IDENTIFICATION OF CONCANAVALIN A RECEPTORS AND GALACTOSE-BINDING PROTEINS IN PURIFIED PLASMA MEMBRANES OF *DICTYOSTELIUM DISCOIDEUM*

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ABSTRACT

Two techniques have been modified to provide simple means for the identification of molecules which bind concanavalin A (Con A). Crossed immunoelectrophoresis was altered by replacing antibody with Con A, and receptors were identified by the precipitin arcs which they produced. Con A, tagged with fluorescein isothiocyanate, was also diffused into prefixed sodium dodecyl sulfate (SDS)-polyacrylamide gels, and additional receptors identified by fluorescence. More than 35 molecules in the plasma membranes of the cellular slime mold *Dictyostelium discoideum* which bind Con A were identified with these techniques. At least 12 of these diminish and 12 increase in importance as receptors during differentiation of the cells from the vegetative to the preculmination stage of development. In the course of these experiments, it was possible to confirm the presence of the galactose-binding protein discoidin, in the plasma membrane, by electrophoresing membrane proteins into an agarose gel. This lectin regains its sugar-binding activity after denaturation and electrophoresis in SDS.

The molecules of the plasma membrane of the cellular slime mold, *Dictyostelium discoideum*, are potentially involved in many aspects of development. Considerable evidence indicates that the membrane is involved in cellular adhesion, induction of developmentally coupled enzymes, the production and response to chemotactic signals, and several other areas of development (see reference 15 for review). Therefore, we have examined two particular classes of molecules of the plasma membrane, concanavalin A (Con A) receptors and agarose-binding proteins.

Con A induces a plethora of responses at the cell surface of *D. discoideum*. Living cells can be agglutinated by Con A and this agglutinability decreases during development so that 15 times more Con A is required to agglutinate maximally preculmination cells as compared to vegetative cells (32, 33). Con A postpones the onset of aggregation, but this effect saturates at a much higher concentration of Con A than does agglutination (8, 9, 32). All of the Con A receptors on the surface of *D. discoideum* cells patch in the presence of Con A and are translocated to a cap which is distinguished by an accumulation of all the microvilli on the cell surface (9, 17). Treatment with Con A results in a fourfold increase in intracellular cAMP phosphodiesterase activity within 30 min (8). This result is especially interesting because a model for cellular position in *D. discoideum* posited such a regulation of cAMP phosphodiesterase activity by receptors on the plasma membrane (14). Other effects of Con A on *D. discoideum* are a delay in the appearance of large intramembranous particles (37), and inhibition of the appearance of extracellular cAMP

phosphodiesterase inhibitor, and extracellular *N*-acetyl glucosaminidase activities during development (6, 8).

A number of additional effects of Con A have been characterized in other cell types. In addition to its mitogenic properties and its ability to return normal growth inhibition to transformed cells (23), Con A has been found to affect membrane enzyme activities such as 5′-nucleotidase (22, 26), Na^+, K^+-stimulated, Mg^{+2}-dependent ATPase (28, 21) and glycosyltransferases (20).

Most studies have suggested and even assumed that the number of Con A receptor species is small. For instance, binding studies of radiolabeled Con A suggest that there is only one class of binding sites in vegetative cells from *D. discoideum* (34). We have determined, however, that there are over 35 different kinds of receptors for Con A in the plasma membrane of *D. discoideum*.

Another lectin, discoidin, occurs naturally in *D. discoideum*. This protein, which binds to galactose residues, has been suggested to be involved in adhesion of aggregating *D. discoideum* (4, 24, 25). One of the methods used to identify receptors for Con A proved also to be a sensitive assay for agarose-binding proteins and was used to verify the presence of discoidin in the plasma membrane.

Preliminary accounts of a portion of this work have appeared elsewhere (35, 36).

MATERIALS AND METHODS

General Methods

D. discoideum, strain A3, was grown axenically (15). Development was induced by plating on filter paper for 12 h (aggregation stage) or 18 h (preculmination stage) as described (15). There are some biochemical differences between strain A3 cells grown axenically or on bacteria (13), but cells grown under both regimes have a similar course of morphogenesis. Plasma membranes were purified by isopycnic centrifugation of cell-free extracts in sucrose and renografin gradients as described by McMahon et al. (15, 18), unless otherwise indicated. This preparation is normally contaminated to the extent of 5% with mitochondrial inner membrane (18). A mixture of discoidins 1 and 2 (prepared according to reference 7) was the generous gift of D. Lesikar and S. Barondes (University of California, San Diego, La-Jolla, Calif.).

