Identification of Glycoproteins, Using Lectins As Probes, in Plasma Membranes from Dictyostelium discoideum and Human Erythrocytes*

(Received for publication, April 4, 1977)

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The glycoproteins of plasma membranes from axenically grown Dictyostelium discoideum and human red blood cells (O−) were characterized according to their apparent molecular weights in sodium dodecyl sulfate-polyacrylamide gels and their ability to bind lectins. This was achieved by diffusing each of several fluorescein-conjugated lectins into sodium dodecyl sulfate-polyacrylamide gels which contained the purified plasma membranes. Subsequent to identifying fluorescent bands the gels were stained with Coomassie blue to relate the lectin receptors to known proteins. In D. discoideum plasma membranes over 25 macromolecules, ranging in apparent molecular weight from 8,000 to 95,000, were identified with fluorescein wheat germ agglutinin. The binding of wheat germ agglutinin was specific and reflected the presence of receptors containing N-acetyl-D-glucosamine. Some of these receptors are distinct from those which bind concanavalin A (West, C. M. & McMahon, D. (1977) J. Cell Biol. 74, 264-273). The concanavalin A receptors and all but one of the wheat germ agglutinin receptors were shown to be glycoproteins since they were hydrolyzed by proteolytic treatment. In contrast, Ricinus communis agglutinins 60 and 120, soybean agglutinin and Ulex europeus agglutinin I, and lectins with specificities directed toward the other common simple sugars of the plasma membrane failed to label any glycoproteins in the sodium dodecyl sulfate-polyacrylamide gel. The absence of glycoproteins recognized by the R. communis agglutinins on the plasma membrane was confirmed by conjugating a mixture of the two lectins labeled with fluorescein isothiocyanate and microspheres, and assaying for binding to cells in a fluorescent or scanning electron microscope. Consequently, D. discoideum plasma membrane glycoproteins seem to be restricted to classes which bind concanavalin A and/or wheat germ agglutinin. This pattern persisted during the course of development, although within each class of glycoproteins there were many developmental changes.

In comparative experiments, the glycoproteins of the human erythrocyte ghost were also identified, using lectins with specificities against each of the preponderant simple sugars of the ghost. In contrast to D. discoideum, there were glycoproteins capable of binding, specifically, all of the lectins tested. In most cases glycoproteins had been known from previous experiments, but a few new glycoproteins were identified as well.

The cell surface of the cellular slime mold Dictyostelium discoideum is becoming described in increasing detail with respect to its structure, function, and role in development (for review see Ref. 1). There are over 80 polypeptides which have been identified in the isolated plasma membrane (2-5); at least 14 of these are located on its external surface (2, 3). More than 40 of the plasma membrane molecules are glycoproteins which bind concanavalin A (6-8); some of these had previously escaped detection as proteins or glycoproteins (2) using conventional staining procedures. Concanavalin A-binding proteins total more than 1 × 10^7 per cell surface (8-12). A significant fraction of the proteins and glycoproteins are modified or replaced during the course of development (2, 3, 5-7) and have been shown shown to be under developmental control.1

Glycoproteins have been suggested to play an important role in plasma membrane function in D. discoideum. For example, an antigen(s) (contact sites A) in the plasma membrane is known which if blocked with the Fab fragment of an appropriate antibody will result in a loss of adhesion between aggregation stage cells. This antigen is carbohydrate in nature (13, 14). Isolated plasma membranes induce a change in activity of enzymes in whole cells (4, 15) and this effect can be prevented by pretreatment of the membranes with periodate, under conditions which are relatively specific for destruction of carbohydrate (4).2 An enzyme on the cell surface, acid phosphatase, is known to be a glycoprotein which binds Con

2 D. McMahon, unpublished results.
A2 (16). Con A (17, 18) and wheat germ agglutinin, lectins which bind to specific classes of glycoproteins (19), interfere with the normal course of D. discoideum development. Finally, a D-galactose (or N-acetyl-D-galactosamine)-specific lectin, dinitol, has been identified on the cell surface (20, 21) and in purified plasma membranes (7) and has been postulated to play a role in cell adhesion (22, 23). The validity of this hypothesis is contingent upon the presence of receptor-glycoconjugates, possibly glycoproteins, in the plasma membrane.

