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Wheat germ agglutinin binds to the contact site A glycoprotein of *Dictyostelium discoideum* and inhibits EDTA-stable cell adhesion

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Wheat germ agglutinin (WGA), a lectin that primarily reacts with N-acetylglucosamine residues, specifically inhibits the EDTA-stable type of intercellular adhesion of aggregation competent *Dictyostelium discoideum* cells. The major WGA-binding protein of these cells is a developmentally-regulated glycolipoprotein of 80 kd apparent mol. wt., designated as contact site A. This glycoprotein is a target site of antibody fragments that block the EDTA-stable cell adhesion, and is characterized by sulfated carbohydrate residues. WGA does not significantly bind to glycoproteins of a mutant, HL220, which produces a 68-kd component in place of the 80-kd glycoprotein. Inhibition of N-glycosylation by tunicamycin causes wild-type cells to produce a WGA-binding but unsulfated 66-kd component and a non-binding 53-kd component. These results indicate that the 80-kd glycoprotein contains two classes of carbohydrate residues, a WGA-binding one that is defective in HL220, and another, sulfated, one that is absent from the 66-kd wild-type product; both are missing in the 53-kd protein. WGA and a monoclonal antibody that is blocked by N-acetylglucosamine were further used to probe for glycoproteins in the multicellular slug stage that share carbohydrate structures – and possibly functions – with the contact site A glycoprotein. Glycoproteins in the 95-kd range have previously been implicated in cell-to-cell adhesion during the slug stage. We distinguished a 95-kd glycoprotein that binds WGA from another one that binds antibody.

Key words: lectins/cell differentiation/monoclonal antibody/cell surface carbohydrates

Introduction

Aggregation competent cells of *Dictyostelium discoideum* express on their surfaces a glycoprotein of 80-kd apparent mol. wt. (Müller and Gerisch, 1978). This glycoprotein was discovered as a stage-specific membrane antigen that neutralized adhesion blocking Fab fragments and was designated as contact site A (Beug *et al.*, 1973). The Fab was prepared from antisera raised against whole membranes of aggregation competent cells, and the glycoprotein neutralized specifically the Fab species that blocked the EDTA-stable type of intercellular adhesion. The ability to form EDTA-stable contacts is acquired during development of the cells from the growth phase to the aggregation competent stage. The contact site A glycoprotein is of interest as a potential adhesion site participating in the change from the single cell state to a multicellular organism, and also because of its stringent regulation during development from the growth phase to the aggregation competent stage (Murray *et al.*, 1981; Ochiai *et al.*, 1982a).

The contact site A glycoprotein is distinguished from most of the other membrane glycoproteins of *D. discoideum* by highly sulfated carbohydrate residues (Stadler *et al.*, 1983) and by the incorporation of palmitic acid (Stadler *et al.*, 1984). Results obtained by inhibition of N-glycosylation and antibody labeling indicate that the 80-kd glycoprotein also carries unsulfated oligosaccharide chains (Ochiai *et al.*, 1982b). Most of the monoclonal antibodies raised against the 80-kd glycoprotein react with this second class of carbohydrate moieties and cross-react with other glycoproteins, most of which appear during later stages of development (Gerisch *et al.*, 1984). Binding of some of these antibodies can be blocked with N-acetylglucosamine, indicating that this amino sugar is part of the epitope to which these antibodies are directed.

Wheat germ agglutinin (WGA) is another agent that binds to oligosaccharides containing N-acetylglucosamine. This lectin labels certain glycoproteins in *D. discoideum*, most of them being developmentally regulated and expressed at or after the aggregation stage (West *et al.*, 1978; Burridge and Jordan, 1979). These glycoproteins represent only a small fraction of the glycoproteins in *D. discoideum* membranes, since after SDS-polyacrylamide electrophoresis many more protein bands are visualized with concanavalin A than are labeled with WGA. Here we show (i) that the contact site A glycoprotein is the major WGA-binding protein of aggregation competent cells, (ii) that WGA binding to living cells inhibits specifically the EDTA-stable cell adhesion, (iii) that a mutant, HL220 (Murray *et al.*, 1984), produces a 68-kd equivalent of the 80-kd glycoprotein that lacks WGA binding sites; and wild-type cells treated with tunicamycin, an inhibitor of N-glycosylation, produce a 66-kd equivalent that contains WGA binding sites. Furthermore, we compare in the multicellular slug stage the patterns of glycoproteins that are labeled with WGA and with mAb 20-121-1, an anti-contact site A antibody that is blocked with N-acetylglucosamine. A WGA-binding glycoprotein of 95-kd apparent mol. wt. has been implicated in intercellular adhesion during the slug stage (Saxe and Sussman, 1982). The question was whether this glycoprotein shares more than its WGA binding activity with the contact site A glycoprotein.

Results

Binding of the contact site A glycoprotein to wheat germ agglutinin (WGA)

Contact sites A were extracted, together with other glycoproteins, from a particulate fraction of aggregation competent cells with butanol-water. The extract was fractionated on a column of WGA-Sepharose, and the bound material was eluted with N-acetylglucosamine. Fractions of unbound and bound material were assayed in spot tests for the contact site A glycoprotein using a monoclonal antibody, mAb 20-6-4, specific for this glycoprotein, and were subjected to SDS-polyacrylamide gel electrophoresis. Most of the antibody binding activity was retained on the WGA column and eluted with N-acetylglucosamine (Figure 1A). Silver staining of the gels showed a protein with an apparent mol. wt. of 80 kd to be enriched in the WGA-bound fractions (Figure