In some experiments, plasma membranes were purified by a modified method which reduced contamination by inner mitochondrial membrane although this method gives a lower yield of material. Cells were broken in a French press with a pressure of 56.4 kg/cm² (170 kg/cm² for cells in aggregation or preculmination) and a mitochondrial pellet was obtained according to the methods of Stuchell et al. (27). The post-mitochondrial supernate was then pelleted at 40,000 *g* for 30 min at 2°C, and resuspended in homogenization buffer (18). Plasma membranes were purified from this fraction in the normal way. At the final step of purification, when the membranes were collected from the renografin gradient, care was taken to collect only the upper portion of the turbid region. This region is white in color. The ratio of succinate dehydrogenase (12) to 5′-nucleotidase (18) was 0.05 of that found in the standard method of purification, suggesting that the content of mitochondrial inner membrane was reduced to 0.25%.

Following solubilization in boiling sodium dodecyl sulfate (SDS) and β-mercaptoethanol, membranes (60–260 μg of protein per gel lane) were electrophoresed in discontinuous polyacrylamide slab gels (8–15% exponential gradient of polyacrylamide) containing 0.1% SDS, modified slightly (11) from reference 5. Polypeptides were stained with Coomassie blue (31) and glycolipids and glycoproteins by the periodic acid-Schiff's base technique (PAS) according to Hoffman and McMahon (11) as modified from reference 10.

Lectin-Electrophoresis

Lanes, sliced from the slab gel and frozen in SDS electrophoresis buffer (30), were thawed and lectin-electrophoresed by a procedure adapted from reference 5. Two glass plates cleaned in Chemsolve (Mallinckrodt Inc., St. Louis, Mo.) were clamped around three 1.4-mm thick plexiglass spacers, and several molten agarose solutions were successively poured. For contact with the cathode, a solution (90°C) of 1.33% (wt/vol) agarose (electrophoresis grade, Nutritional Biochemicals, Cleveland, Ohio) dissolved in electrophoresis buffer ([EB]:80 mM Tris Base, 40 mM sodium acetate, 100 mM NaCl, 2 mM $CaCl_2$, to pH 7.4 with glacial acetic acid) was poured to a depth of 4 cm in the prewarmed (60°C) mold and cooled. In a similar way, a 0.5-cm layer of 0.01% trypan blue in 1.33% agarose solution was cast. The third layer was 0.8 cm of a solution (55°C) consisting of 0.15 part of 10% (wt/vol) Lubrol PX (Sigma Chemical Co., St. Louis, Mo.) in EB and 0.85 part of 1.33% agarose in EB. For the next layer (4 cm), the mold was not preheated. The Con A-agarose solution (1.2 ml of 0.004% [wt/vol] Con A and 0.012% [wt/vol] NaCl in EB at 25°C mixed quickly with 4.8 ml of 1.33% agarose in EB at 50°C) was poured with a warm pipette immediately after it was mixed. Finally, the mold was filled (5 cm) with a 1.33% agarose solution in EB to contact the anode. After cooling, the plates were separated, exposing one face of the gel. A strip of agarose equal in width to a lane of the SDS gel was carved from the gel leaving only a narrow strip of the layer containing the trypan blue abutting the Lubrol layer. The SDS gel lane was inserted, moved into contact, and the agarose gel extending beyond the ends of the polyacrylamide gel was trimmed away. The agarose gel was covered with a

square of cellophane except at the points of contact with the wick. The buffer reservoirs contained EB. Electrophoresis was performed in the cold (4°C) at a potential of 7–10 v/cm for 2–4 h. The gels were washed in two changes of 0.075 M NaCl for 15 h to remove nonprecipitated proteins, dried underneath a sheet of Whatman No.1 paper in a 55°C incubator, and then stained with Coomassie blue for 5 min and destained (31). When hapten sugars were present, they were dissolved in the Lubrol and Con A solutions before dilution with agarose. The concentrations given are those after dilution. Conclusions were based on at least three independent experiments.

