Studies on Cell Adhesion and Recognition I. Extent and Specificity of Cell Adhesion Triggered by Carbohydrate-reactive Proteins (Glycosidases and Lectins) and by Fibronectin

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ABSTRACT The extent and the specificity of the initial cell attachment induced by various proteins coated on plastic surfaces have been studied with the following results: (*a*) Cell adhesion on the surfaces coated with sialidase and β-galactosidase was as strong as on concanavalin A and *Limulus* lectin-coated surfaces and the reactions were strongly inhibited by glycosidase inhibitors or by competitive substrates. The adhesion on sialidase was inhibited by 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid and by polysialoganglioside (GT_{1b}) at low concentration (0.05–0.1 mM). The cell adhesion on β-galactosidase coat was inhibited by 1,4-D-galactonolactone and β-methylgalactoside but not by α-methylgalactoside. Thus, the initiation of cell adhesion on glycosidase surfaces could be mediated through the interactions of the specific binding sites of the enzyme surface with the cell surface substrates under physiological conditions. (*b*) Cell adhesion on various lectins could be blocked by various competing monosaccharides at the concentrations similar to the inhibitory concentrations for binding of lectins from solution to the cells. (*c*) Cell adhesion on fibronectin surfaces as well as on gelatin-coated surfaces was equally inhibited by GT_{1b} at relatively high concentrations (0.25–0.5 mM). Lower concentrations of GT_{1b} (0.05–0.1 mM) inhibited the cell adhesion on surfaces of *Limulus* lectin and sialidase. It is suggested that the cell adhesion mediated by fibronectin is based on yet unknown interactions in contrast to a specific cell adhesion through glycosidases and lectins.

The complex carbohydrates at the cell surface have been implicated to play an essential role in determining the specificity and the reactivity of cell to cell or cell to substratum (e.g., basement membrane) interaction in multicellular system and in tissue. A remarkable change of the carbohydrate structure at the cell surface, associated with oncogenic transformation (24, 65) and differentiation (16, 25, 47), and the presence of lectins at the animal cell membranes (reviewed in references 1 and 59) have supported this concept. However, the biochemical mechanism of cell-cell interaction and adhesion is far from being clear, and the topic has received much discussion in current studies, particularly in relation to the function of fibronectin, which promotes cell adhesion and spreading (23, 27, 68; reviewed in references 11, 22, and 67).

Cell adhesion has been studied on lectins coated on nylon and plastic surfaces (22, 29, 56), on fibronectin and gelatin coated on plastic plates (11, 13, 22, 23, 27, 67, 68), and on galactose-gel particles (66). These assay systems are sensitive and can be used as a good model to study the mechanism of cell-to-cell or cell-to-substratum adhesion. This paper describes the intensity and the specificity of cell adhesion on two classes of carbohydrate-binding proteins (lectins and glycosidases) as compared with the adhesion on fibronectin-coated surfaces.

MATERIALS AND METHODS

Materials

Fibronectin was purified from hamster plasma and from conditioned medium of BALB/c 3T3 cells by gelatin affinity chromatography (13). The isolated

THE JOURNAL OF CELL BIOLOGY · VOLUME 88 JANUARY 1981 127–137
 · 0021-9525/81/01/0127/11 $1.00

proteins were >90% pure, as estimated by polyacrylamide gel electrophoresis. The radioiodinated proteins used for adsorption studies were analyzed by polyacrylamide gel electrophoresis, which revealed radioactive bands comigrating with the nonlabeled proteins without any signs of degradation. Lectins from *Ricinus communis*, peanut, *Dolichos biflorus*, and *Lotus tetragonolobus* were purified by various affinity-chromatography techniques (21). *Limulus* lectin was a gift from Professor Michel Monsigny (University of Orléans, France). Concanavalin A was purchased from Sigma Chemical Co. (St. Louis, Mo.) and wheat-germ lectin from Vector Laboratories (Burlingame, Calif.). β-galactosidase, purified from jack bean meal according to Li and Li (37), was kindly donated by Dr. Michiko Fukuda. Commercially available jack bean meal β-galactosidase (Sigma Chemical Co.) gave similar results, and was used in some experiments. *Clostridium perfringens* sialidase (type IX, affinity purified) was purchased from Sigma Chemical Co. Examination of this sample by use of polyacrylamide gel electrophoresis gave a single band with molecular weight of ~70,000, in agreement with the data of Nees et al. (48). The crystalline trypsin was purchased from Worthington Biochemical Corp. (Freehold, N. J.) and trypsin inhibitor of soybean from Sigma Chemical Co. Asialofetuin was prepared from fetuin (Sigma Chemical Co.; type III) according to the method of Schmid et al. (57). Gelatin (from swine skin, type I; Sigma Chemical Co.) was solubilized by heating at 100°C for 5 min in Salt/Pi[1] before coating on plastic plates. α-Methylgalactoside, β-methylgalactoside, 1,4-D-galactonolactone, α-methylmannoside, and *N*-acetylneuraminic acid were purchased from Sigma Chemical Co. Glycophorin was a gift from Dr. Minoru Fukuda. 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid, the sialidase inhibitor (41), was kindly donated by Professor Roland Schauer (Christian Albrecht University, Kiel, W. Germany). Gangliosides GT_{1b} and GM_1 (shorthand nomenclature according to reference 62) from bovine brain were fractionated according to the method of Momoi et al. (45). The preparations were homogeneous on thin-layer chromatography, and were chemically characterized.

Labeling of Proteins

Fibronectin, concanavalin A (Con A), soybean agglutinin, *Clostridium perfringens* sialidase, fetuin, bovine serum albumin, and glycophorin were iodinated, using the chloramine-T method (15). Briefly, 1 mCi (10 μl of carrier-free iodine in NaOH solution; Amersham Corp., Arlington Heights, Ill.) of [^{125}I]Na was added to protein solution (1 mg/ml in 200 μl of 0.2 M phosphate buffer, pH 7.6) followed by two additions with 2 min intervals of 50 μl of 4 mM chloramine-T in 0.2 M phosphate, pH 7.6. The reaction was stopped by adding 500 μl of sodium bisulfite (0.1 mg/ml in the phosphate buffer). The labeled proteins were dialyzed extensively at 4°C against Salt/Pi/water 1:1 (vol/vol) containing 0.1 mM phenylmethylsulfonyl fluoride and finally against. Salt/Pi. ^{14}C-labeling of Con A and of soybean lectin was carried out using [^{14}C]formaldehyde reductive methylation (54). Wheat-germ agglutinin was tritiated with [^{3}H]acetic anhydride (44).

Biological Activity of Protein after Radiolabeling

All iodinated proteins were analyzed by use of polyacrylamide gel electrophoresis, and were intact on this basis. The iodinated fibronectin and neuraminidase were able to cause a similar cell adhesion as the intact compounds. To determine the activity of iodinated Con A, it was tested by chromatography on Sephadex G-100. About 60% of the radioactivity could bind to Sephadex G-100 and elute specifically with 0.1 M α-methylmannoside.

Adsorption of Adhesion-mediating Proteins on Plastic Surfaces

^{125}I-labeled fibronectin, Con A, soybean agglutinin (SBA), neuraminidase, fetuin, bovine serum albumin (BSA), glycophorin, and ^{3}H-labeled wheat-germ agglutinin (WGA) were incubated at protein concentrations of 3–100 μg/ml in 50 μl of Salt/Pi, pH 7.4, at 37°C for 2 h in flat-bottom polystyrene microtiter wells, devised for high-efficiency protein binding (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.). The multiwell plates were washed three times with 100 μl of Salt/Pi, and the adsorbed protein was solubilized by rinsing the wells three times with 100 μl of 1% SDS in 0.5 N NaOH. The amounts of adsorbed protein were calculated from the recovery of adsorbed radioactivity.

Hydrolysis-catalyzing Activity of Sialidase and β-Galactosidase after Adsorption on Plastic Plates

The enzyme activities after adsorption on plastic plates were tested by incubating [^{3}H]sialyllactitol or *p*-nitrophenyl-β-D-galactoside, according to the method as described in the legends of Tables I and II.

Cells and Cell Culture

BALB/c 3T3 and hamster embryo (NIL) fibroblasts were cultured in Dulbecco's Modified Eagle's Medium supplemented with 5 or 10% fetal calf serum, 10^2 U penicillin G/ml and 0.1 mg streptomycin/ml in an atmosphere of 5% CO_2. Wistar rat liver cells were prepared by collagenase perfusion technique (58), and were cultured using Leibovitz's medium (35; L-15 medium, Grand Island Biological Co., Grand Island, N. Y.).

Binding of Soluble Lectins to the Cells

Confluent cultures of NIL cells were washed three times with Salt/Pi and then digested with trypsin and washed with Salt/Pi containing soybean trypsin inhibitor as described under Adhesion Assays. The washed, trypsinized cells were suspended in Salt/Pi containing 100 μg/ml of BSA (BSA-Salt/Pi) at a cell concentration of 25 × 10^6 cells/ml.

