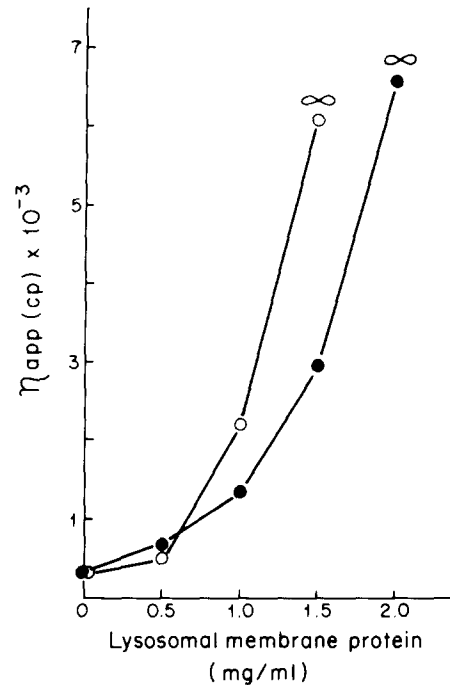


FIGURE 3 Effect of lysosomal membrane protein concentration on actin viscosity. Rabbit muscle G-actin (1 mg/ml) was added to lysosomal membranes in polymerization buffer as described in Materials and Methods. Samples were incubated for 1 h at room temperature prior to viscosity measurements. Viscosities represent averages of four samples.  $\circ$ , high-salt washed membranes;  $\bullet$ , low-salt washed membranes;  $\infty$ , indicates that samples gelled at higher protein concentrations.

### Viscometric Analysis of Lysosomal Membrane-Actin Interaction

The falling ball viscometer was used to determine if there was an interaction between the highly purified lysosomal membranes and actin. When lysosomal membranes and actin were mixed, a marked rise in viscosity was observed that was not seen with either actin or membrane alone. The dependence of the viscosity of the lysosomal membrane-actin mixture on membrane protein concentration can be seen in Fig. 3. The low-salt washed membranes at 1.5 mg/ml considerably increased the viscosity of actin to  $\sim 3,000$  cp with gelation occurring when membrane concentrations were over 2.0 mg/ml. Membranes washed with 2 M KCl also induced a dramatic increase in actin viscosity. High-salt washing of these membranes caused a small increase in the actin cross-linking as compared with low-salt treated membranes. In the absence of actin both the high-salt washed and low-salt washed lysosomal membranes displayed very low viscosities usually in the range of 4–5 cp. When lysosomal membranes that had been prepared in the absence of protease inhibitors were added to actin, no increase in viscosity was seen (results not shown). These results suggested to us that the actin cross-linking factor in the lysosome membranes might be a membrane protein. We therefore examined the effect of Pronase on the actin-lysosomal membrane interaction. When 2.6 mg/ml of high-salt washed lysosomal membrane was pretreated with Pronase and assayed for actin cross-linking activity, an increase in viscosity to only 219 cp was observed. This increase was essentially identical to the viscosity measured for actin alone (230 cp). Thus Pronase treatment caused a dramatic decrease

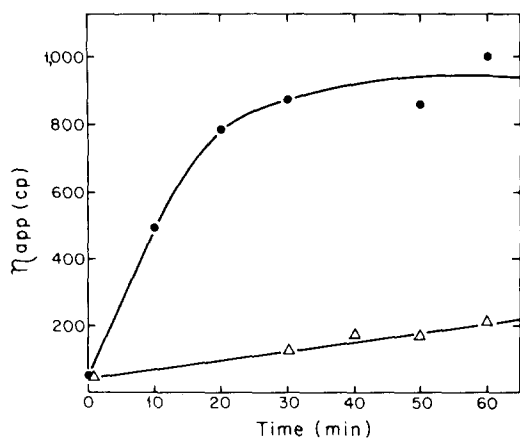


FIGURE 4 Increase in viscosity as a function of time. Low-salt washed lysosomal membrane (1 mg/ml) was mixed with actin (0.8 mg/ml) in polymerization buffer described under Materials and Methods. Samples were incubated at room temperature for the indicated period of time and assayed. ●, low-salt washed lysosomal membrane with actin; Δ, actin alone.

