Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4779–4783, July 1986
Cell Biology

Junctional plasma membrane domains isolated from aggregating *Dictyostelium discoideum* amebae

(contact regions/glycoprotein gp80/cell adhesion/Triton-insoluble residue/cytoskeleton)

HILARY M. INGALLS, CATHERINE M. GOODLOE-HOLLAND, AND ELIZABETH J. LUNA

Department of Biology, Princeton University, Princeton, NJ 08544

Communicated by J. T. Bonner, March 6, 1986

ABSTRACT **Regions of plasma membrane involved in *Dictyostelium discoideum* intercellular adhesion resist solubilization with the nonionic detergent Triton X-100. Electron microscopy shows that these regions of the plasma membrane adhere to each other, forming many bi- and multilamellar structures. NaDodSO$_4$/polyacrylamide gels of these regions contain major polypeptides at 225 kDa (residual myosin), 105 kDa, 88 kDa, 84 kDa, 47 kDa (residual actin), and 34 kDa. These membranes contain a subset of the total plasma membrane proteins, as analyzed by labeling of electrophoretically fractionated and blotted membrane proteins with radioiodinated Con A and by electrophoresis of membrane proteins from surface-labeled cells. Antibodies specific for gp80, a glycoprotein implicated in intercellular adhesion, intensely stain the 88-kDa and 84-kDa bands. Since these membrane regions resist Triton extraction, they appear to be stabilized by protein–protein interactions. Such stabilizing interactions may involve multivalent linkages with adjacent cells, or associations with intracellular actin and myosin, or both. Since these membranes appear to represent regions of intercellular contact, we call them "contact regions."**

Cellular slime molds possess distinct nonsocial and social phases during their life cycle. Log-phase (vegetative) cells exist as single, free-living amebae. Upon starvation, cells develop the ability to adhere to each other in multicellular aggregates. Several developmentally regulated, cell surface proteins are implicated in the formation and maintenance of cell contacts in aggregates. However, the composition, structure, and mechanism of assembly of these cell contacts are unknown.

One approach to the identification of surface molecules involved in adhesion, pioneered by Gerisch and coworkers (1, 2), involves a broad-spectrum antiserum that disrupts cell–cell interactions. These workers have prepared univalent antibody fragments (Fabs) directed against cell homogenates of aggregating *Dictyostelium discoideum*, have adsorbed the Fabs against vegetative cells, and have found that the unadsorbed (remaining) Fabs inhibit the intercellular adhesion that appears in early development (1–3). Preadsorption of the adhesion-blocking Fabs with different membrane proteins has implicated a cell surface glycoprotein, gp80*, in cell–cell adhesion at this stage of development (5). Although this immunological approach is invaluable for implicating polypeptides involved in adhesion, proteins with low immunogenicity or proteins not exposed at the cell surface may be missed. In addition, this approach cannot determine the number of different proteins involved or how they interact with each other. In another approach, tissues are extracted with low concentrations of nonionic or zwitterionic detergents. Proteinaceous assemblies stabilized by protein–protein interactions resist solubilization by these detergents and thus can be isolated from nonstabilized membranes (6). This approach has been used to isolate cytoskeletons from erythrocytes (7) and other cells (8), membranes from aggregating platelets (9), and morphologically distinguishable junctional complexes such as desmosomes (10) and gap junctions (11).

Previously, we have used Con A treatment and Triton extraction to isolate highly purified plasma membrane domains from vegetative *D. discoideum* amebae (12). In this technique, plasma membranes apparently are stabilized by exogenous Con A, by cytoskeletal associations, or by both. In the absence of Con A, only small amounts of plasma membrane are recovered after Triton extraction of vegetative cells.

Although it has long been known that adhesive properties are retained by *D. discoideum* plasma membrane ghosts (13), no definable adhesive structures, such as gap junctions or desmosomes, have been reported (14). However, recent studies have shown that actin is concentrated at regions of contact between aggregating amebae (15). Reasoning that cell-contact regions in aggregating amebae might be stabilized by intracellular and/or extracellular adhesive interactions, we have looked for membrane domains that resist Triton solubilization. Such plasma membrane domains should be enriched in molecules involved in cell adhesion. In this paper, we report that plasma membrane domains, apparently derived from regions of intercellular contact, can be isolated from aggregating amebae. Here, we present the initial characterization of these plasma membranes, which we call "contact regions."