Glass tubes, 100 × 7.5 mm, were soaked in BSA-Salt/Pi for 60 min. Each tube received the radioactive lectin (50 μg) followed by soluble carbohydrate hapten inhibitors (0–50 μmol) and BSA-Salt/Pi to a final volume of 500 μl. Cell suspension, 500 μl, containing 12.5 × 10^6 cells total was added to each tube, mixed, and incubated at room temperature for 30 min with occasional mixing.

The cells were washed three times with 1-ml aliquots of BSA-Salt/Pi by centrifugation at 800 *g* for 10 min. The washed cell pellets were dissolved in 200 μl of 1% SDS containing 0.5 M NaOH, quantitatively transferred to liquid scintillation vials and counted.

TABLE I

Hydrolysis of [^{3}H]Sialyllactitol by Plastic-adsorbed Sialidase

pH	Lactitol released	
	cpm × 10^{-3}	*nmol*
5.0	8.56	1.3
7.4	0.89	0.1

Microtiter well surfaces were coated with sialidase (10 μg/ml in 50 μl Salt/Pi, pH 7.4) for 2 h at room temperature, followed by coating with BSA (100 μg/ml) for 0.5 h. The wells were washed two times with 100 μl of Salt/Pi and once with Salt/HEPES at the pH used for the activity determination. NaB-[^{3}H]$_4$-reduced sialyllactose (6,600 cpm/nmol) at 1 mM concentration in 50 μl Salt/HEPES at pH 7.4 or 5.0 was added to the wells, and the multiwell plate was shaken at 100 rpm at 37°C for 15 min. Formation of lactitol was assayed from increase of radioactivity (as compared to the samples incubated in the buffer only) in the neutral fraction of DEAE-Sephadex chromatography (50). The values given are averages from two determinations.

TABLE II

Hydrolysis of p-Nitrophenyl-β-D-galactoside by Plastic-adsorbed β-Galactosidase

pH	*p*-Nitrophenol released	
	Increase in A_{400}	nmol
	nm	
3.0	0.185	26
7.4	0.002	2

Polystyrene surfaces (3.5-cm-diameter wells) were coated with β-galactosidase (10 μg/ml in 1.0 ml Salt/Pi, pH 7.4) for 2 h at room temperature. The wells were washed two times with Salt/Pi and once with the hydrolysis buffer (Salt/Pi, pH 7.4, and Salt/Pi further buffered with 50 mM Na acetate, pH 3.0). After the washings, 1 mM *p*-nitrophenyl-β-D-galactoside in 1.0 ml of the hydrolysis buffer was added, and the wells were shaken at 80 rpm for 15 min at 37°C. The incubation mixture was transferred to 2.0 ml of 0.2 M Na_2CO_3, and the absorbances at 400 nm were measured. Values from incubations in the buffer only were subtracted from the measured values. The values are averages from two determinations.

[1] ***Abbreviations used in this paper:*** **BSA, bovine serum albumin; Con A, *Canavalia ensiformis* (jack bean) lectin; DOL, *Dolichos biflorus* lectin; LOT, *Lotus tetragonolubus* lectin; PHA, *Phaseolus vulgaris* agglutinin; PNA, peanut agglutinin; RCA, *Ricinus communis* agglutinin; SBA, soybean agglutinin; WGA, wheat-germ agglutinin; Salt/Pi, 137 mM NaCl/2.7 mM KCl/0.7 mM $CaCl_2$/0.5 mM $MgCl_2$/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4; Salt/HEPES, 110 mM NaCl/3.5 mM KCl/1.0 mM $CaCl_2$/1.0 mM $MgSO_4$/0.23 mM Na_2HPO_4/0.29 mM KH_2PO_4/30 mM HEPES. The shorthand nomenclature of Svennerholm (62) is used for gangliosides.**

Adhesion Assays

The adhesion surfaces were prepared by adsorbing different proteins on microtiter wells (see above). Unless otherwise indicated, fibronectin and different lectins were adsorbed at 10 μg/ml. The enzyme concentrations were 0.2 U/ml of *Clostridium perfringens* sialidase and 0.9 U/ml of β-galactosidase (37). In assays specified in the text, the plates were saturated with BSA-Salt/Pi after coating with the adhesion-mediating proteins. Freshly confluent 3T3 or NIL cell cultures were dispersed with 10 μg/ml crystalline trypsin in Ca^{++} and Mg^{++} free Salt/Pi at 37°C for 20 min. The cells were pipetted gently, and an equal volume of soybean trypsin inhibitor solution (40 μg/ml) was added. The cells were centrifuged at 800 *g* for 5 min, and washed two times with the soybean inhibitor solution and two times with Salt/Pi. In assays for the study of the effect of sialic acid–containing components, the buffering activity of Salt/Pi was insufficient to maintain the pH of the adhesion medium. Therefore, the cells were washed with a balanced salt solution buffered with 30 mM HEPES (modified from the Tris-citrate-buffered balanced salt solution of reference 49 by replacing the dicarboxylic acids and Tris-citrate with 30 mM HEPES).

The assays were started by adding 7.0×10^4 cells in 50 μl of Salt/Pi or Salt/HEPES solution to 50 μl of the same buffer in microtiter wells. Each data point is based on two to four determinations. In inhibition studies with sugars, the inhibitor was included in the buffer added to the microtiter wells ~10 min before addition of cells (the concentrations given refer to final concentrations in the adhesion medium). Unless otherwise indicated, the cell incubation was continued for 1 h at 37°C. The adhesion medium was removed, and the nonattached cells were washed off by rinsing the wells three times with 100 μl of Salt/Pi, using a multiwell pipette (Titertek; Finnpipette, Helsinki, Finland). The wells were examined by microscopy for cell attachment and spreading. For quantification of cell attachment, the cells were labeled before the assays either with 1 μCi/ml [^{3}H]thymidine or 2 μCi/ml [^{3}H]proline (New England Nuclear, Boston, Mass.) in complete culture medium for 20 h. Routinely, labeled cells possessed variable specific activities, resulting in variability in the amount of radioactivity bound to the same protein surface in different experiments. However, in any one experiment, the same cell suspension was always used, so direct comparisons of results obtained in any one experiment are reliable and reproducible. Radioactivity from the attched cells was solubilized by rinsing the microtiter wells two times with 100 μl of 1% SDS in 0.5 N NaOH. The solubilized radioactivity was counted in 6 ml of an aqueous counting scintillant (ACS; Amersham Corp.). Adhesion assays with liver cells were carried out in the same way, except that L-15 medium (without serum) was used instead of Salt/Pi, and the reaction time was 2 h.

Analytical Methods

Protein was determined by fluorescamine assay (63). Sialic acid was determined, using the method of Svennerholm (61) as modified by Miettinen and Takki-Luukkainen (43). Linear polyacrylamide gradient (5–14%) slab gels containing 0.1% SDS were prepared following the basic stacking SDS gel procedure of Laemmli (34). Cell samples (75 μg of protein) were dissolved in the sample buffer containing 2% SDS and 5% 2-mercaptoethanol and heated in a boiling water bath for 5 min. Slab gels were stained with Coomassie Blue R-250 (14). Fluorography of slab gels followed the procedure of Bonner and Laskey (4). Protein standards for relative molecular weight estimation in SDS-PAGE were as follows: hamster skeletal muscle myosin, 200,000; bovine serum albumin, 68,000; hamster skeletal muscle actin, 45,000; *Dolichos biflorus* lectin subunit, 27,000. Iodinated proteins were analyzed in a similar way, except that an 8% polyacrylamide gel was used instead of the gradient gel.

RESULTS

Basis of the Assay System

The assay system as described in Materials and Methods requires full information on a few important factors, namely (*a*) the amount of protein adsorbed on the plastic plates, (*b*) the activity of the plastic-adsorbed proteins, (*c*) the stability of the adsorbed protein layer on the plastic plate, (*d*) the reactivity of cell surface glycoproteins with various lectins, and (*e*) the effect of trypsinization on surface glycoproteins of cells used in the attachment assay. This basic information related to the assay system has been studied, and the results are reported in this section and in Tables I–IV, and Figs. 1 and 2.

ADSORPTION OF PROTEIN ON PLASTIC SURFACE: The adsorption of ^{125}I-labeled proteins on plastic surfaces at different concentrations during 2-h incubations is shown in Fig. 1.

