Defective Glycoproteins in the Plasma Membrane of an Aggregation Minus Mutant of *Dictyostelium discoideum* with Abnormal Cellular Interactions*

(Received for publication, June 27, 1977)

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Experiments involving the co-incubation of wild type (A3) cells of *Dictyostelium discoideum* and a spontaneous aggregation-minus mutant (HW 2) suggested that the mutant was defective in cellular interactions. The inhibition of A3 development by HW 2 cells and the differentiation of a small fraction of HW 2 cells which is allowed by A3 cells, both depend on cell contact. Therefore, we compared cell surface molecules in vegetative A3 and HW 2 cells by a variety of techniques to determine whether defects in HW 2 could be found prior to the inhibition of development in vegetative amoebae. Antigenic defects, or differences in binding of concanavalin A, or both, were localized to three plasma membrane macromolecules using glutaraldehyde-fixed sodium dodecyl sulfate gels of plasma membranes. Two periodic acid-Schiff-positive glycoproteins, and one glycolipid also differed in HW 2. Three glycoproteins had an increased sensitivity to pronase in isolated plasma membranes suggesting an alteration in their topography. Glycoprotein E, the major glycoprotein of vegetative plasma membranes is abnormal in topography, altered as a concanavalin A receptor, and is antigenically abnormal.

Developmental mutants have frequently been used to study the biochemical parameters associated with development in *Dictyostelium discoideum*. When development is blocked many normal changes do not occur including the accumulation of stage-specific enzymes (1), the appearance of cAMP receptor and phosphodiesterase (2, 3), and of contact sites A (4). Thirteen normal developmental alterations in the spectrum of macromolecules and five developmental changes in poly-peptide topographical location in the plasma membrane of *D. discoideum* are blocked in a developmental mutant. Although several biochemical failures are associated with developmental mutants, many of these effects could be secondary results of the inhibition of the developmental program.

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* This paper is Number 6 in a series entitled "The Role of the Plasma Membrane in the Development of *Dictyostelium discoideum*". Paper 5 is Footnote 1. This work was supported by United States Public Health Service Grant GM 06965. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 S. Hoffman and D. McMahon (1978) Arch. Biochem. Biophys., in press.

2 The abbreviations used are: Con A, concanavalin A; WGA, wheat germ agglutinin; FBP, L-fucose-binding protein (Ulex europaeus agglutinin I); RCA-60, Ricinus communis agglutinin 60; SDS, sodium dodecyl sulfate; PAS, periodic acid-Schiff; FITC, fluorescein isothiocyanate conjugated. E(Ag) is read antigen E. E(Con A) is read Con A receptor E. E(gp) is read glycoprotein E.

3 D. McMahon, unpublished results.
ated with particulate cellular components found only on aggregating cells (18, 19). An antigen exists that is found specifically on spore cells (20). Gregg and Trygstad found that antiserum against wild type amoebae fail to agglutinate some aggregation minus mutants and vice versa (21). Therefore, it is clear that antigens on the cell surface of *D. discoideum* change during development and that some mutants unable to aggregate may have defective cell surfaces. However, the molecular nature of developmentally controlled antigens has not previously been determined.

In order to find a defect or defects in an aggregation minus mutant that was unlikely to be a secondary effect of the inhibition of development, and therefore more likely to be a direct result of the primary genetic lesion, we examined the plasma membrane of vegetative cells of such a mutant by a variety of independent techniques. A spontaneous mutant was used to minimize the possibility of there being mutations present irrelevant to the developmental defect. We found six differences between the mutant and wild type plasma membrane, at least five of which are carbohydrate-containing molecules.

**EXPERIMENTAL PROCEDURES**

**Cells**

Cells used are *Dictyostelium discoideum* strain A3, and a spontaneous aggregation mutant, HW 2, isolated from A3 cells by D. McMahon.

**Development of Cells in Suspension and Assay of Contact Site A Formation** – To test for the ability of cells to form contact sites A (4), 5 ml of A3 or HW 2 cells in suspension at 1 x 10^9 cells/ml in aggregation buffer (22) are rotated at 20 to 24 rpm in a screw-top culture tube (25 x 200 mm) about the tube’s long axis in a device built according to Gerisch’s description (23).

After 12 h at 22°C, aliquots of cell suspension were photographed in the presence or absence of 10 mM EDTA. If cells remained aggregated in the presence of EDTA, they were assumed to have formed contact sites A (4).

