Journal of Cellular Biochemistry 18:169–180 (1982)
Cellular Recognition 575–586

Immunochemical Analysis of Discoidins I and II at the Cell Surface in Wild Type and Aggregation-Defective Mutants of Dictyostelium discoideum

D. Randall Armant and Edward A. Berger

Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

The endogenous lectins discoidins I and II are believed to be primary components of the morphogenetic cell cohesion system of D discoideum. We have developed two immunochemical methods to analyze the association of the discoidins with the cell surface. One method is a two-stage specific antibody binding assay in which intact cells are incubated on ice with rabbit serum (either control serum or antidiscoidin I and II), washed, then incubated with ^{125}I-Protein A. Specific antibody binding is defined as the difference between percent radioactivity bound with antidiscoidin versus control serum during the first stage. Substantial specific binding was observed with developed A3 cells but not with vegetative cells, and nearly all of the activity could be removed by preadsorption of the antiserum with discoidin-Sepharose. As a complementary method, quantitative immunoadsorption analysis was performed in which we tested the ability of intact cells to remove antibodies reactive with purified ^{125}I-discoidin I or II. Developed cells, but not vegetative cells, were capable of adsorbing antibodies reactive with discoidin I as well as those reactive with discoidin II. This represents the first demonstration that both lectins are present on the surface of cohesive cells.

These procedures, coupled with other methods to analyze soluble discoidin in cell extracts, were used to study discoidin expression in wild type cells and in two newly isolated aggregation-defective mutants. Strain EB-32 fails to aggregate and displays little or no discoidin in cell extracts or at the cell surface. On the other hand, strain EB-18 forms loose amorphous mounds, and expresses substantial quantities of the discoidins, both in cell extracts and at the cell surface. These mutants should prove valuable in studying the organization and regulation of discoidins I and II at the surface of aggregating cells.

Key words: discoidins I and II, lectins, surface-localized, aggregation-defective mutants, cell cohesion

Abbreviations: PBS, phosphate-buffered saline; RIA, radioimmunoassay; NP40, nonidet P40; SDS, sodium dodecyl sulfate.

Received April 30, 1981; accepted September 1, 1981.

0730-2312/82/1802-0169$03.50

As D discoideum amoeba aggregate from the unicellular to the multicellular state, they acquire the capacity for mutual cell surface recognition and cohesion [1]. Specific cell contact serves not only to organize the aggregating cells during their morphogenetic assembly to form the fruiting body, but also to trigger new programs of gene expression for the later stages of development [2, 3].

The acquisition of cell cohesiveness is accompanied by the synthesis of two galactose-binding lectins, discoidins I and II [4–6]. Although evidence has accumulated to suggest an obligatory role for these molecules in the cell cohesion machinery [7–10], their precise functions are currently unknown [7, 10]. It should be noted that two other molecules, gp 80 (contact site A) [11] and gp 150 [12], have also been implicated as primary cohesion components, yet the structural and functional relationships of these glycoproteins to the discoidins remain to be determined [7, 11]. Clearly, the formulation of a precise cohesion mechanism requires a detailed understanding of how these molecules are associated with the cell membrane and with each other, and how their expression at the cell surface is regulated. These questions are of particular importance for the discoidins in view of reports that only a small fraction of the slime mold lectins are surface-localized, the remainder being sequestered in an intracellular pool [13–15].

We have undertaken a combined biochemical and genetic approach to this problem. In this report, we describe the development of two immunochemical assays to measure selectively the discoidins at the surface of intact cells. These methods have enabled us to identify new aggregation-defective mutants showing altered patterns of discoidin expression, which promise to aid our investigations of the organization and regulation of discoidins I and II at the cell surface.

MATERIALS AND METHODS

Materials

Routinely used buffers are designated as follows: 1) phosphate buffer: 3.2 mM Na_2HPO_4 + 12.8 mM KH_2PO_4, pH 6.4; 2) phosphate-hemoglobin buffer: phosphate buffer + 5 mg/ml hemoglobin; 3) PBS-azide: 20 mM potassium phosphate, 150 mM NaCl, 3.1 mM sodium azide, pH 7.2; 4) RIA buffer: PBS-azide supplemented with 0.4 M D-galactose, 10 mM KI, 1 mg/ml hemoglobin, and 0.05% (v/v) NP40.

