

# A Membrane Cytoskeleton from *Dictyostelium discoideum*.

## III. Plasma Membrane Fragments Bind Predominantly to the Sides of Actin Filaments

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**ABSTRACT** The binding between sonicated *Dictyostelium discoideum* plasma membrane fragments and F-actin on Sephacryl S-1000 beads was found to be competitively inhibited by myosin subfragment-1. This inhibition is MgATP-sensitive, exhibits a  $K_i$  of  $\sim 5 \times 10^{-8}$  M, and is reciprocal, since membranes inhibit the binding of  $^{125}\text{I}$ -heavy meromyosin to F-actin on beads. These experiments demonstrate that membrane binding and S-1 binding to F-actin on beads are mutually exclusive and, therefore, that the membrane fragments bind predominantly to the sides, rather than to the ends, of the actin filaments. This conclusion is supported by electron micrographs that show many lateral associations between membrane fragments and bead-associated actin filaments. Such lateral associations could play an important role in the organization and lateral movement of membrane proteins by the cytomusculature.

Two types of interactions between membranes and actin filaments have been proposed. The first type is the binding of filament ends to cytoplasmic membrane surfaces, an interaction that has been documented by the decoration of membrane-associated actin filaments with myosin fragments (1, 22, 24). The "arrowheads" of decorated actin filaments associated with plasma membranes from many different cell types point away from the cytoplasmic face of the membrane and toward the center of the cell (1, 10, 22, 24, 35, 39). Therefore, it is often assumed that the physiologically preferred mode of attachment involves interactions between membranes and the "barbed" ends of actin filaments. However, a second type of F-actin-membrane interaction, the binding between membranes and the sides of actin filaments, has recently attracted attention. This mode of attachment is supported by electron micrographic observations of membranes associated with undecorated actin filaments (20, 34, 35, 41), by the magnitude of the viscosity increases induced by membrane vesicles in solutions that contain F-actin (15, 16, 28), and by the observation of lateral associations between F-actin and membrane-associated actin-binding proteins in solution (6, 7, 14, 21, 23).

The present study was initiated to explore the mode of attachment of F-actin to the plasma membranes of the cellular slime mold, *Dictyostelium discoideum*. In the preceding paper (29), we demonstrated that acetamidofluorescein-F-actin on antfluorescein Sephacryl S-1000 beads (F-actin beads) specif-

ically binds  $^{125}\text{I}$ -labeled, sonicated membrane fragments from *D. discoideum*. Since myosin fragments also interact specifically with F-actin beads (31), we examined the effect of myosin fragments on the interactions between F-actin beads and sonicated *D. discoideum* plasma membranes. We found that myosin subfragment-1 (S-1)<sup>1</sup> and sonicated membranes compete for mutually exclusive binding sites along the sides of actin filaments. This result suggests a mechanism for lateral movement of membrane proteins. Furthermore, we suggest that caution should be exercised in the interpretation of electron microscopic images of decorated actin filaments associated with membranes.

This work was presented in preliminary form at the Annual Meeting of the American Society for Cell Biology in November, 1982 (30).

### MATERIALS AND METHODS

**Chemicals:** Dithiothreitol (DTT), BSA, imidazole (Grade I), and Tris, were supplied by Sigma Chemical Co. (St. Louis, MO). Ovalbumin was secured from Worthington Biochemical Corp. (Freehold, NJ). Ultrapure ammonium sulfate was obtained from Schwarz/Mann, Div. of Becton Dickinson (Orangeburg, NY) and fluorescein-5-isothiocyanate was obtained from Molecular Probes (Junction City, OR). Both Sephacryl S-1000 and Sephadex G-25 were

<sup>1</sup> *Abbreviations used in this paper:* Con A, concanavalin A; DTT, dithiothreitol; HMM, heavy meromyosin; S-1, myosin subfragment-1.

purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Other reagents used were procured from the sources listed in the accompanying paper (29).

**Myosin and Myosin Fragments:** Heavy meromyosin (HMM) and S-1 were produced by chymotryptic digestion of rabbit skeletal muscle myosin as reported previously (31). 1 mM ATP was routinely added to the HMM in storage buffer to improve its shelf life. Both 1 mM ATP and 1 mM DTT were added to the S-1 before storage. Occasionally, S-1 and HMM were stored as precipitates in 50% saturated ammonium sulfate, 5 mM EDTA, 10 mM KCl, 0.5 mM DTT, 0.01% sodium azide, 5 mM imidazole-HCl, pH 7.0. Before use, DTT, ATP, and ammonium sulfate were removed from the myosin fragments by dialysis against a buffer appropriate to assay requirements. HMM and S-1 were used within 2 wk of preparation to ensure maximum binding activity.

**Sedimentation Binding Assays:** Myosin fragments, F-actin beads (29), and sonicated, purified *D. discoideum* plasma membrane fragments from which concanavalin A (Con A), actin, and myosin had been removed (29) were used in binding assays for kinetic analysis of the interactions among these components. For some of the controls, we used antiluorescein IgG-Sepharyl S-1000 beads bound to ovalbumin labeled with fluorescein-5-isothiocyanate. These beads, as well as F-actin and control beads (antiluorescein IgG-Sepharyl S-1000 without bound fluorescent protein) were stored as a 25% suspension in 50 mM KCl, 1 mM MgCl<sub>2</sub>, 20 mM Tris-acetate, pH 7.0 (Buffer A) containing 0.5 mM MgATP, 0.5 mM DTT, 0.02% NaN<sub>3</sub>, and 1–20 μM phalloidin. In general, <sup>125</sup>I-labeled, sonicated plasma membrane fragments (29) were used in sedimentation assays with beads and unlabeled myosin fragments. In some assays, <sup>125</sup>I-labeled HMM was mixed with beads and unlabeled membranes. HMM was radiolabeled with [<sup>125</sup>I]Bolton-Hunter reagent (2,000 Ci/mmol, Amersham Corp., Arlington Heights, IL; reference 3) as previously described (31), desalted into Buffer A over Sephadex G-25, and stored in Buffer A containing 0.4 mM ATP, 0.4 mM DTT, and 0.02% NaN<sub>3</sub>. Binding assays were performed as described previously (29). Incubation buffers (total volume, 110 μl) were as described in the figure legends. Beads, either S-1 or HMM, and sonicated membranes were mixed in rapid succession at 0°C in plastic test tubes. Mixtures were incubated at 300 rpm on a gyrotory shaker for 2 h at 21–23°C.