In this paper we have continued our study of the glycoprotein structure of the plasma membrane using lectins as probes. We and others have developed a procedure where glycoprotein receptors can be conveniently identified in situ after electrophoresis in sodium dodecyl sulfate-polyacrylamide gel (7, 24), and we have extended previous observations to identify a number of other glycoproteins. For comparative purposes we also studied glycoproteins in erythrocyte ghosts.

MATERIALS AND METHODS

Lectins and Lectin Derivatives—FITC-Con A, FITC-WGA, FITC-RCA-60, FITC-RCA-120, and FITC-SBA were obtained from Miles. Hapten sugars for these lectins are, respectively, D-glucose or D-mannose, D-galactose or D-galactosamine, D-ribose, L-fucose, and L-fucose binding protein (Ulex europeus agglutinin I); FITC-RCA, FITC-WGA, FITC-RCA-60, FITC-FBP, L-fucose binding protein (Ulex europeus agglutinin I); RCA-120, R. communis agglutinin 120 (I); SBA, soybean agglutinin; FBP, L-fucose binding protein (Ulex europeus agglutinin I); FITC-RCA was prepared by reacting 10 mg of RCA with 0.5 mg of FITC according to Ref. 25 but not separated. The mixture, containing 0.5 mg of FITC, was dialyzed against 1 liter of 0.05 M sodium phosphate buffer, pH 7.4. FITC-RCA-60 and FITC-RCA-120, generous gifts from Mr. H. Huang, were purified according to Ref. 25. For cell surface labeling experiments the two RCA lectins were purified as in Ref. 25 but not separated. The mixture, containing 1 mg of FITC, was dialyzed against 1 liter of 0.05 M sodium phosphate buffer, pH 7.4. FITC-RCA was prepared by reacting 10 mg of RCA with 0.5 mg of FITC in 0.1 M sodium carbonate buffer, pH 9.0, containing 0.05 M d-galactose and 0.02 M NaCl. The total volume was 10 ml. The mixture was carried out at 4° for 12 h with constant stirring and unbound fluorescein was removed by exhaustive dialysis against NaCl/P, (0.8 g of NaCl, 0.2 g of KCl, 1.15 g of Na,HPO,, and 0.2 g of NaCl/P, (8.0 g of NaCl, 0.2 g of KCl, 1.15 g of Na,HPO,, and 0.2 g of NaCl/P, 1 liter with H,O, pH 7.4). Precipitated protein was removed by centrifugation at 12,000 x g for 10 min. The FITC-RCA had an agglutination titer of 2 against human red blood cells.

Fluorescein-labeled copolymer methacrylate microspheres approximately 40 nm in diameter were prepared as previously described (26). The fluorescein microspheres were derivatized with disuccinimidyl suberate (DSS) (6a) by first adding 1.7 g DSS to 10 ml of 0.1 M sodium phosphate buffer, pH 7.4 containing 0.05 M d-galactose and 0.02 M NaCl. The total volume was 10 ml. The mixture was carried out at 4° for 12 h with constant stirring and unbound fluorescein was removed by exhaustive dialysis against NaCl/P, (8.0 g of NaCl, 0.2 g of KCl, 1.15 g of Na,HPO,, and 0.2 g of NaCl/P, 1 liter with H,O, pH 7.4). Precipitated protein was removed by centrifugation at 12,000 x g for 10 min. The FITC-RCA had an agglutination titer of 2° against human red blood cells.