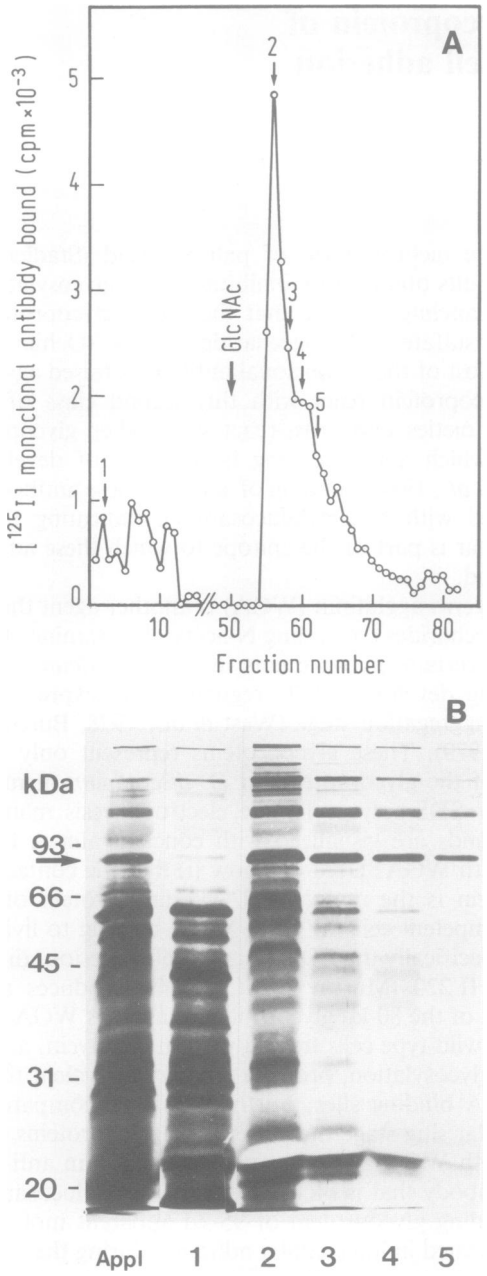

Fig. 1. Chromatography of glycoproteins of *D. discoideum* on WGA-Sepharose. **(A)** A plasma membrane enriched fraction of aggregation competent cells was extracted with butanol-water and separated on a WGA-Sepharose column. The bound glycoproteins were eluted with 450 mM N-acetyl-D-glucosamine (GlcNAc). Fractions were assayed in spot tests for binding of [^{125}I]mAb 20-6-4, an antibody highly specific for the contact site A glycoprotein, and data plotted on the ordinate. **(B)** Material that was applied to the column (Appl), and samples 1–5 as indicated in **(A)** by arrows, were subjected to SDS-polyacrylamide gel electrophoresis and silver staining. Data show that, among other proteins, an 80-kd glycoprotein (arrow on the left) was retained on the column and eluted with N-acetyl-D-glucosamine. Total amount of protein applied to the column was 1.4 mg. A 20 μg aliquot of the applied material, and one-third of the eluted fractions was applied to the gel.

1B). The amount of antibody bound in the spot tests coincided with the staining intensity of this band. To demonstrate that the 80-kd band of the WGA-bound fractions was that of the contact site A glycoprotein, proteins were labeled with mAb 20-6-4. The labeled band coincided with the silver stained one (Figure 2).

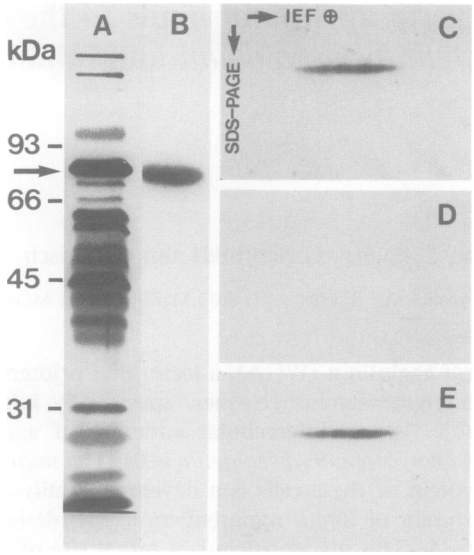

Fig. 2. Identification of the contact site A glycoprotein in the WGA-bound fraction with monoclonal antibody **(A,B)**, and labeling of the glycoprotein after two-dimensional electrophoresis with WGA and antibody **(C,D,E)**. **A,B:** proteins of the peak fraction (sample 2 in Figure 1A) were separated by SDS-polyacrylamide gel electrophoresis and stained with silver **(A)** or blotted onto nitrocellulose filter, labeled with mAb 20-6-4, and autoradiographed **(B)**. The arrow indicates position of the contact site A glycoprotein. **C,D,E:** proteins of a plasma membrane enriched fraction were separated by two-dimensional electrophoresis, blotted onto nitrocellulose, labeled with [^{125}I]WGA and autoradiographed **(C)**. The WGA was removed with 100 mM N-acetyl-D-glucosamine, the blot autoradiographed **(D)**, labeled with mAb 20-121-1 for the contact site A glycoprotein and autoradiographed again **(E)**. Only the contact site A region of the blot is shown. Time of exposure was ~17 h for **C**, **D**, and **E**.

To exclude the possibility that retention of the 80-kd glycoprotein on WGA-Sepharose was due to its association with glycolipid or another glycoprotein that binds to the column, proteins of a particulate fraction of aggregation competent cells were separated by 2D-electrophoresis, blotted and labeled directly with [^{125}I]WGA. In the contact site A region of the blot a series of isoelectric variants was labeled, as typical of this glycoprotein (Figure 2,C). After removal of the WGA by washing the nitrocellulose with N-acetylglucosamine (Figure 2,D), the blot was labeled with mAb 20-121-1, a monoclonal antibody against the contact site A glycoprotein. The pattern of labeled spots in the contact site A region was exactly the same as with WGA (Figure 2,E).

The pattern of WGA-labeled proteins changed during development from the growth phase to the stage of aggregation competence. In growth phase cells primarily a protein of ~125 kd was labeled (Figure 3;0 h). The major change during early development was the appearance of the contact site A glycoprotein, which in the aggregation competent stage, at 8 h of starvation, became the prominent WGA-binding protein (Figure 3;8 h). In addition, a protein of ~130 kd became detectable and increased in quantity during development. To exclude unspecific binding, WGA was applied together with an excess of N-acetylglucosamine, with the result that none of the proteins was labeled (Figure 3;8 h, GlcNAc).