The rabbit antidiscoidin serum employed in this study has been shown to react with both discoidins I and II, but with no other proteins in D discoideum extracts [6]. For binding and adsorption experiments with intact cells, the serum was preheated at 56°C for 30 min to inactivate complement, then clarified by centrifugation at 10,000g for 5 min. Control rabbit serum was purchased from Microbiological Associates. Formalin-fixed S aureus (Pansorbin) was purchased from Calbiochem. Prior to use, the adsorbent was washed once with PBS-azide plus 0.5% (v/v) NP40, then suspended in RIA buffer. Purified Protein A was obtained from Pharmacia.

Sepharose 4B and 6B were purchased from Pharmacia. The latter was acid-treated to enhance discoidin binding capacity [16]. BioGel P-6 (100–200 mesh) was supplied by Bio-Rad. $Na^{125}I$ (carrier-free) was obtained from Amersham. All other reagents were of the highest grades available commercially.

Purification of the Discoidins

The discoidins were purified from sonic lysates of axenically grown A3 cells by affinity chromatography on Sepharose 4B [5]. To obtain homogenous preparations of each lectin, we exploited the observation (unpublished) that discoidin I appears slightly ahead of II upon elution of the affinity column with D-galactose. In some preparations, the earliest fractions contained essentially homogenous discoidin I, whereas the latest contained pure discoidin II.

Growth and Development of Cells

Strain A3 was the wild type strain used throughout these studies. Cells were grown on nutrient agar plates (Falcon, 100 × 20mm) at 22°C in conjunction with K aerogenes [17], and were harvested prior to detectable clearing of the bacterial lawn ($\leq 5 \times 10^7$ cells per plate). To obtain vegetative cells the plates were harvested in phosphate buffer and the amoebae washed by centrifugation at 700g 3 min until free of bacteria. To obtain developed cells, the vegetative amoebae were suspended at 2×10^7 cells per ml in phosphate buffer and incubated at 22°C on a rotary shaker at 150 rpm for 16 h. The developed cells were then pelleted, resuspended in phosphate buffer containing 20 mM EDTA, and dissociated for counting by trituration (ten times) with a 10-ml pipette. Dissociation of cells by this method was found to solubilize less than 0.5% of the total cellular discoidin, as judged by RIA of both the cell extracts and the supernatants remaining after pelleting the dissociated cells.

Measurement of the Discoidins at the Cell Surface

Two procedures were developed to measure the discoidin antigens at the surface of intact cells (see "Results"). The first is a specific antibody binding assay in which surface bound immunoglobulin is detected by ^{125}I-Protein A. Dissociated cells were washed free of EDTA by three washes with phosphate-hemoglobin buffer. Reaction mixtures (0.25 ml total volume, in phosphate-hemoglobin buffer) contained 6.25×10^5 cells plus the indicated volumes of either control rabbit serum or rabbit antidiscoidin serum. Following a 60-min incubation on ice, the cells were washed once with phosphate-hemoglobin buffer and suspended in 0.25 ml phosphate-hemoglobin buffer containing 0.5 μg/ml ^{125}I-Protein A (0.2 μCi/μg). Following a 30-min incubation on ice, duplicate 0.1 ml aliquots of reaction mix were layered onto 0.2 ml cushions of 10% sucrose in phosphate buffer in 0.4 ml plastic microfuge tubes. The tubes were centrifuged 5 min in a Beckman model B microfuge (approximately 10,000g), quick-frozen in a dry ice-ethanol bath, and cut just above the cell pellets. Cell-associated radioactivity was determined by counting the pellets and supernatants separately in a Beckman 300 Gamma counter (68% efficiency for ^{125}I). Specific antibody binding, defined as the difference in percent radioactivity bound with control serum vs antidiscoidin serum during the first incubation, is a measure of relative amounts of surface-localized discoidin. Results are expressed either as percent CPM bound, or Protein A molecules specifically bound.