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Growth of Cells and Isolation of Plasma Membranes—Dictyostelium discoideum, strain A3 (obtained from Dr. F. Rothman, Brown University) was grown axenically (4, 70) and developed on filter paper as described (4). Ax-2 cells were also examined for the presence of RCA-60 and RCA-120 receptors. Total cell protein was measured as described in the legend to Fig. 2 in Ref. 27. Plasma membranes were isolated from these cells by either of two methods. Plasma membranes purified by the first method (28) were characterized by biochemical, ultrastructural, and histochemical methods and were found to contain a 5% inner mitochondrial membrane contamination, but to be free of other identifiable membranes (28). In order to rule out a mitochondrial source for the lectin receptors

\(^3\) The abbreviations used are: Con A, concanavalin A; WGA, wheat germ agglutinin; RCA-60, R. communis agglutinin 60 (II); RCA-120, R. communis agglutinin 120 (I); SBA, soybean agglutinin; FBP, L-fucose binding protein (Ulex europeus agglutinin I); FITC, a fluorescein derivative; SBA, soybean agglutinin; RCA, lectin from R. communis agglutinin 60 (II); SBA, soybean agglutinin; FITC, fluorescein isothiocyanate; NaCl/P, sodium diode sulfate; FITC, fluorescein isothiocyanate; NaCl/P, sodium chloride.

\(^4\) C. West, unpublished results.

RESULTS

Lectin Receptors in Erythrocyte Ghosts—Erythrocyte ghost proteins and the glycoproteins which stain by the periodic acid-Schiff technique are depicted in Fig. 1F after electrophoretic separation on a SDS-polyacrylamide gel. The components in the membrane which bind FITC-RCA-60 are visible in Fig. 1A. Those bands in which the intensity of fluorescence was markedly reduced in the presence of the hapten inhibitor 0.3
FIG. 1. Identification of glycoproteins using fluorescent lectins in human erythrocyte ghosts. Purified ghosts (200 μg of protein) were electrophoresed in SDS-polyacrylamide gels and the gels were fixed. Gel lanes were incubated with an FITC-lectin and a hapten sugar, if appropriate, and washed 2 days (as described under "Materials and Methods"). In A to E, staining by the FITC-lectin alone is shown on the left; staining by the FITC-lectin in the presence of a hapten inhibitor, included during incubation and the first washing, is on the right. A, FITC-RCA-60 + 0.3 M N-acetyl-α-galactosamine; B, FITC-RCA-120 + 0.3 M α-galactose (in the control gel the glycolipid region is not stained at the bottom of the gel because it was broken off); C, FITC-WGA + 0.3 M N-acetyl-β-glucosamine; two M N-acetyl-β-galactosamine (Fig. 1A) were considered to represent specific receptors for RCA-60. A similar pattern of receptors was found after labeling with FITC-RCA-120, although the fluorescence intensity was somewhat less (Fig. 1B). A hapten for RCA-120, galactose, also inhibited the binding of this lectin, FITC-RCA-60 and 120 receptors are present in the high molecular weight region of the gel greater than 260,000, the band 3 region, band 75,000 (4.1, the band 4.5 region periodic acid-Schiff 1 and 2 (see legend to Fig. 1). The periodic acid-Schiff bands bind FITC-RCA-60 relatively weakly if at all. Our results are similar to those of Steck and Dawson (33) and Gahmberg (34) where galactose-terminated glycoproteins have been identified by treatment of erythrocyte ghosts with galactose oxidase, followed by reduction with sodium borotritide and autoradiography after electrophoresis on SDS-polyacrylamide gels. In contrast, however, band 4.1 was not labeled by that technique (33). Possibly we are identifying a glycoprotein which co-migrates with this species. A similar pattern of receptors was also found when radiiodinated RCA was diffused into SDS-polyacrylamide gels, but the resolution was much poorer (24).