To ensure that actin remained associated with beads in the presence of S-1, we analyzed F-actin beads with bound S-1 by SDS polyacrylamide gels (SDS PAGE) after performing a binding assay similar to those described above. 6 μl of a 25% suspension of F-actin beads were mixed at 0°C with 0–44 μl of 500 μg/ml S-1 in Buffer A containing 0.02% NaN<sub>3</sub>. The reaction mixture (total volume, 110 μl) consisted of S-1, 47.5 μg/ml of bead-bound F-actin, Buffer A, 1.5 μM phalloidin, 27 μM ATP, 27 μM DTT, 0.001% NaN<sub>3</sub>, and 45 μg/ml BSA. After a 2-h incubation at 21–23°C, 100 μl of the reaction mixture was layered onto 250 μl of 5% sucrose in Buffer A as described previously (31) and centrifuged at 4,124 g for 5 min at 21–23°C. The top 100 μl from each tube (supernatant) was removed and saved for SDS PAGE. After the remaining solution was discarded, the walls of the tubes were gently dried with a tissue and the bead pellets were resuspended in 50 μl of Buffer A. Pellets and supernatants were denatured in gel sample buffer (12) and heated for 10 min at 70°C. All of each supernatant or pellet was loaded onto a gel lane.

**Electron Microscopy:** Con A-stabilized plasma membranes with associated actin and myosin were isolated from *D. discoideum* essentially as described (29) except that the Sorensen's buffer and the 20 mM sodium phosphate (Buffer B) were titrated to pH 7.0. For S-1 decoration of membrane-associated actin, a 100-μl aliquot of freshly prepared membranes was mixed at 0°C with S-1 in Buffer A such that the final concentration of S-1 was 0.5 mg/ml. A duplicate membrane aliquot was mixed with a similar volume of Buffer A without S-1. Samples were incubated at 21–23°C for 1½ h with occasional mixing and centrifuged at 21,450 g for 10 min through 250 μl of 5% sucrose in Buffer B. The resulting pellets were processed for transmission electron microscopy as described (29). Samples of both the supernatants and the pellets were retained and denatured for SDS PAGE as described above.

F-actin beads or an equal volume of control beads were incubated for 1½ h at room temperature with S-1 or with sonicated plasma membrane fragments, or with both membranes and S-1, or with buffer alone. The reaction buffer was Buffer A containing 0.2 mg/ml BSA, 1 μM phalloidin, and 4 mM sodium phosphate. The final concentrations of S-1, membranes, and bead-bound F-actin were 0.5, 0.1, and 0.24 mg/ml, respectively. Beads were centrifuged at 4,124 g for 5 min through 5% sucrose in Buffer B and processed for transmission electron microscopy. No problems were experienced with the beads during processing although plastic infiltration was carried out for 4 d under vacuum to insure an even matrix within the bead. Sepharyl S-1000 beads sectioned well with a diamond knife and were reasonably stable under an electron beam.

**Other Methods:** The concentrations of the assay components were determined prior to mixing by various methods. Membrane protein was determined in the presence of 1% SDS by the procedure of Lowry et al. (27); BSA was used as a standard. To quantify the amount of bead-bound proteins, we

pipetted samples of control beads, F-actin beads, ovalbumin beads, or their supernatants into separate tubes and heated them to 70°C for 1 h in 5% SDS. After total protein was measured (27), the concentration of bead-bound actin or ovalbumin was estimated by difference. The concentrations of S-1 and HMM were usually determined by ultraviolet absorption at 280 nm, assuming the following absorptivity constants: 750 cm<sup>2</sup>/g for S-1 (42) and 650 cm<sup>2</sup>/g for HMM (44). S-1 concentrations were occasionally measured by the Lowry procedure (27); concentrations determined by the two methods correlated well.

6–16% linear gradient SDS slab gels were prepared and run according to the method of Laemmli (25). Details were previously reported (28).

## RESULTS AND DISCUSSION

### S-1 Inhibits Binding of *D. discoideum* Plasma Membrane Fragments to F-Actin Beads

In sedimentation assays containing F-actin beads and <sup>125</sup>I-labeled, sonicated plasma membrane fragments, the inclusion of S-1 substantially inhibits the binding of the membranes to bead-bound F-actin (Fig. 1). This inhibition is caused by a specific interaction between S-1 and F-actin since it is reversed by MgATP, an agent that dissociates actin-S-1 complexes (17, 40). MgATP, in the absence of S-1, does not affect the binding of sonicated membranes to F-actin beads (Fig. 1). Inhibition is not due to the presence of residual chymotrypsin in our highly purified S-1 since chymotryptic digestion of the membranes or bead-bound F-actin would not be MgATP sensitive.