Lectin-Diffusion

SDS polyacrylamide gels were prepared as above, sliced into lanes, then fixed with methanol, glutaraldehyde, and sodium borohydride (29). For labeling, gel lanes were rocked for three days in a small plastic tray containing 16 ml of a solution composed of 0.1 M NaCl, 0.033 M sodium phosphate, pH 8.0, 0.2% bovine hemoglobin, 0.05% sodium azide, 0.4–0.6 mg Con A conjugated with fluorescein isothiocyanate per gel (Miles Laboratories, Elkhart, Ind. or Vector Laboratories, Ignacio, Calif.; OD_{495}:OD_{280} = 0.95) in the presence or absence of 0.25 M *α-methyl*-D-mannopyranoside (αMM). The gel was washed for 2 days in two changes of 200 ml per lane of a solution containing 0.1 M NaCl, 0.033 M sodium phosphate, pH 8.0, 0.05% sodium azide, and 0.25 M αMM if appropriate. Gels illuminated from below with a short wavelength ultraviolet light box were photographed for 5–10 min with a Wratten filter No. 65. Conclusions were based on two or three independent experiments.

Some gels were postfixed overnight with 6.5% (vol/vol) 2-aminoethanol in place of the $NaBH_4$, or, alternatively, not fixed and postfixed at all but labeled with Con A immediately following a ½ h incubation in 25% isopropanol. The pattern of Con A bands was essentially the same, but in the absence of fixation there was some loss of resolution.

Molecular weight positions were calibrated in the lectin diffusion gels by poststaining with Coomassie blue and, in the case of lectin-electrophoresis, by staining nearby lanes of the same SDS polyacrylamide gel with Coomassie blue.

RESULTS

Identification of Con A Receptors by Crossed Lectin-Electrophoresis

Con A receptors were identified in two ways after prior separation in SDS polyacrylamide gels. The first technique was a modified form of crossed immunoelectrophoresis (5) substituting a lectin for antibody. The feasibility of the system was explored with a glycoprotein which contains mannose, avidin, and a nonglycosylated protein, cytochrome c. In Fig. 1, avidin is located to the right of cytochrome c in the SDS polyacrylamide gel. After electrophoresis in the second dimension gel, which contained Con A, a precipitate (stained with Coomassie blue) was produced by avidin but not by cytochrome c. The precipitation was reduced in the presence of 0.1 αMM and not present in the absence of Con A (not shown). It is probable that only multivalent Con A receptors would form precipitates with this method. Such receptors might contain a branched polysaccharide, multiple polysaccharides attached to a single polypeptide, or aggregates of polypeptides each linked to a minimum of a single polysaccharide.

This method was used to analyze plasma membranes isolated from vegetative cells and resolved by SDS polyacrylamide gel electrophoresis. The gels in Figs. 2*a* and 2*b* illustrate the molecular weight profile of proteins and glycoproteins, respectively, as they were stained with Coomassie

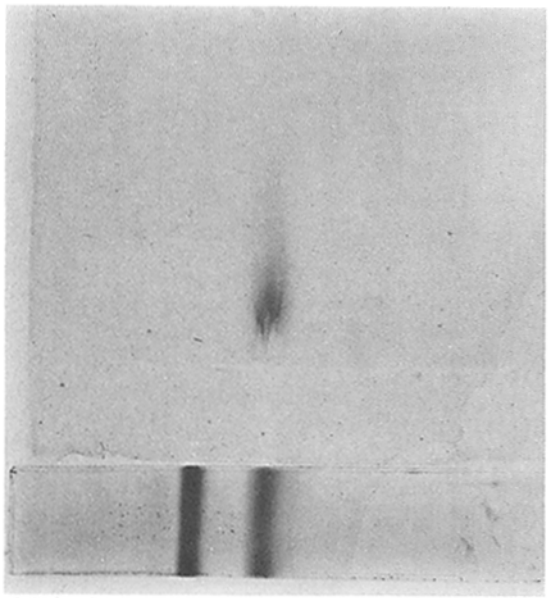

FIGURE 1 Crossed lectin-electrophoresis of avidin and cytochrome c. 2 μg each of avidin (on right), a glycoprotein containing mannose, and cytochrome c (on left), a non-glycosylated protein, were electrophoresed on SDS polyacrylamide gels and then electrophoresed into an agarose gel containing, successively, Lubrol and Con A. A composite picture containing a duplicate of the original polyacrylamide gel (bottom) and the agarose gel (top), each stained with Coomassie blue, is presented.