In order to determine the threshold number of RCA-60 receptors required for producing a detectable response in the polyacrylamide gel, a series of decreasing amounts of red blood cell ghost protein was electrophoresed and labeled. In Table I, an analysis of densitometric scans of the fluorescent labeling of bands migrating in the band 3 region and at molecular weight positions of approximately 75,000 (band 4.1 position) and 61,000 is presented. The percentage of stained protein in the polyacrylamide gel which these glycoproteins comprise is given in Table I. Assuming 0.57 pg of protein/ghost (32), we estimate there to be 8.5 × 10⁶, 1.1 × 10⁶, and 0.45 × 10⁶ protein chains/ghost, respectively, which migrate at these molecular weight positions in the SDS-polyacrylamide gels (see Ref. 32). Because band 3 is known to be heterogeneous, and because the others may be heterogeneous, these are maximum numbers of chains which bind RCA-60. Specific binding of RCA-60 to each of these bands is readily detectable when 20 μg of ghost protein are electrophoresed per gel lane. Consequently, in 200 μg of ghost protein/gel lane, as in Figs. 1, 3, and 4, receptors present at a frequency of 0.5 to 8.4 × 10⁶ chains/ghost should also be detectable. Band 3 receptors could be detected at this threshold, even though they migrated as a broad band 0.8 cm long.
Red blood cell ghosts were electrophoresed in SDS-polyacrylamide gels, labeled with FITC-RCA-60, photographed, and the photograph scanned and integrated, as described under "Materials and Methods." Nonspecific background fluorescence in the presence of 0.3 M lactose has been subtracted from the integration values presented. The percentage of stained protein, derived as in Ref. 32, is the fraction of Coomassie blue absorption in the whole gel which is contained in the band, as determined from an integral of a densitometric scan of Coomassie blue staining.

The pattern of labeling with FITC-WGA is shown in Fig. 1C. In the center panel, staining of receptors migrating with periodic acid-Schiff 1, 2, 4, and 3, the region of spectrin, and the band 3 and 75,000 (position of band 4.1) regions was evident. In the left panel of Fig. 1C, at least 15 additional receptors were identified when the photographic negative was printed under conditions which blanched the higher molecular weight region of the gel. Binding of FITC-WGA by these minor bands was also inhibited with N-acetyl-D-glucosamine.

Binding of WGA to periodic acid-Schiff 1 has previously been documented using affinity column chromatography (35), but binding to the other glycoproteins has not been reported. The possibility that WGA can bind to sialic acid-bearing glycoproteins (such as the periodic acid-Schiff bands) as well as to glycoproteins containing the hapten inhibitor used here, N-acetyl-D-glucosamine (36), should be noted (35, 37, 38).

FBP had a binding specificity restricted to the band 3 region, periodic acid-Schiff 1, band 75,000 (position of band 4.1), and the region of the spectrin bands (Fig. 1D). Although there was binding to band 4.2, this was not inhibited by t-fucose. FBP specifically agglutinates type O erythrocytes (39) and periodic acid-Schiff 1 has previously been identified as a type O blood group determinant (40). The other glycoproteins have not previously been suggested to be type O determinants.

Con A bound only to the band 3 region and this binding was inhibited with a-methyl-D-mannoside (Fig. 1E), in agreement with previous results (41, 42).

Each FITC-lectin exhibited specific binding to at least one membrane glycoprotein. This is consistent with the hemagglutinating ability of each of these lectins (19, 39). All of the lectins bound to receptors in the band 3 region and this is consistent with the sugar composition of this family of glycoproteins (43). The method used in this paper did not fail to identify known receptors and identified some new receptors as well. These new receptors may not have been identified in previous experiments using affinity (lectin) column chromatography, or other techniques, because of (a) failure to be solubilized from the ghost, (b) failure to elute from the column, (c) failure to be resolved in the SDS-polyacrylamide gel, or (d) lack of sensitivity. Since lectins were utilized which had specificities against the typical anomeric configurations of each of the sugars believed to exist in ghost glycoproteins (44), Fig. 1 probably identifies the apparent molecular weight of all of the glycoproteins which reside in the ghost, except for any which might be present at extremely low levels.