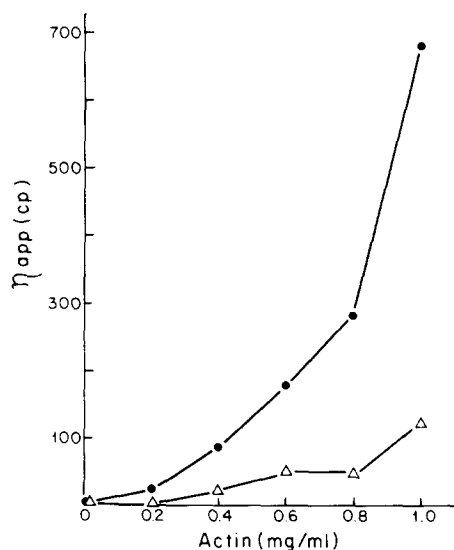


FIGURE 5 Effect of actin concentration on the apparent sample viscosity. Actin at the indicated concentrations was added to 1 mg/ml of low-salt washed lysosomal membrane in polymerization buffer. Samples were incubated for 30 min at room temperature prior to viscosity measurements. ●, lysosomal membrane plus actin; Δ, actin alone.

in cross-linking ability when compared with untreated high-salt washed membranes which caused gelation of actin above 1.5–2.0 mg/ml.

The membrane-actin cross-linking interaction is fairly rapid, attaining half maximal viscosity within 10 min at room temperature (Fig. 4). Maximal viscosities were reached by ~30 min and remained stable with incubations up to 1 h. Viscosities did not increase significantly after 60-min incubation. Fig. 5 illustrates the actin concentration effect on the apparent viscosity of the membrane-actin complex seen after 30 min at room temperature. The increase in viscosity of actin induced by lysosomal membranes was dependent on an increase in the concentration of actin available to bind to the membrane. Viscosity measurements showed variability when measurements taken on different days were compared (for

example, see Figs. 4 and 5: 0.8 mg/ml actin with 1 mg/ml lysosomal membrane). These variations were sometimes due to differences in actin preparations, although even the same actin and lysosome membrane preparations reached different maximal viscosities on different days. Because of these variations a single viscosity value cannot be accurately compared between experiments, however, the overall viscosity trends (i.e., pH optima, time, and concentration dependence, etc.) were all consistent and reproducible even when carried out on different days with different actin and membrane preparations.

The ability of lysosomal membranes to cross-link actin was moderately dependent on the ionic conditions. The actin cross-linking activity of the membranes was optimal at pH 6.4, however there was less than a 50% decrease in viscosity between 6.4 and 7.2 (Fig. 6). This implies that there is a fairly broad physiological pH dependence in the membrane induced actin cross-linking activity. A decrease in viscosity was observed with free  $\text{Ca}^{++}$  concentrations above  $0.5 \times 10^{-7}$  M and partially inhibited when  $\text{Ca}^{++}$  concentrations were below  $0.5 \times 10^{-8}$  M (Fig. 7). The viscosity of actin alone was not significantly affected by these changes in  $\text{Ca}^{++}$  concentration. In contrast, KCl concentrations between 0 and 200 mM at pH 7.3, had no significant effect on the ability of lysosomal membranes to cross-link actin, indicating a negligible KCl dependence (results not shown).

#### Sedimentation Analysis of Actin Binding

To corroborate the falling ball viscometry data, we directly assayed for the binding of actin by lysosomal membranes using a sedimentation technique similar to that previously used to measure the association of actin with chromaffin

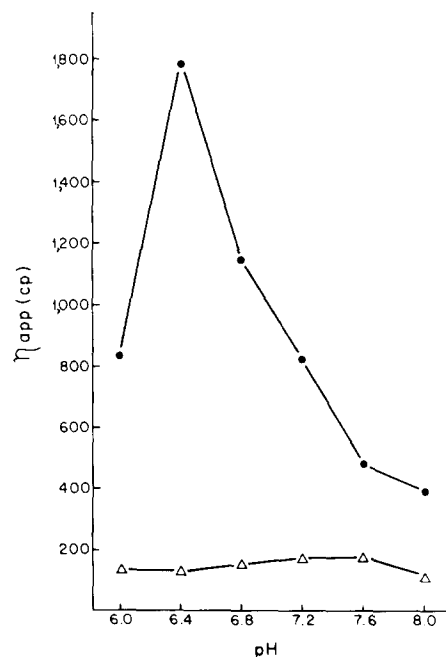


FIGURE 6 pH dependence of the actin cross-linking ability of lysosomal membrane. Actin at a final concentration of 1 mg/ml was added to low-salt washed lysosomal membrane (2.25 mg/ml) in polymerization buffer at the indicated pH. The samples were incubated at room temperature for 1 h. A different  $\text{Ca}^{++}$ /EGTA stock solution was prepared for each pH so that the free  $\text{Ca}^{++}$  concentration would be about  $10^{-8}$  M (see Materials and Methods). ●, lysosomal membranes plus actin; Δ, actin alone.