A second method to measure surface discoidin antigens employed quantitative immunoadsorption analysis. Incubation mixes contained cells plus rabbit antidiscoidin serum in the indicated amounts, adjusted to a total volume of 0.25 ml with phosphate-hemoglobin buffer. Following a 30-min incubation on ice, the cells were removed by centrifugation, and the residual antidiscoidin activities of the superna-

tants were determined by measuring their ability to immunoprecipitate purified radioiodinated discoidin I or II. Assay mixes (adjusted to 0.25 ml with RIA buffer) contained 5 ng of ^{125}I-discoidin I or II, plus aliquots of the adsorbed supernatants. The volumes of supernatants were chosen such that the binding of labelled discoidin by the unadsorbed control was within the range which was linear with antiserum volume. Following overnight incubation at 4°C, 5 μl of fixed S aureus (2%, v/v) was added and the incubations continued for an additional hour at 22°C. Duplicate 0.1 ml aliquots were then centrifuged through cushions of 20% sucrose in PBS-azide, and the radioactivity in the pellets and supernatants was determined as described above for the specific antibody binding assay. Results are expressed either as antidiscoidin activity of the adsorbed supernatant (% of unadsorbed control), or antidiscoidin activity adsorbed (% of total activity).

Measurement of Total Cellular Discoidins in Cell Extracts

Soluble discoidin antigens in cell extracts were quantitated using competition RIAs in which unlabeled discoidin competes for the immunoprecipitation of purified radioiodinated discoidin I or II. To prepare cell extracts, aliquots of EDTA-dissociated developed cells were frozen and thawed, then diluted 10–500- fold with RIA buffer containing 0.5% (v/v) Triton X-100. Supernatants were obtained by centrifugation 5 min at 10,000g. For RIA, incubation mixes contained 5 ng of radioiodinated discoidin I or II, 4 ng of the heterologous unlabeled discoidin (to block binding of the labeled probe to possible cross-reacting antibodies [see reference 18]), and 1×10^{-4} μl of rabbit antidiscoidin serum. In addition, varying amounts of cell lysates or purified discoidin standards were included, and the reaction volumes were adjusted to 0.24 ml with RIA buffer. Following overnight incubation at 4°C, 10 μl of fixed S aureus (2%, v/v) was added, and duplicate 0.1 ml aliquots were centrifuged through cushions of 20% sucrose as described for the specific antibody binding assay. Radioactivity in the pellets and supernatants was determined. Percent radioactivity specifically precipitated was calculated by subtracting the percent precipitated using control serum instead of antidiscoidin. The discoidin I and discoidin II assays were highly specific, since under these conditions competition occurred only with the homologous pairs of labeled and unlabeled lectins (Fig. 1). The concentration of each discoidin in cell lysates could thus be determined by comparing the ability of the lysates with that of standard discoidin solutions to compete for immunoprecipitation of the labeled probes.*

To assess the carbohydrate binding activity of discoidin in cell extracts, we performed hemeagglutination assays with formalinized rabbit erythrocytes according to the method of Barondes et al [19], modified by pretreating the microtiter wells with 10 mg/ml bovine serum albumin for 10 min. Under these conditions, the combined activity of discoidin I plus II is measured [5].

Isolation of Mutant Strains

The parental A3 strain was cloned on a bacterial lawn immediately prior to use. For mutagenesis, cells harvested during the exponential phase of growth were washed free of bacteria and suspended at 1×10^6 cells per ml in phosphate buffer

*Displacement RIAs also indicated that the level of cross-contamination of the purified discoidin I and discoidin II standards is less than 0.2% in each case.