**Table 1** Sensitivity of detection of receptors for FITC-RCA-60 in red blood cell ghosts

<table>
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<tr>
<th>Glycoprotein</th>
<th>Amount of fluorescent lectin binding (arbitrary units)</th>
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<td>Stained protein in gel</td>
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<td></td>
<td>100 µg</td>
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<td>Band 3 region</td>
<td>21.5</td>
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<tr>
<td>Band 75,000 (4.1)</td>
<td>2.5</td>
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<tr>
<td>Band 61,000</td>
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FITC-WGA labeled many receptors at each of three stages of development (Fig. 2B), and the binding was specific for the sugar binding site of WGA (Fig. 2C). The majority of WGA receptors on vegetative cells disappeared or decreased greatly in amount (or affinity for WGA) when the cells differentiated to the pseudoplasmodial stage of the life cycle, and a new set of receptors appeared (Fig. 2D). The plasma membrane of aggregating cells appears to be mainly transitional in its composition, but there were some receptors peculiar to this stage as well (e.g. with apparent molecular weights of 19,000, 40,000, and 50,000). One vegetative stage receptor (or group of
receptors) had a very broad and continuous distribution in the gel between the molecular weight positions 48,000 and 62,000. This species seemed to have less affinity for FITC-WGA than normal receptors, since an additional 1.0 day of washing diminished the relative fluorescence intensity of this band considerably when compared to other receptors (compare Figs. 2 and 3). As described below, it contained little, if any, protein.

In order to increase the sensitivity of the technique, twice as much membrane protein was applied to the gel and the photographic negative was exposed twice as long as normal. A densitometric scan of a gel labeled and photographed in this way exhibited the major receptors seen in Fig. 2 and demonstrated at least 25 receptors at each stage of development (Fig. 3). The additional receptors identified in this manner might be lower affinity receptors or receptors which are present in small amounts.

When gels containing plasma membranes from either the vegetative, aggregation, or preculmination stages of development were labeled with either FITC-RCA-60, FITC-RCA-120, or SBA, there was no detectable binding of fluorescent lectin. Because of the negative result only examples of membranes from the aggregation stage are shown for these lectins (Fig. 4, A, B, and C). This suggests that receptors for these lectins are relatively rare on the cell surface. Consequently, it was of interest to estimate the maximum number of RCA receptors in a single band which could have escaped detection. The data from red blood cell ghosts suggested that an RCA-60 receptor can be detected at a frequency of $0.5 \times 10^4$ to $8.4 \times 10^4$ per cell ghost (see above) depending on the receptor and the nature of its migration in the SDS-polyacrylamide gel. In D. discoideum, the cell protein content was measured as 89 pg of protein/vegetative cell and 2% of this, or 1.8 pg, is located in the plasma membrane (28). There is 0.57 pg (32), or one-third of this amount of protein, in the red blood cell ghost. Thus we can estimate that any plasma membrane RCA-60 receptors similar to those in red cell ghosts must be present in less than $1.5 \times 10^4$ to $25 \times 10^4$ copies/cell. The number of RCA receptors on the cell surface was examined more directly as described below.

FITC-FBP also failed to specifically label any glycoproteins in plasma membranes from either the vegetative (Fig. 4D) or pseudoplasmodial (Fig. 4E) stage of development. Although FITC-FBP strongly labeled several peptides this was not inhibited by L-fucose. While these might be very high affinity sites for FITC-FBP which are not competed effectively by the concentration of L-fucose employed, this is unlikely in view of the agglutination assays which are presented below.