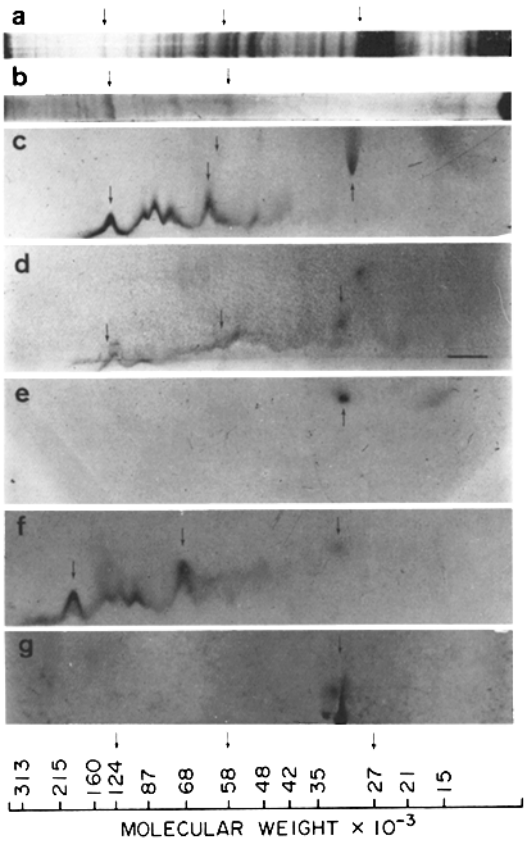

FIGURE 2 Components of the plasma membrane (60 μg protien) of *D. discoideum*. (*a*) Polypeptides from vegetative stage plasma membranes resolved on an SDS polyacrylamide gel and stained with Coomassie blue. (*b*) Glycoproteins and glycolipids on an equivalent gel stained with PAS. (*c–g*) Crossed lectin-electrophoresis of the components of the SDS polyacrylamide gel as given in the legend to Fig. 1. The Con A-containing portion of the gel is shown. (*c*) Plasma membranes from vegetative amoebae were in the polyacrylamide gel and Con A (40 μg) was in the agarose gel. (*d*) Plasma membranes from cells in the preculmination stage of development in the polyacrylamide gel were electrophoresed into agarose containing Con A (40 μg protein). In this case, part of the Lubrol gel is shown. The bar marks the bottom of the Con A part of the gel. (*e*) Plasma membranes from vegetative cells were electrophoresed into an agarose gel containing Con A (40 μg) and 0.15 M αMM. (*f*) As in (*e*), except that αMM was replaced with 0.5 M D-fucose. (*g*) As in (*c*), but Con A was replaced by bovine serum albumin (40 μg). The arrows mark components with apparent molecular weights of 124,000, 58,000 and 27,000 daltons, from left to right, respectively.

blue and PAS. Fig. 2*c* shows the stained profile of precipitation arcs which developed when 60 μg of plasma membrane protein, resolved on an SDS gel, was lectin-electrophoresed. Only the part of the gel which contained Con A is shown. At least six receptors with apparent molecular weights of 124,000, 87,000, 76,000, 68,000, 58,000, and 42,000 daltons are visible. Several other relatively minor arcs indicate that there are potentially more Con A receptors.

We examined the specificity of the reaction in several ways. The precipitin arc reactions depended on Con A since they were absent when Con A was replaced by the same concentration of bovine serum albumin (Fig. 2*g*). Furthermore, the reactions were the result of Con A's sugar-specific binding properties since, which the hapten sugar αMM (0.15 M) was present in Lubrol and Con A layers of the gel, the arcs did not form (Fig. 2*e*). On the other hand, the same or higher concentration of L-fucose (not shown) or D-fucose (Fig. 2*f*) did not affect the pattern of arcs. However, a spot of adsorbed protein, of molecular weight 27,000 daltons, was not removed by αMM or L-fucose, and was present when the Con A was replaced by bovine serum albumin. This will be discussed later.

At this relatively low concentration of membrane protein, the major Con A receptors comigrated with several major glycoproteins in the SDS gel (compare Figs. 2*b* and 2*c*). It appears that the majority of glycoproteins revealed by the PAS method are Con A receptors.

It was of interest to determine whether there were any changes in the Con A receptors when cells had differentiated. When similar concentrations of preculmination stage plasma membranes were studied, the precipitin pattern was consistently very weak and difficult to interpret (Fig. 2*d*). Higher concentrations of protein gave clear patterns.