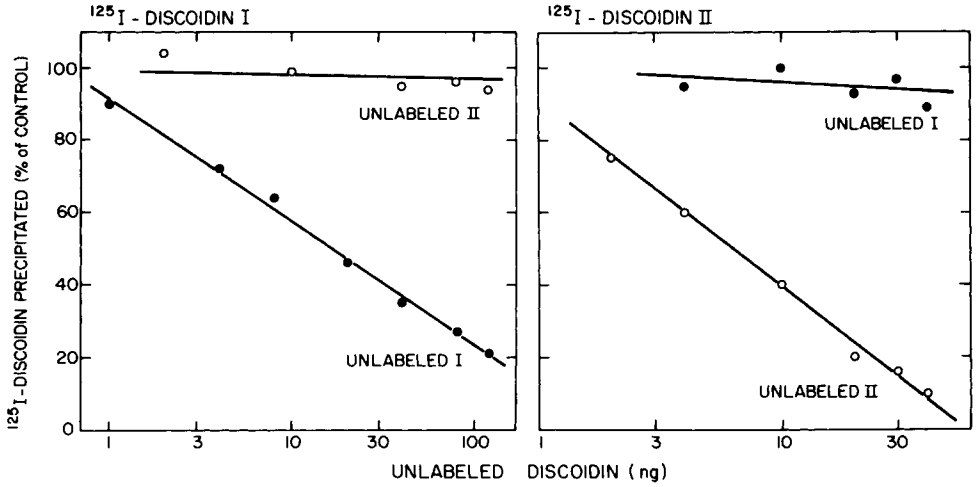


Fig. 1. Standard curves for RIA of soluble discoidin I and discoidin II. The ability of unlabeled discoidin I (closed circles) or discoidin II (open circles) to compete for the immunoprecipitation of ^{125}I-discoidin I (left) or ^{125}I-discoidin II (right) was tested, as described in Materials and Methods. The percent radioactivity specifically precipitated in the absence of unlabeled competitor is defined as 100%.

containing 0.4 mg/ml N-methyl-N'-nitro-N-nitrosoguanidine. After rotating 30 min at room temperature the cells were washed twice in ice-cold phosphate buffer and plated onto bacterial agar plates at several densities in the range of 300–3,000 cells per plate. After 8–9 days at 22°C, plaques showing aberrant aggregation were picked, subcloned twice on bacterial agar plates, and maintained on bacterial stock plates for subsequent study. Based on the plating efficiencies of control and mutagenized populations, the survival frequency after mutagenesis was calculated to be approximately 7%. Of the 351 independent plaques observed from the mutagenized population, 26 (corresponding to 7%) were judged to be blocked at or before the aggregate stage. By contrast, no morphogenetic mutants were detected among 244 plaques screened from the nonmutagenized population.

Additional Methods

Discoidins I and II and Protein A were radioiodinated in solution using chloramine T [20]. Unbound iodine was removed by gel filtration on BioGel P-6. Cell counts were obtained using a hemeacytometer.

RESULTS

Assays for the Discoidins at the Cell Surface

In order to investigate the association of discoidins I and II with the cell surface in wild type and mutant strains, we have developed two procedures to measure the lectins at the surface of intact cells. The first procedure is a two-stage specific antibody binding assay in which surface discoidin is detected with antidiscoidin serum followed by ^{125}I-Protein A. Several parameters were optimized in preliminary experiments with developed cells (not shown). Antibody binding in the first stage is complete within 60 min, whereas Protein A binding in the second stage occurs even more rapidly. Sixty and thirty minutes were therefore chosen as the standard in-

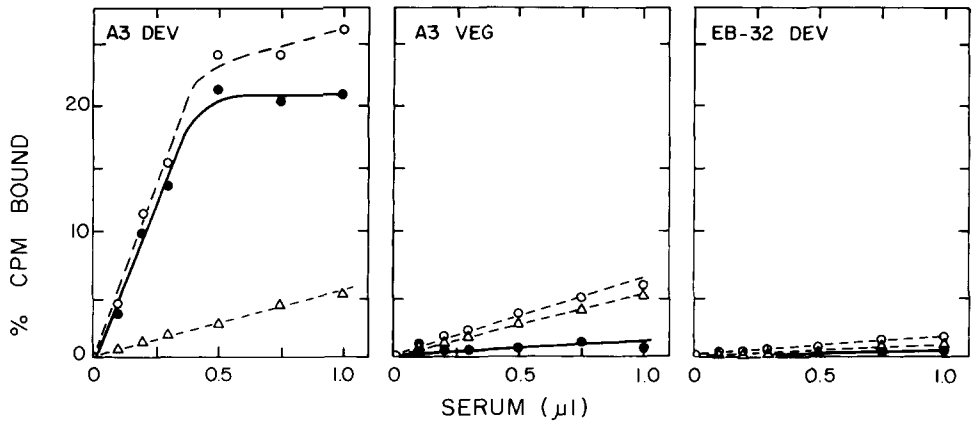


Fig. 2. Specific antibody binding assay for cell surface discoidin. The indicated cells were incubated with sera, washed, then incubated with ^{125}I-Protein A, and cell-associated radioactivity was determined as described in Materials and Methods. The values plotted were obtained by subtracting the per cent radioactivity bound in incubation mixtures without serum. Open triangles: control serum during first incubation; open circles: antidiscoidin serum during first incubation; closed circles: specific antibody binding.