The reduction in binding of <sup>125</sup>I-labeled, sonicated membrane fragments to F-actin beads observed in the presence of

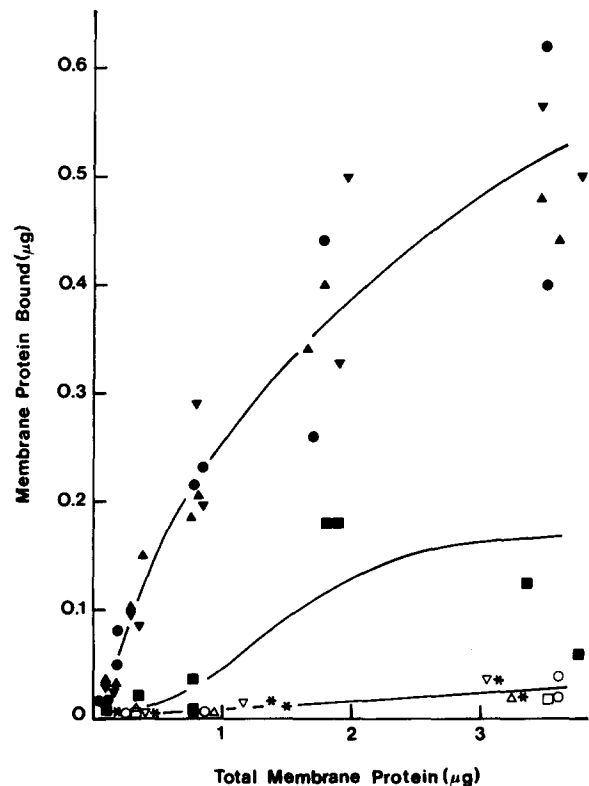


FIGURE 1 Binding curves of <sup>125</sup>I-labeled, sonicated plasma membrane vesicles to antiluorescein beads containing 0.78 μg of bound fluorescein-conjugated F-actin (solid symbols), 0.1 μg of bound fluorescein-conjugated ovalbumin (asterisks), or no bound fluorescent protein (open symbols). Assays contained either beads alone (●, ○) or 2.3 mM MgATP (▲, △), 23.9 μg of S-1 (■, □), or 2.3 mM MgATP and 23.9 μg of S-1 (▼, ▽). Assay mixtures also included Buffer A, 0.54 μM phalloidin, 45 μM ATP, 45 μM DTT, 0.002% NaN<sub>3</sub>, and 0.45 mg/ml ovalbumin.

S-1 is not caused by an S-1-mediated release of actin from the beads. SDS PAGE of pellets and supernatants from sedimentation assays containing S-1 and F-actin beads shows that the amount of actin in the supernatant does not increase as the beads become saturated with S-1 (Fig. 2). Similar analyses of sedimentation assays containing sonicated membrane fragments and F-actin beads show that the membranes also are incapable of releasing actin from the beads (not shown).

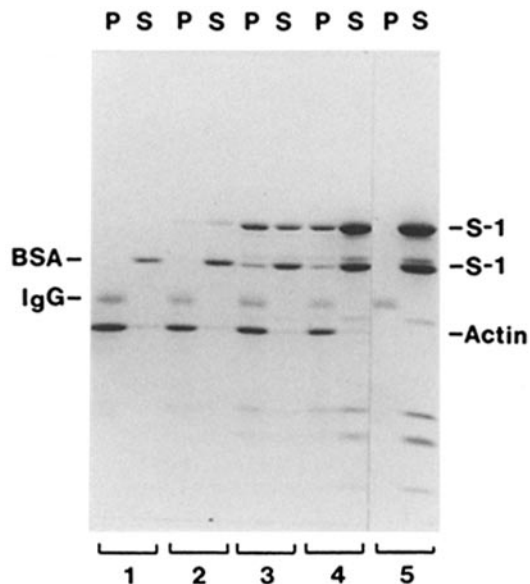


FIGURE 2 SDS PAGE of pellets (P) and supernatants (S) from sedimentation assays containing 5.3  $\mu\text{g}$  of bead-bound F-actin. F-actin beads alone (lane 1) or F-actin beads plus 2.5  $\mu\text{g}$  (lane 2), 11  $\mu\text{g}$  (lane 3), or 22  $\mu\text{g}$  of S-1 (lane 4). SDS PAGE of the pellet and supernatant from an assay containing control beads and 22  $\mu\text{g}$  of S-1 (lane 5). Actin, BSA, S-1, and IgG heavy chain are labeled. A component of the S-1 preparation co-migrates with BSA.

### Myosin Fragments and Membrane Vesicles Compete for Common Binding Sites on F-Actin Beads

Both unlabeled HMM (not shown) and unlabeled S-1 (Fig. 3a) compete the binding between  $^{125}\text{I}$ -labeled, sonicated membrane fragments and F-actin beads to levels just above those observed with control beads. When averaged data from the low ends of these competition curves are plotted according to the method of Dixon (Fig. 3b; reference 9), apparent inhibition constants ( $K_i$ s) are obtained that can be compared with reported equilibrium constants for the S-1 association with F-actin. For example, the apparent  $K_i$  in Fig. 3b is  $\sim 5 \pm 3 \mu\text{g}/\text{ml}$  or  $\sim 4 \pm 2 \times 10^{-8} \text{ M}$ , assuming a molecular weight of 120,000 for S-1. In independent determinations, we have obtained the following approximate  $K_i$ s:  $2.5 \times 10^{-8}$ ,  $4 \times 10^{-8}$ , and  $8 \times 10^{-8} \text{ M}$ . These values are consistent with the dissociation constant ( $K_d$ ) for the interaction between S-1 and F-actin. Using various techniques and assay conditions, other researchers have measured the following  $K_d$ s for S-1 binding to actin:  $2 \times 10^{-8} \text{ M}$  (33),  $5 \times 10^{-8} \text{ M}$  (43),  $20 \times 10^{-8} \text{ M}$  (18),  $30 \times 10^{-8} \text{ M}$  (32), and  $50 \times 10^{-8} \text{ M}$  (19). The differences among these reported  $K_d$ s are probably due to the different temperatures, ionic strengths, and divalent cation concentrations used by the different experimenters.

