

DIFFERENTIAL INVOLVEMENT OF CELL SURFACE SIALIC ACID RESIDUES IN WHEAT GERM AGGLUTININ BINDING TO PARENTAL AND WHEAT GERM AGGLUTININ-RESISTANT CHINESE HAMSTER OVARY CELLS

PAMELA STANLEY, TADASHI SUDO, and JEREMY P. CARVER

From the Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461, and the Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada. Dr. Sudo's present address is the Institute for Virus Research, Kyoto University, Sakyo-Ku, Kyoto, Japan.

ABSTRACT

Two Chinese hamster ovary (CHO) cell mutants selected for resistance to wheat germ agglutinin (WGA) have been shown to exhibit defective sialylation of membrane glycoproteins and a membrane glycolipid, GM₃. The mutants (termed Wga^{R_{II}} and Wga^{R_{III}}) have been previously shown to belong to different genetic complementation groups and to exhibit different WGA-binding abilities. These mutants and a WGA-resistant CHO cell mutant termed Wga^{R_I} (which also possesses a surface sialylation defect arising from a deficient *N*-acetylglucosaminyltransferase activity), have enabled us to investigate the role of sialic acid in WGA binding at the cell surface. Scatchard plots of the binding of ¹²⁵I-WGA (1 ng/ml to 1 mg/ml) to parental and Wga^R CHO cells before and after a brief treatment with neuraminidase provide evidence for several different groups of sialic acid residues at the CHO cell surface which may be distinguished by their differential involvement in WGA binding to CHO cells.

The carbohydrate structures present at the surface of animal cells are currently of great interest because of their possible role in recognition phenomena. Thus, lectins which exhibit specific interactions with carbohydrate moieties have been extensively used to monitor cell surface carbohydrate differences (10). However, the structural specificities of lectin-cell interactions are little understood, for two major reasons. Firstly, the different types of carbohydrate structures and their relative proportions at the cell surface are not known for any animal cell. Secondly, the specificities of lectins for complex carbohydrates attached to glycoproteins and glycolipids are not well defined. In an

attempt to obtain information relevant to both of these questions, we have examined the lectin-binding properties of a number of Chinese hamster ovary (CHO) cell lines which possess structural alterations in surface carbohydrate (20-22).

Previously, we have shown that the binding of wheat germ agglutinin (WGA) to parental CHO cells gives a complex Scatchard plot indicative of positive cooperativity amongst high-affinity binding sites (21, 22). This positive cooperativity does not appear to arise from interactions between mobile WGA-binding sites but rather to reflect WGA-"receptor" or WGA-WGA positively cooperative interactions (23). Two WGA-resistant

(Wga^R) CHO cell lines (Wga^{R1} and Wga^{R11}) were shown to exhibit reduced binding of WGA over unique regions of this binding curve, whereas a third Wga^R cell line (Wga^{R111}) was shown to bind WGA in a manner essentially identical to that for parental cells (22).

The present report correlates certain of the WGA-binding properties of the Wga^R mutant lines and the structural carbohydrate alterations that have occurred at their respective cell surfaces. As other authors have reported that the removal of surface sialic acid residues via neuraminidase treatment results in a reduced ability to bind WGA (1, 4, 8, 17), we have also examined the effect of a brief neuraminidase treatment on the WGA-binding properties of parental and mutant cell lines. Our results suggest that there are several "classes" of sialic acid residue at the CHO cell surface, only certain of which are involved in WGA binding.

MATERIALS AND METHODS

Materials

WGA, bovine serum albumin (BSA), galactose oxidase (EC 1.1.8.9), and *N*-acetylneuraminic (sialic) acid were obtained from Sigma Chemical Co. (St. Louis, Mo.); neuraminidase from *Vibrio cholerae* (EC 3.2.1.18) from Calbiochem-Behring Corp., American Hoechst Corp. (San Diego, Calif.); [³H]borohydride (>330 mCi/mmol) from New England Nuclear (Boston, Mass.); ¹²⁵I from Amersham Corp. (Arlington Heights, Ill.); Chloramine-T from British Drug Houses Chemicals Ltd. (Poole, England); fetal calf serum (FCS) from Flow Laboratories, Inc. (Rockville, Md.); and alpha medium from Grand Island Biological Co. (Grand Island, N. Y.). All other chemicals were reagent grade.

CELL LINES: The CHO cell lines Pro⁻⁵ (proline-requiring), Gat⁻² (glycine-, adenosine-, and thymidine-requiring), Pro⁻⁵Wga^{R1}3C, Gat⁻²Wga^{R1}1N, Pro⁻⁵Wga^{R11}6A, Gat⁻²Wga^{R11}4C, and Pro⁻⁵Wga^{R111}4B were cultured at 34°C in suspension in alpha medium containing 10% FCS as previously described (20, 25). The derivation of the mutant lines is described in reference 20 and their nomenclature and complementation groups in reference 25. Most experiments were performed with two independent isolates from each complementation group. For simplicity, however, the results for each complementation group have been combined and the Wga^R cell lines referred to as Wga^{R1}, Wga^{R11}, and Wga^{R111} which represent complementation groups I, II, and III, respectively (25).

Surface Labeling via

Galactose Oxidase:[³H]Borohydride

Cells in exponential growth were washed twice with cold phosphate-buffered saline (PBS) and resuspended at about 5 × 10⁷ cells/ml in warm PBS. Neuraminidase at 50 U/10⁸ cells was added to one-half of the cell suspension. Both control and neuraminidase-treated cells were incubated for 5 min at 37°C.