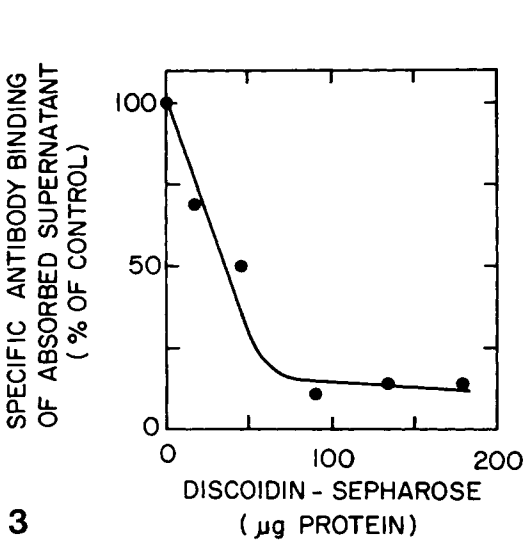
cubation times for the first and second stages respectively. A single wash after initial antibody binding was found to be optimal for measuring specifically bound immunoglobulin. Experiments in which the concentration of ^{125}I-Protein A was varied revealed that 500 ng/ml was optimal for saturating the cell-bound immunoglobulin, with a minimum of non-specific Protein A binding.

Figure 2 shows the results of this assay applied to vegetative cells from the wild type strain (A3) and to developed cells from either strain A3 or strain EB-32, a newly isolated aggregation-defective mutant blocked in development prior to the synthesis of the discoidins (see below). With control and antidiscoidin sera, vegetative A3 cells display low levels of binding that are linear with serum concentration throughout the range tested. By contrast, differentiated A3 cells show a low, linear level of binding with control serum, but a greatly enhanced binding curve with antidiscoidin. These results clearly indicate that the antidiscoidin serum is detecting developmentally regulated antigens on the surface of differentiated cells. As expected, binding was negligible with EB-32 cells. Since it is reasonable to assume that the binding observed with antidiscoidin contains both nonspecific and specific components, "specific antibody binding" is defined as the difference between binding observed with antidiscoidin vs normal serum, and is judged to be a good estimate of immunoglobulin specifically bound to cell surface antigens. The specific antibody binding curve is saturable, with 1 μl of antiserum being sufficient under these conditions (250 μl total volume) for detecting most of the reactive cell surface antigen with a minimum of nonspecific binding. Specific antibody binding was also found to be linear with cell number up to 2×10^6 cells (not shown).

Since this antiserum has been shown to react only with the discoidins among the proteins in cell lysates [6], it seemed likely that specific binding reflects only these molecules at the cell surface. To test this, we incubated the antiserum with Sepharose to which purified discoidins I and II had been bound. Figure 3 demonstrates that most of the specific antibody binding activity is removed by such an ad-

sorption. We therefore conclude that the specific antibody binding assay is a valid measure of the relative quantities of the discoidins at the cell surface. Because the antiserum used reacts with both discoidins I and II, this assay does not permit individual assessments of the quantities of each lectin.

As an alternate method, we performed quantitative immunoadsorption analyses in which intact cells were tested for their ability to remove antibodies reactive with purified radioiodinated discoidin I or discoidin II. Because this assay can distinguish between residual antibodies reactive with either lectin, this approach provides a means to separately quantitate discoidin I vs II at the cell surface. Figure 4 depicts the ability of intact vegetative or developed cells to adsorb antidiscoidin antibodies. Developed cells are capable of removing most of the antibodies reactive with discoidin I, as well as those reactive with discoidin II. As expected, vegetative cells have no effect. These results confirm the developmental regulation of cell surface discoidin expression and demonstrate that both lectins are present on the surface of developed cells.