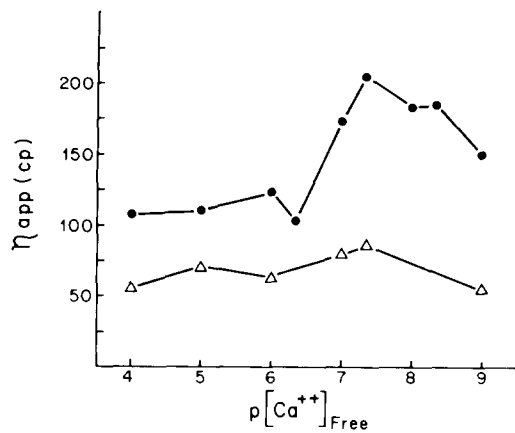


FIGURE 7 Effect of  $\text{Ca}^{++}$  on the interaction of lysosomal membrane with actin. Actin at a final concentration of 1 mg/ml was mixed with 1.5 mg/ml low-salt washed lysosomal membrane in pH 7.0 polymerization buffer at the indicated free  $\text{Ca}^{++}$  concentrations expressed as  $p[\text{Ca}^{++}]_{\text{Free}}$ . Each sample was titrated individually as described in Materials and Methods. ●, low-salt washed lysosomal membrane plus actin; Δ, actin alone.

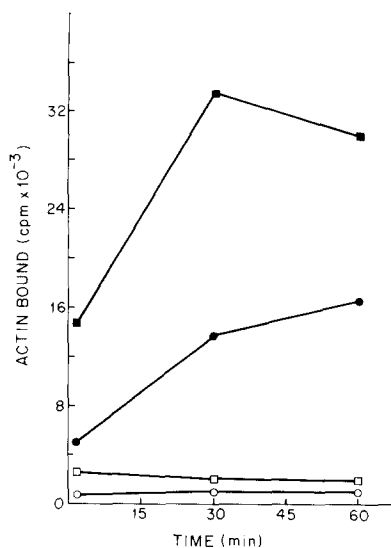


FIGURE 8 Lysosome membrane-dependent sedimentation of  $^{125}\text{I}$ -labeled actin as a function of time. Lysosome membrane (1.5 mg/ml) was mixed with  $^{125}\text{I}$ -labeled actin (0.8 mg/ml or 0.4 mg/ml) in polymerization buffer, pH 6.8 as described in Materials and Methods. ■, actin (0.8 mg/ml) + membranes; □, actin (0.8 mg/ml) alone; ●, actin (0.4 mg/ml) + membranes; ○, actin (0.4 mg/ml) alone. Points are averages of duplicate assays. Variations averaged <5%.

granule membranes (31). Lysosomal membranes bound  $^{125}\text{I}$ -labeled actin in a time- and concentration-dependent manner (Fig. 8). Lysosomal membranes purified on metrizamide gradients (11) and by a bulk isolation procedure (30) displayed similar actin binding properties. Results obtained by sedimentation paralleled those of the viscometry assay in several ways: (a) actin sedimentation was membrane dependent; (b) there was a similar time course; (c) a similar pH optimum (results not shown) and; (d) high salt washing of membranes did not reduce the actin binding. We also carried out the sedimentation assay using lysosomal membranes that had been extracted with KCl, urea, and membranes extracted with NaOH.

KCl, urea-extraction of the membranes caused less than a 30% reduction in actin binding. Extraction of lysosomal membranes with NaOH considerably altered their sedimentation properties resulting in poor recovery of membranes. This was probably due to the removal of 97% of the membrane protein. However the extracted membranes still retained ~20% of their original actin binding activity.

## DISCUSSION

Actin polymer cross-linking by actin binding proteins has been shown in a number of systems to alter the viscoelastic properties of the actin network. A number of these interactions have been measured using low-shear viscometry which allows a semi-quantitative characterization (7, 21). Spectrin, a major peripheral protein of the erythrocyte membrane was shown to be associated with actin in a network that underlies the membrane and is responsible for erythrocyte shape and deformability. In the presence of band 4.1 protein, spectrin can bind actin causing a sharp increase in viscosity of the sample (8, 22–24). The spectrin-actin-band 4.1 gelation was shown to be optimal at ~pH 6.0 at free calcium concentrations between  $10^{-8}$  and  $10^{-7}$  M and inhibited at KCl concentrations above ~10–20 mM.