The excellent agreement between published equilibrium constants and the apparent  $K_i$ s obtained from competition experiments (Fig. 3) suggests that the binding of S-1 to F-actin beads is directly responsible for the diminution of membrane binding to these beads. However, experimental error and the low  $1/B_{\text{max}}$  for the membrane interaction with F-actin beads preclude the use of Dixon plots to distinguish between the various types of binding inhibition. To ascertain whether S-1 is a true competitive inhibitor of membrane binding to F-actin beads, we measured the binding of  $^{125}\text{I}$ -labeled, sonicated membrane fragments to F-actin beads in the presence

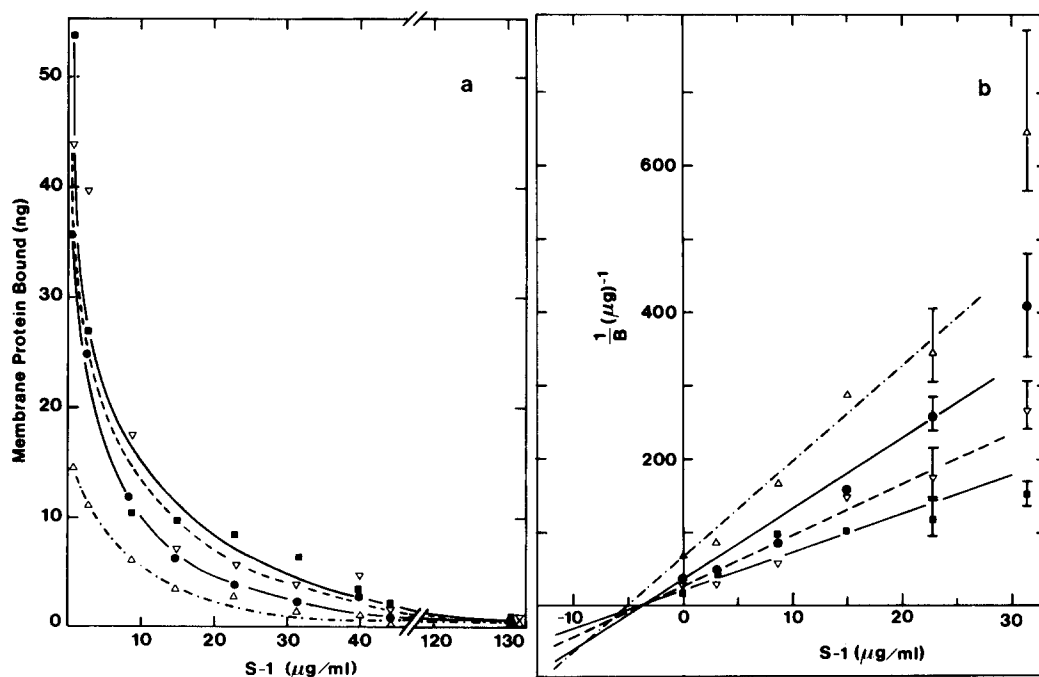


FIGURE 3 Competitive displacement by S-1 of  $^{125}\text{I}$ -labeled membrane binding to F-actin beads. (a) 0.3  $\mu\text{g}$  of bead-bound F-actin incubated with increasing amounts of S-1 and different, fixed amounts of membrane protein: 1.78  $\mu\text{g}$  (■—■), 1.34  $\mu\text{g}$  (▽—▽), 0.83  $\mu\text{g}$  (●—●), or 0.4  $\mu\text{g}$  (△—△) of membrane protein. Assay mixtures contained Buffer A, 2.27  $\mu\text{M}$  phalloidin, 2.3  $\mu\text{M}$  ATP, 45  $\mu\text{M}$  DTT, 0.002%  $\text{NaN}_3$ , and 0.45 mg/ml ovalbumin. Each data point is an average of two independent assays. Nonspecific binding to control beads has been subtracted. (b) Averaged data from a plotted according to Dixon (9). Error bars represent reciprocals of individual data

points. The error bars that were omitted for the sake of clarity are no larger than those shown and tended to decrease in size with decreasing  $1/B$ .  $B$ , membrane protein bound. The apparent  $K_i$  is  $\sim 5 \pm 3 \mu\text{g}/\text{ml}$  or  $\sim 4 \pm 2 \times 10^{-8} \text{ M}$ .