TABLE III

Inhibition of [^{125}I]Fibronectin Binding and of Fibronectin-mediated Cell Attachment by Precoating of the Adhesion Surfaces with Fetuin and Asialofetuin

Precoating	Fibronectin bound*	Cells bound‡
	% control	
Control (Salt/Pi)	100	100
Fetuin	15	38
Asialofetuin	23	29

Tables III and IV show experiments demonstrating the stability of protein coats on plastic surfaces. For Table III, precoating of the adhesion surfaces was carried out at 50 μg/ml of different proteins for 2 h as described in Materials and Methods. Controls were incubated in buffer. After washing of the wells, fibronectin was adsorbed at 10 μg/ml for 2 h, and the surfaces were studied for adsorption of iodinated fibronectin and for cell attachment activity. In the controls, 5,945 cpm [^{125}I]fibronectin and 536 cpm [^{3}H]-thymidine-labeled 3T3 cells were bound to the surfaces.

* Averages of three determinations.

‡ Averages of two determinations.

TABLE IV

Percent of Adsorbed Protein That Can Be Eluted under Various Conditions **

Adhesion surface	Incubation solution: Salt/Pi	BSA-Salt/Pi (100 μg BSA/ml)	NIL cells in Salt/Pi‡
Con A	0.4	1.1	2.1
SBA	0.5	2.1	5.7

* [^{125}I]-labeled proteins were adsorbed on petri dishes (35 × 10 cm, #1008 Falcon plastic; Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) in 1 ml Salt/Pi buffer at a concentration of 20 μg protein/ml for 3 h at room temperature and washed with Salt/Pi. 1 ml of the indicated incubation solutions were added, mixed, and incubated 60 min at room temperature. The plates were mixed and aliquots of the supernate removed and counted. The number of counts detected in the supernate were divided by the number of counts present on untreated plates, in determining the percentage of radioactivity eluted by each solution. Radioactivity on plates was determined by solubilization with 1 ml of 1% SDS in 0.5 M NaOH.

‡ NIL cells were suspended by trypsinization as described in Materials and Methods and diluted with Salt/Pi to a concentration of 2.3×10^6 cells/ml.

The microtiter plate adsorbed various proteins effectively. At the lowest concentrations (3–6 μg/ml), 50–70% of the added protein (except glycophorin) was adsorbed on plastic plate. The amount of protein adsorbed on the plastic plate as a function of the concentration of protein in solution rises steeply up to the concentration 10–20 μg/ml, at which concentration the surfaces are almost saturated, but adsorption of fibronectin increases somewhat more after this concentration. The adsorption curves for different proteins were similar, except that for glycophorin (Fig. 1).

ACTIVITY OF THE PLASTIC-ADSORBED ENZYMES: As shown in Table I, the plastic-adsorbed sialidase was able to hydrolyze sialyllactitol into sialic acid and lactitol. At the protein concentration (10 μg/ml) used to coat the surfaces, 8% of the applied enzyme activity was found adsorbed to the plastic surface when the activity was measured in Salt/HEPES, pH 5.0 (see also legends, Tables I and II). On the basis of adsorption studies using ^{125}I-labeled enzyme (Fig. 1), 18% of protein was adsorbed to the wells at the protein concentration of 10 μg/ml. Therefore, the apparent specific activity of the plastic-adsorbed sialidase was 44% of that measured in solution in the same salt and pH conditions. However, quantitative calculations based on measurements of immobilized enzyme activities must be taken with caution, because the values given

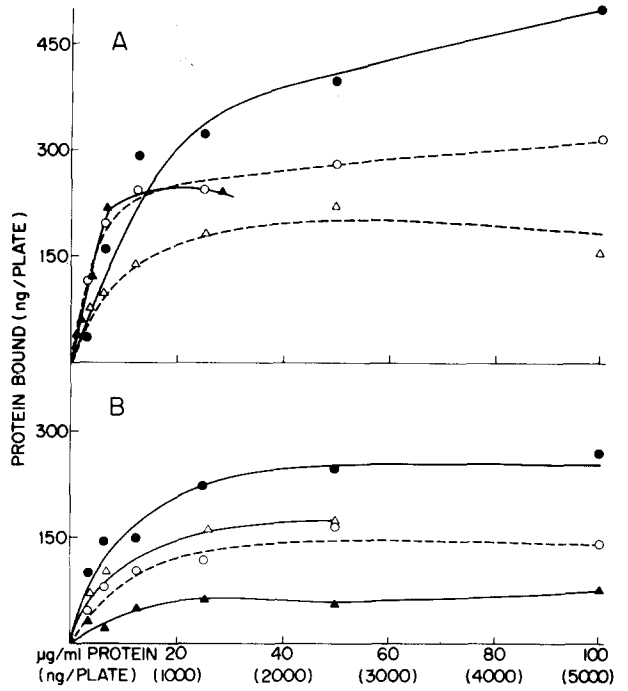

FIGURE 1 Adsorption of proteins on plastic surfaces as the function of added amount of protein (in 50 μl Salt/Pi). (*A*) ●, fibronectin; ○, Con A; ▲, WGA; △, SBA. (*B*) ●, fetuin; ○, neuraminidase; ▲, glycophorin; △, bovine serum albumin.

by such determinations are a function of various physical parameters of the assay (40). Qualitatively the plastic-adsorbed sialidase is highly active, and also seems to retain its pH specificity, because little hydrolysis could be detected in Salt/HEPES, pH 7.4 (Table I).

Measurements of plastic-adsorbed activity of β-galactosidase gave comparable results to those observed in neuraminidase determinations. The adsorbed activity could be easily detected, using *p*-nitrophenyl-β-D-galactoside (see Table II). At the protein concentration 10 μg/ml (1.0 ml volume used to coat 3.5-cm-diameter dishes), 16% of the applied activity was detected as adsorbed to the surface. The adsorbed activity could be demonstrated at pH 3.0 and 4.0 but not at pH 7.4 (Table II).

THE STABILITY OF THE PROTEIN COAT ON PLASTIC PLATE: The adsorbed protein coat on plastic plate was found to be very stable; no labeled protein adsorbed on plastic can be released by repeated washing with Salt/Pi. For example, ^{125}I-labeled fibronectin with 4,400 cpm activity adsorbed after the routine washing procedure was unchanged after a 30-min incubation followed by washing three times with Salt/Pi.

Although the protein coat adsorbed on plastic surface was found to be stable to washing procedures with Salt/Pi, the protein coat may become unstable on addition of a second protein or cells. This possibility was tested by addition of the second protein: the second protein added could not be coated if the first protein coat on plastic surface were stable. In fact, the adsorption of radiolabeled fibronectin on the plastic surface was greatly reduced, if the plastic well was preincubated with fetuin or asialofetuin (Table III). Fibronectin-induced cell attachment was also greatly reduced when the plastic plate was preincubated with fetuin or asialofetuin (see Table III). No release of iodine-labeled fibronectin (adsorbed at 10 μg/ml) could be observed by incubating the well with BSA (0.5 mg/ml) or GT_{1b} (0.5 mM) ganglioside solutions for 0.5 h. To further test the stability of the protein coat upon incubation with cells, plastic surfaces coated with radiolabeled Con A and SBA were incubated with NIL cells and the released activity was examined. The majority of the activity was not released upon incubation with Salt/Pi, BSA-Salt/Pi, and with cells (Table IV). These findings suggest that protein film coated on the plastic surface was fairly stable and was hardly released by cells or exchanged with any second protein or glycolipid subsequently incubated. These are important basic findings for cell attachment assays on protein-coated surfaces, which were not extensively investigated previously.

PROPERTIES OF SURFACE GLYCOPROTEINS OF CELLS USED FOR ASSAY: Properties of cell surface glycoproteins and their reactivities with various lectins have been studied for NIL cells. Five major cell surface–labeled glycoproteins termed galactoprotein a (Gap a, LETS, or fibronectin) (6–9, 17, 18, 20, 30, 33), GP170 (37), Gap b, Gap bT, and GP100 (6–8), have been labeled by galactose oxidase-NaB[^{3}H]$_4$ followed by polyacrylamide gel electrophoresis and fluorography (6–9, 17, 18,