Crossed lectin-electrophoretic analysis of higher concentrations (190 μg protein) of plasma membranes from vegetative cells showed several additional Con A receptors (Fig. 3*a*). Examination of preculmination stage plasma membranes with this higher concentration also showed a complex set of Con A receptors (Fig. 3*c*). The majority of the receptors of the two developmental stages have similar molecular weights, but some are clearly different. It appeared that the receptor at 58,000 daltons decreased or disappeared from the plasma

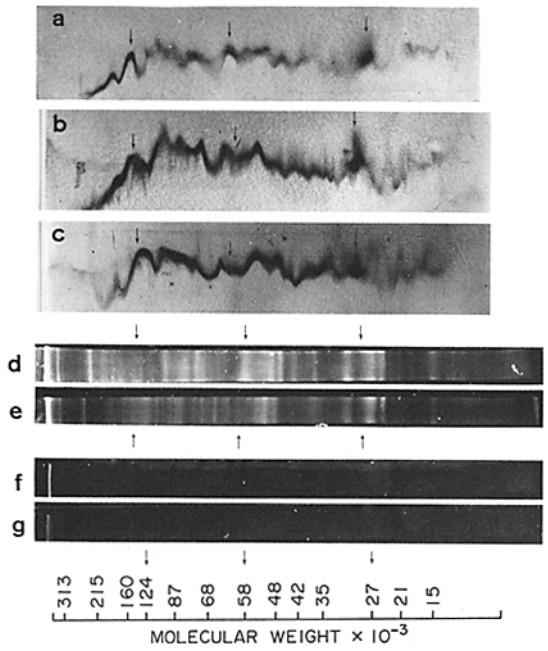

FIGURE 3 Alteration of Con A receptors during development. The components of the SDS polyacrylamide gel were electrophoresed into agarose gels containing Con A (40 μg). (*a*) Vegetative stage plasma membranes (190 μg protein). (*b*) Vegetative stage plasma membranes (190 μg protein) plus preculmination stage plasma membrane (190 μg protein). (*c*) Preculmination stage plasma membranes (190 μg protein). In the four panels on the bottom, fluorescent Con A was allowed to diffuse into prefixed SDS polyacrylamide gels. (*d*) Vegetative plasma membranes (260 μg protein). (*e*) Preculmination stage plasma membranes (260 μg protein). (*f*) As in (*d*) plus 0.25 M αMM. (*g*) As in (*e*) plus 0.25 M αMM. Arrows are described in legend to Fig. 2.

membrane as development progressed from the vegetative to the preculmination stage, while a new one with an apparent molecular weight of 115,000 daltons replaced a receptor with an apparent molecular weight of 124,000 daltons. In pseudoplasmodial plasma membranes, new precipitin arcs were produced by receptors whose apparent molecular weights were 63,000 and 48,000 daltons. A comparison of membranes from the two developmental stages was least ambiguous when vegetative and preculmination plasma membranes were co-electrophoresed together in the SDS gel and then lectin-electrophoresed (Fig. 3*b*). The exchange of the 124,000-dalton receptor for one of 115,000 daltons is supported by the presence of two superimposed arcs in the mixture. Furthermore, a complex set of changes occurred in the molecular weight region between 58,000 and 124,000 daltons.

In summary, crossed lectin-electrophoresis with Con A showed a complicated pattern of receptors between 30,000 and 160,000 daltons. Below 30,000, the pattern of arcs was ill-defined and detection of potential receptors above 160,000 daltons was hindered because they were trapped in the polyacrylamide gel, although this could be overcome by electrophoresis for longer times (data not presented).

Identification of Con A Receptors by Lectin-Diffusion

Another technique for detecting Con A receptors was adapted in order to complement the findings of crossed lectin-electrophoresis. This procedure, modified from that used by Tanner and Anstee (29), used glycoproteins resolved on SDS polyacrylamide gels and then fixed. After washing, fluorescein isothiocyanate-conjugated Con A (FITC-CON A) was diffused into the gel. Fig. 3*d* illustrates the bands which stain with FITC-Con A. The presence of 0.25 M αMM prevents labeling (Fig. 3*f*). The residual apparent labeling with αMM present is visible even when the Con A is not added and is presumably due to autofluorescence. Preculmination stage plasma membranes examined in a similar way are presented in Figs. 3*e* and 3*g*.

These Con A receptors were detected in a plasma membrane preparation which contained about a 5% contamination with inner mitochondrial membrane. In order to determine whether the Con A receptors came from the plasma membrane, we studied plasma membranes purified by a modified procedure which contained mitochondrial contamination reduced by twentyfold.