**Development of Cells on Filters** – A3, or HW 2 cells, or both, were washed and resuspended in lower pad solution (24). The indicated numbers of A3 cells, HW 2 cells, or a combination of the two were uniformly spread on a 47-mm diameter Millipore filter on a pad saturated with lower pad solution and incubated for development at 22°C.

In experiments where cells were incubated on opposite sides of Millipore filters from each other, the appropriate number of A3 or HW 2 cells were spread onto each filter and the filters were clamped together back to back with a stainless steel ring and suspended horizontally 5 mm above a pad soaked in lower pad solution.

**Triton X-100 Treatment**

Spores were treated with 0.2% Triton X-100, and suitable dilutions were mixed with bacteria, and plated on SM agar (25). Fewer than 1 in 10^9 amoebae survive this treatment although it has no effect on the viability of spores.

**Plasma Membrane Preparation and SDS-Gel Electrophoresis**

Cells were grown, plasma membranes prepared (26), and analytical SDS-gels were run and stained as previously described (14). The Coomassie blue-stained gel in Fig. 10 contains 50 μg of protein per lane and the PAS-stained gel in Fig. 6 contains 200 μg of protein per lane as estimated by the Lowry method (27). Pronase treatment of intact cells and isolated plasma membrane was also as previously described (14).

**Scanning Electron Microscopy**

Cells were fixed with glutaraldehyde, postfixed with OsO₄, dehydrated with an ethanol series, and critical-pointed dried. Before viewing the samples were shadowed with gold.

**Lectin and Antibody Labeling of Gels**

SDS slab gels were run (14) and then cut into 0.7-cm-wide gel strips containing 120 μg of plasma membrane protein. The strips were then fixed as described in Ref. 28. For lectin binding, gel strips were incubated for 3 days in 8 ml of infuson solution (28) per gel strip plus 0.25 mg of FITC-lectin per gel strip. They were then washed for 2 days in phosphate-buffered saline (0.9% NaCl solution) (28) with two changes of solution and photographed over a short wavelength (predominantly 254 nm) UV light box through a Wratten type 65 filter (14). For antibody binding, gel strips were incubated for 3 days with 5 ml of rabbit serum plus 3 ml of 2.67 times infuson solution per gel strip. The gel strips were then washed as above to remove unbound antibody and incubated for 3 days with 8 ml of infuson solution plus 5 mg of FITC-goat anti-rabbit immunoglobulin per gel strip. Finally the gels were washed and photographed as above.

**Gel Scanning**

Photographic negatives of gels were scanned on a Syntax AD-1 Autodensitometer.

**Preparation, Titering, and Adsorption of Sera**

Rabbits were inoculated with A3 vegetative plasma membrane (10 mg of protein content) suspended 1:1 (v/v) with complete Freund’s adjuvant in a final volume of about 2 ml. Equi aliquots were injected intramuscularly, intradermally, and subcutaneously. Similar inoculations were given 4 weeks later and weekly for 3 weeks thereafter. Starting at Week 5, the rabbits were bled about 25 ml weekly from the ear. After 3 h at room temperature the serum was separated from the blood clot by centrifugation for 10 min at 4200 x g.

To titer sera, vegetative A3 cells were washed and resuspended in 0.15 x NaCl plus 2 mM EDTA (pH 6.0) at 4 x 10^8 cells/ml. One-tenth milliliters of cells were mixed with 0.1 ml of a range of serum dilutions and the highest dilution to give complete agglutination was determined. Typically titers were 64 for immune serum and 2 for preimmune serum.

Adsorption of a titer 64 serum with 3.2 x 10^8 A3 or HW 2 cells/ml for 30 min completely removes all agglutinating activity. However, when pooled immune sera from two rabbits (average titer 64) was adsorbed with a sufficient number of A3 cells to completely absorb 128 units of agglutinating titer, detectable antibody binding to fixed gels of A3 vegetative plasma membrane remained (data not presented). Repeating this adsorption a total of three times removed almost all antibody binding to fixed gels. Therefore, A3 and HW 2 adsorption of anti-A3 vegetative plasma membrane antisera with cells of A3 or HW 2 was routinely done by three rounds of adsorption, each with 6.4 x 10^8 cells/ml of serum.