Partially glycosylated contact site A equivalents

In the presence of tunicamycin, an inhibitor of N-glycosylation, synthesis of the fully glycosylated 80-kd protein is

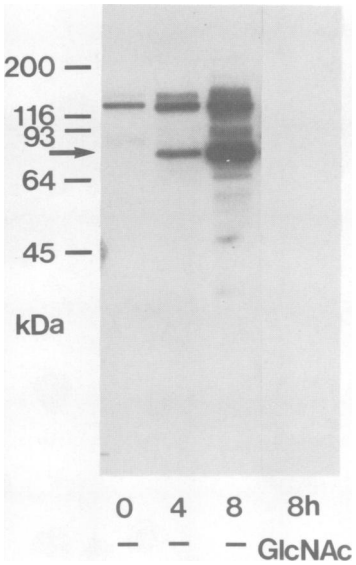

Fig. 3. Expression of WGA-binding glycoproteins during development from the growth phase to the aggregation competent stage. Wild-type AX2 cells were harvested at the end of exponential growth (0 h) or at 4 and 8 h of development, and plasma membrane enriched fractions were subjected to SDS-polyacrylamide gel electrophoresis. The proteins were blotted onto nitrocellulose, labeled with [^{125}I]WGA, and the blots were autoradiographed. The right lane shows absence of labeling when 300 mM N-acetyl-D-glucosamine (GlcNAc) was added to the WGA. Postion of the contact site A glycoprotein is indicated by arrow. 20 μg of protein were applied per slot.

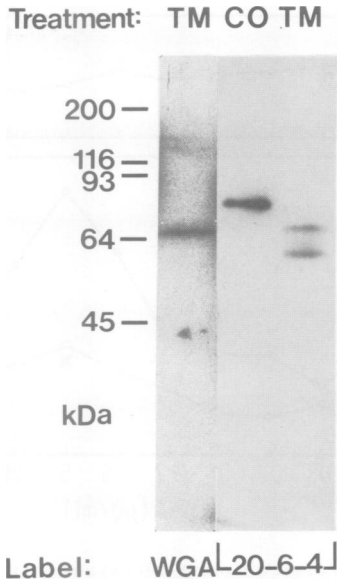

Fig. 4. WGA-labeling of proteins from tunicamycin-treated wild-type AX2 cells, and labeling of proteins from control **(CO)** and tunicamycin treated **(TM)** cells with mAb 20-6-4. Cells were harvested after 8 h of development for the control, and after 14 h for the tunicamycin-treated cells. Crude particulate fractions, equivalent to 1 x 10^6 cells per slot, were subjected to SDS-polyacrylamide gel electrophoresis and blotting of the proteins. WGA- and antibody-labeled samples from tunicamycin-treated cells were aliquots of the same preparation. The antibody indicates absence of the 80-kd glycoprotein and presence of both the 66-kd and 53-kd components in the tunicamycin-treated cells.

blocked. Instead two proteins with apparent mol. wts. of 66 kd and 53 kd are synthesized, both of which are labeled with certain monoclonal antibodies against the 80-kd glycoprotein (Ochiai *et al.*, 1982b). Antibodies which recognize the 53-kd protein are most likely directed against the polypeptide core of the glycoprotein. With [^{125}I]WGA the 66-kd protein was labeled, but not the 53-kd protein, indicating that the 66-kd product of tunicamycin-treated cells carried a carbohydrate moiety that was absent from the 53-kd component (Figure 4).

In a mutant of *D. discoideum*, HL220 (Murray *et al.*, 1984), the 80-kd glycoprotein is replaced by a 68-kd protein (Gerisch *et al.*, 1984). This protein lacks an epitope of the 80-kd glycoprotein that is recognized by a series of monoclonal antibodies including mAb 20-121-1. As shown later, this antibody is blocked by N-acetylglucosamine, indicating that it reacts with carbohydrate residues. To test whether HL220 lacks WGA-binding carbohydrate residues, proteins of the mutant were incubated with [^{125}I]WGA. No labeling of the 68-kd protein was detected, and also no other membrane protein was significantly labeled in the mutant (Figure 5).

Inhibition of EDTA-stable cell adhesion by WGA

Since Fab fragments against the contact site A glycoprotein inhibit EDTA-stable cell adhesion (Müller and Gerisch, 1978; Müller *et al.*, 1979; Murray *et al.*, 1981) and WGA binds to the same glycoprotein, an effect of WGA similar to the Fab effect appeared to be possible, although we expected WGA-mediated cell agglutination to obscure this effect.

Growth phase cells form only EDTA-sensitive contacts, aggregation competent cells adhere to each other by EDTA-stable as well as sensitive contacts (Gerisch, 1961). Cells of both stages were incubated in an agglutinometer under conditions of constant shear in the presence and absence of

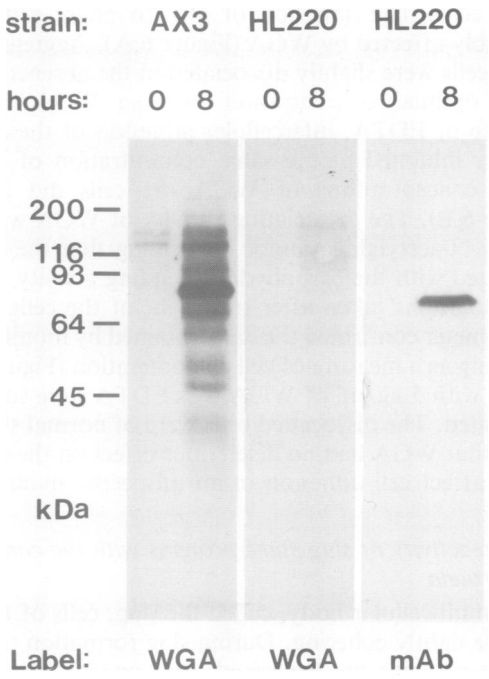

Fig. 5. Defective WGA-binding sites in mutant HL220. Cells of mutant HL220 and its parent wild-type, AX3, were harvested during exponential growth (0 h) or at 8 h of development. Crude particulate fractions were applied to SDS-polyacrylamide gel electrophoresis, blotting, labeling with [^{125}I]WGA and autoradiography. For comparison, a section of the same blot was labeled with an anti-contact site A antibody, mAb 33-294-17 (mAb), to visualize the developmentally regulated 68-kd component of the mutant. Equivalents of 2 x 10^6 cells were applied per slot. All lanes were from the same blot, and exposure times for autoradiography were the same for each one.