The chemical composition of the plasma membrane lectin receptors was examined by exhaustively digesting dissolved membranes with pronase prior to assay for lectin receptors. Proteolysis effectively hydrolyzed all of the receptors for Con A in vegetative stage plasma membranes to low molecular weight material (Fig. 5B) and had a similar effect on WGA receptors in red blood cell ghosts (Fig. 5A). This indicated that the receptors are predominantly glycoprotein in composition. Most of the WGA receptors in D. discoideum plasma membranes were also glycoproteins (Fig. 5C). However, one vegetative stage receptor, with a broad apparent molecular weight distribution ranging from 48,000 to 62,000 was not a glycoprotein.

**Lectin Receptors on Cell Surface** - Analysis of plasma membrane glycoproteins indicates that there are relatively large numbers of receptors and receptor species for WGA and Con
A and relatively few receptors, if any, for RCA-60, RCA-120, SBA, and FBP, on the surfaces of axenically grown D. discoideum cells at any stage of development (Figs. 2, 3, and 4). Because of previous reports that RCA-120 and lectins of similar specificities could agglutinate and/or bind to another strain of D. discoideum cells (20, 21, 23), this inference was investigated more directly by testing the ability of lectins to agglutinate the cells and by visualizing the binding of RCA, coupled to fluorescein and microspheres, to whole cells.

RCASO, RCA-120, SBA, and fucose-binding protein (from L. tetragonolobus) were unable to agglutinate heat-killed D. discoideum cells (all three stages of development) at the highest concentrations tested (0.5 to 2.5 mg/ml). Similar results were found with living cells as well at the vegetative and aggregation stages of development. On the other hand, WGA and Con A agglutinated the cells at 0.08 and 0.06 mg/ml, respectively, and this was inhibited by the appropriate hapten sugars. The minimal level of lectin which could agglutinate did not change more than 2-fold during the course of development. This pattern of lectin-agglutinability corroborates the pattern of lectin-binding to receptors in SDS-polyacrylamide gels as described above.

The presence of RCA-60 and RCA-120 receptors on D. discoideum cells was also examined by fluorescent light or scanning electron microscopy using FITC-RCA and FITC-RCA-microspheres. When living cells in either the vegetative or aggregation stage of development were treated with these fluorescent reagents, no labeling of cells was observed (Fig. 6). Likewise, cells stabilized with formaldehyde prior to labeling exhibited no fluorescence. When red blood cells were added as an internal control, only the red blood cells were fluorescent (Fig. 6). Labeling of red blood cells was inhibited if 0.1 M β-galactose was present (not shown).

Scanning electron microscopy was used to detect cell surface RCA receptors after glutaraldehyde fixation, as shown in Fig. 7 (upper), in order to increase the sensitivity of detection of ricin receptors. The D. discoideum cell surface, including the microvilli, bound less than 50 RCA-microspheres/cell. This binding was largely nonspecific, since the same degree of labeling was observed in the presence of 0.1 M β-galactose (Fig. 7, upper right). In contrast, red blood cells labeled with RCA-microspheres (not shown) and vegetative or aggregation stage D. discoideum cells labeled with Con A or WGA coupled microspheres (Fig. 7, lower) displayed a uniform tight packing of these markers when viewed under the scanning electron microscope (10).

**Discussion**

Glycoproteins can be conveniently identified in SDS-polyacrylamide gels by employing fluorescent lectins as probes (7). In contrast to chromatographic methods for assaying receptors, there is little danger of identifying a molecule as a lectin receptor on the basis of noncovalent association with an authentic receptor, because of denaturation of membrane proteins in SDS and β-mercaptoethanol. In addition, a lectin receptor is unlikely to be undetected because it is not solubilized in preparation for chromatography or is protected from reaction with a chemical probe such as lactoperoxidase (see Ref. 45). The lectins WGA, FBP, Con A (6, 7), RCA-120, RCA-60, and SBA were employed to recognize, in plasma membrane glycoproteins, the pyranose forms of the following sugars, in the given anomeric configurations, respectively: N-acetyl-β-glucosamine, α or β (36, 46); L-fucose, α (39); D-mannose or D-glucose, α or β (47); β-galactose, α or β (25, 48); and β-galactose or N-acetyl-β-galactosamine, α or β (48, 49) for the last two. Glycoproteins which specifically bind these lectins probably contain the hapten sugar at the nonreducing
Plasma Membrane Glycoprotein Identification and Structure