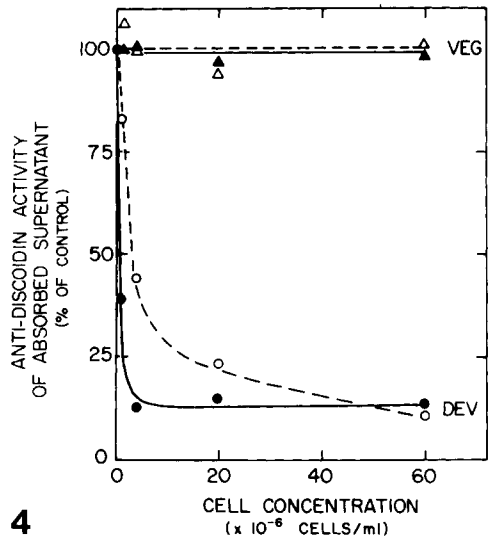


Fig. 3. Adsorption of antidiscoidin serum with discoidin-Sepharose. A mixture of discoidins I and II (approximately 1:1) was affinity bound to acid-treated Sepharose 6B (1 mg protein per ml packed Sepharose). Each incubation mix contained control or antidiscoidin serum (0.5 μl), the indicated quantity of discoidin-Sepharose 6B, plus sufficient untreated Sepharose 6B to bring the total packed volume of resin to 50 μl. The mixes were diluted to 0.25 ml total volume with phosphate-hemoglobin buffer. Following a 30-min incubation, the resin was removed by centrifugation and the supernatants containing unbound antibody were used to determine specific antibody binding. The volumes of supernatants used (100 μl per mix, corresponding to 0.2 μl of undiluted serum) were chosen to fall in the range which is linear with serum concentrations (see Fig. 2). The specific antibody binding activity is reported as percent of the activity observed in control incubations containing only untreated Sepharose 6B.

Fig. 4. Measurement of surface-localized discoidin I and discoidin II by immunoadsorption. Antidiscoidin serum (0.1 μl) was adsorbed with the indicated concentrations of vegetative (triangles) or developed (circles) A3 cells in a total volume of 0.25 ml, as described in Materials and Methods. The supernatants were then tested for their ability to immunoprecipitate ^{125}I-discoidin I (closed symbols) or ^{125}I-discoidin II (open symbols). The relative discoidin specifically precipitated by supernatants from control incubations without cells is defined as 100%.

Altered Patterns of Discoidin Expression in Aggregation-Defective Mutants

As part of our effort to study the nature of the discoidins' association with the cell surface, we have analyzed several newly isolated aggregation-defective mutants for discoidin expression. At the outset, we felt it was essential to distinguish between total cellular pools and surface-localized pools of these lectins, particularly in view of reports that only a fraction of the cellular discoidin resides at the surface [14, 15]. We therefore analyzed cell extracts to quantitate the total cellular discoidin pool (using competition RIAs and hemeagglutination assays), as well as intact cells to measure surface-localized discoidin (using specific antibody binding and immunoadsorption analysis).

The properties of two such mutants are compared with the parental A3 strain in Figures 5 and 6 and Table I. When developed on an agar surface, strain EB-32 shows no signs of aggregation long after the wild type cells have formed fruiting bodies (Fig. 5). No discoidin antigen is detected either in cell extracts or at the cell surface (Fig. 6 and Table I). Consistent with this, no hemeagglutination activity is found in cell extracts (Table I). Although it is possible that the primary defect in EB-32 is a direct and specific block in the production of the discoidins, it seems more likely that this strain is a pleitropic "program" mutant, blocked early in the developmental cascade prior to the aggregation stage and the synthesis of the discoidins. In any case, it serves as a useful control (Fig. 2), but like most similar mutants described [21] provides little information on the nature of the discoidins' association with the membrane or the factors controlling their surface expression. A very different pattern emerges with strain EB-18. When developed on agar, this mutant forms loose amorphous mounds that do not progress to fruiting body formation (Fig. 5). Specific antibody binding assays confirmed the absence of discoidin on the surface of vegetative cells (not shown). Unlike EB-32 however, EB-18 cells display substantial, though reduced levels of discoidins I and II, both in cell extracts and at the cell surface (Fig. 6 and Table I). Clearly this strain can proceed through development at least to the stage of aggregation and the initiation of discoidin synthesis. Furthermore, hemeagglutination assays suggest that the discoidins in this strain retain carbohydrate binding activity (Table I).

DISCUSSION

The immunochemical assays described here have enabled us to measure directly the relative amounts of the discoidins at the surface of intact D discoideum cells. The validity of the specific antibody binding assay is established by the known specificity of the antidiscoidin serum used [6], the absence of activity from vegetative cells (Fig. 2), the ability of discoidin-Sepharose to adsorb out the activity (Fig. 3), and the absence of activity in strain EB-32, a mutant displaying no discoidin in cell extracts (Figs. 2 and 6, Table I). The validity of the immunoadsorption analysis is demonstrated by the absence of adsorption activity from vegetative cells (Fig. 4) and from strain EB-32 (Table I). It should be noted that in experiments in which increasing numbers of cells were incubated with fixed volumes of antiserum, there was a very close reciprocal relationship between specific antibody binding and residual immunoreactivity in the adsorbed supernatants (not shown). The agreement between the two assays is also observed in the analysis of mutant strains (Table I). This suggests that potential artifacts, such as those arising from