After centrifugation for 10 min at 1,200 rpm in a Sorvall G1-2 centrifuge (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.), the supernates were frozen at -20°C, and the cell pellet was washed once with 10 ml of cold PBS/10⁸ cells and resuspended at 5 × 10⁷/ml in warm PBS. Galactose oxidase was added (15 U/10⁸ cells) and the cell suspension was incubated for 5 min at 37°C. After centrifugation, the cells were washed once with 10 ml of cold PBS and incubated on ice with ~1 mCi of [³H]borohydride. After 5 min, the cells were washed three times with 10 ml of cold PBS containing 1% BSA. Control experiments showed that the concentrations used of neuraminidase, galactose oxidase, and [³H]borohydride were necessary to achieve maximal labeling under these reaction conditions. The cells were 85–90% viable after neuraminidase treatment, 80–85% viable after galactose oxidase, and 75–80% viable after the [³H]borohydride step. To reduce contamination from internally labeled components, we prepared plasma membranes from the labeled cells according to the method of Brunette and Till (7). A comparison of the TGA-precipitable cpm per mg protein assayed by the method of Lowry et al. (15) showed that the plasma membrane fraction was enriched approximately sixfold compared with the cell homogenate after the partial-purification step. The specific activity (TCA-precipitable cpm/mg protein) of each membrane preparation was routinely determined.

Gel Electrophoresis

Membrane samples (100 µg of protein; 25,000–30,000 cpm) were solubilized in an equal volume of 0.3 mM Tris-HCl (pH 6.5) containing 3% SDS, 5% β-mercaptoethanol, and 10% glycerol. These samples (in a volume of 50–100 µl) were analyzed on 20-cm polyacrylamide gels with the buffer system of Laemmli (14) and a 3–17% gradient of polyacrylamide made with a Harvard apparatus model 1203 gradient maker (Harvard Instrument Co., Ayer, Mass.). Electrophoresis was commenced at 70 V and increased to 160 V after the bromophenol blue dye entered the running gel. After ~7 h, the gel was fixed in ethanol:acetic acid:water (5:2:20) for 2 h, stained in 0.25% Coomassie Blue for 15 min, and destained in the fix solution. After photography, the gel was impregnated with 2,5-diphenyloxazole, dried onto filter paper, and exposed to x-ray film at -70°C (5).

Identification of Sugars Labeled by Galactose Oxidase:[³H]Borohydride

Membrane samples in SDS gel electrophoresis digestion buffer were hydrolyzed by boiling for 4 h in 1.5 N HCl (glass-distilled). The hydrolysates were applied to 4-ml coupled columns of Dowex 50-X4(200–400) *H above Dowex 1-X8(200–400) formate (Dow Chemical Co., Midland, Mich.), and neutral and amino sugar fractions were eluted as described by Adamany and Spiro (2). Neutral sugars and acetylated amino sugars were separated by paper chromatography and identified according to their co-migration with unlabeled sugars (2).

Thiobarbituric Acid Assay

The supernate from cells treated with neuraminidase as described above were stored at -20°C. After ~1 yr, they were concentrated 10-fold via lyophilization and subsequent reconstitution in distilled H₂O. Samples of 0.2 ml were assayed in

duplicate for free sialic acid with the thiobarbituric acid (TBA) assay (3). Standard curves were obtained with sialic acid dissolved in PBS at 10 times the usual concentration. These standard curves were identical to those obtained with sialic acid in H₂O.

Extraction of Glycolipids and Thin-Layer Chromatography

Approximately 10⁹ exponentially growing cells were washed twice with cold PBS, resuspended in 40 ml of cold PBS, and counted. After centrifugation, the cells were resuspended in 40 ml of 10 mM Tris-HCl, pH 7.4, in a graduated glass centrifuge tube and centrifuged at 2,000 rpm for 15 min at 4°C in an International PR2 centrifuge (Damon/IEC Div., Damon Corp., Needham Heights, Mass.). The cell pellet was extracted with chloroform:methanol (C:M) according to the method of Suzuki (26). Briefly, 20 vol of C:M (2:1) was mixed with the cells for 2 min in a Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.) (low speed). The extract was filtered through sintered glass, and the residue was re-extracted with 10 vol of C:M (1:2). The combined filtrates were roto-evaporated to dryness and redissolved in C:M (1:1) at 10⁹ cells/ml (based on cell counts obtained from the cells suspended in 40 ml of PBS before extraction). The lipid extract was stored at -20°C and was stable for many months. It was noted that storage of frozen cell pellets at -20°C before lipid extraction resulted in extensive desialylation of the major ganglioside GM₃.

The glycolipids in crude lipid extracts were examined by thin-layer chromatography (TLC) on precoated silica gel 60 plates (0.25 mm, E.M. Laboratories, Inc., Darmstadt, Germany) with solvent A (C:M:H₂O = 60:30:4.5) or solvent B (C:M:NH₄OH:H₂O = 60:35:1:7). Purified glycolipids GM₂, GM₃, lactosylceramide (LC), and glucosylceramide (GC) were kindly provided by Dr. Samar Kundu (Albert Einstein College of Medicine). The plates were stained with α -naphthol/sulphuric acid for the detection of lipids containing neutral sugars (19) or with resorcinol which specifically stains gangliosides (27). The extract from approximately 3 × 10⁷ washed cells was compared in a typical TLC plate.