An actin binding activity associated with *Dictyostelium discoideum* plasma membrane has been described by Luna et al. (25). Large increases in viscosity are seen when membranes depleted of peripheral proteins are incubated with actin. The studies suggest that an integral protein complex associated with the plasma membrane directly interacts with cytoskeletal components and confers on *D. discoideum* the ability to exhibit ameboid movement, endocytosis and exocytosis and maintain cell shape. Unlike the spectrin-actin-band 4.1 interaction, the *D. discoideum*-actin interaction did not appear to be calcium sensitive nor was it particularly sensitive to inhibition by concentrations of KCl above 20 mM (25).

The interaction of cytoskeletal elements with subcellular organelles may be necessary for the translocation of vesicles either to or from the plasma membrane. Actin has been implicated in the movement of chromaffin granules of the adrenal medulla (4, 5). Large increases in viscosity have been measured when purified chromaffin granule membranes and actin are combined. Various ionic parameters (pH, salts, nucleotides) have been shown to influence the extent of the actin-chromaffin granule-membrane interaction under conditions resembling the intracellular environment.

Treatment of fibroblasts with cytochalasin B, which disrupts microfilaments, was shown to block endocytosis and secretion of lysosomal enzymes and block endocytosis of glycosaminoglycans (26). Electron microscopy of polymorphonuclear leukocytes has revealed an apparent lysosome-microfilament interaction and one biochemical study indicated the co-purification of an actin-like inhibitor of DNAase I with partially purified lysosomes from bovine thyroid (27). To our knowledge the present study represents the first direct evidence for a lysosomal membrane-actin interaction. Previous studies of lysosome-cytoskeletal interactions have for the most part centered on the role of microtubules. The addition of the antimicrotubule agents colchicine, vinblastine, and vincristine to cultured fibroblasts reduced the uptake of lysosomal enzymes and sulfated glycosaminoglycans (28). The drugs did not appear to interfere with lysosome function, rather the inhibition appeared to be at the level of lysosome-

endosome fusion. Colchicine and vinblastine inhibited endogenous protein degradation and secretion in rat hepatocytes as well as degradation of exogenously internalized protein (29) also by a mechanism that appeared to involve inhibition of organelle fusion.

The actin cross-linking factor that we have characterized in the lysosomal membrane is presumably an intrinsic membrane protein based on the following observations: (a) The ability of membranes to cross-link actin is not lost with very extensive low or high-salt treatment which depletes membranes of loosely bound soluble factors as well as peripheral membrane proteins. (b) Membranes prepared in the absence of protease inhibitors do not display any actin cross-linking activity. (c) The increases in viscosity of actin induced by lysosomal membranes are not observed if the membranes are pretreated with Pronase. (d) Binding of  $^{125}\text{I}$ -labeled actin to membranes was not lost following a harsh KCl, urea-extraction and only 80% inhibited when greater than 97% of the membrane proteins were removed by a NaOH extraction. The lysosomal membrane-actin interaction shows some similarities to the spectrin-actin-band 4.1 interaction mentioned above, particularly with regard to pH optimum and calcium sensitivity. Unlike the spectrin-band 4.1 system, cross-linking of actin by lysosomal membranes was not dependent on low concentrations of KCl, a property reminiscent of *D. discoideum* (25) and chromaffin granule membranes (5).

It is unlikely that the actin cross-linking activity of the lysosomal membranes was due to the presence of nonspecifically associated actin. We were unable to detect actin in SDS gels of our purified membrane preparations even when the gels were visualized with a sensitive silver staining technique. Furthermore, the lack of cross-linking by membranes either prepared in the absence of protease inhibitors or treated with Pronase suggests that a nonspecific association of these membranes with actin does not occur. In addition, high-salt washing of the lysosomal membranes caused a small increase in actin cross-linking over that seen with low-salt treated membranes, this could have been due to further exposition or accessibility of actin binding sites on the membranes to actin. However, alternative explanations are possible such as an increase in specific activity due to the removal of significant amounts of proteins that do not bind actin. Maximal actin binding to *D. discoideum* plasma membranes occur only if the membranes are first depleted of peripheral proteins (25).

The interaction of lysosomal membranes and actin that we have characterized may be physiologically significant since it is optimal under conditions that are presumably present in the cytosol; however, a definitive biochemical role for a lysosomal membrane-actin binding protein requires additional studies.

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