**Preparation of Lipids and Thin Layer Chromatography**

Total cell or plasma membrane lipids were prepared as described in Ref. 29, p. 227, and partitioned once as described. The upper phase was dried, diazylated 24 h against H₂O₂, dried, resuspended in chloroform/methanol (2:1, v/v), and insoluble material was removed by centrifugation. The lower phase was dried and resuspended in chloroform/methanol (2:1, v/v). The samples were analyzed by thin layer chromatography on precoated 0.25-mm silica gel plates (EM Laboratories, Darmstadt). Plates were developed with chloroform/methanol/H₂O (60:35:8, v/v/v). The samples were analyzed by thin layer chromatography on precoated 0.25-mm silica gel plates (EM Laboratories, Darmstadt). Plates were developed with chloroform/methanol/H₂O (60:35:8, v/v/v).

**Gas-Liquid Chromatography**

A3 and HW 2 vegetative plasma membranes were resuspended at about 1 mg/ml of membrane protein in 0.03 M ammonium bicarbonate, pH 7.9. Ten-microliter samples were dried, hydrolyzed in methanolic, derivatized with trifluoroacetic acid, and analyzed for carbohydrate by gas-liquid chromatography.

**RESULTS**

**Development of Mutant, HW 2** – The spontaneous mutant, HW 2, fails to develop normally on plates of bacteria, in suspension, or on filters resting on buffer-saturated pads. When wild type, A3, cells are gently rotated for at least 6 h in aggregation buffer (22), large, tight aggregates are formed (Fig. 1A) that are resistant to EDTA (Fig. 1B). Similarly incubated HW 2 cells from only smaller, loose aggregates

* M. Wrann and C. W. Todd submitted for publication.
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**FIG. 1.** Assay of contact sites A formation by developing cells. A3 or HW 2 cells at a concentration of $1 \times 10^4$ cells/ml were rotated for 12 h as described under "Experimental Procedures" and photographed in the presence or absence of 10 mM EDTA. A, A3 cells without EDTA; B, A3 cells plus EDTA; C, HW 2 cells without EDTA; D, HW 2 cells plus EDTA. All magnifications are $\times$ 364.

280 cells/ml were rotated for 12 h as described under "Experimental Procedures" and photographed in the presence or absence of 10 mM EDTA. A, A3 cells without EDTA; B, A3 cells plus EDTA; C, HW 2 cells without EDTA; D, HW 2 cells plus EDTA. All magnifications are $\times$ 364.

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**FIG. 2.** Comparative scanning electron microscopy of A3 and HW 2 cells. A3 and HW 2 cells were incubated for 12 h and prepared for scanning electron microscopy as described under "Experimental Procedures." A, A3 cells (magnification $\times$ 424); B, HW 2 cells (magnification $\times$ 154); C, A3 cells (magnification $\times$ 1446); D, HW 2 cells (magnification $\times$ 1735). Globular protrusions (arrows) and the edge of the aggregate (agg) are indicated; D, HW 2 cells (magnification $\times$ 1735).
The A3 and HW 2 cells are separated by Millipore filters. When A3 and HW 2 cells in a ratio of 1:3 are incubated so that each strain is separated by Millipore filters, 10 times as many spores form as when a similar ratio of A3 and HW 2 cells are co-incubated (compare Experiment 1E with 1H in Table I). A slight reduction in the number of spores formed is still observed as compared to an equivalent concentration of A3 cells (Experiment 1B), but this difference may be a result of the placement of the filters in this experiment since the number of spores formed was similarly reduced when A3 cells were plated transfilter to A3 cells (Experiment 2). Therefore, the inhibitory effect of HW 2 cell on A3 development appears to depend on cell contact.

Co-incubation with A3 cells results in the development of a small fraction of HW 2 cells. Twelve individual sorocarps were picked from Experiment 1E (A3 and HW 2 cells in a ratio of 1:3), plated with bacteria, and the resultant plaques scored for the HW 2 (aggregation-minus) phenotype. As indicated in Table I, Experiment 2, about 0.5% of the plaques formed were aggregation-minus, suggesting that HW 2 was rescued in its ability to develop into spores but was selected against in the ratio of 150:1. Two control experiments substantiate the suggestion that the aggregation-minus plaques were due to HW 2 spores which are produced by association with A3. No aggregation-minus plaques were observed when pure A3 sorocarps were plated with bacteria. Treatment of spores with Triton X-100 to destroy any HW 2 amoebeae which might have accidentally contaminated the spores did not decrease the frequency of aggregation-minus plaques (Table I, Experiment 2). No spores were found when pure HW 2 cultures or HW 2 cultures transfilter from A3 culture were incubated under conditions which allow development of A3. Therefore, the rescue of HW 2 also appears to depend on cell contact.