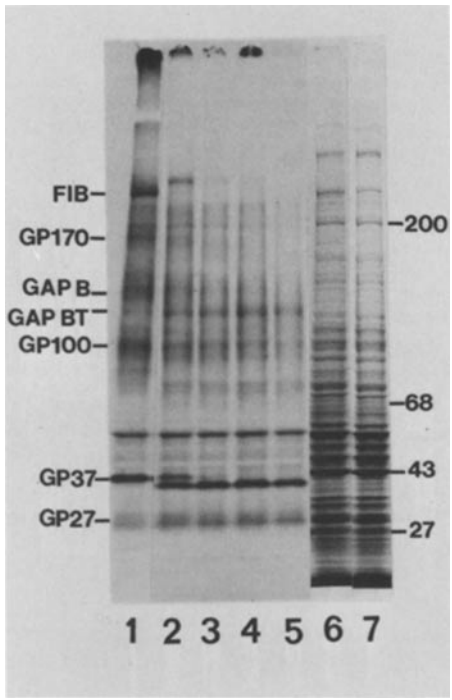

FIGURE 2 Trypsin digestion of surface glycoproteins of NIL cells. Confluent cultures of NIL cells were subjected to cell surface labeling, using the periodate-NaB[^{3}H]$_4$ method (19) for labeling of sialic acid residues. The cells were periodate oxidized on the culture plates, washed, and scraped with a rubber policeman. The detached cells were then reduced with NaB[^{3}H]$_4$ and washed. The labeled cells were suspended in calcium- and magnesium-free Salt/Pi buffer, pH 7.4, and digested with 10 μg/ml trypsin (Worthington Biochemical Corp., 2X crystallized) at 37°C. After different time periods (0–40 min), an aliquot of cell suspension was removed and mixed with 4 vol of Salt/Pi buffer containing soybean trypsin inhibitor (40 μg/ml). The cell pellets were washed by centrifugation and then dissolved in 1% SDS containing 1 mM phenylmethylsulfonyl fluoride. The trypsin-digested cells were analyzed on polyacrylamide gel electrophoresis in the presence of SDS under reducing conditions by protein staining and fluorography (see Materials and Methods for details). Direction of protein migration is from top to bottom. Gels *1–5* are fluorographs of cells digested for the following times: *1*, 0 min; *2*, 5 min; *3*, 10 min; *4*, 20 min; and *5*, 40 min. Gels *6* and *7* are protein stains of cells digested for 0 min (*6*) and 40 min (*7*).

20). Labeling of cell surface sialyl residues with the periodate-$NaB[^3H]_4$ method (19) detects the same five glycoproteins as well as two additional highly sialylated glycoproteins termed GP37 and GP27. Fibronectin and Gap b were previously reported to react with SBA, WGA, Con A, *Ricinus communis* agglutinin (RCA), and *Phaseolus vulgaris* agglutinin (PHA) but not peanut agglutinin (PNA), *Lotus tetragonolobus* lectin (LOT), or *Dolichos biflorus* lectin (DOL) on immobilized lectin columns and/or by double diffusion against lectins (6, 7, 9). Purified Gap bT has similar reactivities with these lectins by double diffusion in agarose.[2] GP170 and GP100 were both reactive to RCA (7, 8). GP37 reacts strongly with WGA but not RCA; however, after desialylation, GP37 is a major cell surface receptor for PNA.[2] Isolation and properties of these lower molecular weight glycoproteins will be described elsewhere.[2]

CELL SURFACE MODIFICATION BY TRYPSINIZATION: Trypsinized cells (see Adhesion Assay, under Materials and Methods) were used in the adhesion assay, rather than EDTA-liberated cells, for the following reasons: (*a*) homogeneous, well-separated cell suspension can only be obtained by trypsinization, (*b*) EDTA treatment followed by mechanical agitation may be even harsher than trypsinization in terms of membrane disruption, (*c*) EDTA-liberated cells attach on various protein coats to the same degree as trypsinized cells, and (*d*) trypsinization eliminates a complex factor attributable to the presence of fibronectin at the cell surface, because trypsinization deletes fibronectin completely, but modification of other glycoproteins is slight (see below). A time-course study on the effect of trypsin treatment on cell surface glycoproteins is shown in Fig. 2. Labeled fibronectin was rapidly removed from the cell surface, whereas GP170, Gap b, and GP100 showed a partial removal during the 20-min digestion period. GP37 decreased slightly in molecular weight, but the intensity of sialic acid label was unchanged after trypsinization. Other glycoproteins, Gap bT and GP27, were not affected by trypsinization. In general, trypsinization alters the migration of various cell surface glycoproteins; however, the majority of lectin receptors are not released from the cell surface by trypsinization.

Extent of Cell Adhesion on the Plastic Matrix Surfaces Coated with Fibronectin and Various Lectins and Glycosidases

Adhesion of NIL, 3T3, and rat hepatocytes on the surfaces coated with fibronectin, glycosidases, lectins, and various other proteins is compared in Table V. Extensive adhesion of all these cells was induced by surfaces coated with fibronectin, Con A, and sialidase prepared from 2–10 μg/ml of these protein solutions (see Figs. 3 and 4). However, despite the similarities in extent of cell attachment on various surfaces, there was a clear difference in the kinetics of cell attachment on lectins and other proteins (see reference 10 [accompanying paper II]). Cell spreading proceeded rapidly on fibronectin and lectin surfaces and at a somewhat slower rate on glycosidase surfaces, whereas cells adsorbed on BSA, polystyrene, or tissue culture plate surfaces remained completely round for >1 h. Cell spreading on various adhesion surfaces will be more extensively discussed in the following paper (10). The number of cells attached on the coated surfaces of glycophorin (prepared from 50 μg/ml), BSA (50 μg/ml), fetuin, asialofetuin, and a plain polystyrene

[2] Carter, W. G., and S. Hakomori. Unpublished data.

TABLE V

Adhesion-promoting Activity of Different Proteins Adsorbed on Plastic Surfaces

Adhesion surface	Cell type: 3T3	NIL	Hepatocyte
Enzymes			
Neuraminidase	+++	++*	++
β-Galactosidase	+++	++	ND
Galactose oxidase‡	ND	+++	ND
Lectins			
Con A	+++§	+++§	+++
Succinyl Con A	+++	+++	ND
Monovalent Con A	+++	+++	ND
WGA	+++	+++*	ND
Limulus	+++	++*	ND
SBA	+++	+++	ND
LOT	ND	+++	ND
RCA	ND	+++	ND
PNA	ND	++	ND
DOL	ND	+	ND
Other proteins			
Fibronectin	+++	+++	++
Gelatin	++	++	ND
BSA	–	–	–
Fetuin	–	–	–
Asialofetuin	–	–	+
Glycophorin	–	–	ND
Ovalbumin	–	++‖	–
Plastic surface without proteins	–	–	–

The number of cells attached and saturated on one well is ~4×10^4 during 1 h. Proteins stimulating a cell attachment, which is 75–100% of plate saturation, are designated +++; 50–75% plate saturation is designated ++; and 25–50% plate saturation is designated +. Values below 25% are designated –. The estimations apply to the protein concentration (50 μg/ml) used to coat the surfaces. The estimations for fibroblasts are based on the recovery of radioactivity from attached cells, which was in agreement with the microscope finding. The estimations for liver cells are based on evaluation by microscope. *ND*, no determination.

* The adhesion is stimulated by additional coating with BSA.

‡ Further studies on cell adhesion to galactose oxidase-coated surfaces are presented in the accompanying paper (10).

§ The numbers of cells attached are higher for Con A (95–100% saturation) than for other proteins designated +++ (75–90% saturation).

‖ The mechanism of the cell attachment on ovalbumin-coated surfaces will be described in the third paper of this series (53).

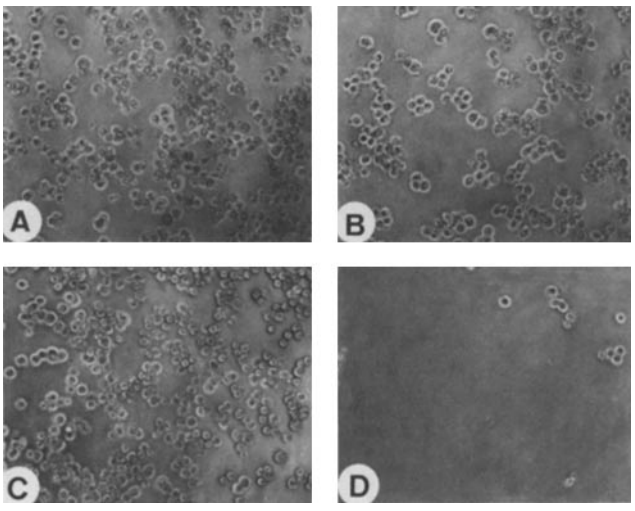

FIGURE 3 Adhesion of liver cells on different protein-coated surfaces. *A*, fibronectin (10 μg/ml); *B*, neuraminidase (4 μg/ml); *C*, Con A (2 μg/ml); *D*, plain plastic incubated with Salt/Pi.