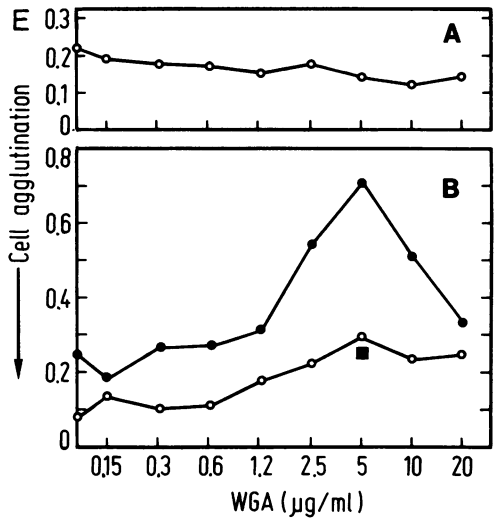

Fig. 6. Cell adhesion of growth phase cells **(A)** and of aggregation competent cells **(B)** as a function of WGA concentration. Cells were incubated with (●) or without (○) 10 mM EDTA in an agglutinometer under conditions of constant shear for ~1 h, until stable light scattering values (E) were obtained. To one sample (■) 5 μg WGA/ml plus 200 mM N-acetyl-D-glucosamine were added together with 10 mM EDTA. Light scattering was used as a measure of cell agglomeration, low values indicating large cell clumps. Data show unaffected cell adhesion of growth phase cells over the whole range of WGA concentrations, strong inhibition of the EDTA-stable cell adhesion of aggregation competent cells at an optimal concentration of 5 μg WGA/ml, and neutralization of the WGA by N-acetyl-D-glucosamine. The *D. discoideum* strain used was V12M2.

WGA, and of EDTA in the case of the aggregation competent cells. The adhesion of growth phase cells was not detectably affected by WGA (Figure 6,A). Aggregation competent cells were slightly dissociated in the absence of EDTA at an optimal concentration of 5 μg WGA/ml. In the presence of EDTA, intercellular adhesion of these cells was strongly inhibited at the same concentration of WGA. At higher concentrations of WGA the cells did agglutinate (Figure 6,B). The dissociating activity of WGA was neutralized by N-acetylglucosamine, indicating that the effect was associated with the carbohydrate binding activity of WGA.

Photographs taken after treatment of the cells in the agglutinometer confirmed the data obtained by monitoring light scattering as a measure of cell agglomeration (Figure 7). Cells treated with 5 μg/ml of WGA plus EDTA were substantially dissociated. The dissociated cells were of normal shape, indicating that WGA had no deleterious effect on the cells which would affect cell adhesion in an unspecific manner (Figure 7,H).

Cross-reactivity of slug stage proteins with the contact site A glycoprotein

In the multicellular body, called the slug, cells of *D. discoideum* are tightly cohering. During slug formation new WGA-binding proteins are expressed, and one of them, with an apparent mol. wt. of 95 kd, has been implicated in slug cell cohesion (Saxe and Sussman, 1982). In a search for glycoproteins of slugs that share carbohydrate epitopes with the contact site A glycoprotein, we have used the monoclonal antibody 20-121-1 in addition to WGA. Binding of mAb 20-121-1 to glycoproteins was blocked by N-acetylglucosamine (Figure 8), which was taken as evidence that the antibody reacted with carbohydrate.