**Table I**

| Synergistic and antagonistic developmental interactions between A3 and HW 2 cells |
|---|
| In Experiment 1, the indicated number of A3 cells, HW 2 cells, or a combination of the two were incubated together or transfilter from each other as described under "Experimental Procedures." The fact that the number of spores recovered under optimal conditions is greater than the original number of cells plated is consistent with previous results (39) indicating increase in cell number during development. In Experiment 2, the spores from 12 sorocarps from Experiment 1E were harvested and suitable dilutions incubated with bacteria on SM agar and the development of the plaques formed were scored. Some spores were treated with 0.2% Triton X-100 before plating to destroy any contaminating amoebeae. |

| Experiment 1 |  |
|---|---|
| Cells | Spores/filter | % Experiment 1/A |
| A. 4 x 10^7 | 4.7 x 10^4 | 100 |
| B. 1 x 10^7 | 7.7 x 10^3 | 16 |
| C. 5 x 10^6 | 2.4 x 10^3 | 5.1 |
| D. 2 x 10^7 | 2 x 10^2 | 57 |
| E. 1 x 10^7 | 3 x 10^1 | 0.78 |
| F. 5 x 10^6 | 3.5 x 10^0 | 0.04 |
| G. 4 x 10^5 | 0 | 0 |
| H. 1 x 10^7 | 3.6 x 10^0 | 7.7 |
| I. 1 x 10^7 | 3.3 x 10^0 | 7.0 |

| Experiment 2 |  |
|---|---|
| Sorocarp | Aggregation minus plaques |
| Untreated | +0.2% Triton X-100 |
| A | 2/578 | 2/514 |
| B | 3/245 | 1/102 |
| C | 2/123 | 1/107 |
| D | 2/107 | 0/74 |
| E | 0/203 | 2/134 |
| F | 1/192 | 0/64 |
| G | 0/163 | 2/110 |
| H-L | 0/255 | 0/403 |
| Total | 10/2209 | 8/1546 |
| Frequency aggregation-minus plaques | 0.45% | 0.51% |

The lectin binding of plasma membrane glycoproteins—The total carbohydrate compositions of A3 and HW 2 plasma membranes are very similar, with the exception of fucose (Table II). Although there appears to be about twice as much fucose in HW 2 plasma membranes, interpretation of this point must remain tentative since the difference is not statistically significant. The major carbohydrates found in Dictyostelium discoideum plasma membranes are very similar, with the exception of fucose (Table II). Although there appears to be about twice as much fucose in HW 2 plasma membranes, interpretation of this point must remain tentative since the difference is not statistically significant. The major carbohydrates found in Dictyostelium discoideum.
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Fig. 3. Antigenic comparison of mutant and wild type plasma membranes. Plasma membrane macromolecules were separated by SDS-gel electrophoresis, fixed in the gels, and reacted with either preimmune rabbit serum or immune serum prepared against vegetative A3 plasma membranes. The antigens were then detected with fluorescent goat anti-rabbit antibodies. The details of this technique are presented under "Experimental Procedures." A, A3 plasma membrane macromolecules reacted with immune serum; B, HW 2 plasma membrane macromolecules reacted with immune serum. The two major discrete antigenic differences between A3 and HW 2 plasma membranes are indicated as E(Ag) because this antigen co-migrates with E(gp) (13) and 150(Ag) because the molecular weight of the antigen is 150,000. Also note that the broad continuous band of antigen seen in both A3 and HW 2 plasma membranes has a smaller migration in HW 2.

Fig. 4. Identification of antigenic differences between A3 and HW 2 plasma membranes. Antiserum against vegetative A3 plasma membranes was adsorbed with either A3 or HW 2 cells and reacted with A3 or HW 2 plasma membranes after SDS-gel electrophoresis as described under "Experimental Procedures." A, HW 2 adsorbed serum reacted with A3 plasma membrane macromolecules; B, HW 2 adsorbed serum reacted with HW 2 plasma membrane macromolecules; C, A3 adsorbed serum reacted with A3 plasma membrane macromolecules; and D, A3 adsorbed serum reacted with HW 2 plasma membrane macromolecules. The position of E(Ag) and 150(Ag) are indicated.