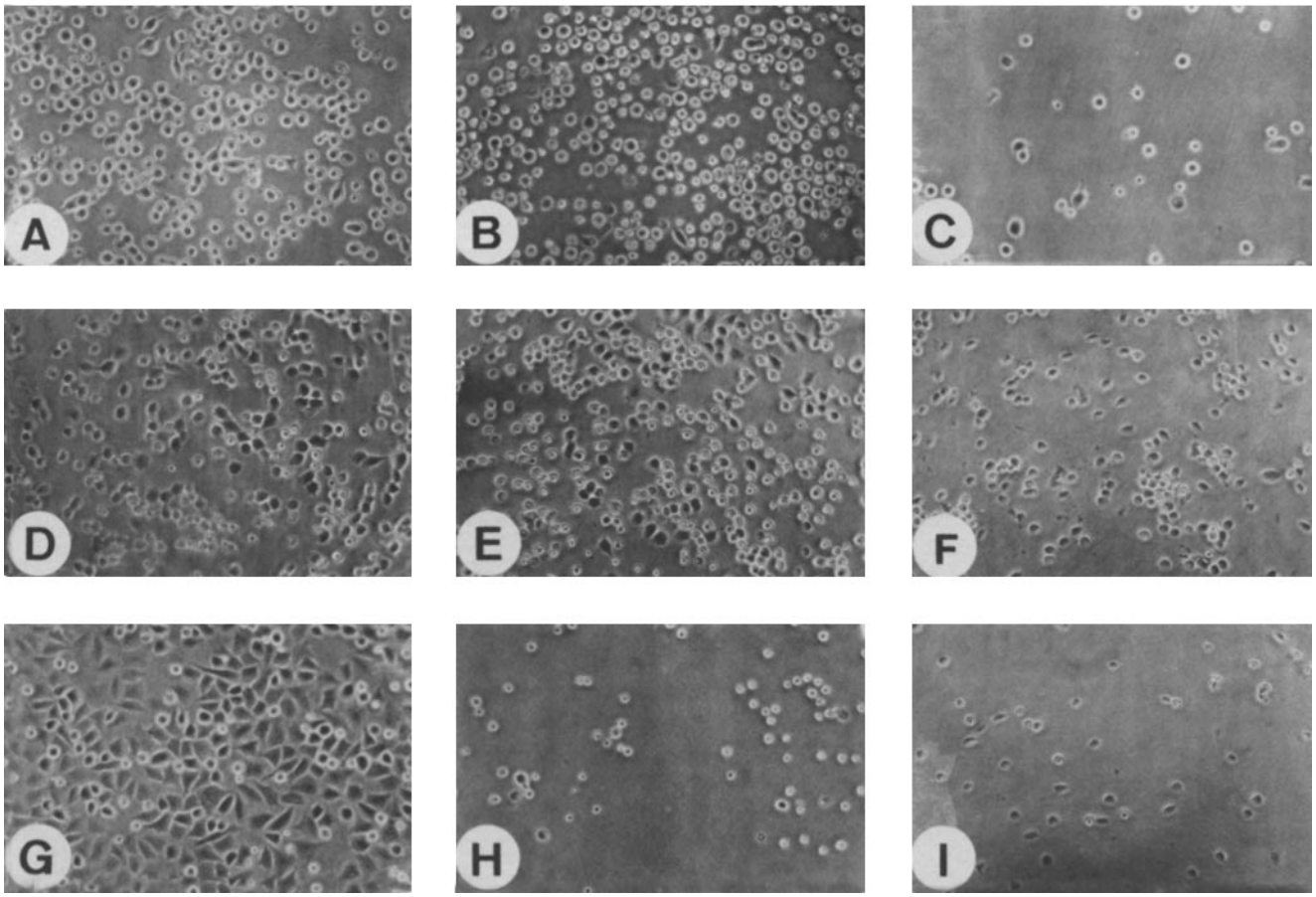

FIGURE 4 Adhesion of fibroblasts on glycosidase-coated surfaces. Top three, 3T3 cells on β-galactosidase-coated surfaces. *A*, control cells without inhibitor; *B*, cells in the presence of 100 mM α-methylgalactoside; *C*, cells in the presence of 100 mM β-methylgalactoside (for more details on sugar inhibitions, see Fig. 8). Middle three, NIL cells on surfaces coated with sialidase. *D*, control cells without inhibitor; *E*, cells in the presence of 10 mM *N*-acetylneuraminic acid; *F*, cells in the presence of 5 mM 2-deoxy-2,3-dehydro- *N*-acetylneuraminic acid (for more details, see Fig. 7). Bottom three, control surfaces; *G*, NIL cells on fibronectin-coated surfaces; *H*, NIL cells on BSA-coated surfaces; *I*, NIL cells on a plastic surface incubated with Salt/Pi.

surface were clearly much lower as compared with the numbers of cells attached on the surfaces of fibronectin, various lectins, and glycosidases (Figs. 3, 4, and 5; Tables III–V). Only the viable, active cells display these adhesive reactions irrespective of the kind of substratum and the specificity of the reactions involved (see the following paper [10]).

The adhesion reaction cannot be explained only in terms of specific ligand-receptor interactions. Wheat-germ lectin was adsorbed on plastic surfaces as effectively as Con A and fibronectin (Fig. 1), and the trypsin treatment used for preparation of cells for the adhesion assay did not cause extensive removal of cell surface glycoproteins reactive to wheat-germ lectin (Fig. 2). Nevertheless, a low adhesion reaction was observed on wheat-germ-lectin-coated surfaces. However, the adhesion became as strong as on Con A surfaces (Fig. 5) when the wheat-germ-coated plastic surface was incubated with BSA; in other words, successive coating with BSA greatly enhances wheat-germ-lectin-mediated cell attachment. A similar effect was observed on sialidase and *Limulus* lectin–coated surfaces (Table VI). It is noteworthy that a strong enhancement of cell attachment by coating with BSA was only observed with the sialic acid–binding proteins, such as WGA (3). A successive coating with BSA on Con A and fibronectin surface did not enhance cell adhesion (Table VI). On the other hand, it should be noted that the adjuvant effect of BSA on the sialidase surface is different between NIL and 3T3 cells. The sialidase-dependent adhesion proceeded well for 3T3 cells without addition of BSA, whereas the attachment of NIL cells on sialidase surface was greatly promoted by additional coating with BSA.

Specificity of Cell Adhesion on Glycosidase and Lectin Surfaces

INHIBITION OF LIMULUS LECTIN- AND SIALIDASE-MEDIATED CELL ADHESION BY POLYSIALOGANGLIOSIDE: Cell attachment on *Limulus* lectin surface was inhibited by GT_{1b} ganglioside and to a lower degree by GM_1 ganglioside (Fig. 6*A*). Cell attachment and spreading on *Clostridium* sialidase surfaces was strongly affected by GT_{1b} ganglioside but not by GM_1 ganglioside (Fig. 6*B*). The effective dose of GT_{1b} for 50% cell attachment inhibition was 0.05–0.1 mM. These results suggest that the catalytic site of sialidase may be involved in this adhesion reaction. The inefficient inhibition observed for the GM_1 ganglioside is in accordance with the low reactivity of this glycolipid with *Clostridium perfringens* sialidase (51), whereas GT_{1b} ganglioside was a strong inhibitor, at somewhat lower concentrations than for *Limulus* lectin (Fig. 6).

INHIBITION OF SIALIDASE-MEDIATED CELL ATTACHMENT BY 2-DEOXY-2,3-DEHYDRO-N-ACETYLNEURAMINIC ACID: The specific sialidase inhibitor 2-deoxy-2,3-dehydro-

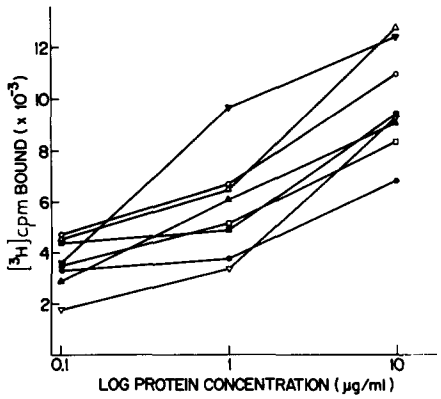

FIGURE 5 The effect of protein concentration on attachment of [^{3}H]proline-labeled NIL cells to various lectin and fibronectin surfaces. Fibronectin and lectin solutions were prepared at 0.1, 1.0, and 10 μg/ml concentrations. The proteins were adsorbed on microtiter wells, at the concentrations indicated, in 75 μl of Salt/Pi as described in Materials and Methods. The wells were analyzed for adhesion-promoting activity at room temperature. △, WGA; ▼, Con A; ○, SBA; ■, LOT; ▽, fibronectin; ▲, RCA; □, PNA; ●, DOL.

TABLE VI

Effect of BSA on Cell Adhesion to Fibronectin-, Lectin-, and Sialidase-coated Surfaces

First coating	Second coating	Cells attached	% Salt/Pi Control
		cpm	
None	Salt/Pi	797	100
None	BSA	754	95
BSA	Salt/Pi	1,079	100
BSA	BSA	633	59
FN	Salt/Pi	3,970	100
FN	BSA	3,776	95
Con A	Salt/Pi	5,570	100
Con A	BSA	5,093	91
WGA	Salt/Pi	1,811	100
WGA	BSA	4,792	265
Sialidase	Salt/Pi	2,789	100
Sialidase	BSA	4,725	169
Limulus	Salt/Pi	1,272	100
Limulus	BSA	4,139	325

Microtiter wells were coated with Salt/Pi or with BSA (100 μg/ml, 1 h) in Salt/Pi after coating with different adhesion-mediating proteins (10 μg/ml) and tested for cell attachment activity, using [^{3}H]proline-labeled NIL cells. The values given are averages from two determinations. *FN*, fibronectin.