WGA-Binding Assay

The binding of WGA to parental and Wga^R CHO cells was performed as described previously (21-23). Briefly, WGA was iodinated by the chloramine-T method to a sp act of ~12-15 μ Ci/ μ g and used within 3 d of its preparation (23). Washed cells were incubated with ¹²⁵I-WGA, unlabeled WGA, BSA (2%), and PBS for 1 h at room temperature. Cells treated with neuraminidase were obtained in exactly the manner described above. In many cases the same cell preparations were examined simultaneously for WGA binding and via SDS gel electrophoresis after galactose oxidase/[³H]borohydride labeling. WGA concentrations in the binding assay ranged from 1 ng/ml to 1 mg/ml. Each point was performed in duplicate, and five to six points were determined with two WGA preparations of different specific activities in each experiment. Unbound ¹²⁵I-WGA was removed by filtration through GF/C filters soaked in 10% BSA at least 2 h. The Scatchard parameters *r* (amount WGA bound per cell) and *A* (amount of free WGA) were calculated from the cpm in the reaction tubes before the addition of cells, the cpm on the filters, and the cpm in the washed reaction tubes. Nonspecific binding of WGA is negligible under these assay conditions (22).

RESULTS

Surface Carbohydrate Changes Exhibited by Wga^{R_I}, Wga^{R_{II}}, and Wga^{R_{III}} CHO Cells

Each of the Wga^R cell lines that we examined has previously been shown to possess alterations in surface carbohydrate (21) and to belong to distinct genetic complementation groups (25). Wga^{R_I} cells have lost a specific *N*-acetylglucosaminyltransferase activity (GlcNAc-T₁) which appears to provide the biochemical basis of their mutant phenotype (16, 24). These cells synthesize a partially completed asparagine (Asn)-linked oligosaccharide Man₅GlcNac₂Asn (18) which appears to be an intermediate in the pathway to "complex" Asn-linked oligosaccharides terminating in GlcNAc-Gal-sialic acid sequences (28). This is reflected by the reduced ability of Wga^{R_I} cells to be surface-labeled by the galactose oxidase method (~10% compared with parental CHO cells; 13). By contrast, little is known about the carbohydrate alterations that have occurred at the surfaces of Wga^{R_{II}} and Wga^{R_{III}} CHO cells. Therefore we examined the surface-labeling properties of these Wga^R mutants in the presence of galactose oxidase and [³H]borohydride before and after a pretreatment with neuraminidase. This approach enables the qualitative comparison of certain Gal, GalNAc, and sialic acid residues present at the cell surface (9).

Fig. 1*a* compares the Coomassie Blue-stained gel patterns of membranes from parental and Wga^R CHO cells after surface labeling with and without a neuraminidase pretreatment. Within the limits of detection of this gel system, it can be seen that there are no significant profile differences. This shows that the protein complements of the parental mutant cell membranes are very similar and also provides evidence that significant proteolysis does not occur during the labeling procedures. Other experiments showed that, under identical incubation conditions, the neuraminidase and galactose oxidase preparations exhibited negligible proteolysis of BSA or [¹⁴C]casein hydrolysate.

An autoradiogram of cells surface-labeled by galactose oxidase is shown in Fig. 1*b*. In the absence of a neuraminidase treatment, parental and Wga^{R_I} cells exhibit very little labeling of surface glycoproteins. However, Wga^{R_{II}} and Wga^{R_{III}} cells exhibit many well-labeled bands of various molecular weights. (Though apparently of reduced