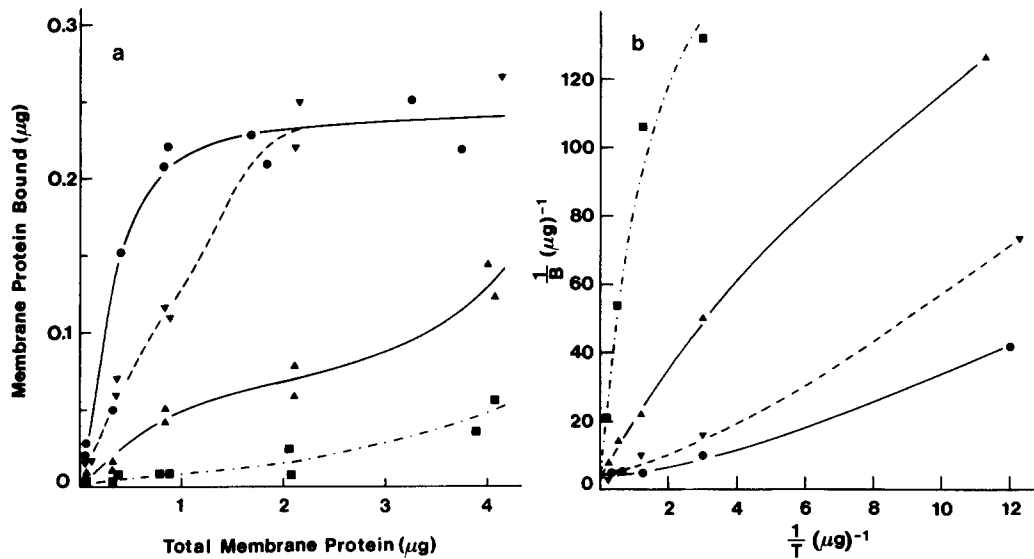


FIGURE 4 Binding curves of  $^{125}\text{I}$ -labeled, sonicated plasma membranes to F-actin beads in the presence of myosin S-1. (a) 0.8  $\mu\text{g}$  F-actin incubated with increasing amounts of membrane protein and either without S-1 ( $\bullet$ ) or with different, fixed concentrations of S-1: 3.4  $\mu\text{g}$  ( $\blacktriangledown$ ), 7.8  $\mu\text{g}$  ( $\blacktriangle$ ), or 27  $\mu\text{g}$  ( $\blacksquare$ ) of S-1. Other assay components were Buffer A, 0.55  $\mu\text{M}$  phalloidin, 45  $\mu\text{M}$  ATP, 45  $\mu\text{M}$  DTT, 0.002%  $\text{NaN}_3$ , and 0.45 mg/ml ovalbumin. Nonspecific binding to control beads has been subtracted. (b) Averaged data from a plot-

ted according to Lineweaver and Burk (26). In general, error bars approximate the size of the symbols and have been omitted.  $B$ , membrane protein bound;  $T$ , total membrane protein.

of different, fixed concentrations of S-1 (Fig. 4a). In the presence of low concentrations of S-1, inhibition is observed with low amounts, but not with relatively large amounts, of added  $^{125}\text{I}$ -labeled, sonicated membrane fragments. As the S-1 concentration is increased, ever larger amounts of membranes must be added before the S-1-induced inhibition is overcome (Fig. 4a).

When averaged data from binding curves such as those in Fig. 4a are plotted according to the method of Lineweaver and Burk (26), curves are obtained that intersect on the  $y$ -axis at  $1/B_{\text{max}}$  (Fig. 4b). This result indicates that S-1 and membranes do compete for the same, or overlapping, sites on F-actin (38). In addition, the curvature changes observed with increasing S-1 concentration suggest that at least one other phenomenon may occur in this system. One possibility is that S-1 reduces the apparent Hill coefficient (29) of the F-actin-membrane interaction from  $\sim 1.1$ – $1.2$  to  $< 1.0$ . A more likely possibility is that interactions between F-actin and different membrane fragments, or proteins within a membrane fragment, are unequally sensitive to inhibition by S-1 (38). Specifically, the inhibition of binding induced by S-1 (Figs. 1, 3, and 4) may have both competitive and noncompetitive components.

As expected when two ligands compete for the same or overlapping sites, we observe competition for binding sites regardless of which ligand is radiolabeled. Although  $^{125}\text{I}$ -labeled S-1 binds poorly to F-actin either in solution or on beads (not shown),  $^{125}\text{I}$ -labeled HMM with only one label per molecule binds to F-actin beads with saturation kinetics (31). The binding of  $^{125}\text{I}$ -labeled HMM to F-actin beads is substantially reduced by the inclusion of unlabeled, sonicated membrane fragments in the assay (Fig. 5). Since about half of the  $^{125}\text{I}$ -labeled HMM binding is competed by sonicated membrane fragments and about half the binding is not (Fig. 5), this inhibition of binding also appears to have both competitive and noncompetitive components (see reference 38). However, the noncompetitive component in this situation is probably an artifact caused by the differential penetration of  $^{125}\text{I}$ -labeled HMM and sonicated membrane fragments into the bead interiors (see below). In general, when using gel filtration

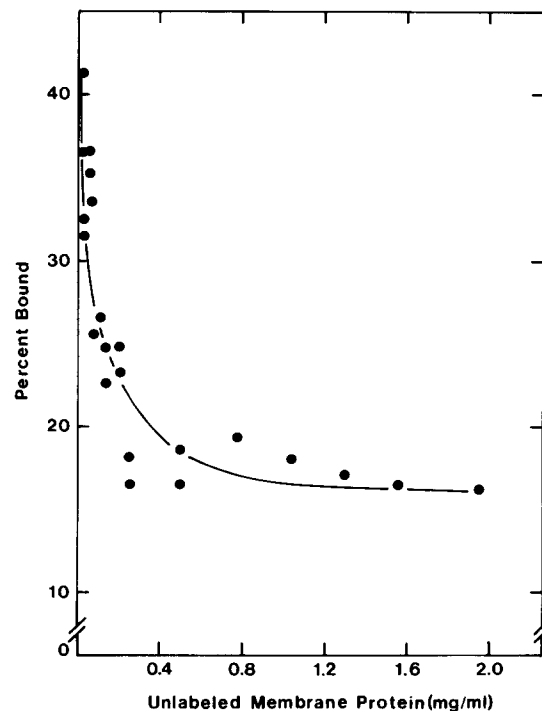


FIGURE 5 Competitive displacement of  $^{125}\text{I}$ -labeled HMM binding to F-actin beads by unlabeled, sonicated plasma membrane fragments. Assay mixtures contained 2.8  $\mu\text{g}$  of  $^{125}\text{I}$ -labeled HMM, 0.7  $\mu\text{g}$  of bead-bound F-actin, Buffer A, 1.1  $\mu\text{M}$  phalloidin, 6.8  $\mu\text{M}$  ATP, 68  $\mu\text{M}$  DTT, 0.003%  $\text{NaN}_3$ , and 0.45 mg/ml ovalbumin. Nonspecific binding to control beads has been subtracted.

beads as affinity matrices in competition studies, it is obvious that small molecules will compete more effectively with large structures than will large structures with small molecules.