Identification of lectin receptors as glycoproteins. Plasma membranes were denatured and dissolved in SDS and β-mercaptoethanol, and treated with pronase, a group of proteases, as described under "Materials and Methods." Lectin receptors were assayed as in the legend to Fig. 2. In each section (A to C) the panel on the left shows an untreated control, and the panel on the right shows the result after proteolysis. A, red blood cell ghosts labeled with FITC-WGA. B, vegetative stage D. discoideum plasma membranes labeled with FITC-Con A. C, same as B except labeled with FITC-WGA. Electrophoresis is from top to bottom.

Fig. 5. Identification of lectin receptors as glycoproteins. Plasma membranes were denatured and dissolved in SDS and β-mercaptoethanol, and treated with pronase, a group of proteases, as described under "Materials and Methods." Lectin receptors were assayed as in the legend to Fig. 2. In each section (A to C) the panel on the left shows an untreated control, and the panel on the right shows the result after proteolysis. A, red blood cell ghosts labeled with FITC-WGA. B, vegetative stage D. discoideum plasma membranes labeled with FITC-Con A. C, same as B except labeled with FITC-WGA. Electrophoresis is from top to bottom.

terminus of one of their oligosaccharide chains, or at an internal position where its glycosidic linkages do not interfere with lectin binding.

Dictyostelium discoideum (A3 + Ax-2) plasma membranes and cell surfaces are relatively rich in glycoproteins which bind WGA and Con A, but have few, if any, receptors for RCA-60, RCA-120, SBA, and FBP. This was most clearly demonstrated by the lack of binding of FITC-RCA-microspheres to whole cells, which indicated that there were less than 50 binding sites/cell, under conditions where Con A and WGA-microspheres cover the entire cell surface (10). These results support the finding that RCA-60, RCA-120, SBA, and FBP failed to bind specifically to glycoproteins in plasma membranes subjected to SDS-polyacrylamide gel electrophoresis, under conditions which revealed numerous species of Con A and WGA receptors. If any RCA-60 receptors were undetected by this method, they would have to be located on the interior face of the plasma membrane and number less than 1.5 × 10^4 to 25 × 10^4 copies/plasma membrane. This calculation was based on the known frequency of receptors detected in erythrocyte ghosts and consequently presumes an avidity of D. discoideum receptors for RCA-60 similar to that of erythrocyte receptors.

There may be a conflict between our results and previous work. RCA-120 was reported to agglutinate aggregation stage NC-4 cells grown on bacteria at a concentration much lower than concentrations at which we could find no effect on axenic cells (23). Another lectin which, as far as has been examined, also has a sugar binding specificity similar to RCA-60 (48, 50), has been isolated from D. discoideum and its binding to cells also studied (23). This lectin, discoidin, was found to bind to NC-4 cells through 5 × 10^5 high affinity sites/cell, a level of binding which should have been easily detected by RCA-60 in our cells. This might be due to a very specific difference in recognition properties between RCA-60 and discoidin or to a difference in strains and culture conditions.

In contrast to D. discoideum the spectrum of lectin receptors was very different in the erythrocyte plasma membrane. There were no similarities in the molecular weight profiles of identified glycoproteins from the two species. Receptors for RCA-60 and RCA-120 were richest in variety in red blood cell plasma membranes, whereas Con A and WGA receptors were richest in variety in D. discoideum. The red cell ghost contained receptors to lectins of all six of the lectin specificity classes studied, while the D. discoideum plasma membrane was devoid of receptors to four of these classes. There apparently are glycoproteins in the red cell ghost which could bind...
FIG. 7. Scanning electron micrographs of *D. discoideum* cells in the aggregation stage of development. Cells were allowed to develop on filters for 12 h, dissociated from filters, washed in NaCl/P, fixed in 0.25% glutaraldehyde NaCl/P, for 30 min, and incubated in NaCl/P containing 0.01 M glycine for 1 hr. Cells in the upper panels were treated with RCA-microspheres in the absence (left) or presence (right) of 0.1 M α-galactose. Arrows show microspheres on cell surface. (× 27,000). Cells in the lower panel were treated with FITC-WGA coupled to microspheres in the absence (left) or presence (right) of 0.01 M N-acetylchitobiose. (× 38,000).