Table II

| Carbohydrates | A3      | HW 2     |
|---------------|---------|----------|
| Fucose        | 0.039 ± 0.012 | 0.076 ± 0.027 |
| Mannose       | 0.036 ± 0.001 | 0.039 ± 0.010 |
| Glucose       | 0.027 ± 0.001 | 0.039 ± 0.009 |
| Glucosamine   | 0.122 ± 0.013 | 0.124 ± 0.005 |
| Galactose     | <0.005   | <0.005   |
| Galactosamine | <0.001   | <0.001   |
| Sialic acids  | <0.001   | <0.001   |

Values given are mean ± standard deviation.

be detected. The monosaccharide inhibitors for these lectins are, respectively: d-mannose or d-glucose, N-acetyl-d-glucosamine, l-fucose, and d-galactose or N-acetyl-d-galactosamine. This method can detect about 10⁴ receptors per cell (17) and is therefore more sensitive than the analysis of sugars by gas-liquid chromatography. With the exception of FBP, the lectin-binding patterns shown here are inhibited by the appropriate monosaccharide haptens (17). There are two differences in lectin receptors between A3 and HW 2 detectable with Con A (Fig. 5A). Receptor E(Con A) binds more lectin in HW 2, while receptor 113(Con A) binds more Con A in A3. The difference in lectin binding to E(Con A) was apparent over a wide range of concentrations of Con A. In other experiments with a concentration of Con A 5 times the standard amount,
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Fig. 5. Identification of lectin receptors in vegetative A3 and HW 2 plasma membranes. A3 (left lane of each pair) and HW 2 (right lane of each pair) plasma membrane glycoproteins were resolved by SDS-gel electrophoresis, fixed in the gels, and reacted with fluorescent lectin as described under "Experimental Procedures." The lectins used were A, Con A; B, FBP; C, WGA; D, RCA-60; and E, no lectin (autofluorescence control). The major differences in Con A receptors are indicated as E(Con A) because this receptor co-migrates with E(gp) (13), and 113(Con A) because the molecular weight of this receptor is 113,000.

the difference in E(Con A) was accentuated and the difference was still obvious at one-fifth the routine concentration of Con A (data not shown).

No difference between A3 and HW 2 plasma membranes were detected with the other lectins used (Fig. 5, B to E). This indicates that extensive modification of glycoprotein side chains has not occurred in HW 2.

Plasma Membrane Glycoprotein Composition and Topography—PAS staining of plasma membranes indicated two additional differences in HW 2 (Figs. 6 and 7). J(gp) and K(gp) are present in lesser amounts in the HW 2 plasma membrane. Although E(gp) was defective antigenically and in Con A binding in HW 2, it was not altered in PAS staining. Therefore, the defect in E(gp) in HW 2 does not significantly affect the number of periodate-oxidizable carbohydrate residues.

We have previously (14) used treatment with pronase to assay the topography of plasma membrane molecules during development. Topographical differences between A3 and HW 2 PAS-positive glycoproteins are also detectable. As in A3 (14) no glycoproteins are sensitive to pronase treatment of intact cells (Fig. 6, Lane 2). However, glycoproteins C, E, and G are sensitive to pronase treatment of isolated HW 2 plasma membranes (Fig. 6, Lane 3), although they are relatively insensitive to similar treatment of A3 plasma membranes (Fig. 4, Lane 8, Ref. 14). Therefore, E(gp), besides being defective in Con A binding and antigenicity in HW 2, is also in an aberrant topographical location or configuration within the HW 2 plasma membrane. At the present we do not know whether the altered sensitivity of glycoproteins C and G represents an alternation in the nature of their insertion into the plasma membrane, their structure, or a change in shielding of these components by an altered molecule such as E(gp).

Plasma Membrane Polypeptide Composition and Topography—We were concerned with the possibility that the defects observed above in the HW 2 plasma membrane were secondary effects of a general disruption in plasma membrane composition or organization. Therefore, plasma membrane polypeptide composition and topography were examined.