This work was presented in preliminary form at the 25th Annual Meeting of the American Society for Cell Biology, Atlanta, GA, November 18–22, 1985 (16).

MATERIALS AND METHODS

Chemicals. Bovine serum albumin (BSA) and Triton X-100 (TX-100) were from Sigma. Cyclic AMP (cAMP) was from Calbiochem. Sulfosuccinimidobiotin (SNHS-biotin) and avidin were from Pierce. Reagents used for electrophoresis were from Bio-Rad. Other chemicals were reagent grade.

Isolation of Contact Regions. *D. discoideum* amebae (strain Ax-3; 10^7 cells per ml) were harvested from HL-5 medium (17) at 1000 × *g* for 2 min. The amebae were washed three times by suspension in Sorensen's buffer (14.6 mM KH_2PO_4/2 mM Na_2HPO_4), pH 6.1, followed by centrifugation at 1000 × *g* for 2 min and finally were resuspended to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BSA, bovine serum albumin; SNHS-biotin, sulfosuccinimidobiotin; TX-100, Triton X-100.

*To avoid confusion, we use the original designation gp80 to refer to this developmentally regulated glycoprotein, even though in our NaDodSO$_4$/PAGE system, it migrates with an apparent molecular mass of ≈84 kDa. The apparent molecular mass of gp80 depends on the porosity of the gel (1). An apparent molecular mass of 84 kDa is consistent with reported values (1, 4).

$1.5–2 \times 10^7$ cells per ml in Sorensen's buffer. A peristaltic pump driven by an electronic timer (ChronTrol, Lindburg Enterprises, San Diego, CA) was used to pulse the cells with cAMP (50 nM final concentration) every 6 min for 12 hr (18). The pulsed cells were pelleted at $1000 \times g$ for 2 min and resuspended to $5–7.5 \times 10^7$ cells per ml in ice-cold buffer 1 (12). After the addition of TX-100 to 0.2% (vol/vol), the cell suspension was mixed on ice for 1 min and centrifuged at $4000 \times g$ for 10 min at 2°C. The fluffy part of the pellet was resuspended in fresh buffer 1, centrifuged at $14,500 \times g$ for 5 min at 2°C, and washed in 1 mM EGTA/5 mM Tris·HCl, pH 7.6, at $14,500 \times g$ for 10 min. The pellet was resuspended in this buffer with gentle homogenization and was centrifuged at $120,000 \times g$ for 1 hr at 2°C into 40–60% sucrose gradients containing 0.02% NaN_3 and 20 mM sodium phosphate (pH 6.8). The band at about 52–53% sucrose was collected and washed twice with 20 mM sodium phosphate (pH 6.8).

Cytoskeletal components were removed by extraction at low ionic strength as described (19), except that the pelleting, resuspension, and dialysis steps were repeated at least three times. The final pellet was centrifuged into 26–51% linear sucrose gradients, and the band at about 35–42% sucrose was collected and washed as described (12).

Isolation of Plasma Membranes. Crude plasma membranes were prepared both by the method of Das and Henderson (20) and by a modification (19) of the method of Spudich (21). Highly purified plasma membranes were isolated using an extension (12) of a Con A-stabilization and TX-100-extraction procedure (22, 23).

Cell Surface Labeling. Cell surfaces were labeled with SNHS-biotin according to the procedure of Goodloe-Holland and Luna (24). In brief, suspension-developed cells were pelleted and resuspended to $\approx 4 \times 10^8$ cells per ml in Sorensen's buffer, pH 8.0 (Sorensen's buffer, pH 6.1, raised to pH 8.0 with NaOH). The cells were shaken gently with SNHS-biotin (0.2 mg/ml) for 30 min at 4°C. The cells were washed four times with Sorensen's buffer, pH 6.1, and lysed, and contact regions or membranes were isolated as described above.

Isolated membranes also were labeled with SNHS-biotin. Membranes resuspended to 0.2 mg/ml in Sorensen's buffer, pH 8.0, were incubated with SNHS-biotin (0.4 mg/ml) at 4°C for 30 min. Membranes were washed four times with 20 mM sodium phosphate (pH 6.8) and stored in this buffer with 0.02% NaN_3.

Electron Microscopy. Samples were processed for transmission electron microscopy as described (12), except that whole cells were fixed for 1.5 hr. In brief, samples were fixed in 50 mM cacodylate (pH 7.0) containing 1% glutaraldehyde and 2% tannic acid and were postfixed in buffered 0.8% $K_4Fe(CN)_6$ and 0.5% OsO_4. The samples then were stained in 2% uranyl acetate, dehydrated, and embedded.