Proteins of a particulate fraction from slugs were separated

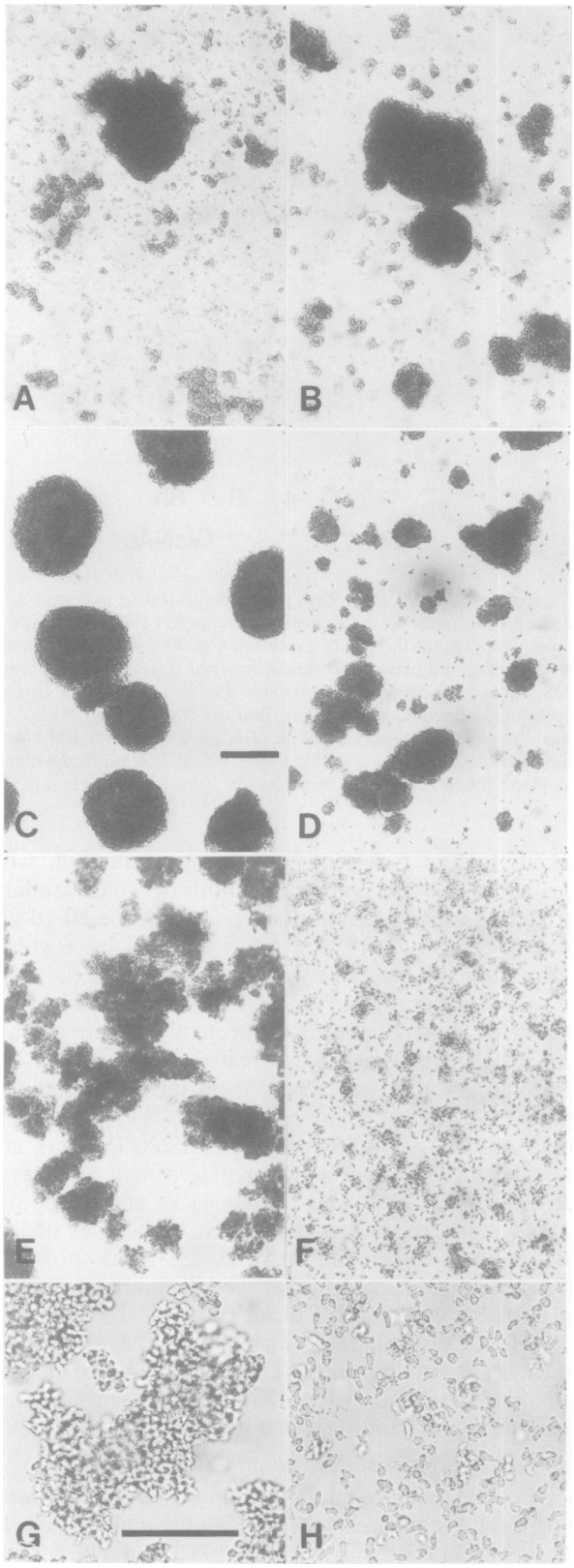

Fig. 7. Agglomeration of cells after 1 h exposure to constant shear. Photographs at low **(A–F)** and high **(G,H)** power of magnification were taken from the same samples as used for measuring light scattering (Figure 6). **A,B:** growth phase cells without EDTA. **C,D:** aggregation competent cells without EDTA. **E–H:** aggregation competent cells with 10 mM EDTA. **A,C,E:** without WGA. **B,D,F,H:** with 5 μg/ml of WGA. **G:** with 5 μg/ml of WGA plus 200 mM N-acetyl-D-glucosamine. The scale in **(C)** indicates 200 μm and refers to **A–F**, that in **(G)** indicates 100 μm and refers to **G** and **H**.

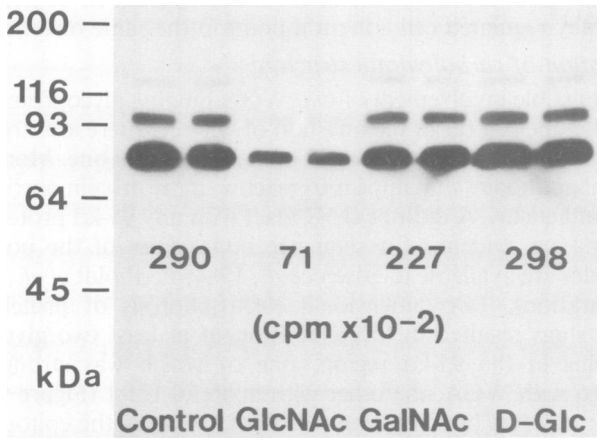

Fig. 8. Inhibition of mAb 20-121-1 binding by N-acetyl-D-glucosamine. Total homogenate from 1 x 10^6 aggregation competent cells of wild-type AX2 was applied to each slot, the proteins were separated by SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose filter. Duplicates were labeled with ^{125}I-antibody without additions (Control) or with 100 mM N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc) and D-glucose (D-Glc). After autoradiography the 80-kd bands of the contact site A glycoprotein were cut out from the filter and the bound antibody quantified in a γ-counter. Numbers are means from the duplicates after subtraction of background measured below the bands.

by two-dimensional electrophoresis, and labeled with WGA or, after removal of the bound WGA, with mAb 20-121-1. Figure 9 shows that the labeled glycoproteins fell into three classes: those strongly labeled with both WGA and antibody, those strongly labeled with WGA alone, and those strongly labeled with antibody only. The contact site A glycoprotein, still present in the slug, belonged to the first class. In the 95-kd range two glycoproteins or – because of their isoelectric heterogeneity – two clusters of glycoproteins were separated: a more acidic one that was labeled with WGA, and another one that was spread over a broad pH range, and was labeled with mAb 20-121-1. Binding of the WGA to the antibody-labeled 95-kd glycoprotein, and *vice versa*, was negligible, if it occurred at all. These results indicate that WGA and the antibody recognized different epitopes, although both were blocked with N-acetylglucosamine, and that these epitopes occurred together or separately depending on the glycoprotein.

Discussion

WGA reacts most strongly with β(1→4) linked di- and trisaccharides of N-acetylglucosamine (Nagata and Burger, 1974; Goldstein *et al.*, 1975). It also binds to sialic acid containing carbohydrate residues (Greenaway and Le Vine, 1973; Monsigny *et al.*, 1980). However, in *D. discoideum* no sialic acids have been found (Wilhelms *et al.*, 1974), so that the WGA binding sites are most likely N-acetylglucosamine containing carbohydrate sequences. N-acetylglucosamine is the most abundant sugar in *D. discoideum* glycoproteins, indicating that most, if not all glycoproteins of this organism contain N-acetylglucosamine (Gilkes and Weeks, 1977). Nevertheless, and quite in contrast to concanavalin A that binds to many glycoproteins or glycolipids of *D. discoideum* (West and McMahon, 1977; Burridge and Jordan, 1979), WGA binds rather selectively to a small group of glycoproteins.

In this paper we have investigated the binding of WGA to contact sites A and other glycoproteins of *D. discoideum,* and