No significant differences were found between A3 (Fig. 8, Lane 5) and HW 2 vegetative plasma membranes (Fig. 8, Lane 4) in polypeptide composition or cell surface exposure of polypeptides as determined by pronase treatment of intact cells (compare Fig. 8, Lane 2, with Fig. 3, Lane 2, Ref. 14). As previously observed in HW 2 cells incubated for development, the number of polypeptides resistant to pronase treatment of purified HW 2 plasma membranes (Fig. 8, Lane 3) is less than in similarly treated A3 cells (Fig. 3, Lane 3, Ref. 14).
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FIG. 6. SDS-gel electrophoresis of plasma membrane glycoproteins from vegetative wild type and mutant cells identified with the PAS method. Lanes from left to right are: 1, vegetative HW 2 plasma membranes; 2, plasma membranes from pronase-treated vegetative HW 2 cells; 3, pronase-treated vegetative HW 2 plasma membranes; 4, as in Lane 1; 5, vegetative A3 plasma membranes. White letters indicate glycoproteins deficient in HW 2. Black letters indicate glycoproteins sensitive to pronase treatment of isolated HW 2 plasma membranes that were insensitive to similar treatment in A3 (13). The lettering system is the same as used in Ref. 13.

Plasma Membrane and Whole Cell Lipid Composition—Since defects in several glycoproteins occur in HW 2, we thought it possible that glycolipids might also be defective. Therefore, we examined plasma membrane lipid composition. HW 2 plasma membranes contain a glycolipid not found in A3 plasma membranes (Fig. 9, Lane 6). Since it has a similar \( R_f \) to cerebroside (data not shown) and since it partitions into the lower phase (21) it may be cerebroside but it has not been characterized. The remaining glycolipids found in both A3 (Fig. 9, Lane 3) and HW 2 (Fig. 9, Lane 5) probably have more extensive carbohydrate domains since they partition primarily into the upper phase (29) (in some preparations a minor portion partitioned into the lower phase) and have smaller \( R_f \) values indicating they are more polar. Therefore, it is possible that the HW 2-specific glycolipid is an incomplete form of the glycolipids common to both A3 and HW 2. These low \( R_f \) glycolipids are found only in the plasma membrane as would be expected if the lipids bear complex oligosaccharide chains (29). Whole cells and plasma membranes from a similar number of cells contain a similar amount of glycolipids (Fig. 9, Lanes 1 and 3). Glycolipids were below detection (Fig. 9, Lane 2) in the whole cell protein equivalent to the protein content of the plasma membranes shown in Fig. 9, Lane 3. Although the low \( R_f \) glycolipids are identical in lipid preparations prepared in parallel from A3 and HW 2 plasma membranes, they are extremely sensitive to slight differences between preparations. For example, heating samples to 50° during rotary evaporation of the upper phase destroys these glycolipids.
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50
30
20
10

Fig. 8. SDS-gel electrophoresis of plasma membrane polypeptides from vegetative wild type and mutant cells. Lanes from left to right are: 1, vegetative HW 2 plasma membranes; 2, plasma membranes from pronase-treated vegetative HW 2 cells; 3, pronase-treated plasma membranes from vegetative HW 2 cells; 4, as in Lane 1; 5, vegetative A3 plasma membranes. Letters indicate polypeptides sensitive to pronase treatment of intact HW 2 cells. The same lettering system is used as in Ref. 13.

The cell surface of HW 2 is not only defective in the amoebae but is clearly defective during development. When incubated alone, HW 2 cells do not collect into aggregates or form the cohesion-mediating contact sites A. Concurrently, we have detected the occurrence of several defects in plasma membrane composition and topography. Several normal developmental changes in plasma membrane composition and topography do not occur in HW 2. These aberrations include the absence of changes in the amount of some of the macromolecules identified as defective in vegetative HW 2 plasma membranes in this study which normally occur during slimes mold development. For instance, E(gp) is not detectable in preculture A3 plasma membranes, however, it is still present in similarly incubated HW 2 cells. Therefore, defects are constantly present in the HW 2 cell surface that may continue to participate abnormally in the interaction between A3 and HW 2 cells inhibiting the development of A3 cells.