The overall qualitative fluorescence intensity produced by labeling these plasma membrane glycoproteins with FITC-Con A (Fig. 4*b*) was similar to that seen before with less purified plasma membranes (Fig. 3*d*). The greater amount of fluorescence intensity associated with vegetative stage plasma membrane glycoproteins compared with that from pseudoplasmodial plasma membrane glycoproteins was also preserved in the more purified plasma membranes. Most of the receptors were found in both preparations, but there were distinctive differences as well. It was concluded that those bands which were reduced in amount in Fig. 4 either do not reside in the plasma membrane or reside in a portion of the plasma

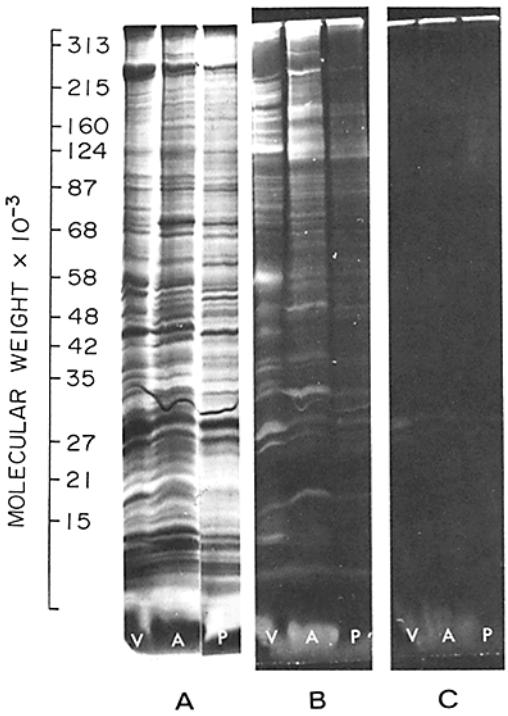

FIGURE 4 Con A receptors in highly purified plasma membranes. 100 μg of plasma membrane protein per gel lane were electrophoresed into polyacrylamide gels in the presence of SDS and 2-mercaptoethanol. After fixation in methanol, glutaraldehyde and $NaBH_4$, the gels were incubated in FITC-Con A followed by washing in buffer. After photography of fluroescent staining, the gel was stained with Coomassie blue and destained. (*A*) Gel shown in part *B* stained with Coomassie blue. (*B*) Gel stained with FITC-Con A. (*C*) Gel stained with FITC-Con A and washed, both in the presence of 0.25 M αMM. *V*, *A*, and *P* refer to plasma membranes isolated from cells in the vegetative, aggregation and preculmination stages of development, respectively. The irregular band with a molecular weight of approximately 30,000 daltons is an artifact. Full size copies of the figures will be provided by the authors upon request.

membrane which does not co-purify with the marker enzyme 5'-nucleotidase. In vegetative stage plasma membranes, there were five differences, including the pair of Con A receptors migrating at 48,000 daltons and another band at about 215,000 (Fig. 3*d*). The pair of major bands migrating near 24,000 daltons was relatively diminished in intensity of fluorescence and staining by Coomassie blue (compare Fig. 4*a* and Fig. 2*a*).

The major conclusions from crossed lectin-electrophoresis were corroborated by the results from lectin-diffusion shown in Fig. 4. In both cases, the pattern of Con A receptors was very complex. The changes described to take place during the course of development were also observed in lectin-diffusion. However, the lectin-diffusion technique was more sensitive and had greater resolution, and this permitted a number of additional receptors to be identified (Fig. 4*b*). A list of Con A receptors in the vegetative stage plasma membranes is given in Table I. There are at least 8 additional unlisted minor bands which do not photograph well. Many of the Con A receptors from aggregation and preculmination stage plasma membranes were similar on the basis of their comigration with vegetative stage receptors (Fig. 4*b*). There were differences as well, and these are summarized in Table I.