### Electron Microscopy

To visualize the interactions between F-actin, S-1, and plasma membrane fragments, we present transmission elec-

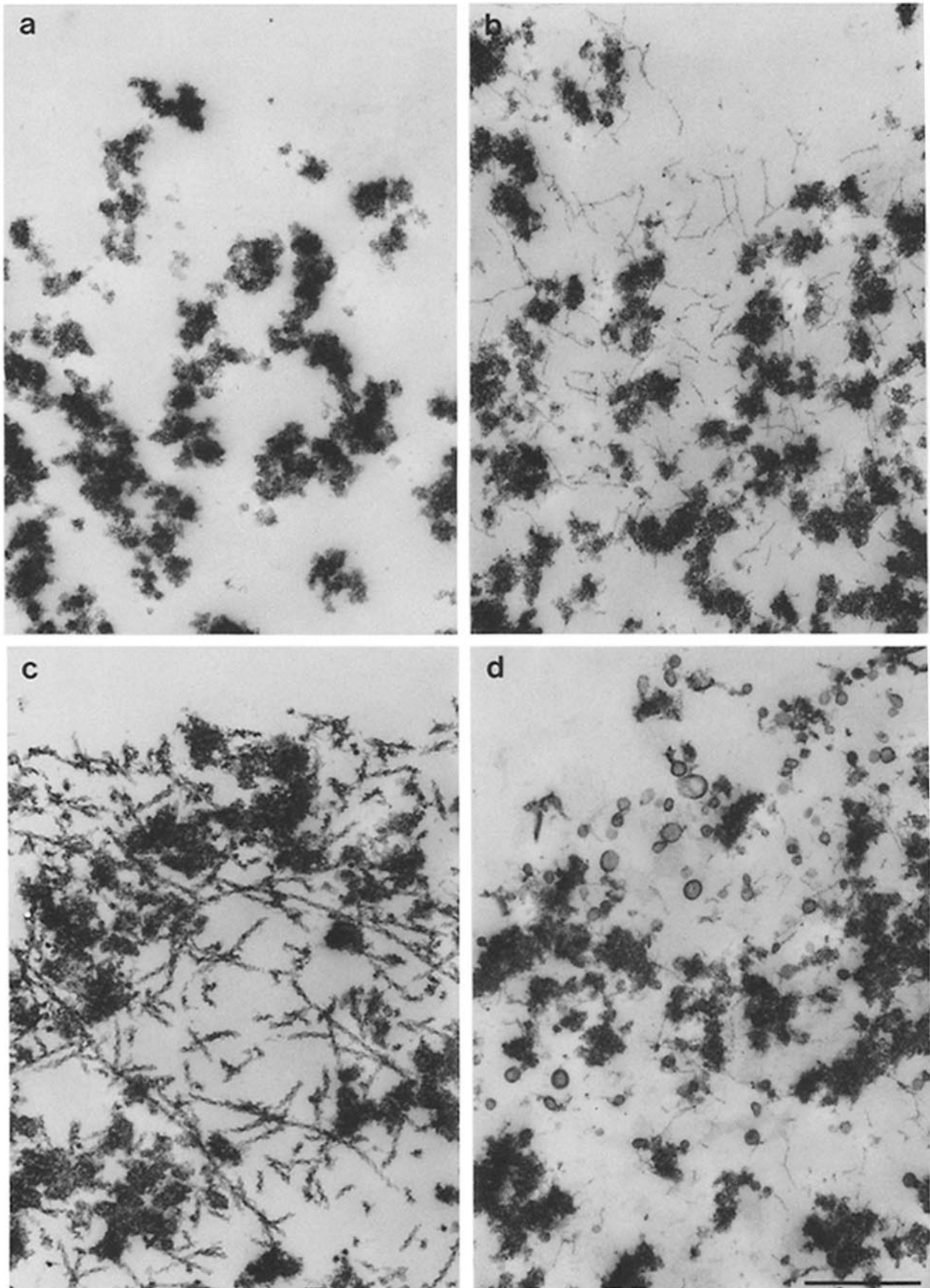


FIGURE 6 Transmission electron micrographs of the surfaces of antifluorescein IgG-Sephacryl S-1000 beads taken from the same experiment. (a) Control bead, (b) F-actin bead, (c) F-actin bead with bound S-1, and (d) F-actin bead with bound plasma membrane vesicles. The external surfaces of the beads are at the tops of the micrographs. Bar,  $0.5 \mu\text{m}$ .  $\times \sim 40,000$ .

tron micrographs of anti fluorescein IgG-Sephacryl S-1000 beads bound to one or more of these moieties (Figs. 6 and 7). Thin sections of control beads show scattered regions of electron-dense, amorphous material surrounded by extensive electron-lucent pores (Fig. 6*a*). In sections of F-actin beads, filaments are observed weaving around the electron-dense regions and extending into the bead pores (Fig. 6*b*). Only an occasional filament extends beyond the bead periphery. When S-1 is added to F-actin beads, the filaments in the pores assume the "arrowhead" appearance characteristic of S-1-decorated actin filaments (Fig. 6*c*). After the addition of sonicated plasma membrane fragments to undecorated F-actin beads, vesicular and linear membrane profiles are observed at or near the bead surface, apparently in close association with the actin filaments (Fig. 6*d*). Control beads bind relatively few membrane fragments (not shown).