As suggested by the biological experiments, the cell surface of vegetative HW 2 cells is defective in several molecules. Two antigens, present on the A3 cell surface, as demonstrated by the ability of intact A3 cells to adsorb antibodies with these specificities, are defective in structure or expression on HW 2 cells. One of these, 150(Ag), may only be present in decreased amount or cryptic in HW 2. The other, E(Ag), although present at the cell surface, must be altered in structure in HW 2 since HW 2 adsorbed anti-A3 plasma membrane antiserum binds to E(Ag) in A3 but not in HW 2. HW 2 E(Ag) does bind unadsorbed antiserum, in fact, it binds more antibody from unadsorbed anti-A3 plasma membrane antiserum than does E3 E(Ag).

Two independent models have been constructed for the general nature of the antigenic relationships between E(Ag) and 150(Ag) from A3 and HW 2 cells. Other, more complicated, but supportable models are possible. In the first model, glycoprotein E from A3 cells is suggested to bear a unique antigen not found elsewhere on A3 cells or at all on HW 2 cells. Glycoprotein E in HW 2 cells bears an antigen that is also found on another molecule (X) in HW 2 cells and on X in A3 cells. The 150(Ag) is structurally similar in A3 and HW 2 cells but a greater amount of the antigen is expressed on A3 cells. Therefore, A3 adsorption removes antibody against the A3 E(Ag), the X antigen, and the 150(Ag). HW 2 adsorption removes antibody against the X antigen (or HW 2 E(Ag)) and a small part of the antibody against the 150(Ag). The model thus predicts that A3 adsorption will remove all antibodies against glycoprotein E and the 150,000-dalton macromolecule, while HW 2 adsorption will leave antibodies against the glycoprotein E in A3 cells and the 150,000-dalton macromolecule in A3 and HW 2 cells.

The second model suggests that both macromolecules detectable on A3 plasma membranes by HW 2 adsorbed serum (glycoprotein E and the 150,000-dalton macromolecule) bear a common antigen (the 150-E antigen) and that glycoprotein E from A3 cells has an additional unique antigenic site (the E antigen) in close proximity to the location of the 150-E site on E(gp). In this model, glycoprotein E from A3 and HW 2 cells bear the E antigen in common. In addition, glycoprotein E plasma membranes from 2 x 10^6 A3 cells; 4, lower phase lipids from plasma membranes from 2 x 10^6 A3 cells; 5, upper phase lipids from plasma membranes from 2 x 10^6 HW 2 cells; and 6, lower phase lipids from plasma membranes from 2 x 10^6 HW 2 cells. The positions of the sample origins and solvent front are indicated.

Fig. 9. Plasma membrane and whole cell glycolipids from vegetative A3 and HW 2 cells. Plasma membrane and whole cell lipids were extracted, partitioned, chromatographed on silica gel thin layer plates, and stained for carbohydrate as described under "Experimental Procedures." The following samples were run and the results traced: 1, upper phase lipids from 2 x 10^6 A3 cells; 2, upper phase lipids from 2 x 10^6 A3 cells; 3, upper phase lipids from 2 x 10^6 A3 cells; 4, lower phase lipids from plasma membranes from 2 x 10^6 A3 cells; 5, upper phase lipids from plasma membranes from 2 x 10^6 HW 2 cells; and 6, lower phase lipids from plasma membranes from 2 x 10^6 HW 2 cells. The positions of the sample origins and solvent front are indicated.
from A3 cells has an antigen which is found only on the 150,000-dalton macromolecule of HW 2 cells (the 150-E antigen). Since there appear to be less 150,000-dalton macromolecule exposed in HW 2 than in A3 cells, A3 adsorption is assumed to remove antibody against both of these antigens, while HW 2 adsorption leaves most of the antibody against the 150-E antigen.

We also detected several defects in HW 2 using techniques sensitive only to carbohydrate structure. Two Con A receptors were different in A3 and HW 2 plasma membrane. E(Con A), although detectable in A3, bound more lectin in HW 2. 113(Con A) was detectable only in A3. No differences were detected between A3 and HW 2 plasma membrane in receptors for the lectins WGA RCA-60, or FBP. Two PAS-positive glycoproteins were diminished in amount in HW 2. Three other PAS-positive glycoproteins, including glycoprotein E, which was also detected as an antigenic and Con A-binding defect, were more sensitive to pronase treatment of isolated plasma membrane in HW 2 than in A3. A glycolipid was present in HW 2 that was not detectable in A3. This glycolipid may be an incomplete form of more complex glycolipids common to both A3 and HW 2 but must be examined in more detail.