Plasma Membrane Glycoprotein Identification and Structure

WGA bound to more than 25 different receptor species in plasma membranes from each stage of development and is probably binding to N-acetyl-d-glucosaminyl residues since sialic acids are not present in this organism (51). α- or β-linked N-acetyl-d-glucosamine could be located in either terminal (46, 52) or internal (46) positions for binding of WGA. The actual number of macromolecular species in the plasma membrane which bind WGA may be less, since subunits of different molecular weights could combine to form multimeric proteins. The more important species (those with the heaviest lectin-binding) present at the vegetative stage were absent and replaced by new species in the pseudoplasmodial stage of development, and the converse was also true. Most of the WGA receptors were glycoproteins as evidenced by hydrolysis by treatment with proteases in the presence of SDS. One receptor was not a glycoprotein. This may correspond to glycosphingolipids which are located on the cell surface, contain large amounts of N-acetyl-d-glucosamine, and migrate as a diffuse band in SDS-polyacrylamide gels (53).

Most WGA-binding proteins and Con A-binding proteins are probably different glycoproteins in the *D. discoideum* plasma membrane. There is limited correspondence between the apparent molecular weight and developmental changes of glycoproteins from these two classes of lectin receptors. At least some WGA receptors do not co-cap with Con A receptors when Con A is added to cells (10). The change in the molecular weight profile of WGA receptors during development is much more profound than that of Con A receptors (6–8). Finally, except for material which does not enter the polyacrylamide gel, all of the WGA receptors are restricted to the apparent molecular weight region below 95,000, while Con A receptors as large as 313,000 have been detected (7).

Chemical analyses of the sugars of *D. discoideum* plasma membranes are relevant to the interpretation of glycoprotein structure. The sugar composition of vegetative stage plasma membranes isolated from an axenically grown strain was recently determined by Gilkes and Weeks (51). There were very small amounts, if any, of galactose (<1 nmol/mg of membrane protein), corresponding to less than 10% galactose molecules/plasma membrane. This is consistent with the absence of detectable receptors for RCA-60, RCA-120, and SBA (N-acetyl-d-galactosamine is also not detectable in the plasma membrane3). Likewise, there was no detectable sialic acid3 (51), although sialic acid commonly terminates glycopro-

tein oligosaccharides in other systems (54). The occurrence of both of these sugars may not be surprising, since sialic acid, when present, is glycosidically linked to galactose, N-acetyl-D-galactosamine, or another sialic acid in systems where this has been studied (55). N-Acetyl-D-glucosamine, D-mannose, and D-glucose, possibly D-contaminant (51), were the common est sugars analyzed. This confirms with our observation that the only lectins which bind to these sugars (WGA and Con A) have receptors in the plasma membrane. L-Fucose was also the only lectin which bind to these sugars (WGA and Con A), which conforms with our observation that L-Fucose was also the only lectins which bind to these sugars (WGA and Con A). Since the only detectable sugars found in the vegetative stage plasma membrane, when the plasma membrane was found in the plasma membrane of D. discoideum (51). Since the only detectable sugars found in the vegetative stage plasma membrane, when the plasma membrane was found in the plasma membrane of D. discoideum (51).

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Identification of glycoproteins, using lectins as probes, in plasma membranes from Dictyostelium discoideum and human erythrocytes.

C M West, D McMahon and R S Molday