Radioiodination. Con A (Miles–Yeda, Rehovot, Israel) was radiolabeled with ^{125}I, using chloramine-T, and purified on Sephadex G-150 (12). Protein A (Pharmacia) and avidin were radioiodinated with ^{125}I-labeled Bolton–Hunter reagent (New England Nuclear) (25) in 20 mM sodium phosphate (pH 7.8) for 3 hr at 2°C. The iodinated proteins were separated from unreacted reagent on a Sephadex G-25 column.

Antibodies. gp80 was prepared by the method of Müller *et al.* (1) and was electrophoresed in 6–12% gradient NaDodSO$_4$/polyacrylamide gels. Bands corresponding to gp80* were excised from the gels, neutralized, and homogenized. The homogenate was mixed with an equal volume of Freund's complete adjuvant (Miles) and ≈ 175 μg of gp80 was injected into each of two rabbits. The rabbits were given booster injections after 1 month and 4 months with the same amount of gp80 mixed with Freund's incomplete adjuvant (Miles). They were bled 10–14 days after each boost. Anti-gp80 IgG was purified (26) and adsorbed four times against fixed vegetative cells (4). Anti-discoidin was a generous gift from D. Cooper and S. Barondes (Department of Psychiatry, University of California at San Diego).

Electrophoresis and Nitrocellulose Blots. Samples were denatured (19) and run in 6–16% polyacrylamide gradient NaDodSO$_4$ slab gels, using the discontinuous system of Laemmli (27). Molecular weight determinations were based on the apparent molecular weights (28) of Bethesda Research Laboratories Prestained HMW Standards.

Nitrocellulose blots were prepared (29), and proteins were detected as described below. Con A: Visualization with ^{125}I-labeled Con A was performed as described (12). Anti-gp80: Blots were soaked overnight at 4°C in 2% (wt/vol) BSA/150 mM NaCl/10 mM sodium phosphate, pH 7.4, and were incubated for 2 hr at room temperature with anti-gp80 IgG (50–100 μg/ml) in 4% BSA/150 mM NaCl/0.2% (vol/vol) TX-100/1 mM EDTA/10 mM sodium phosphate, pH 7.2 (30). Blots were washed for a total of 2 hr with four changes of 150 mM NaCl/0.02% TX-100/1 mM EDTA/10 mM sodium phosphate, pH 7.2, and four changes of 150 mM NaCl/0.1% TX-100/1 mM EDTA/10 mM sodium phosphate, pH 7.2. Blots then were incubated for 2 hr at room temperature with 200,000 cpm (≈ 0.5 μg) of ^{125}I-labeled protein A per ml of solution. Washes were repeated as described above. Avidin: Blots were soaked overnight at 4°C in 2% BSA/150 mM NaCl/50 mM sodium borate, pH 10.5, and then incubated for 2 hr at room temperature in 4% BSA/150 mM NaCl/0.02% TX-100/50 mM sodium borate, pH 10.5, containing 200,000 cpm (1–2 μg) of ^{125}I-labeled avidin per ml. Blots were washed for a total of 2 hr with four changes of 150 mM NaCl/0.01% TX-100/50 mM sodium borate, pH 10.5, and four changes of 0.1% TX-100/50 mM sodium borate, pH 10.5 (24). Blots were dried and autoradiographed on Kodak XAR-2 film.

Assays. Membrane protein was assayed in the presence of 1% NaDodSO$_4$ using BSA as the standard (31).

Biotinylated membranes were incubated with 0.5 μg of ^{125}I-labeled avidin and centrifuged through a 5% sucrose cushion at $24,000 \times g$ for 30 min at 20°C to separate membranes (with bound avidin) from unbound avidin (24). The amount of avidin bound per μg of membrane protein was graphed. Binding curves were used to estimate the biotin content of membrane preparations, so that samples containing equal amounts of biotin could be loaded onto gel lanes for ^{125}I-labeled avidin blots.

RESULTS

Suspension Development. Starvation and pulsing with cAMP stimulates cells in suspension to begin development (32, 33). These cells increase in cohesiveness and form clumps. When plated onto agar, cells developed for 12 hr in suspension culture rapidly form large aggregates. The time required for development to the "finger" stage is only 1–2 hr more than for cells developed on plates from time zero (data not shown). Contact regions can be isolated from plate-developed cells (data not shown) as well as from suspension-developed cells. However, because larger numbers of cells are easily obtainable from suspension culture, the use of 12-hr suspension-developed cells is preferred.