Although many associations between sonicated membrane fragments and the sides of actin filaments are observed in micrographs of beads containing undecorated F-actin (Fig. 6*d*), most of the F-actin-membrane interactions are ambiguous with respect to F-actin orientation, probably because the actin filaments are weaving in and out of the plane of section. The orientation of membranes on F-actin is more readily identified when F-actin beads are incubated with both membranes and S-1 at concentrations such that membranes and S-1 both bind to the F-actin on the beads (Fig. 7). Near the surface of such a bead (Fig. 7*a*), many plasma membrane fragments are observed bound to the decorated actin filaments. While some membrane fragments may be bound to filament ends, many more membrane fragments appear to

bind along the sides of the filaments (Fig. 7, *a* and *inset*). In the core of the bead (Fig. 7*b*), few membrane fragments are observed although the actin filaments are completely decorated with S-1. This observation supports the hypothesis, proposed above, that myosin fragments and membrane fragments penetrate the bead to different extents.

#### *F-Actin Associations with Intact D. discoideum Plasma Membranes*

Using sonicated plasma membrane fragments and phalloidin-stabilized, rabbit F-actin on anti fluorescein IgG Sephacryl S-1000, we have shown that most of the binding between these membranes and the F-actin can be inhibited by S-1. To show that this observation is applicable to native associations between plasma membranes and actin, we present electron micrographs of freshly prepared *D. discoideum* plasma membranes before (Fig. 8*a*) and after (Fig. 8*b*) decoration with S-1. These plasma membrane domains, obtained by Con A stabilization and detergent extraction, are associated with endogenous actin and myosin (8, 29, 37). Electron micrographs of undecorated plasma membrane domains show filamentous material in close association with the cytoplasmic surfaces of the membranes (Fig. 8*a*). In contrast, the external membrane surfaces, coated with Con A and visualized as described in the accompanying paper (29), are not associated with the filamentous material (Fig. 8*a* and reference 8). After decoration with S-1, little or no endogenous actin is released from the membranes in sedimentation assays, as quantified by SDS PAGE (not shown), but far fewer contacts are ob-

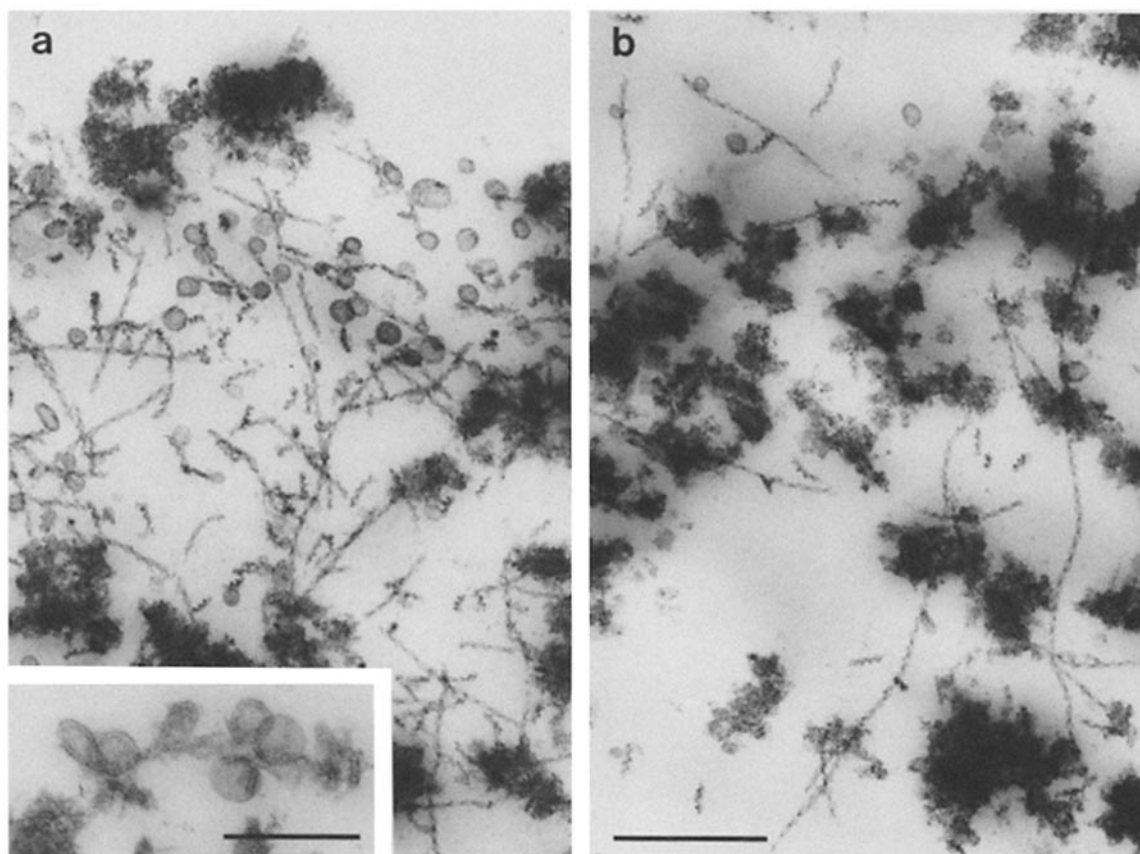


FIGURE 7 Transmission electron micrograph of the surface (a) and interior (b) of an F-actin bead after incubation with both S-1 and sonicated plasma membrane fragments. Bar, 0.5  $\mu\text{m}$ .  $\times \sim 40,000$ . The inset shows a region of the surface of a similar bead at a higher magnification. Bar, 0.2  $\mu\text{m}$ .  $\times \sim 100,000$ .