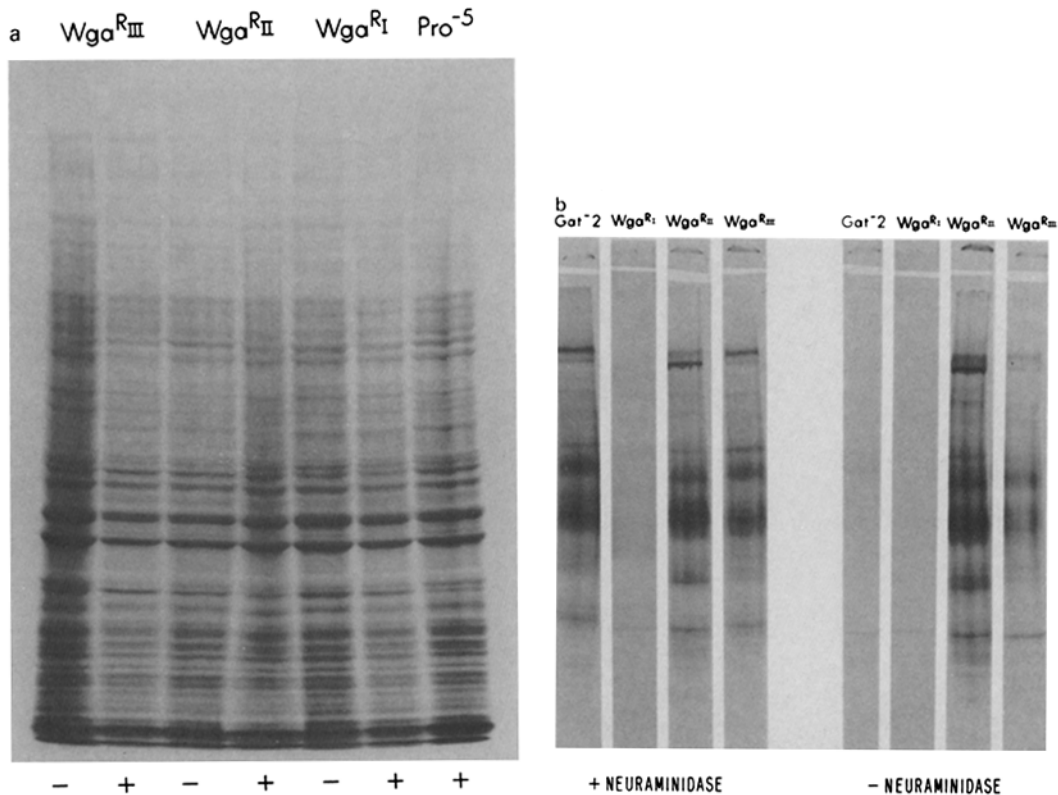


FIGURE 1 (a) SDS slab gel of parental and Wga^R CHO cells labeled via galactose oxidase: 3H borohydride before (-) and after (+) a treatment with neuraminidase. This gel was stained with Coomassie Brilliant Blue before autoradiography. The autoradiography gave results essentially identical to those shown in Fig. 1b. The individual samples from left to right are as follows: $Pro^{-5}Wga^{RII}4B$, $Pro^{-5}Wga^{RII}6A$, $Pro^{-5}Wga^{RII}3C$, and Pro^{-5} . (b) Glycoproteins labeled via galactose oxidase: 3H borohydride in parental and Wga^R CHO cells. Washed cells were labeled via galactose oxidase: 3H borohydride before or after a treatment with neuraminidase. Plasma membranes were partially purified from the labeled cells, assayed for TCA-precipitable cpm and protein, solubilized in electrophoresis sample buffer, and analyzed in 3-17% gradient SDS slab gels (see Materials and Methods). The results presented were obtained from one slab gel except for the Wga^{RI} samples. The individual samples from left to right are as follows: Gat^{-2} , $Gat^{-2}Wga^{RI}IN$, $Gat^{-2}Wga^{RII}4C$, $Pro^{-5}Wga^{RII}4B$, Gat^{-2} , $Gat^{-2}Wga^{RI}IN$, $Gat^{-2}Wga^{RII}4C$, and $Pro^{-5}Wga^{RII}4B$.

intensity in this experiment, the decreased labeling of Wga^{RIII} glycoproteins was not consistently reflected in the specific activities of Wga^{RII} compared with Wga^{RIII} membranes in a number of independent experiments.) Acid hydrolysis of labeled membrane samples showed that 80-90% of the recovered label was associated with Gal residues. The remaining label eluted in the amino sugar fraction from Dowex 50 (H^+) as would be expected of labeled GalNAc. However, the cpm recovered were too low for chromatographic identification. Thus, the results in Fig. 1b show that untreated parental and Wga^{RI} CHO cells possess compara-

tively few Gal residues accessible to galactose oxidase. By contrast, untreated Wga^{RII} and Wga^{RIII} cells possess "exposed" Gal residues distributed amongst a broad variety of membrane glycoproteins.

The surface labeling of parental CHO cells is increased ~15-fold by neuraminidase pretreatment, and the gel patterns obtained are very similar to those of untreated Wga^{RII} and Wga^{RIII} cells (Fig. 1b). This suggests that Wga^{RII} and Wga^{RIII} cells possess a defect in the sialylation of certain membrane glycoproteins. They are not completely deficient in surface sialic acid, however, because

TABLE I
Sialic Acid Released by Neuraminidase from
Parental and Wga^R CHO Cells

Cell line	No. expts.	µg Sialic acid released/10 ⁶ cells
Parental	4	25 (20–28)
Wga ^{R1}	3	19 (14–24)
Wga ^{R11}	3	2 (1–6)
Wga ^{R111}	3	8 (5–11)

Sialic acid released into the supernate after neuraminidase treatment of washed cells was assayed by the TBA method as described in Materials and Methods. Figures in parentheses correspond to the range of values obtained. The values given are per 10⁶ CHO cells but the samples measured were obtained from 2–3 × 10⁶ cells in each case.

neuraminidase treatment releases sialic acid from each of the Wga^R mutants (Table I) and results in an increase (~1.4-fold) in their respective abilities to be surface labeled by galactose oxidase:[³H]-borohydride. The final specific activities of neuraminidase-treated parental, Wga^{R11}, and Wga^{R111} cells are similar (~2.4 ± 0.8 × 10⁵ cpm/mg protein) as would be predicted if the surface defects in Wga^{R11} and Wga^{R111} cells are confined to sialic acid residues. As observed previously (13), Wga^{R1} cells do not label well even after neuraminidase treatment (Fig. 1*b*), presumably because of the lack of Gal residues in certain of their Asn-linked oligosaccharides.

GLYCOLIPIDS: A deficiency in the transfer of sialic acid to glycoproteins may also affect the addition of sialic acid to glycolipids, depending on the primary metabolic site of the defect. Because the major glycolipid in CHO cells is the ganglioside GM₃ (6, 30) which contains one residue of sialic acid, it was important to compare the GM₃ contents of the parental, Wga^{R11} and Wga^{R111} cell lines. Wga^{R1} cells were examined as a control because it has previously been reported that their glycolipid complement is essentially identical to that of parental CHO cells (24).

The glycolipids of parental CHO cells and the three Wga^R mutants were extracted and analyzed by TLC (Fig. 2). The identity of each glycolipid was established by three criteria: (a) co-migration with purified glycolipids in solvent A (Fig. 2*a*) and solvent B (data not shown); (b) conversion of putative GM₃ material to LC after neuraminidase treatment (Fig. 2*b*); and (c) specific staining of neutral hexoses by α-naphthol (Fig. 2*a* and *b*) and of gangliosides by resorcinol (Fig. 2*c*).