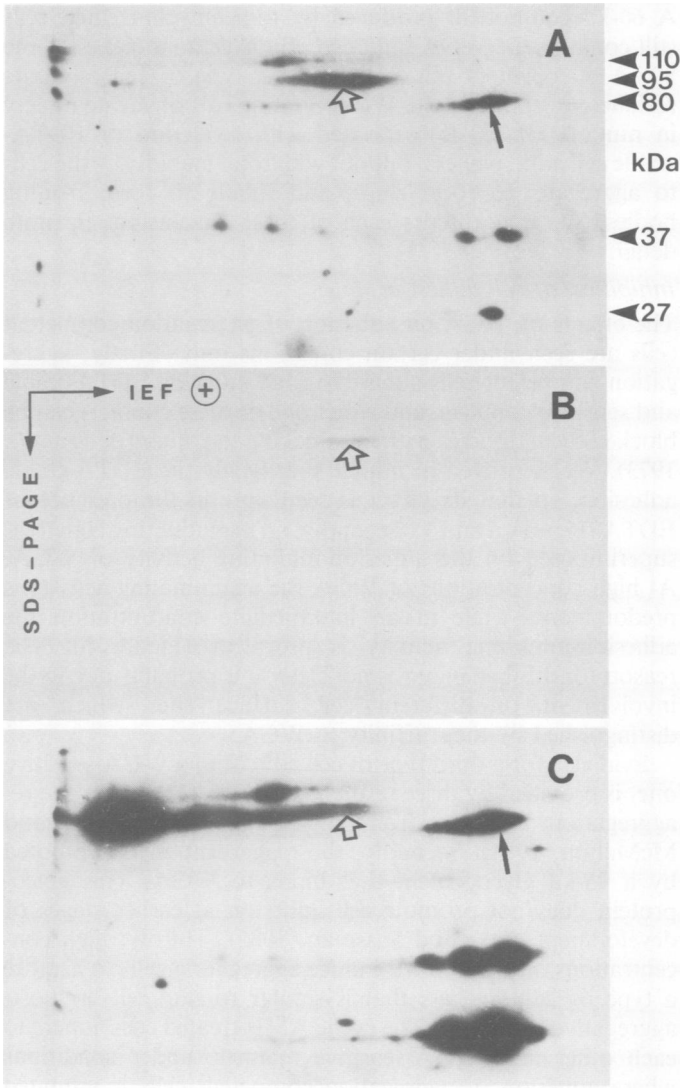

Fig. 9. Labeling of glycoproteins of the slug stage with WGA **(A)**, removal of WGA with N-acetyl-D-glucosamine **(B)** and labeling with mAb 20-121-1 **(C)**. Proteins of a crude particulate fraction prepared from slugs were separated by two-dimensional electrophoresis and blotted onto a nitrocellulose filter. After autoradiography **(A)** the blot was treated with 100 mM N-acetyl-D-glucosamine, re-exposed for **(B)** to show that only traces of the WGA-label remained detectable on the 95-kd glycoprotein, before the antibody was added and the blot autoradiographed again for **(C)**. Arrows on the autoradiograms point to the major 95-kd WGA-binding protein (⇧) and to the contact site A glycoprotein (↑); arrows on the right side indicate apparent mol. wts. as determined in one-dimensional runs. Exposure times were 3 days for **(A,B)** and 4 days for **(C)**.

its consequences on intercellular adhesion of aggregation competent cells. The conclusions from our results and those of others may be summarized as follows. (i) WGA inhibits two different types of cell adhesion, typical of aggregation competent and of 'post-aggregative' or slug cells. The blockage is nevertheless specific in the sense that a third type of adhesion, the EDTA-sensitive one, is not blocked. (ii) The majority of WGA-binding proteins, or at least their WGA-binding activities, are developmentally regulated, suggesting a function during establishment of multicellularity. (iii) The structural requirements for binding of WGA and for binding of a monoclonal antibody that is blocked by N-acetylglucosamine are different, and slug stage glycoproteins vary from each other with respect to WGA *versus* antibody binding. (iv)

A 66-kd component produced by tunicamycin-treated cells still contains the WGA-binding carbohydrate moieties of the 80-kd glycoprotein but lacks another type of carbohydrate residues. (v) Lack of the WGA-binding carbohydrate moiety in mutant HL220 is associated with reduction of EDTA-stable cell adhesiveness, but not with total inability of the cells to aggregate, to form slugs, and finally to form fruiting bodies. We now discuss each of these conclusions in more detail.

Inhibition of cell adhesion

The effects of WGA on adhesion of aggregation competent cells are seen under certain conditions only. Firstly, aggregation competent cells adhere to each other by EDTA-stable and sensitive contacts, and either one of these contacts can be blocked by antibodies without blocking the other (Beug *et al.*, 1973). WGA proved to inhibit specifically the EDTA-stable adhesion, so that its effect is seen only in the presence of EDTA (Figures 6 and 7). Secondly, an agglutinating activity is superimposed on the adhesion-inhibiting activity of WGA. At high concentrations of WGA the agglutinating activity is predominant, while at an intermediate concentration the adhesion-inhibiting activity is prevalent (Figure 6). The reason for this behaviour is not clear; it is probably due to the involvement of different cell surface sites which are distinguished by their affinity to WGA.

Evidence for a third type of cell adhesion, a WGA-sensitive one, is provided by three results. Firstly, WGA blocks the reaggregation of cells from dissociated slugs (West and McMahon, 1981). Secondly, the reaggregation is promoted by a 95-kd glycoprotein that binds to WGA. This glycoprotein does not promote cell adhesion at earlier stages of development (Saxe and Sussman, 1982). Thirdly, high concentrations of cyclic AMP induce aggregating cells to acquire a type of adhesiveness that is similar to slug cell or 'post-aggregative' adhesion. The cyclic AMP-treated cells adhere to each other in a WGA-sensitive manner, under conditions where adhesion of aggregation competent cells is inhibited (Oyama *et al.*, 1982).

Developmental regulation

With one or a few exceptions WGA-binding glycoproteins become detectable in the course of development, either early at the aggregation competent stage (Figure 3) or at later stages (Burridge and Jordan, 1979). These results indicate either *de novo* synthesis of WGA-binding glycoproteins at specific stages of development, or modification of their carbohydrate moieties in a way that makes WGA binding possible. The major WGA-binding protein of aggregation competent cells, the contact site A glycoprotein, differs by its low pI of 4.5–5.2 (Stadler *et al.*, 1983) from an 80-kd WGA-binding protein found by West and McMahon (1979) in slugs, which had a pI of 7.4. The contact site A glycoprotein has not been detected in growth phase cells using 20 monoclonal antibodies for labeling (Bertholdt *et al.*, in preparation). Since several of these antibodies appear to react with the protein moiety of the 80-kd glycoprotein, developmentally-regulated expression, rather than modification, seems to be the case for this glycoprotein.

Their absence from growth phase cells suggests that most of the WGA-binding proteins are not essential for growth and maintenance of the cells, but play a role in development. Results suggesting the involvement of the contact site A glycoprotein (Müller and Gerisch, 1978) and of a 95-kd slug-specific glycoprotein (Saxe and Sussman, 1982) in developmentally-regulated cell adhesion point in the same direction.