Yield. Significant amounts of membrane can be isolated from Triton-extracted aggregating amebae in the absence of Con A (Table 1, preparation c). The amount of membrane protein recovered is comparable to that obtained when using a Con A-stabilization, Triton-extraction isolation method (Table 1, preparations a and b). Triton extraction of vegetative cells without bound Con A yields only minute amounts of membrane protein (Table 1, preparation d). Contact regions isolated from aggregated cells comprise 7–25% of the total plasma membrane protein. Compared with crude plasma membrane preparations (Table 1, preparations e and f),

Table 1. Average membrane protein yield from different plasma membrane preparations

Preparation	Membrane protein, mg per 10^{10} cells	*n*
(a) Veg. Con A/TX-100*	1.8 ± 0.31	6
(b) 12-hr Con A/TX-100*	2.6 ± 0.18	3
(c) 12-hr TX-100 Extracted†‡	2.1 ± 0.51	5
(d) Veg. TX-100 Extracted‡	0.18 ± 0.11	2
(e) 12-hr Das–Henderson§	25 ± 3	2
(f) 12-hr Spudich§	28 ± 0.0	2

Veg., preparation from vegetative (late log-phase) cells; 12-hr, preparation from 12-hr suspension-developed cells. *n*, Number of preparations.
*Membranes prepared using Con A stabilization followed by Triton extraction.
†Contact regions.
‡Membranes prepared by lysing cells with Triton (no Con A added).
§Crude plasma membranes prepared as described in *Materials and Methods*.

contact regions represent only about 7–10% of the membrane protein. But, because crude preparations contain membranes other than plasma membrane, this is a minimum value. Based on avidin binding, the contact regions contain as much as 25% of the total biotin bound to the surface-labeled cells (data not shown). However, this value may be an overestimate, since most of the biotin label is on proteins concentrated in contact regions (see below).

Physical Appearance. Suspension-developed amebae form large aggregates with much of their surface areas involved in intercellular contact (Fig. 1*a*). The space between the plasma membranes in the regions of cell contact is 24 ± 3 nm wide and contains an amorphous electron-dense material (Fig. 1*a*, *Inset*). The inner leaflet of the plasma membrane does not stain as densely as the outer leaflet but is visible in the inset (arrow). The crude TX-100-insoluble residue consists of filamentous cytoskeletal material associated with a mixture of single-bilayer vesicles (Fig. 1*b*, arrow) and sheets of two bilayers running in parallel (arrowheads). Most of the vesicles are sectioned obliquely, obscuring the familiar trilaminar structure of the plasma membrane. Two parallel, apparently adherent plasma membrane sheets, ≈23 nm apart, appear in the lower third of Fig. 1*b*. In isolated membranes, both leaflets stain equally well, although Triton extraction results in an irregular interleaflet spacing. Centrifugation on the first sucrose gradient removes much of the filamentous material and results in the formation of multilayered vesicles (Fig. 1 *c* and *d*). After removal of most of the cytoskeletal components and purification on the second sucrose gradient, the sample looks homogeneous, containing membranes that appear to adhere to each other, forming many structures consisting of two or more bilayers (Fig. 1*e*). The distance between the adherent membranes is the same as that between intact cells (23 ± 4 nm), and the area between the adherent membranes exhibits much of the electron density observed between adhering cells (Fig. 1 *a* and *e*, *Insets*). We call these structures "contact regions."

Protein Composition. We observe large differences in protein composition between highly purified plasma membranes prepared from vegetative cells (Fig. 2, lanes 2) and highly purified plasma membranes from aggregating cells (lanes 4). Similar differences have been reported (34) for developmentally regulated lectin-binding proteins, but in general, differences have not been observed in Coomassie blue-stained profiles. A recent study (24) suggests that this lack of observed differences is due to the large number of contaminants in most plasma membrane preparations. Con A/TX-100 membranes (Fig. 2 *a* and *b*, lanes 4) contain decreased amounts of polypeptides of apparent molecular mass 180, 160, 140, 130, 105, and 32–34 kDa and increased