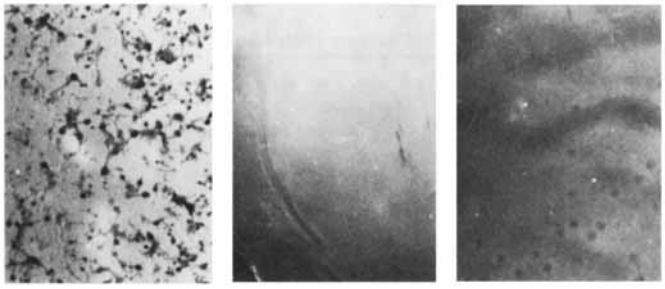


Fig. 5. Developmental morphologies of wild type and mutant D discoideum strains. Cells were inoculated onto agar plates in conjunction with K aerogenes and incubated at 22°C to allow the amoebae to consume the bacterial lawn. Photographs were taken 72 h after the food source had been exhausted. The wild type A3 strain completed fruiting body construction within 20 h of starvation. By contrast, strain EB-32 showed no sign of aggregation, whereas strain EB-18 formed occasional amorphous mounds of cells.

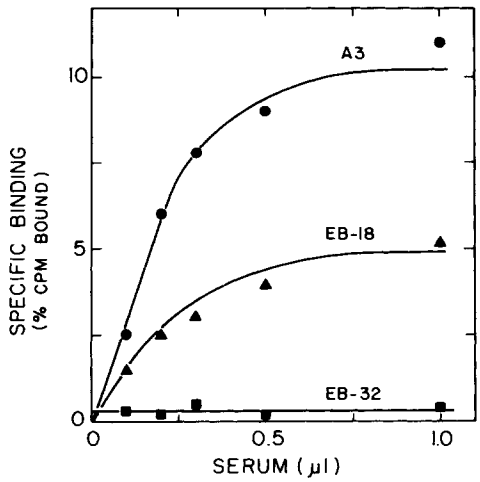


Fig. 6. Measurement of cell surface discoidin in wild type and aggregation-defective mutants by specific antibody binding. Specific antibody binding was determined as detailed in Figure 2 for developed cells of strains A3 (circles), EB-32 (squares), and EB-18 (triangles).

cell lysis during the assay or from possible contaminating antibodies in the antisera, are of little consequence, since they would be expected to affect the two assay procedures quite differently. By contrast, some methods employed by others, including hemeagglutination studies with intact cells [4], immunocytochemical methods [13, 22], cell surface radioiodination [21], and displacement radioimmunoassays with intact cells [14], are either semiquantitative at best or quite sensitive to intracellular discoidin released from a small number of lysed cells. More recently, other workers have employed both antibody binding and quantitative immunoadsorption analyses to measure lectins at the surface of intact slime mold cells [15, 23].

Whereas the assays described here provide valid means for measuring the relative amounts of the discoidins at the cell surface, difficulties arise when the data

TABLE I. Total Cellular Discoidin and Cell Surface Discoidin in Wild Type and Mutant Strains

		Strain		
Total cellular discoidin		A3	EB-32	EB-18
RIA:				
I	molecules/cell ($\times 10^{-6}$)	6.4	< 0.01	1.5
II	molecules/cell ($\times 10^{-6}$)	0.8	< 0.01	0.3
I and II	molecules/cell ($\times 10^{-6}$)	7.2	< 0.01	1.8
Hemagglutination:[a]				
I and II	$titer^{-1}$	16	< 1	4
Cell surface discoidin				
Specific antibody binding:[b]				
I and II	Protein A molecules/cell ($\times 10^{-6}$)	0.32	< 0.01	0.15
Immunoadsorption:[c]				
I	Anti-I adsorbed (% of total)	79	< 1	32
II	Anti-II adsorbed (% of total)	25	< 1	5

Total cellular discoidin and cell surface discoidin of developed cells were measured by performing the indicated assays on cell lysates or intact cells respectively, as described in Materials and Methods. The RIAs, hemeagglutination assays, and specific antibody binding assays were performed with the same batch of cells. In all of the assays, no discoidin was detected in EB-32. The values presented for this strain represent the lower limits of detectability for each assay.