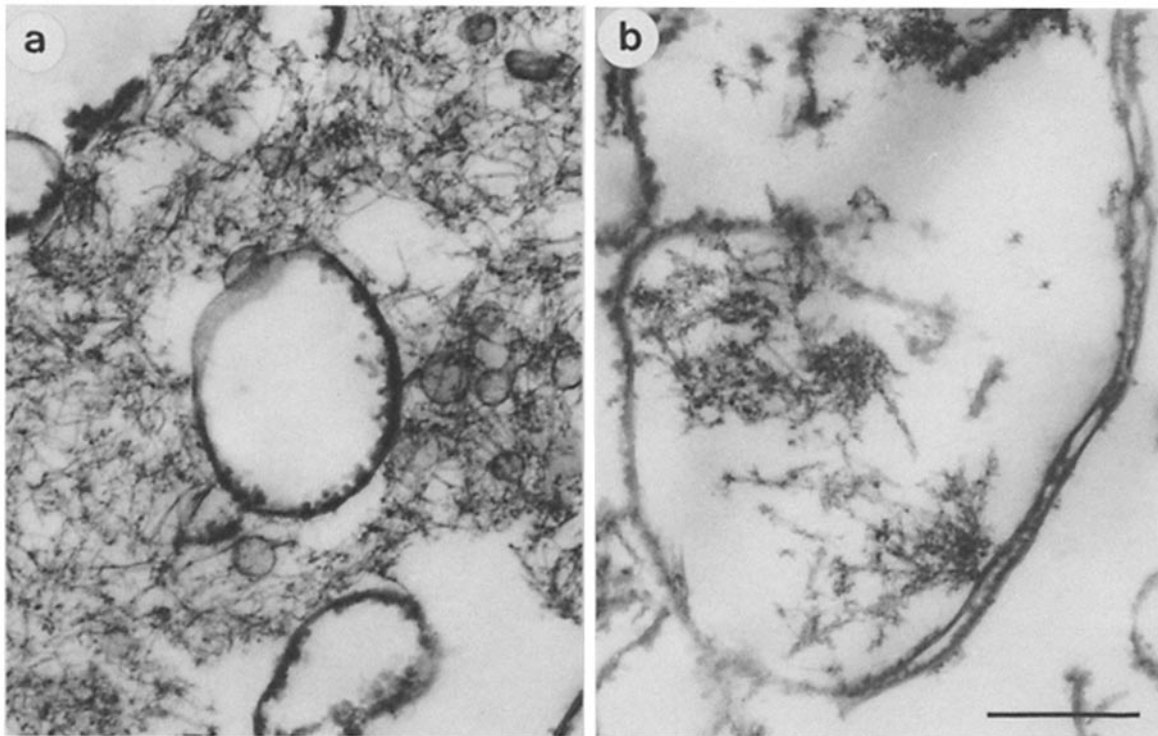


FIGURE 8 Transmission electron micrographs of Con A-isolated plasma membranes with associated actin and myosin before (a) or after decoration with 0.5 mg/ml of myosin S-1 (b). The electron-dense spherical structures inside the membrane vesicle are surface-bound Con A after mordanting with tannic acid, ferrocyanide, and osmium tetroxide (29). Bar, 0.5  $\mu\text{m}$ .  $\times \sim 40,000$ .

served between the decorated filaments and the cytoplasmic membrane surfaces (Fig. 8b). Although we have not attempted to quantify the number or type of actin-membrane associations before and after S-1 decoration, Bennett and Condeelis (2), using stereo microscopy, report that several types of attachment exist for both lateral and end-on binding of actin filaments to *D. discoideum* membranes and that S-1 decoration diminishes the number of filament-membrane interactions. This result is in gratifying agreement with the predictions made on the basis of our competition binding assays (Figs. 3 and 4).

## CONCLUSION

Our results show that S-1 competitively inhibits most of the binding between sonicated plasma membrane fragments and F-actin beads (Figs. 1, 3, and 4). This inhibition is abolished in the presence of MgATP (Fig. 1) and exhibits an inhibition constant that is essentially identical to the reported dissociation constant of the interaction between S-1 and F-actin (Fig. 3). In addition, sonicated membrane fragments compete with  $^{125}\text{I}$ -HMM for binding to F-actin beads (Fig. 5). From these experiments, we conclude that most of the binding of the *D. discoideum* membrane fragments is along the sides of the actin filaments at or near the well-characterized S-1 binding sites (36). This conclusion is supported by electron micrographs of sonicated membrane fragments bound to F-actin beads. These micrographs show many lateral interactions between the membrane fragments and bead-associated actin filaments (Figs. 6 and 7). Lateral interactions between endogenous actin and plasma membranes are consistent with the observation that S-1 disrupts many of the native interactions between Con A-stabilized plasma membranes and actin filaments (Fig. 8). These results agree with the suggestion, based

on low shear viscosity data, that associations between plasma membranes and the sides of actin filaments are extensive in *D. discoideum* (28).

The demonstration of binding to the sides of actin filaments does not exclude binding to filament ends. Indeed, end-on associations may be responsible for that component of the binding between  $^{125}\text{I}$ -labeled, sonicated membranes and F-actin beads that is not competitively inhibited by S-1 (Figs. 3 and 4). End-on associations may also explain the binding of endogenous actin to Con A-stabilized plasma membranes that persists in the presence of relatively high concentrations of S-1 (Fig. 8).

It is noteworthy that the membrane fragments used in this study are derived from plasma membrane domains involved in Con A-induced patching and capping. In conjunction with the observation that integral, possibly transmembrane, proteins mediate much of the binding between F-actin and *D. discoideum* plasma membrane fragments (29), our data suggest that *D. discoideum* plasma membranes contain one or more integral membrane proteins that bind to the sides of actin filaments. The laterally bound actin filaments may then interact with cytoplasmic myosin filaments during the capping process, as previously proposed (4, 5, 8, 11, 13). In fact, a lateral association between membranes and actin filaments is intuitively the most efficient orientation for the lateral movement of membrane proteins by the cytomusculature.

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