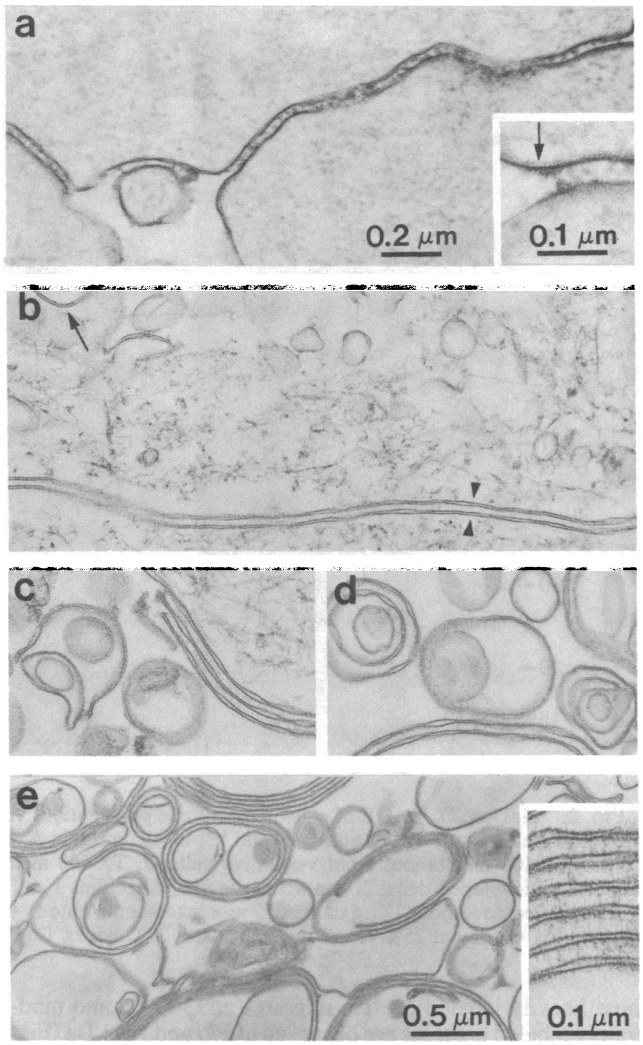

FIG. 1. Electron micrographs of contact regions between intact adhering cells (*a*), crude contact regions after TX-100 extraction (*b*), contact regions after first sucrose gradient (*c* and *d*), and purified contact regions (*e*). Arrows: inner leaflet of plasma membrane (*a Inset*); single-bilayer vesicle (*b*). Arrowheads: paired bilayers (*b*). Bar in *a* applies to *a–d*.

amounts of polypeptides at 84–88 and 53 kDa. TX-100-extracted vegetative membranes (Fig. 2 *a* and *b*, lanes 3) consist primarily of myosin (≈225 kDa) and proteins of 130, 105, 47 (actin), 32–34, and 16 kDa. The purified contact regions (Fig. 2 *a* and *b*, lanes 5) exhibit major polypeptides at ≈225 (residual myosin), 130, 105, 88, 84, 47 (residual actin), and 34 kDa. Since many plasma membrane polypeptides are excluded from contact regions (Fig. 2 *a* and *b*, lanes 5), these regions are a subset of the total plasma membrane.

Two proteins that have been implicated in *D. discoideum* adhesion are gp80 and discoidin (4). In our gels, the polypeptides at 84–88 kDa comigrate with gp80 and the 32/34-kDa doublet comigrates with discoidin (Fig. 2). Antibodies specific for gp80 (Fig. 2*c*, lane 1), intensely stain the 84- to 88-kDa region in 12 hr Con A/TX-100 membranes and in contact regions (Fig. 2*c*, lanes 4 and 5). Antibodies directed against discoidin do not react with any proteins in our preparations (data not shown). This result agrees with the recent suggestion that discoidin is involved in cell–substrate, rather than cell–cell, adhesion (35, 36).

Crude membranes exhibit a large number of surface-biotinylated proteins with a wide range of molecular sizes (Fig. 3, lanes 1–3). In membranes from vegetative cells, the