Variation of carbohydrate structure

The possible involvement of two WGA-binding glycoproteins in cell adhesion raises the question of whether there are structural similarities between the 80-kd and the 95-kd one. Monoclonal antibodies that appear to react with the protein portion of contact sites A did not cross-react with any 95-kd protein, so that no evidence for sequence homologies of the polypeptides are available (Gerisch *et al.*, 1984; Bertholdt *et al.*, in preparation). Two-dimensional electrophoresis of proteins from slugs resulted in the separation of at least two glycoproteins in the 95-kd region, one of which was strongly labeled with WGA, the other with mAb 20-121-1 (Figure 9). This result has two implications. First, as far as the epitopes responsible for WGA and mAb 20-121-1 binding are concerned, three types of glycoproteins exist in *D. discoideum*: those carrying both epitopes on their carbohydrates, and those carrying either one of them. Second, Steinemann and Parish (1980) have prepared Fab fragments from antisera against slugs, which inhibited reassociation of slug cells. The adhesion-blocking Fab was neutralized by antigen(s) of ~95 kd. Independent of whether the neutralizing activity resided in the WGA-binding or in the mAb 20-121-1-binding glycoprotein, the carbohydrates would share some epitope with the contact site A glycoprotein.

The results presented here and elsewhere (Gerisch *et al.*, 1984) indicate that a basically similar carbohydrate exists in different versions on a number of *D. discoideum* glycoproteins. The basic similarity is suggested by the absence of binding sites for WGA and mAb 20-121-1 from all glycoproteins of mutant HL220 (Figure 5). These findings suggest at least one step in common in the carbohydrate biosynthesis of glycoproteins that are labeled by either WGA or the antibody, or by both ligands. It can be speculated whether the variability of the carbohydrate structure is functionally important, e.g., whether glycoproteins equipped with a particular version of the carbohydrate are devised to play a primary role in cell adhesion.

Genealogy of contact site A equivalents in tunicamycin-treated wild-type and mutant cells

In wild-type cells, tunicamycin causes the production of 66-kd and 53-kd equivalents of the 80-kd protein (Ochiai *et al.*, 1982b; see also Figure 4). Monoclonal antibodies against the glycoprotein fell into one of two classes, those labeling the 66-kd and the 53-kd components, and those labeling the 66-kd component only. It was suggested that the 66-kd component still contained a carbohydrate moiety, and that the second class of antibodies was directed against this carbohydrate, the first against the polypeptide core of the glycoprotein (Ochiai *et al.*, 1982b). This suggestion has now been substantiated by the finding that mAb 20-121-1, an antibody of the second class, was blocked by N-acetylglucosamine, and also by the finding that the 66-kd component was labeled with WGA. Taking into account that the 66-kd component was devoid of carbohydrate-linked sulfate residues (Stadler *et al.*, 1983), we conclude that the 80-kd glycoprotein contains two kinds of carbohydrate residues, N-linked carbohydrate I that is sulfated and missing in the 66-kd component, and carbohydrate II that reacts with WGA and antibodies of the second class (Figure 10). Results obtained with HL220 are easily fitted into this scheme. The mutant glycoprotein lacks carbo-

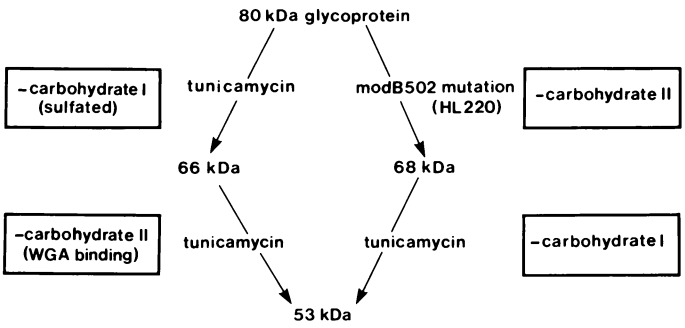

Fig. 10. Scheme showing the suggested defects of contact site A glycosylation in tunicamycin-treated wild-type and mutant HL220 cells that carry the modB502 mutation (Murray *et al.*, 1984). It is not clear whether the shift from 66 kd to 53 kd in the wild-type is caused by a direct effect of tunicamycin on N-glycosylation or whether, more indirectly, absence of carbohydrate I partially suppresses the attachment of carbohydrate II. Attachment of carbohydrate I does not depend on carbohydrate II, as indicated by the mutant.

hydrate II and contains carbohydrate I, which as in the wild-type is still sulfateable (H.P.Hohmann, unpublished). Carbohydrate I is missing in tunicamycin-treated HL220 cells, so that the same 53-kd component is produced as in the wild-type (J.Stadler, unpublished).

The absence of WGA binding seems to be the only similarity between HL220 and a mammalian cell line that lacks WGA binding capacity (Finne *et al.*, 1980,1982). The defect in WGA binding of the F_1 melanoma line was associated with a loss of sialic acid bound to C-3 of galactose and an increased substitution of N-acetylglucosamine residues by fucose, due to an increase in a fucosyltransferase. These changes were correlated with poor metastasizing activity of the cells. As outlined above, HL220 glycoproteins lack a major portion of their carbohydrate.

Relationship between defects of glycosylation and of the potency to aggregate

A crucial question is whether the carbohydrate structure responsible for WGA binding is directly involved in intercellular adhesion. The inhibition of cell adhesion by WGA *per se* does not provide an answer since WGA, with a mol. wt. of 35 kd of the dimer (Nagata and Burger, 1974), will cover a substantial portion of the external part of a membrane glycoprotein, and thus might inhibit adhesion even when it does not exactly bind at the site of intercellular contact.

Cells of HL220 that carry the mutation modB502 responsible for the lack of WGA binding sites, still form EDTA-stable contacts, although their capacity to do so is largely reduced, and they also form slugs and eventually fruiting bodies (Murray *et al.*, 1984; Gerisch *et al.*, 1984). These results indicate that, although the WGA-binding carbohydrate residues might enhance cell adhesion, they are not essential for development.