Identification of Discoidin

As described above, a protein with a molecular weight of 27,000 was detected by staining in the agarose gels after crossed lectin-electrophoresis (Figs. 2*e* and 2*g*). This is discoidin, a galactose-binding protein, known to exist on the surface of *D. discoideum* cells (4, 25). The identification of this spot was established by the following observtions: The protein remained bound to the agarose gel when Con A was replaced by bovine serum albumin (Fig. 2*g*); discoidin binds to galactose and would be expected to bind to agarose; the presence of 0.5 M D-fucose (a hapten of discoidin) in the gel substantially reduced the amount of protein which was bound to the gel (Fig. 2*f*); L-fucose (not shown) and αMM (Fig. 2*e*), sugars which are not haptens for discoidin, did not have this effect; the molecular weight of the bound protein was identical, within experimental error, to the molecular weight of discoidin (26,000) (7, 18); this protein, band S (11) was destroyed when intact cells were treated with pronase (11), agreeing with the presence of discoidin on the surface of the cell; and, finally, an authentic sample of discoidin (7) behaved in a similar manner (Fig. 5). Recently, a second galactose-binding protein (called discoidin II) which co-purifies with discoidin and has a slightly lower molecular weight has been resolved (7; see also Fig. 5). A second agarose-binding protein at a position corresponding to the molecular weight of discoidin II also appeared intermittently in preculmination stage plasma membranes (Fig. 2*d*), and this may be discoidin II. Therefore, discoidin can recover its native specificity after being denatured in boiling SDS

TABLE I

Apparent Molecular Weights of Receptors for Concanavalin A in the Plasma Membrane of D. discoideum

Apparent molecular weight ($\times 10^{-3}$)*	Stage of development		
	Vegetative	Aggregation	Preculmination
313	++‡	++	0
260	0	++	0
250	0	+	0
213	0	++	+
210	++	++	+
205	++	++	+
200	++	+	+
175	++	++	+
165	++	++	+
145	++	++	+
128	+	+	+
124	+++	++	+
115	+	++	+++
93	0	+	+
91	+	+	+
87	+	+	+
84	+	+	0
82	+	+	+
80	+	+	+
76	++	+	+
73	+	++	+
71	+	+	+
70	0	+	0
68	+	+	+
65	+	++	+
63	0	+	+
61	0	+	0
58	+++	+	+
55	0	++	+
52	0	0	+
48	+	++	++
42	++	+	+
39	++	++	+
37	++	+	0
32	++	+	0
30	++	+++	++
19	+	+	++
15	++	++	++
12	++	0	0
11	++	0	0
8	++	++	++

* Molecular weights calibrated with thyroglobulin (335,000 daltons), myosin (220,000 daltons), β-galactosidase (130,000 daltons), phosphorylase A (92,000 daltons), human serum albumin (68,000 daltons), immunoglobulin G (50,000 and 23,000 daltons), avidin (16,000 daltons) and cytochrome c (11,800 daltons).

‡ Relative fluorescence intensity of labeling, on a scale ranging from 0, not detectable, to +++, a relatively large amount of fluorescence.

and β-mercaptoethanol. This procedure is a sensitive assay for the presence of galactose-binding proteins.

DISCUSSION

As discussed in the introduction, Con A has a variety of biochemical effects on plasma membrane-associated activities in many kinds of cells. Because several effects of Con A on *D. discoideum* disrupt the normal program of development, considerable effort has been devoted to analyzing the cellular and biochemical properties of the molecules which bind Con A. These studies produced a picture which suggested that a relatively homogeneous class of Con A receptors, perhaps as few as one to two molecular species, was present on the surface of *D. discoideum* and also that some property(ies) of the receptor(s) changed during development which resulted in lowered affinity for Con A (6, 34).

The techniques used in this paper indicate that a minimum of 35 glycoproteins are Con A receptors in purified plasma membranes of *D. discoideum*. This number is much greater than that determined for two other types of cells, neurons and lymphocytes, which have seven to nine Con A receptors as determined by affinity chromatography (1, 38). This may be due to a higher sensitivity of techniques presented here. Our techniques would detect receptors regardless of whether they were found on the exterior or interior face of the plasma membrane. Some of the Con A receptors are located on the external face of the plasma membrane (9, 17), but it is not known whether all receptors have this orientation. While all Con A receptors seem to be localized on the external rather than the internal face of mammalian (19) and yeast (2) plasma membranes, in an amoeba one is found on the cytoplasmic face (3). Nevertheless, there is no reason to assume that the diverse effects of Con A on *D. discoideum* are mediated by a single receptor.