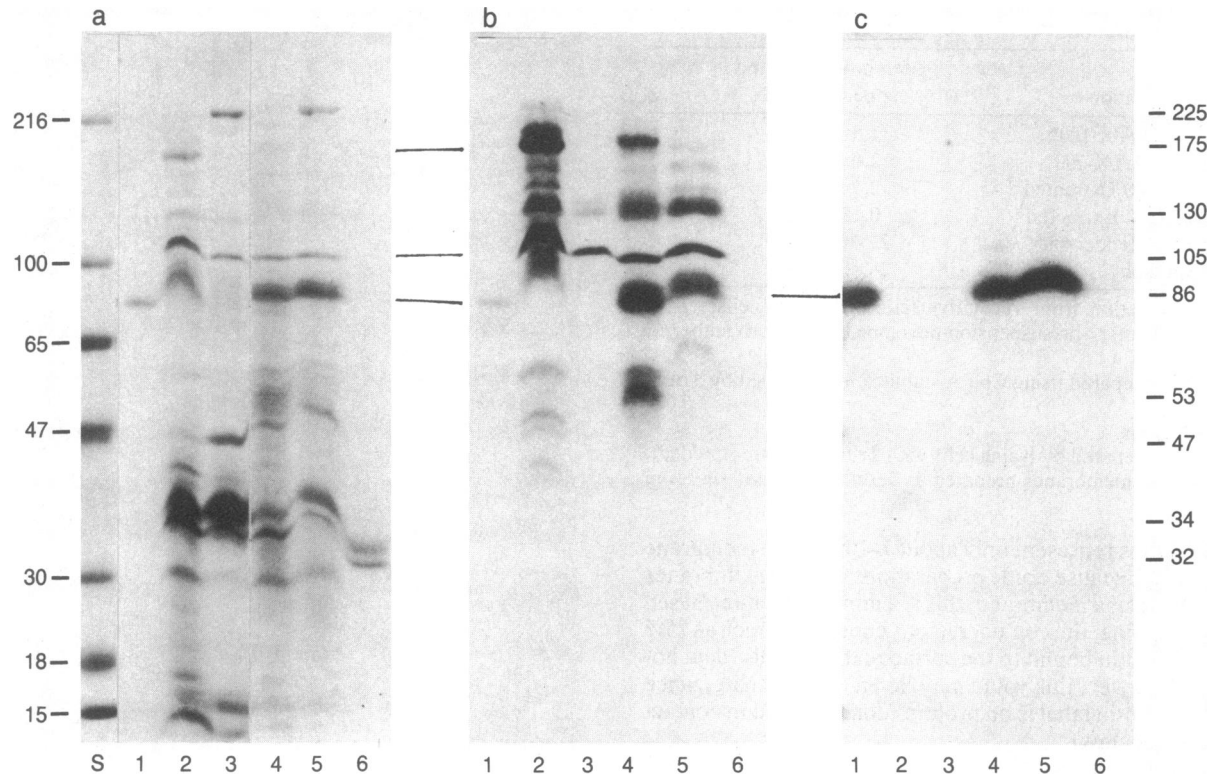

FIG. 2. NaDodSO$_4$/PAGE of plasma membrane polypeptides visualized with Coomassie blue (*a*) and by incubation of nitrocellulose blots with ^{125}I-labeled Con A (*b*) or anti-gp80 and ^{125}I-labeled protein A (*c*). Lanes: S, standards (values in kDa at left); 1, gp80-enriched extract; 2, Con A/TX-100 membranes from vegetative cells; 3, TX-100 membranes from vegetative cells; 4, Con A/TX-100 membranes from 12-hr suspension-developed cells; 5, contact regions; 6, discoidins I and II. Numbers at right indicate apparent molecular mass (in kDa) of major proteins detected. About 12 μg of protein was loaded onto lane 1; 40 μg, onto each of lanes 2–5; and 5 μg, onto lane 6. Autoradiographs were exposed for ≈1 day at 25°C (*b*) or for 2 days at −70°C with a Dupont Cronex Lightning Plus screen (*c*).

most heavily labeled protein migrates at 130 kDa and moderately labeled proteins are observed at 175 and 105 kDa (Fig. 3, lane 1). Membranes from aggregating cells contain major surface-biotinylated proteins between 84 and 88 kDa and between 47 and 50 kDa (Fig. 3, lanes 2 and 3). Other labeled proteins occur at ≈200, 175, 130, and 105 kDa. Purified contact regions contain only a few of the total surface-labeled proteins (Fig. 3, lane 4). The heaviest label in the contact regions is in the band at 84–88 kDa. Minor labeled bands are observed at 175, 130, and 105 kDa. The surface labeling is side-specific, since a number of additional proteins, including actin and myosin, are labeled only when contact regions are biotinylated after isolation (Fig. 3, lane 5). No endogenous biotin is present in contact regions (Fig. 3, lane 6).