In tunicamycin-treated wild-type cells, EDTA-stable cell adhesion was more severely affected than it was by the modB502 mutation (Lam and Siu, 1982; Ochiai *et al.*, 1982b). Tunicamycin might affect adhesion in an indirect way, e.g., the transport of incompletely glycosylated proteins to the cell surface might be disturbed. But an alternative and attractive explanation of the tunicamycin effect would be that carbohydrate I, which is normally sulfated, is essential for EDTA-stable cell adhesion. This would not necessarily mean that the sulfation as such is required for adhesiveness (Stadler *et al.*, 1983).

Materials and methods

Cell culture

Cells of *D. discoideum* strain AX2-214 were grown axenically on a shaker at 23°C, harvested at a density of 5 x 10^6 cells/ml, washed free of nutrient medium and adjusted to 1 x 10^7 cells/ml in 17 mM Soerensen phosphate buffer pH 6.0 (Malchow *et al.*, 1972). Within 6–8 h AX2-214 cells developed in suspension spontaneously to the aggregation competent stage. The cells used for Figure 8 were treated every 6 min with 2 x 10^{-8} M pulses of cyclic AMP, which enhanced contact site A expression, and were harvested at 8 h of development.

Cells of the *D. discoideum* strain AX3 and of mutant HL220 were grown in suspension on *Salmonella minnesota* R595 and washed for starvation (Gerisch *et al.*, 1984). Development of these strains was stimulated by periodic pulses of 2 x 10^{-8} M cyclic AMP applied every 6 min. For the assay of cell agglutination, cells of *D. discoideum* V12M2 were grown in suspensions of 1 x 10^{10} *Escherichia coli* B/r bacteria per ml, and harvested as 'growth phase cells' by washing them when 4 x 10^9 bacteria were left. Aliquots of the starved cells were cultivated in the Soerensen phosphate buffer for 4 h and used as 'aggregation competent cells'. For inhibition of N-glycosylation, tunicamycin (Boehringer Mannheim) was added at 2 h of starvation at a final concentration of 0.5 μg/ml (Ochiai *et al.*, 1982b), development was stimulated by pulses of cyclic AMP as above, and the cells were harvested at 14 h of development.

Slugs were obtained by dispensing growth phase cells of AX2-214 in a streak on agar containing 17 mM Soerensen phosphate buffer pH 6.0, and collecting slugs that had migrated onto the clean agar surface.

Particulate and plasma-membrane enriched fractions

Crude particulate fractions were prepared by freezing and thawing and centrifugation for 20 min at 10 000 *g*. Plasma-membrane enriched fractions were obtained from crude particulate fractions by dextran 500/polyethylene glycol 6000 separation (Brunette and Till, 1971) as described by Müller *et al.* (1979).

Affinity chromatography of butanol-water extracts of membranes on WGA-Sepharose

A plasma-membrane enriched fraction from aggregation competent AX2 cells was extracted with butan-1-ol (Morton, 1950; Müller *et al.*, 1979). Proteins recovered in the water-phase were precipitated by nine volumes of acetone (Stadler *et al.*, 1982) and 1.4 mg of protein were solubilized in 1 ml of 10 mM phosphate buffer, pH 6.5, containing 0.02% NaN_3, 0.2 mM phenylmethylsulfonyl fluoride and 0.1% Triton X-100. This solution was applied to a column (1 x 4 cm) packed with wheat germ lectin-Sepharose 6 MB (Pharmacia, Uppsala) for affinity chromatography. The sample was allowed to adsorb for 30 min before the column was washed with 10 column volumes of the buffer at a flow rate of 3 ml/h. For the elution of bound proteins the buffer was supplemented with N-acetyl-D-glucosamine (final concentration 450 mM), fractions of 0.5 ml were collected and the material precipitated as above for SDS-polyacrylamide gel electrophoresis. Spot tests were performed as described by Stadler *et al.* (1982).

Gel electrophoresis and immunoblotting

SDS-polyacrylamide electrophoresis in 10% gels (Laemmli, 1970) and silver staining (Oakley *et al.*, 1980) were standard methods. For two-dimensional electrophoresis (O'Farrell, 1975) one volume of ampholytes pH 3–10 (Pharmacia, Uppsala) and four volumes of ampholytes pH 4–6 (LKB, Bromma) were used. For transfer to nitrocellulose (BA85, Schleicher and Schüll, Dassel) the method of Towbin *et al.* (1979) was used. Protein was determined according to Lowry with bovine serum albumin (BSA) as standard.

Labeling with antibodies or WGA

Monoclonal antibodies against the contact site A glycoprotein were prepared as described (Ochiai *et al.*, 1982a) and iodinated with ^{125}I using the chloramine T method. WGA was purchased from Calbiochem-Behring and iodinated according to Burridge (1978) in the presence of 0.4 mg N-acetylglucosamine per 200 μl, using 0.5 mCi ^{125}I (Amersham) per 100 μg of WGA. A specific activity of 2.5 x 10^6 c.p.m./μg WGA was obtained. Blots were labeled with antibody as described by Stadler *et al.* (1982). For labeling with WGA, the iodinated WGA was diluted to 3 x 10^5 c.p.m./ml in 10 mM Tris-HCl buffer pH 7.5 containing 150 mM NaCl, 8% BSA and 2% NaN_3. For washing of the blots BSA was replaced by 0.05% Tween 20. The blots were autoradiographed on Kodak X-Omat AR film. WGA was removed from the blots by 1 h incubation with 100 mM N-acetyl-D-glucosamine in 10 mM Tris-HCl buffer pH 7.5, containing 1 M NaCl, 8% BSA and 2% NaN_3.

Assay of cell agglutination

Cell agglutination and its inhibition by WGA was measured according to Beug *et al.* (1973) in 17 mM Soerensen phosphate buffer pH 6.0 with additions as indicated, using a microprocessor-controlled version of the agglutinometer of Beug and Gerisch (1972). Photographs were taken through the glass

walls of the cuvettes used in the agglutinometer immediately after settling of agglutinates and cells.

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