Fig. 2*a* shows that a number of discrete bands appear after staining with α-naphthol. However, parental CHO cell extracts contain only five areas that stain the blue color characteristic of carbohydrate (arrows). Area 1 corresponds to material remaining at the origin and also material co-migrating with free sialic acid. Area 2 contains material that migrates like free glucose or galactose. The bands in regions 3, 4, and 5 co-migrate with the glycolipids GM₃, LC, and GC (data not shown), respectively. Fig. 2*a* shows that parental and Wga^{R1} CHO cells contain mostly material migrating with GM₃ and only a small amount of material migrating with LC and GC. By contrast, Wga^{R11} cells contain mostly material migrating with LC, and Wga^{R111} cells exhibit intermediate amounts of material migrating with both GM₃ and LC. Thus, both Wga^{R11} and Wga^{R111} cells exhibit reduced sialylation of the major CHO cell glycolipid as well as numerous CHO cell glycoproteins.

Evidence that the cell-derived material co-migrating with GM₃ in Fig. 2*a* is in fact GM₃ is provided in Fig. 2*b* and *c*. Fig. 2*c* shows that this material stains with the resorcinol reagent which is specific for sialic acid residues, and Fig. 2*b* shows that it is converted to LC by treatment with neuraminidase. Fig. 2*b* and *c* also provide evidence that there are no appreciable amounts of other gangliosides in parental or mutant CHO cells.

WGA-Binding Abilities of Parental and Wga^R CHO Cells

The WGA-binding properties of Wga^{R1}, Wga^{R11}, and Wga^{R111} CHO cells have previously been described (22). In those experiments a million-fold range of WGA concentrations was examined and, in linear Scatchard plots, many of the data points fell on the *r/A* axis. Therefore the data have been replotted as *r/A* versus log₁₀ *r*, where *r* equals the amount of WGA bound per cell and *A* equals the amount of unbound WGA (Fig. 3). As described previously, Wga^{R1} and Wga^{R11} cells exhibit reduced WGA-binding abilities, whereas Wga^{R111} cells bind WGA essentially the same as parental CHO cells. These binding properties may now be correlated with the surface carbohydrate alterations shown to occur in each mutant. Thus, Wga^{R1} cells which appear to have the most severe surface carbohydrate alteration bind more WGA than Wga^{R11} cells which possess a sialylation defect affecting glycoproteins and glycolipids. On the other hand, Wga^{R111} cells exhibit no change in