[a]Cell extracts (corresponding to 3.5×10^7 cells/ml) were used to prepare two-fold serial dilutions. $Titer^{-1}$ is defined as the reciprocal of the last dilution in which visible hemeagglutination occurred.

[b]Assays were performed as described in Materials and Methods using saturating conditions for antiserum (1 μl).

[c]Assays were performed as described in Materials and Methods using 1 μl antiserum and 5×10^6 cells. Under these conditions, the amount of activity adsorbed was linear with cell concentration.

are used to compute absolute numbers of molecules. The bivalency of the antibodies, the multiplicity of antigenic determinants in each discoidin molecule, the unknown accessibilities of these determinants at the cell surface, the possible differences in immunoreactivities of soluble versus cell surface discoidin forms, and the ability of divalent antibodies to elicit the appearance of additional discoidin molecules at the cell surface [15] all contribute to the error in extracting absolute numbers from these data. Nevertheless, we have determined (Table I) that approximately 3×10^5 molecules of Protein A are bound to cells saturated with antidiscoidin (1 μl antiserum under these conditions). This value is comparable to the number of surface discoidin molecules obtained by competition radioimmunoassays with intact cells (2×10^5) [14], but considerably higher than those obtained either by selective elution of discoidin from the cell surface (0.52×10^5) or by direct binding of radioiodinated monovalent antidiscoidin F_{ab} (0.37×10^5) [15]. It should be noted that in a related species of slime mold, widely divergent estimates have been obtained using different immunochemical procedures to measure absolute quantities of a surface-localized lectin (purpurin) [15]. Some of these differences can be rationalized by proposing elicitation induced by divalent antibodies, but others cannot (ie, the five-fold difference in the number of surface-localized purpurin molecules calculated by selective elution with hapten sugar versus direct binding of radioiodinated antilectin F_{ab}). For these reasons, we feel confident in using the

REFERENCES

1. Beug H, Katz FE, Gerisch G: J Cell Biol 56:647, 1973.
2. Franke J, Sussman M: J Mol Biol 81:173, 1973.
3. Alton TH, Lodish HF: Dev Biol 60:207, 1977.
4. Rosen SD, Kafka JA, Simpson, DL, Barondes SH: Proc Natl Acad Sci USA 70:2554, 1973.
5. Frazier WA, Rosen SD, Reitherman RW, Barondes SH: J Biol Chem 250:7714, 1975.
6. Ma GCL, Firtel RA: J Biol Chem 253:3924, 1978.
7. Rosen SD, Barondes SH: In Garrod D (ed): "Specificity of Embryologial Interactions." London: Chapman and Hall, 1978, p 233.
8. Ray J, Shinnick T, Lerner RA: Nature 279: 215, 1979.
9. Shinnick TM, Lerner RA: Proc Natl Acad Sci USA 77:4788, 1980.
10. Barondes SH: In Curtis A, Pitts J (eds): "Cell Adhesion and Motility." New York: Columbia University Press, 1980, p 309.
11. Gerisch, G: Curr Top Dev Biol 14:243, 1980.
12. Geltosky JE, Weseman J, Bakke A, Lerner RA: Cell 18:391, 1979.
13. Chang CM, Rosen SD, Barondes SH: Exp Cell Res 104:101, 1977.
14. Siu CH, Loomis WF, Lerner RA: In Lerner RA, Bergsma D (eds): "The Molecular Basis of Cell-Cell Interactions." New York: Alan R. Liss, 1978, p 439.
15. Springer WR, Haywood PL, Barondes SH: J Cell Biol 87:682, 1980.
16. Allen HJ, Johnson EAZ: Carbohydr Res 50:121, 1976.
17. Sussman M: Meth Cell Physiol 2:397, 1966.
18. Berger EA, Armant DR: Proc Natl Acad Sci USA (in press).
19. Barondes SH, Rosen SD, Frazier WA, Simpsom DL, Haywood PL: Meth in Enzymol 50:306, 1978.
20. McConahey PJ, Dixon FJ: Int Arch Allergy Appl Immunol 29:185, 1966.
21. Siu CH, Lerner RA, Ma G, Firtel RA, Loomis WF: J Mol Biol 100:157, 1976.
22. Chang CM, Reitherman RW, Rosen SD, Barondes SH: Exp Cell Res 95:136, 1975.
23. Springer WR, Barondes SH: J Cell Biol 87:703, 1980.