N-acetylneuraminic acid (41) inhibited sialidase-mediated attachment and spreading at reasonably low doses, which suggested a specific interaction of the cell surface sialosyl residues with the sialidase substratum (Figs. 4 and 7). In contrast, fibronectin-mediated attachment and spreading was not affected by the sialidase inhibitor. *N*-acetylneuraminic acid did not inhibit sialidase-mediated attachment or spreading (Fig. 4). At 10 mM sialic acid concentration, the number of cells attached was 102% relative to controls (as the mean of four determinations).

INHIBITION OF GALACTOSIDASE-MEDIATED CELL ATTACHMENT BY 1,4-D-GALACTONOLACTONE AND BY α- AND β-METHYLGALACTOSIDES: Inhibition of β-galactosidase-mediated cell adhesion by 1,4-D-galactonolactone is shown in Fig. 8. The inhibitory effect of the lactone in a 1-h experiment at 37°C could be observed from decreased cell spreading and from the lower numbers of cells attached at 5–25 mM sugar concentrations. 1,4-D-Galactonolactone did not inhibit adhesion on sialidase surfaces when tested up to 50 mM concentration (Fig. 8). Cell adhesion on β-galactosidase-coated surfaces was inhibited in a 1-h experiment at 37°C by β-methylgalactoside at 30–100 mM concentrations of the sugar, whereas α-methylgalactoside had no inhibitory effect with various doses (Fig. 4*A–C* and 9*A*). In contrast, cell adhesion on SBA surfaces was inhibited to the same degree by α- and

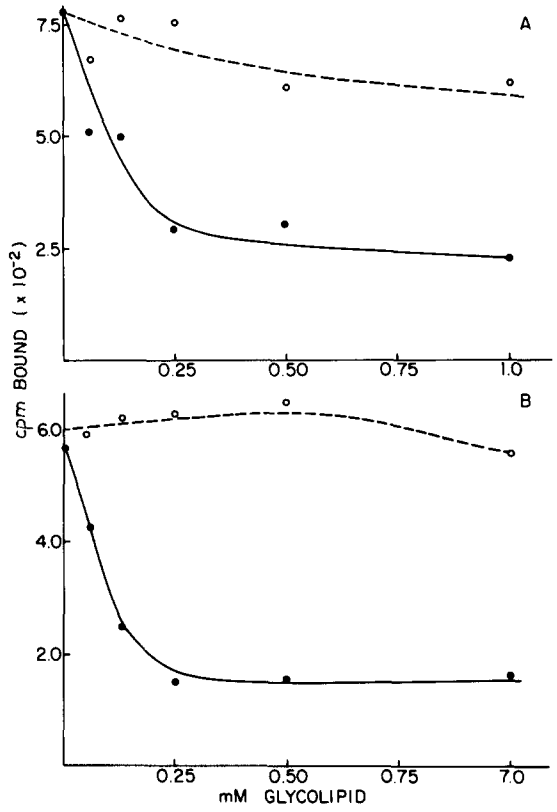

FIGURE 6 Inhibition of 3T3 cell attachment on *Limulus* lectin (*A*) and on *Clostridium perfringens* sialidase (*B*) surfaces by GM_1 ganglioside (○) and by GT_{1b} ganglioside (●). The cells were labeled with [^{3}H]thymidine.

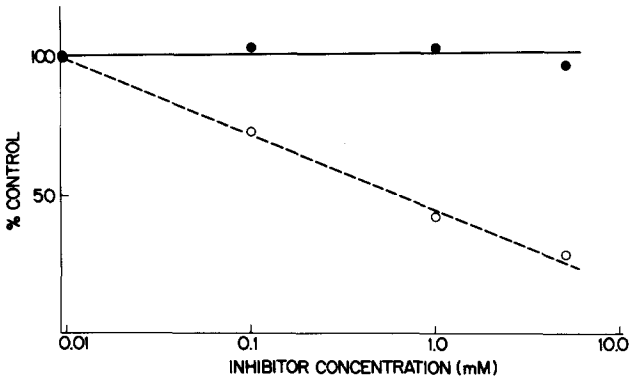

FIGURE 7 Effect of 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid on the adhesion of [^{3}H]proline-labeled NIL cells. Surfaces coated with fibronectin (10 μg/ml) for 2.5 h at room temperature (●). Surfaces coated with sialidase (10 μg/ml) for 2 h at room temperature followed by 0.5 h of BSA coating (100 μg/ml) at room temperature (○). The inhibitor was added on microtiter wells at twice the final concentration in 50 μl of Salt/HEPES, pH 7.4. After 10 min, the cells were added in 50 μl Salt/HEPES, pH 7.4, and the adhesion assays were carried out according to the routine procedure.

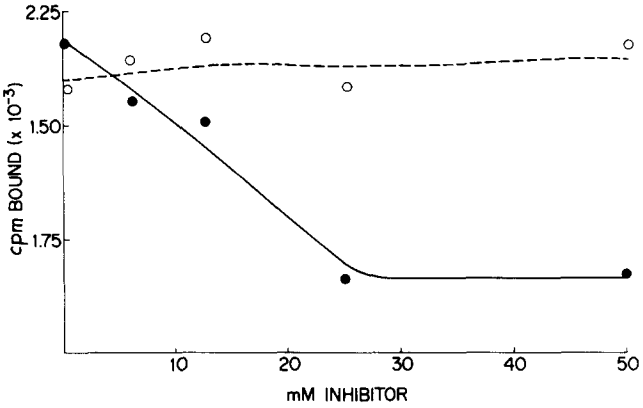

FIGURE 8 Effect of 1,4-D-galactonolactone on cell adhesion on β-galactosidase (●) and sialidase (○) surfaces. Sialidase and β-galactosidase (both at 50 μg/ml) were coated on plastic for 2 h at room temperature, followed by 1 h of coating with BSA (100 μg/ml). Freshly prepared lactone solution at twice the final concentration was added on microtiter wells in 50 μl Salt/Pi, pH 7.4. After 10 min, the cells were added in 50 μl Salt/Pi, and the assays were carried out according to the routine procedure. [^{3}H]proline-labeled 3T3 cells were used in the assay.

β-methylgalactosides (data not shown). Any of the simple sugars tested (α-methylgalactoside, β-methylgalactoside, α-methylmannoside, *N*-acetylgalactosamine) did not inhibit fibronectin-mediated adhesion (Fig. 9*B*). Some stimulatory effect was constantly observed at 25–50 mM sugar concentrations.

INHIBITION OF FIBRONECTIN-MEDIATED CELL ATTACHMENT BY POLYSIALOGANGLIOSIDES: In response to a recent finding by Kleinman et al. (32) that polysialogangliosides inhibit the cell attachment on fibronectin-coated surfaces, a study of ganglioside inhibition on *Limulus* and sialidase coat was expanded to include fibronectin surfaces. As shown in Fig. 10*A*, a higher concentration of GT_{1b} ganglioside was necessary to inhibit fibronectin-mediated cell attachment (0.25–0.5 mM for 50% inhibition) as compared to the GT_{1b} ganglioside concentration necessary for inhibition of cell attachment on *Limulus* and sialidase coat (0.05–0.01 mM for 50% inhibition). With this concentration of GT ganglioside, cell attachment on a gelatin-coated surface was also inhibited in the same way as cell attachment on fibronectin. Soybean lectin–mediated cell attachment was inhibited at higher ganglioside concentrations (Fig. 10*B*). Inhibition of soybean lectin–mediated adhesion by polysialoganglioside cannot be explained by contamination with GM_1 ganglioside (having a terminal galactose residue), because GT_{1b} ganglioside was an even better inhibitor for soybean than was the purified GM_1 ganglioside. Therefore, ganglioside inhibition of fibronectin-mediated cell adhesion cannot be explained only in terms of specific ligand-receptor interactions.

INHIBITION OF LECTIN-MEDIATED CELL ATTACHMENT BY MONOSACCHARIDES: Inhibition of cell attachment on SBA and Con A surfaces by different concentrations of competing sugar haptens is shown in Fig. 11. Binding of lectins from solution to the cells and cell attachment on lectin-coated surfaces are inhibited by similar sugar concentrations. Inhibition of lectin-cell interaction is more complete in the case of SBA than in the case of Con A. This difference suggests a Con A–membrane interaction that is independent of the sugar-binding site of Con A. This type of Con A effect has also been suggested previously (38). 50 mM xylose or galactose had no effect on Con A–mediated adhesion. Inhibition of lectin-mediated cell attachment as a function of time is shown in Fig. 12. Cell attachment on SBA surfaces can be inhibited to a rather constant degree up to at least 90 min at 25°C, whereas Con A–mediated adhesion is strongly inhibited only during the first 10–20 min of the adhesion assay (Fig. 12). Kinetics of cell adhesion on different surfaces and the reversibility of the adhesion reaction will be discussed in the following paper (10).