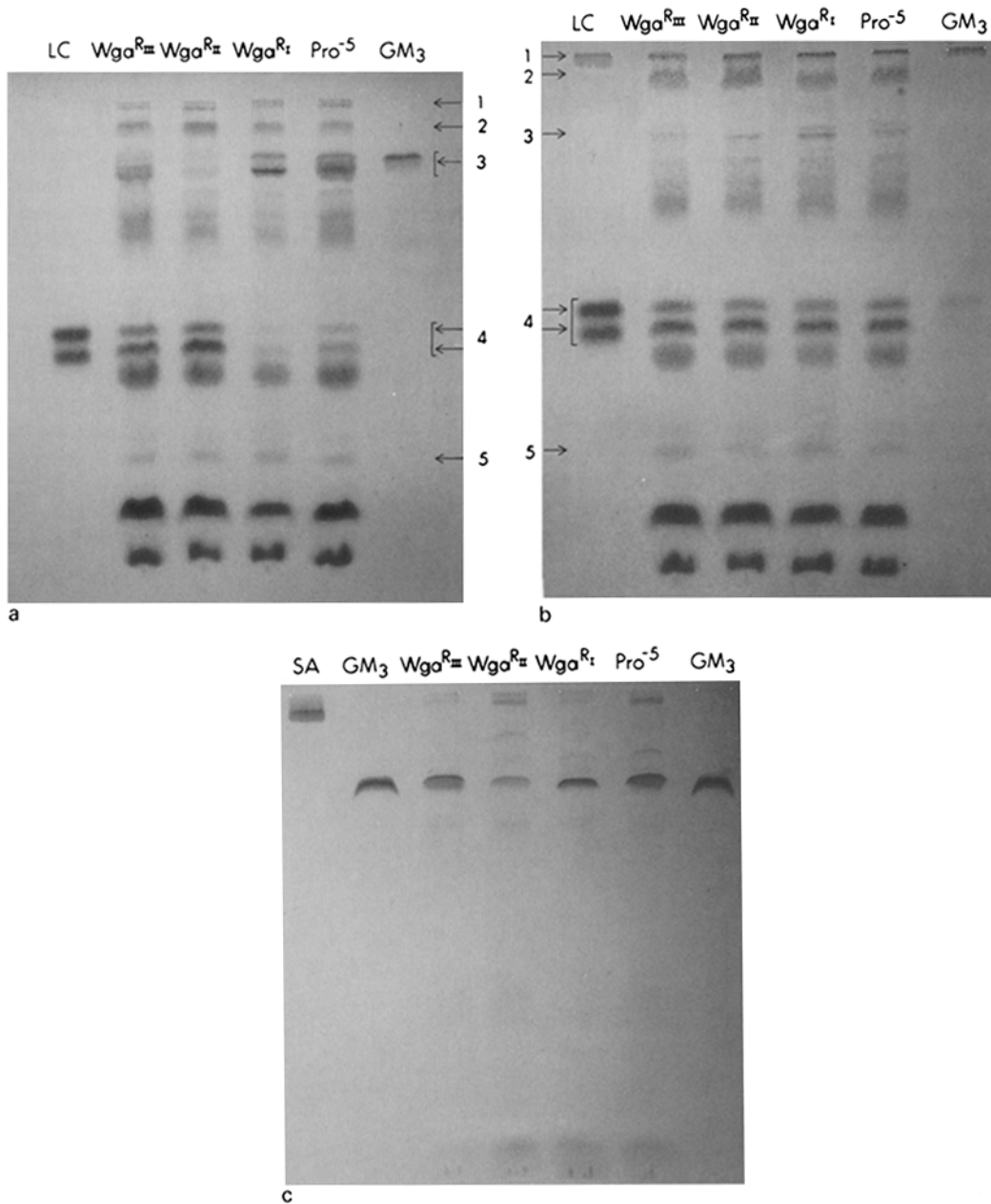


FIGURE 2 (a) Glycolipid composition of parental and Wga^R CHO cells. C:M extracts representing $\sim 3 \times 10^7$ parental and Wga^R CHO cells were analyzed by TLC in solvent A in parallel with the purified glycolipids GM₃ (6 µg) and LC (10 µg). The plate was stained with α -naphthol/sulphuric acid, and the bands which stained blue/purple are marked with arrows and numbered 1–5. All other bands stained yellow, brown, or red. The numbered bands co-migrated in this or in other experiments with marker molecules as follows: region 1, with material remaining at the origin and with free sialic acid; region 2, similarly to free sugars such as glucose and galactose; region 3, with purified GM₃; region 4, with purified LC; region 5, with purified glucosyl ceramide (not shown). Purified GM₂ migrated in the region between bands 2 and 3 (not shown). (b) C:M extracts from $\sim 3 \times 10^7$ parental or Wga^R CHO cells, 6 µg GM₃, and 10 µg LC were dried down under nitrogen and incubated for 19 h with 25 U of *V. cholerae* neuraminidase at 37°C. These samples were dried under nitrogen, redissolved in 15 µl of C:M (1:1), and analyzed by TLC in solvent A. The plate was stained with α -naphthol/sulphuric acid and the bands which stained blue/purple are marked with arrows. (c) C:M extracts from $\sim 3 \times 10^7$ parental and Wga^R cells, 6 µg of GM₃, and 10 µg of sialic acid (SA) were dried under nitrogen, redissolved in 10–15 µl of C:M (1:1), and analyzed by TLC in solvent A. The plate was stained with resorcinol. The faint bands below the bands co-migrating with marker GM₃ did not stain blue, but gray-black. All the bands, from the origin to the bands co-migrating with GM₃, stained blue with the resorcinol reagent.

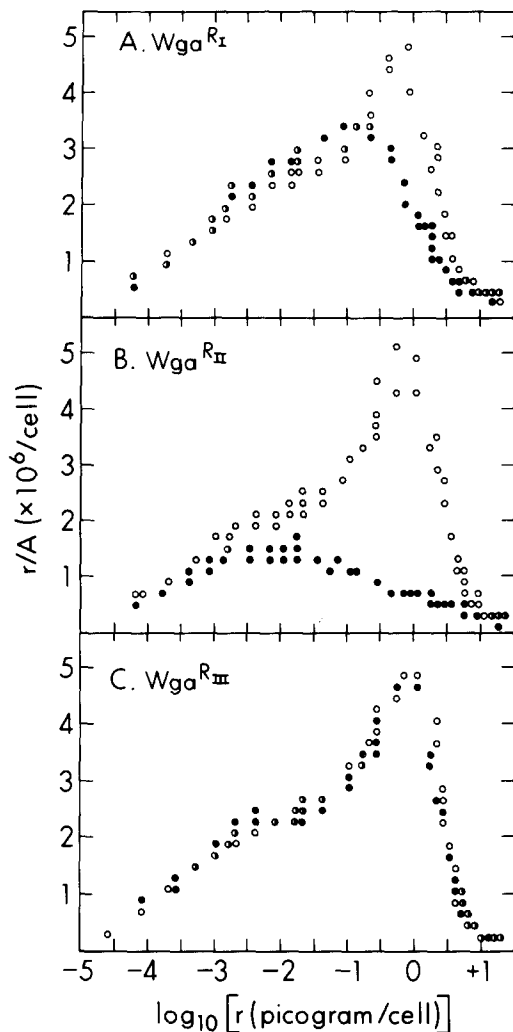


FIGURE 3 WGA-binding abilities of parental (○) and Wga^R (●) CHO cells. WGA binding to parental and Wga^R cells was determined at WGA concentrations from 1 ng/ml to 1 mg/ml and analyzed by the method of Scatchard. These data have previously been presented as a linear plot (22), whereas in this figure r/a is plotted against $\log_{10} r$.

WGA binding despite a sialylation defect similar to that of Wga^{RII} cells. Thus, it must be concluded that the sialic acid residues that are missing from Wga^{RIII} cells are not involved in WGA binding.

Effect of Neuraminidase on the WGA-Binding Abilities of Parental and Wga^R CHO Cells

As previous studies have implicated sialic acid

in the binding of WGA to cell surfaces (1, 4, 8, 17), and as we have clearly demonstrated at least one "class" of sialic acid residues that is not involved in WGA binding, it seemed likely that an examination of the WGA-binding properties of the Wga^R mutants after treatment with neuraminidase might define various classes of sialic acid residues at the cell surface and their involvement in WGA-binding parameters.