DISCUSSION

The existence of membrane regions involved in *D. discoideum* intercellular adhesion is suggested by the uniform distance observed between bilayers of adhering amebae. The width of this intercellular space falls within the range observed for other regions of intercellular adhesion, such as desmosomes (37). Although *D. discoideum* contact regions exhibit less morphological substructure than desmosomes exhibit, one might expect that aggregating amebae contain less-structured adhesive regions, since they must be able to create and destroy intercellular associations with relative ease and speed.

We have isolated membrane regions that retain the ability to adhere to each other with the characteristic uniform distance observed between adhering cells (Fig. 1). Gel electrophoresis shows that these putative contact regions contain a subset of all the proteins in the plasma membrane (Figs. 2 and 3), indicating that these regions are a subdomain of the cell membrane. The most abundant protein in contact regions is gp80 (Fig. 2), a glycoprotein implicated in adhesion during aggregation of *D. discoideum*. Since contact regions resist Triton extraction, they may be stabilized by multivalent linkages with adjacent cells, by associations with intracellular actin and myosin, or by both. Indeed, significant amounts of actin and myosin are present even in highly purified contact regions (Fig. 2*a*).

It has been proposed that the cytoskeleton may "activate" gp80 during the formation of cell–cell contacts (33). This hypothesis is supported by the observation that cytoskeletal inhibitors dissociate aggregating cells (38). Our electron micrographs suggest that, *in vitro*, the cytoskeleton may play a role in maintaining the orientation of membrane-bound adhesion molecules. When the cytoskeleton is present, the membranes in the TX-100-insoluble residue form unilamellar vesicles and sheets of double membranes (Fig. 1*b*). Upon removal of the cytoskeleton, the membranes apparently lose their asymmetry and form multilamellar structures (Figs. 1 *c* and *d*). This loss of asymmetry apparently results from the loss of cytoskeletal stabilization, which may allow membrane fragments to anneal in mixed orientations. Alternatively, residual Triton may upset the nonstabilized bilayer, permitting the "flip-flop" of integral membrane proteins. These questions can be resolved only by further characterization of the associations between the membrane components of contact regions and the cytoskeleton.

We acknowledge C. Mao and G. Barcelo for technical assistance and L. Wuestehube for contributing data to Table 1. We appreciate the use of equipment located in the laboratories of Drs. E. C. Cox and A. Newton and the critical comments of Drs. O. Blaschuk, A.

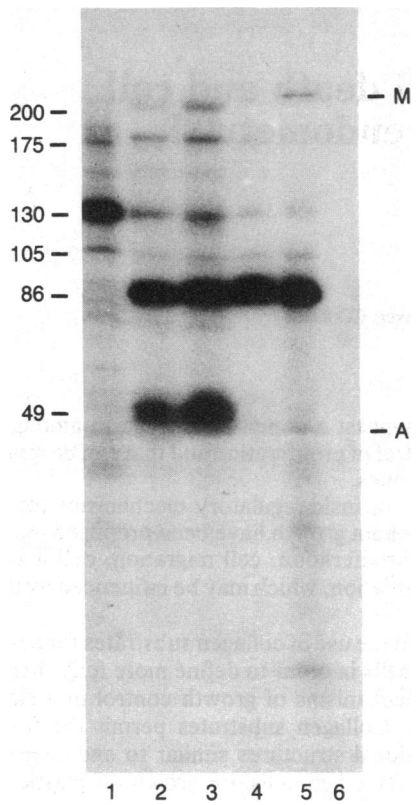

FIG. 3. ^{125}I-labeled avidin blot of membranes labeled with SNHS-biotin. Lanes were loaded for equal avidin-binding activity. Lanes: 1, surface-labeled vegetative Spudich membranes (60 μg of protein); 2, 12-hr surface-labeled Spudich membranes (40 μg); 3, 12-hr surface-labeled Das–Henderson membranes (51 μg); 4, surface-labeled contact regions (18 μg); 5, contact regions biotinylated after isolation (3 μg); 6, unlabeled contact regions (18 μg). Autoradiograph was exposed for 12 days at room temperature. A, actin; M, myosin.

Ross, and R. Manteuffel. H.M.I. was supported in part by National Institutes of Health Grant 5T32 GM7312. This work was supported by National Institutes of Health Grant GM29699, March of Dimes Grant 1-981, and a Faculty Research Award from the American Cancer Society to E.J.L.