The Con A-induced agglutinability of preculmination stage cells has been shown to be reduced when compared to vegetative cells (32, 33). Changes in receptor mobility (17) or number of receptors (34) do not appear to be responsible for this altered agglutinability. Weeks' findings that the affinity of the majority of Con A receptors is decreased in preculmination stage cells is, however, a potential explanation for the altered agglutinability. The changes observed by Weeks could be due to modification of preexisting receptors,

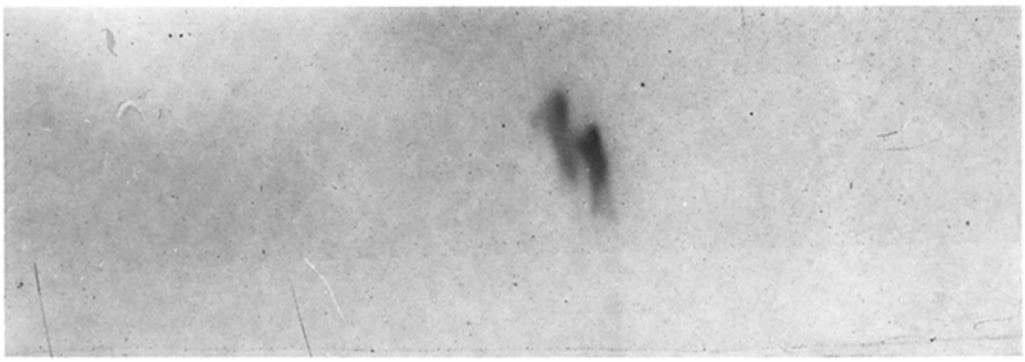

FIGURE 5 Detection of discoidin by electrophoresis into an agarose gel. Seventeen micrograms of a mixture of discoidins I and II were subjected to SDS gel electrophoresis and then electrophoresed into an agarose gel, as described in Materials and Methods, in the absence of Con A.

alterations of their environment, or replacement of receptors. Our results indicate that many vegetative receptors are removed, and that new ones appear during the course of development (Fig. 4*b*). In addition, many of the receptors which seem to remain, during development, bind less fluorescent Con A (Fig. 4*b*). This is consistent with a lowered affinity of receptors on the surface of preculmination cells.

Comparison of the results of lectin-electrophoresis and lectin-diffusion allows inferences to be made regarding structural features of the Con A receptors. Lectin-electrophoresis would probably detect only those receptors that have multiple binding sites which are sterically independent or those which aggregate when they migrate from the SDS gel. Many of the receptors fit into this category (Figs. 3*a* and 3*b*). In contrast, lectin-diffusion should detect receptors of any valency. Comparison of the results of lectin-electrophoresis with those of lectin-diffusion suggests that the receptors migrating below 20,000 daltons are univalent.

The techniques discussed here will greatly facilitate the detection and identification of lectin receptors separated on SDS polyacrylamide gels. The lectin-diffusion technique posesses three important advantages over other techniques for identifying receptors. It requires only small amounts of material and is extremely convenient. Second, its resolution is equal to that of the electrophoresis system which is used. Finally, the method results in a profile of receptors which are stained in proportion to their lectin-binding unlike other methods which may depend on the exposure of a receptor on the membrane, its mass, or its amino acid composition. Electrophoresis in a second dimension into agarose gels was also a sensitive assay for galactose-binding proteins, as shown with discoidin. This technique could potentially be expanded to identify proteins of many types by adding or coupling different ligands to the agarose. Likewise, other kinds of receptors could potentially be identified in prefixed SDS polyacrylamide gels by infiltration with fluorescein-tagged or radiolabeled ligands.

ADDENDUM

After the submission of this work for publication, Geltosky et al. (Geltosky, J. E., C.-H. Siu, and R. A. Lerner. 1976. *Cell.* **8**:391-396) published a paper describing Con A receptors on the *D. discoideum* cell surface, using an alternative method. In the part of the paper relevant to our work, whole cells were iodinated with ^{125}I and lactoperoxidase, solubilized with NP-40, and applied to a Con A affinity column; material was eluted with α-methyl mannoside and electrophoresed in an SDS-polyacrylamide gel which was examined with autoradiography. They observed about 15 cell surface Con A receptor species and suggested that there were two changes during development: one with an apparent molecular weight of 180,000 which decreased in amount or affinity to Con A, and another with an apparent molecular weight of 150,000 which increased. This pair may correspond to the two glycoproteins, with apparent molecular weights of 124,000 and 115,000, found in our work.

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This paper is the third in a series entitled, The Role of

The Plasma Membrane in The Development of *Dictyostelium discoideum*. Paper number two is reference 11.

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