A brief treatment of parental CHO cells with neuraminidase (under conditions identical to those described in Fig. 1) resulted in a reduction in the ability of parental CHO cells to bind WGA (Fig. 4). In fact, neuraminidase-treated parental cells

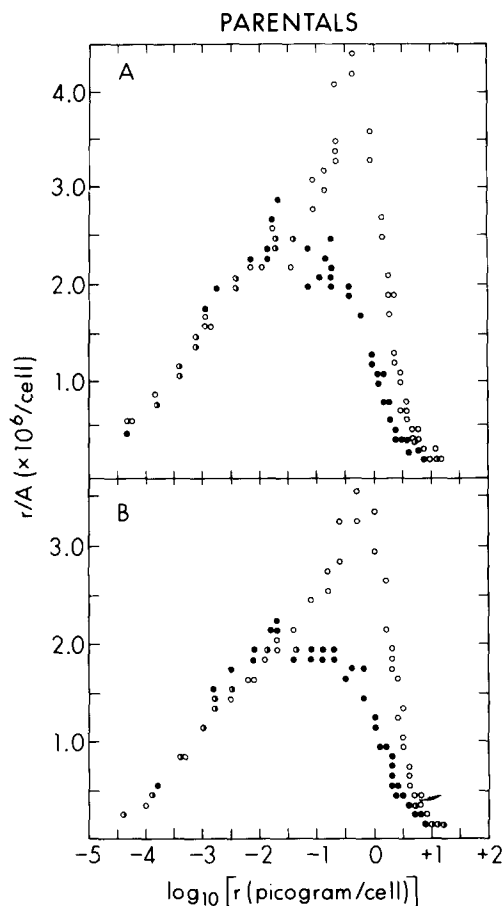


FIGURE 4 Effect of neuraminidase on the binding of WGA to parental CHO cells. Washed Pro^- (A) or Gat^{-2} (B) cells were treated with neuraminidase (50 U/ 10^8 cells; 5 min at 37°C), washed, and their WGA binding abilities were compared with those of parental cells that had been processed identically but in the absence of neuraminidase. Untreated (○) and neuraminidase-treated (●) cells.

resembled Wga^{Ri} mutants in their WGA binding properties. A similar result was obtained when Wga^{Riii} cells were treated with neuraminidase (Fig. 5A). Neuraminidase-treated Wga^{Ri} cells also exhibited reduced WGA binding (Fig. 5B). In this case, the WGA binding curve approaches the type observed with untreated Wga^{Rii} cells. By contrast, the binding properties of Wga^{Rii} cells were not significantly affected by the neuraminidase treat-

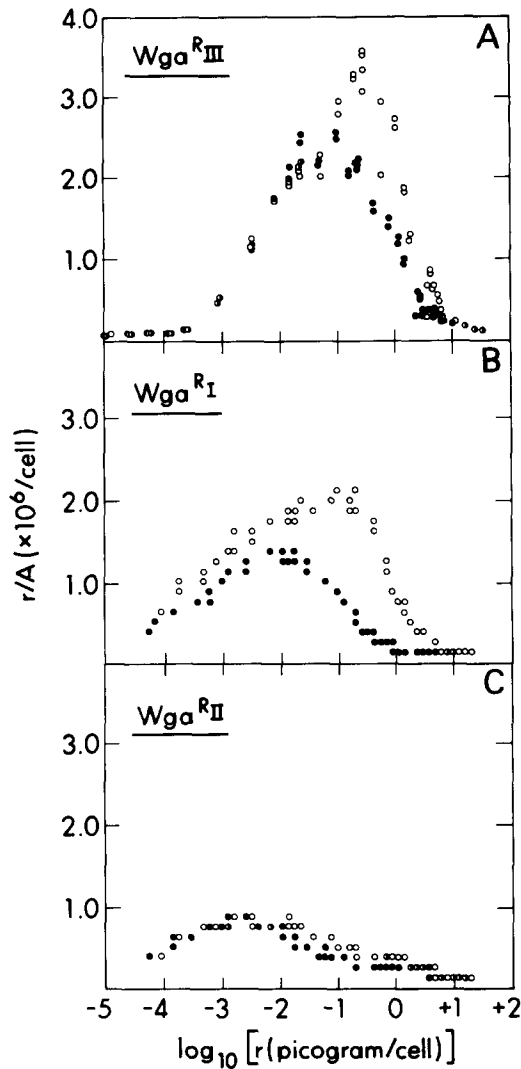


FIGURE 5 Effect of neuraminidase on the binding of WGA to Wga^R CHO cells. Pro⁻⁵ Wga^{Riii} 4B (A), Pro⁻⁵ Wga^{RI} 3C (B), and Pro⁻⁵ Wga^{Rii} 6A, (C) cells were incubated with neuraminidase as described in Fig. 4, and the WGA-binding abilities of untreated (○) and neuraminidase-treated (●) cells were compared. The data were plotted as r/A vs. $\log_{10} r$.

ment (Fig. 5c). This suggests that any sialic acid residues released by neuraminidase from Wga^{Rii} cells are not involved in WGA binding to these cells. In fact, it is shown in Table I that treatment of each of the Wga^R cell lines with neuraminidase results in the release of sialic acid residues. (Although for Wga^{Rii} cells the amount of sialic acid in the supernate was small, it was reproducible and was always correlated with a small (~25%) increase in labeling via galactose oxidase:³H]borohydride consistent with the exposure of surface Gal residues by the action of neuraminidase). Thus, it would appear that a change in WGA binding parameters is not always correlated with the loss of sialic acid residues.