DISCUSSION

Knowledge of molecular mechanisms of adhesive reactions on cell-to-cell and on cell-to-substratum contact is of fundamental importance for understanding various biological phenomena involved in the multicellular system and in tissues. Various hypotheses have been presented to explain adhesive phenomena. In general, two types of interactions have been discussed: one is attributable to nonspecific interactions between similar groupings, and the other is attributable to ligand-receptor-type interactions, such as lectin-carbohydrate interactions (for reviews, see references 1, 2, 12, 39, 42, 46, 60). The role for cell surface glycosyltransferases as adhesion-mediating activities has been suggested by Roseman (55). Fibronectin, previously called galactoprotein a (20) or LETS (30), has been shown to promote cell attachment and spreading when coated on substratum (11, 22, 23, 27, 29, 67, 68).

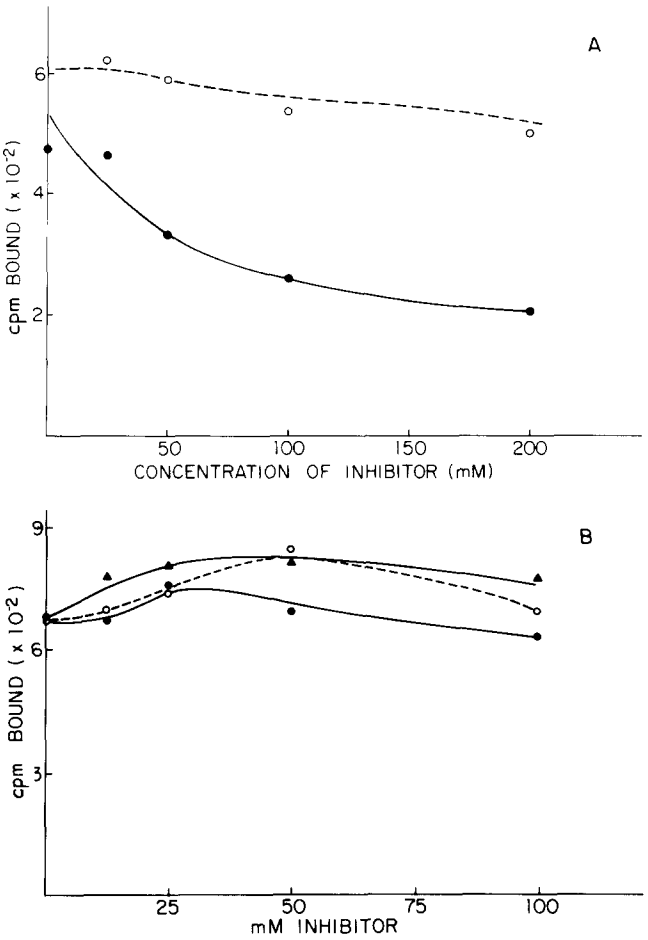

FIGURE 9 Effect of different sugars on the adhesion of [^{3}H]thymidine-labeled 3T3 cells. *A*, β-galactosidase surfaces in the presence of β-methyl-D-galactoside (●) and α-methyl-D-galactoside (○). *B*, fibronectin surfaces in the presence of α-methyl-D-mannoside (▲), α-methyl-D-galactoside (●), and β-methyl-D-galactoside (○).

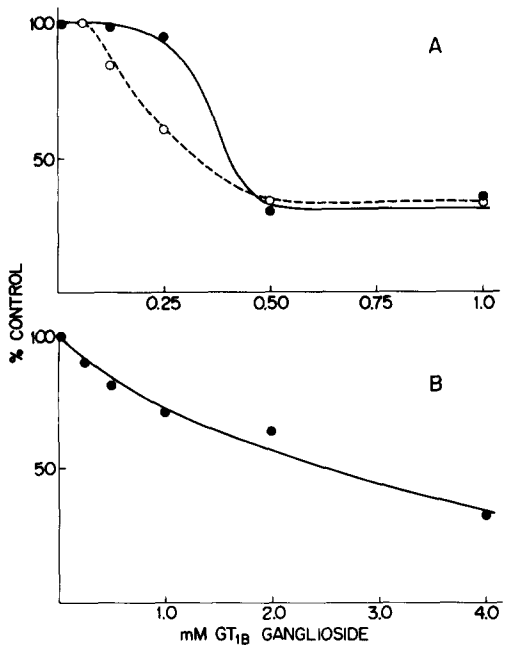

FIGURE 10 Effect of GT_{1b} ganglioside on the attachment of [^{3}H]-thymidine-labeled 3T3 cells. *A*, fibronectin surfaces (●) and gelatin surfaces (○). *B*, SBA surfaces (●).

The extent of cell adhesion on various protein coats is summarized in Table V. A clear difference in cell adhesion is attributable to the specific quality of the substratum rather than the quantity of adsorbed substratum on plastic surfaces. Thus, the quantity of BSA, fetuin, and asialofetuin adsorbed on plastic surfaces (Fig. 1; Tables III and IV) was similar to the quantity of fibronectin, glycosidase, and lectins adsorbed. Yet, these proteins have a low adhesion-promoting activity for various cell types (Table V), with the exception that hepatocytes were reactive on asialofetuin coat and NIL cells were reactive on ovalbumin coat. The former may be attributable to the presence of a galactose-binding protein on the hepatocyte membranes (1). The rationale of the NIL cell reaction on the ovalbumin coat (Table V) is discussed in the third paper of this series (53).

In the present studies several lectins coated on substratum, such as WGA, Con A, SBA, and *Limulus* (Table V), showed an activity comparable to that of fibronectin reported in several studies (reviewed in references 11, 22, and 67). In general, the adhesion-promoting activity of various lectins (Fig. 5) seems to correlate with the presence of specific glycoprotein receptors on the cell surface, and the adhesion reaction is inhibitable with competing monosaccharides within a short period of time. However, in the assay of a longer time period, the specificity may become masked by ill-defined interactions of lower affinity that become more pronounced with increasing time (see the following paper [10]).

Interestingly, β-galactosidase, as well as sialidase, promotes cell adhesion to the same extent as fibronectin and lectins (Table V). The data presented on glycosidases in these studies are, to our knowledge, the first direct evidence that an enzyme surface can promote cell adhesion. We suggest that the cell adhesion on glycosidase-coated surfaces could be determined by interactions between the catalytic sites of the enzyme surface and the substrate sites at the cell surface, based on the following observations: (*a*) The enzyme surface adsorbed on plastic retains its catalytic activity (see the third and fourth paragraphs of Results, and Tables III–V), and the substrate sites are present at the cell surfaces. (*b*) Sialidase and β-galactosidase showed a strong adhesion-promoting activity for various types of cells in a striking contrast to various glycoproteins and proteins, which displayed a low adhesion-promoting activity (Table V). (*c*) The glycosidase-mediated cell adhesion was inhibited strongly by the competitive inhibitors such as 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (41) for sialidase-catalyzed adhesion and 1,4-D-galactonolactone (36) and β-methylgalactoside for the β-galactosidase-mediated cell adhesion. The effect of these adhesion inhibitors is not the result of cytotoxic effects. Fibronectin-mediated adhesion was not affected by the sialidase inhibitor, and sialidase-mediated adhesion was not inhibited by the galactonolactone. The β-galactosidase-mediated adhesion was not inhibited by α-methylgalactoside. (*d*) Desialylation of the cells increased the adhesion on β-galactosidase coat,