These studies indicate that an examination of plasma membrane composition only by one or two methods may mask many important independent structural defects. To survey fully plasma membrane composition, a variety of independent analytical techniques may generally be necessary.

With one exception, the defects found in the HW 2 plasma membrane were detectable only with one of the techniques used. This may be due to different structural requirements for antigenicity, lectin binding, and the PAS reaction. Therefore, 113(Con A) J(gp) and K(gp) may not be antigenic in rabbits or may share their antigenic sites with other membrane molecules and so would not be observed with adsorbed sera. Antibody binding to J(gp) and K(gp) by unadsorbed serum also might have been obscured by antibody binding to the broad, continuous band of antigen observed. The defects in 150(Ag), E(Con A), 113(Con A) need not involve changing the number of PAS-positive carbohydrate residues on a glycoprotein. The fact that no differences in lectin binding were seen at the position of J(gp) and K(gp) suggests that although fewer PAS-positive carbohydrate residues are present in HW 2 than in A3, an equal number of lectin binding sites with equal affinities are available. Alternatively, they may only be co-migrating with other lectin receptors.

The defect in E(gp) in HW 2 was detected both antigenically and by Con A binding. Subsequently we have purified E(gp) and found that both defects seem to be associated with E(gp) and not a co-migrating contaminant. E(gp) from A3 and HW 2 cells have a very similar amino acid composition. Therefore, both the antigenic and lectin-binding defects in HW 2 E(gp) may result from the same defect in carbohydrate structure.

This difference between E(gp) from A3 and HW 2 in carbohydrate structure may be analogous to those responsible for human blood group antigenic specificities. Human A, B, and O blood groups are defined by a large oligosaccharide with a constant core region for all three specificities. The addition of a single carbohydrate unit changes the O antigen to A or B and, despite the extensive structural identity, removes all immunological cross-reactivity (32). Similarly, the complete antigenic difference between A3 and HW 2 A(Ag) could be the result of a single carbohydrate difference. This hypothesis can also explain the difference in Con A binding between A3 and HW 2 E(gp). Con A binds to glycoproteins containing α-d-mannose or α-d-glucose residues with unsubstituted hydroxyl groups at C-3, C-4, and C-6 (33). These residues need not be terminal. However, glycopeptides that do bind Con A can be fractionated on the basis of their avidity for the lectin (34). The less avid class of receptors contains less mannos and a greater variety of other monosaccharides (35). It seems unlikely that the difference in E(gp) Con A binding between A3 and HW 2 is due to more receptors in HW 2 since in that case PAS staining of glycoprotein E in HW 2 and the adsorption of antibody against E might have increased. Therefore, HW 2 E(gp) may be an oligosaccharide chain(s) terminating in α-d-mannose or α-d-glucose, while A3 E(gp) may have I additional carbohydrate molecule not recognized by Con A at the end of an otherwise identical carbohydrate chain. This configuration could cause A3 and HW 2 E(gp) to be antigenically distinct and cause E(gp) from HW 2 cells to bind more Con A than E(gp) from A3 cells.

The defects apparent in the HW 2 vegetative plasma membrane appear to be in carbohydrate-containing molecules. Analogous defects are frequently associated with the transformed state of cells (36). Recently, defective glycosylation of cell surface glycoproteins was directly related to defects in adhesion to substratum, cell shape, number of microvilli, Con A agglutinability, and mobility in 3T3 cells (37) by Pouyssegur et al. In that study, defective glycosylation was related to a defect in glucosamine metabolism. Similar structural defects in HW 2 glycoproteins may be responsible for its inability to develop. The primary defect in HW 2 may be in the activity or substrate specificity of a glycosyltransferase (a defect frequently found in transformed cells (36)), a glycosidase or in an alteration in the cellular concentration of a precursor. Such a defect could alter the structure of oligosaccharide-containing molecules in HW 2.

Acknowledgements—We wish to thank Doctors J. Shively and M. Wran for performing the gas-liquid chromatographic analysis of plasma membrane carbohydrate composition. We also wish to thank Dr. J.-P. Revel for the use of his electron microscopy facilities and Dr. R. Stroud for the use of his autodensitometer. Thanks also go to Chris West for help in antiserum preparation and electrophoresis.

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J. Biol. Chem. 1978, 253:278-287.

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