DISCUSSION

Lectin-resistant cells provide a genetic approach to the eventual delineation of the carbohydrate structures that occur at cell surfaces and of the specificity of the interactions of surface carbohydrate with lectins and other molecules that bind to the cell surface. In this paper, we have partially characterized the surface carbohydrate alterations in three Wga^R CHO cell mutants and described some of the parameters involved in the binding of WGA to CHO cells by comparing the WGA-binding abilities of Wga^R mutant and parental cells. The data reveal the complexity of both WGA/cell surface binding parameters and CHO cell surface structures.

A summary of some of the properties of the three Wga^R mutant phenotypes including the principal findings presented in this paper is given in Table II. The phenotype of Wga^{Ri} cells is so far completely accounted for by its deficient GlcNAc-T1 activity. This phenotype appears to be identical with that of CHO-15B described by Gottlieb et al. (11, 12) and Tabas et al. (29). By contrast, the biochemical bases of the surface alterations that have occurred in Wga^{Rii} and Wga^{Riii} cells have not been uncovered. Preliminary glycosyl transfer studies on a variety of desialylated glycoproteins, mucins, and glycopeptides revealed no significant differences between the sialyl transfer abilities of parental CHO cells and the Wga^{Rii} and Wga^{Riii} mutants (P. Stanley, unpublished observations). A Wga^R CHO cell line (clone 1021) which possesses properties very similar to those of Wga^{Rii} cells has been described by Briles et al. (6). This mutant was shown to exhibit a markedly decreased ability to transfer sialic acid to LC in cell-free extracts. However, this GM₃ synthetase deficiency did not

TABLE II
Summary Wga^R CHO Cell Mutant Phenotypes

Cell line	Wga ^{R1}	Wga ^{R2}	Wga ^{R3}	Parental
D ₁₀ WGA, $\mu\text{g/ml}$	>60	25	11	2
Complementation group	I	II	III	
Surface molecules affected by mutation:				
Glycoproteins	+	+	+	
Glycolipids	-	+	+	
Surface sugars newly exposed	Man	Gal(GalNAc)	Gal(GalNAc)	
Defective enzyme activity	GlcNAc-TI	?	?	
Labeling by galactose oxidase	+	+++	+++	+
Labeling after NM	++	++++	++++	++++
WGA binding after NM	"Wga ^{R1} "	Unchanged	"Wga ^{R3} "	"Wga ^{R1} "
Sialic acid released by NM	+	+	+	+

A summary of some of the properties of the three Wga^R CHO cell lines examined here is presented. It incorporates data obtained in this report and previous publications (13, 20, 21, 24). D₁₀ WGA = concentration of WGA giving 10% survival. Also included is a qualitative description of the surface labeling and WGA binding properties of parental and Wga^R CHO cells from Figs. 1 b, 3, and 4, and the sialic acid released by neuraminidase (NM) from Table I.

appear to account for the complex phenotype of clone 1021 cells. Assuming that these mutants possess single mutations, biochemical lesions that might affect the sialylation of both glycoproteins and glycolipids must be sought.

Certain conclusions concerning the role of sialic acid in WGA binding to cell surfaces may be drawn from the WGA binding properties of parental CHO cells and the Wga^R CHO mutants before and after neuraminidase treatment. In general terms, it is apparent that the loss of surface sialic acid residues (by mutation or following the action of neuraminidase) may or may not correlate with changes in WGA binding parameters. More importantly, our results suggest that the different Wga^R mutants lack different groups or classes of sialic acid residues as defined functionally by their involvement with the binding of WGA at the cell surface.

Clearly, the definition of putative classes of surface sialic acid residues and their direct or indirect role in WGA binding will require extensive structural and quantitative analyses. Such studies are currently underway, but they pose numerous problems. Even the determination of total surface sialic acid content is not straightforward, especially when one is dealing with mutants that possess altered carbohydrate at the cell surface. In fact, wide discrepancies exist between the total surface sialic acid contents of CHO cells reported in the literature. Thus, Yogeewaran et al. (30) found 136 μg of sialic acid/20 mg protein ($\sim 10^8$

cells), Gottlieb et al. (12) found 11 $\mu\text{g}/10^8$ cell membranes, and Briles et al. (6), using the same procedures as Gottlieb et al. (12), reported 28.5 μg of sialic acid/ 10^8 cell membranes. Suffice it to say, the present results clearly demonstrate that sialic acid residues at the CHO cell surface are not equivalent in their abilities to interact with WGA. In addition, it is of interest that the WGA-binding sites of highest affinity remain unaltered by the brief neuraminidase treatment.

The question arises as to whether WGA is interacting directly with sialic acid at the CHO cell surface? Recent evidence supports this possibility because sialic acid has been shown to bind specifically to WGA crystals (C. Wright, personal communication), and recent NMR studies have shown that sialic acid in the α configuration exhibits a high affinity for WGA (Kronis and Carver, manuscript in preparation). However, it is equally possible that the removal of sialic acid from carbohydrate moieties results in conformational changes that indirectly affect WGA binding at the cell membrane. It is known that WGA binds in a specific fashion to certain GlcNAc, GalNAc, and sialic acid residues in glycoconjugates (see reference 4), but the specific interactions occurring at the cell surface remain to be defined. The study of mutants possessing specific carbohydrate lesions at the cell surface together with studies of the interaction of WGA with model glycopeptide binding site analogues will greatly facilitate studies in this area.

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