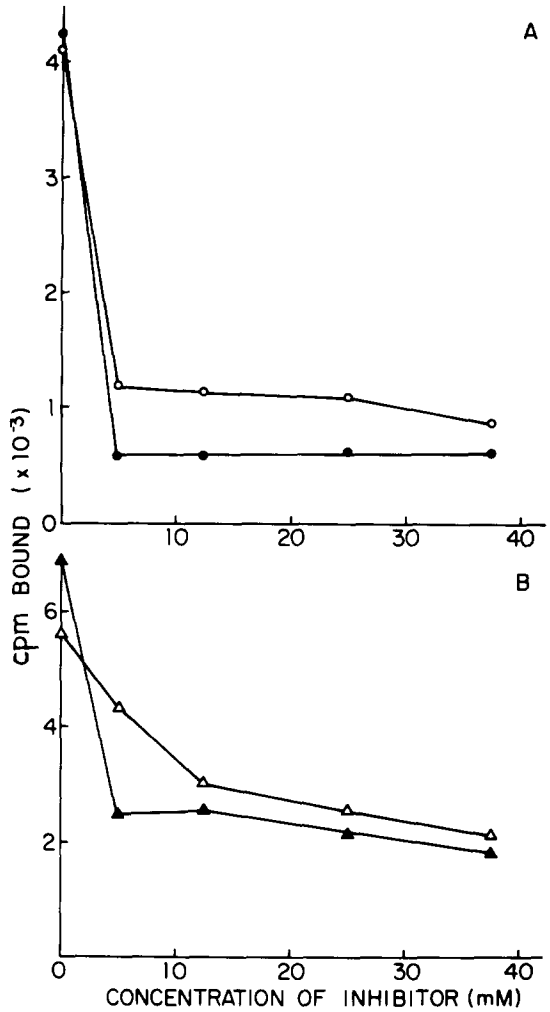

FIGURE 11 Inhibition of soluble lectin binding to NIL cells and of lectin-induced attachment of [^{3}H]proline-labeled NIL cells by monosaccharide hapten inhibitors. The binding of soluble [^{14}C]SBA (*A*, ●) and [^{14}C]Con A (*B*, ▲) to NIL cells in the presence and absence of saccharide hapten inhibitors is compared to cell attachment to SBA- (*A*, ○) and Con A- (*B*, △)coated surfaces in the presence and absence of sugar inhibitors. Monosaccharide inhibitors used were *N*-acetyl-D-galactosamine (0–37.5 mM) for inhibition of SBA and α-methyl-D-mannoside for inhibition of Con A. The adhesion surfaces were prepared by adsorbing the lectins on plastic surfaces (see Materials and Methods), followed by coating with BSA (100 μg/ml, 1 h).

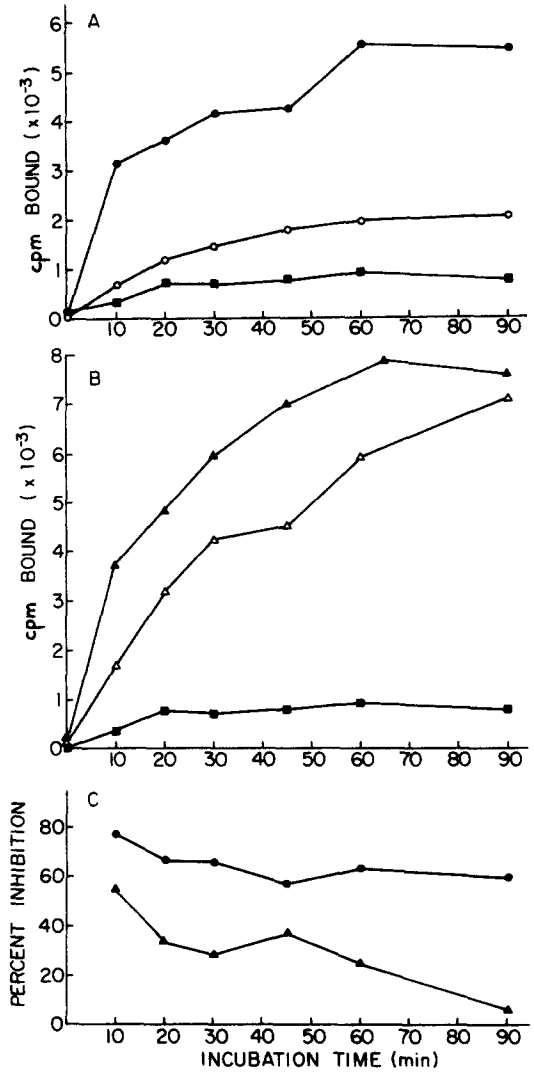

FIGURE 12 Inhibition of NIL cell adhesion on Con A- and SBA-coated surfaces by monosaccharides; effect of incubation time. (*A*) NIL cells, metabolically labeled with [^{3}H]proline, were incubated on SBA-coated surfaces for increasing periods of time (0–90 min) in the presence or absence of 25 mM *N*-acetyl-D-galactosamine. After the indicated incubation period, the nonadherent cells were washed off and the bound radioactivity was determined. ●, Cells bound to SBA-coated surfaces; ○, cells bound to SBA-coated surface in the presence of *N*-acetyl-D-galactosamine; ■, cells bound to BSA-coated surface. (*B*) The adhesion experiment performed in *A* was repeated on Con A-coated surfaces, using 25 mM α-methyl-D-mannoside as an inhibitor. ▲, Cells bound to Con A-coated surface; △, cells bound to Con A-coated surface in the presence of α-methyl-D-mannoside; ■, cells bound to BSA-coated surface. (*C*) The effect of incubation time on the inhibition of cell attachment to SBA-coated surfaces (●) from *A* and on Con A-coated surfaces (▲) from *B* as a result of the presence of monosaccharide inhibitors.

but decreased the adhesion on the sialidase coat (see the kinetic study presented in the following paper [10]).

It should be noted that the adhesion reactions described for the glycosidases take place in conditions resembling physiological environment, buffered at 7.0–7.4, which generally restricts hydrolytic cleavages by animal, bacterial, and viral glycosyl hydrolases. The optimum pH of *Clostridium perfringens* sialidase is about 5 (5, 48, 50) and that of jack-bean β-galactosidase is 3–4 (36). The hydrolysis-catalytic activities of the enzymes adsorbed on plastic surface were greatly reduced at physiological pH (see Tables III–V), yet these glycosidases showed a strong promoting activity for attachment and spreading at physiological pH. Therefore, we believe that the affinity of the plastic-adsorbed glycosidases for the cell surface is sufficiently strong to stimulate cell adhesion, even though their hydrolysis-catalytic activities were strongly diminished at physiological pH. It was not possible to observe cell attachment and spreading on glycosidase surfaces in nonphysiological pH conditions, because variation in pH (including both acidic and basic conditions) strongly inhibits the general adhesion mechanism. Because glycosidase activities are reported to occur at the cell surface (10, 26, 64) and the glycosidases seem to provide sufficient affinities to stimulate cell attachment, the idea that these enzymes play a role in mediating cell adhesion and recognition may be justified. Interestingly, various cells interact with fluorescein-tagged glycolipid-liposomes (28) or with fluorescein-tagged oligosaccharides (31). These results may not necessarily indicate the presence of various lectins on the cell surface, but may be regarded as an indication of glycosidase-catalyzed interaction. The occurrence of mannosidase at the cell surface and cell attachment mediated through this enzyme is presented in the third paper of this series (53; see also reference 52).

Although the cell adhesion on lectin and glycosidase coats is strongly affected by specific protein-sugar interactions, various nonspecific interactions may also be important in cell adhesion to protein-coated surfaces. Thus, BSA enhanced adhesion on wheat-germ lectin, *Limulus* lectin, and sialidase surfaces. It is suggested that BSA stabilizes the adhesion on sialyl-reactive surfaces attributable to favorable ionogenic environment or to increased surface wettability effected by BSA coating.

Fibronectin mediates a strong cell adhesion and cell spreading to the same extent as lectins and glycosylhydrolases. However, fibronectin-mediated cell attachment was not inhibited by various monosaccharides, glycosides, sialic acid, and sialic acid analogues in a striking contrast to the cell attachment mediated by carbohydrate-reactive proteins (lectins and glycosidases), which were specifically inhibited by monosaccharides and glycoside analogues. The only effective inhibitor of fibronectin-mediated cell attachment was the polysialylated ganglioside, in agreement with Kleinman et al. (32). However, polysialosyl gangliosides also inhibit cell attachment on gelatin and soybean lectin-coated surfaces, and therefore the inhibition is nonspecific. Moreover, the ganglioside inhibition of cell attachment on fibronectin and gelatin required a higher concentration of GT_{1b} ganglioside than the specific inhibition for cell adhesion on two sialic acid–binding proteins, *Limulus* lectin and sialidase. Most fibroblast cell lines, including NIL, baby hamster kidney, and chick embryonic fibroblasts, are lacking polysialosyl gangliosides (our unpublished observation). It is assumed, therefore, gangliosides could not be a receptor for fibronectin and the reason for ganglioside inhibition of cell attachment still remains unclear. Further studies on the specificity of fibronectin-cell interactions are warranted.

The authors wish to thank Professor Roland Schauer (Christian Albrecht University, Kiel, Germany) for the sample of 2-deoxy-2,3-dehydroneuraminic acid and Professor Michel Monsigny (University of Orléans, Orléans, France) for the sample of *Limulus* lectin. The technical assistance of Mr. Edward Nudelman and Mr. Mark E. Powell is gratefully acknowledged. Helpful discussions and suggestions by Drs. Kiyotoshi Sekiguchi and Minoru Fukuda are appreciated. The authors wish to thank Mrs. Charlotte Pagni for excellent assistance during the preparation of the manuscript.

This work has been supported by grant CA23907 from the National Institutes of Health (NIH). H. Rauvala is supported by International Fogarty Fellowship 1 FO5 TW02773-01; W. G. Carter is supported by Fellowship 5 F32 GM06588-02 from NIH.

Received for publication 9 June 1980, and in revised form 10 September 1980.

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