1. Müller, K., Gerisch, G., Fromme, I., Mayer, H. & Tsugita, A. (1979) *Eur. J. Biochem.* **99**, 419–426.
2. Beug, H., Gerisch, G., Kempff, S., Riedel, V. & Cremer, G. (1970) *Exp. Cell Res.* **63**, 147–158.
3. Barondes, S. H., Springer, W. R. & Cooper, D. N. (1982) in *The Development of Dictyostelium discoideum*, ed. Loomis, W. F. (Academic, New York), pp. 195–231.
4. Murray, B. A., Yee, L. D. & Loomis, W. F. (1981) *J. Supramol. Struct. Cell. Biochem.* **17**, 387–401.
5. Müller, K. & Gerisch, G. (1978) *Nature (London)* **274**, 445–449.
6. Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79.
7. Yu, J., Fischman, D. A. & Steck, T. L. (1973) *J. Supramol. Struct.* **1**, 233–248.
8. Geiger, B. (1983) *Biochim. Biophys. Acta* **737**, 305–341.
9. Phillips, D. R., Jennings, L. K. & Edwards, H. H. (1980) *J. Cell Biol.* **86**, 77–86.
10. Gorbsky, G. & Steinberg, M. S. (1981) *J. Cell Biol.* **90**, 243–248.
11. Fallon, R. F. & Goodenough, D. A. (1981) *J. Cell Biol.* **90**, 521–526.
12. Luna, E. J., Goodloe-Holland, C. M. & Ingalls, H. M. (1984) *J. Cell Biol.* **99**, 58–70.
13. Sussman, M. & Boschwitz, C. (1975) *Dev. Biol.* **44**, 362–368.
14. Johnson, G., Johnson, R., Miller, M., Borysenko, J. & Revel, J.-P. (1977) *Science* **197**, 1300.
15. Yumura, S., Mori, H. & Fukui, Y. (1984) *J. Cell Biol.* **99**, 894–899.
16. Ingalls, H. M., Goodloe-Holland, C. M. & Luna, E. J. (1985) *J. Cell Biol.* **101**, 63a (abstr.).
17. Cocucci, S. & Sussman, M. (1970) *J. Cell Biol.* **45**, 399–407.
18. Murray, B. A., Niman, H. L. & Loomis, W. F. (1983) *Mol. Cell. Biol.* **3**, 863–870.
19. Luna, E. J., Fowler, V. M., Swanson, J., Branton, D. & Taylor, D. L. (1981) *J. Cell Biol.* **88**, 396–409.
20. Das, O. P. & Henderson, E. J. (1983) *Biochim. Biophys. Acta* **736**, 45–56.
21. Spudich, J. A. (1974) *J. Biol. Chem.* **249**, 6013–6020.
22. Parish, R. W. & Müller, U. (1976) *FEBS Lett.* **63**, 40–44.
23. Condeelis, J. S. (1979) *J. Cell Biol.* **80**, 751–758.
24. Goodloe-Holland, C. M. & Luna, E. J. (1986) in *Methods in Cell Biology*, ed. Spudich, J. A. (Academic, New York), Vol. 28, in press.
25. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539.
26. Reif, A. E. (1969) *Immunochemistry* **6**, 723–731.
27. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
28. Tsang, V. C. W., Hancock, K. & Simons, A. R. (1984) *Anal. Biochem.* **143**, 304–307.
29. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
30. Bennett, V. & Davis, J. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **46**, 647–657.
31. Lowry, O. H., Rosebrough, N., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
32. Coffman, D. S., Leichtling, B. H. & Rickenberg, H. V. (1981) *J. Supramol. Struct. Cell. Biochem.* **15**, 369–385.
33. Stadler, J., Gerisch, G., Bauer, G., Suchanek, C. & Huttner, W. B. (1983) *EMBO J.* **2**, 1137–1143.
34. Murray, B. A. (1982) in *The Development of Dictyostelium discoideum*, ed. Ivatt, R. J. (Plenum, New York), pp. 71–116.
35. Springer, W. R., Cooper, D. N. W. & Barondes, S. H. (1984) *Cell* **39**, 557–564.
36. Gabius, H.-J., Springer, W. R. & Barondes, S. H. (1985) *Cell* **42**, 449–456.
37. Staehelin, L. A. (1974) *Int. Rev. Cytol.* **39**, 191–283.
38. Cappuccinelli, P. & Ashworth, J. M. (1976) *Exp. Cell Res.* **103**, 387–394.