The carbohydrate binding specificities of three sialoadhesins, a subgroup of I-type lectins (immunoglobulin superfamily lectins), were compared by measuring lectin-transfected COS cell adhesion to natural and synthetic gangliosides. The neural sialoadhesins, myelin-associated glycoprotein (MAG) and Schwann cell myelin protein (SMP), had similar and stringent binding specificities. Each required an α2,3-linked sialic acid on the terminal galactose of a neutral saccharide core, and they shared the following rank-order potency of binding: GQ1b > GQ1a > GM2 = GM4 > GQ1b > GD3, GQ1b (nonbinders). In contrast, sialoadhesin had less exacting specificity, binding to gangliosides that bear either terminal α2,3- or α2,8-linked sialic acids with the following rank-order potency of binding: GQ1b > GQ1a > GQ1b = GM2 = GM4 > GD3 = GQ1b > GM1 (nonbinders). CD22 did not bind to any ganglioside tested. Binding of MAG, SMP, and sialoadhesin was abrogated by chemical modification of either the sialic acid carboxylic acid group or glycerol side chain on a target ganglioside. Synthetic ganglioside G43 derivatives further distinguished lectin binding specificities. Deoxy and/or methoxy derivatives of the 4-, 7-, 8-, or 9-position of sialic acid attenuated or eliminated binding of MAG, as did replacement of the sialic acid amido group with a hydroxyl. In contrast, the 4- and 7-deoxy sialic acid derivatives supported sialoadhesin binding at near control levels (the other derivatives did not support binding). These data are consistent with sialoadhesin binding to one face of the sialic acid moiety, whereas MAG (and SMP) may have more complex binding sites or may bind sialic acids only in the context of more restricted oligosaccharide conformations.

Sialoadhesins (1) are a structurally and functionally related family consisting of five immunoglobulin superfamily lectins (I-type lectins) (2) including myelin-associated glycoprotein (MAG), Schwann cell myelin protein (SMP), CD22, CD33, and sialoadhesin. MAG and SMP are found on oligodendroglia and Schwann cells in the nervous system (3, 4). CD22 is expressed on a subset of B lymphocytes, sialoadhesin on a subset of macrophages, and CD33 on cells of myelomonocytic lineage. Sialoadhesins have been proposed to mediate cell-cell recognition, perhaps via their carbohydrate binding activities (5–7). Each sialoadhesin family member has two or more Ig-like domains: an amino-terminal V-set domain followed by one or more (up to 16) C2-set domains (8). Domain deletion and site-directed mutagenesis of sialoadhesin and CD22 localize their carbohydrate-binding sites to the amino-terminal V-set domain, with contributions (for CD22) from the adjoining C2-set domain. These first two domains share very high amino acid sequence similarity between MAG and SMP (>70%) and significant similarity across all I-type lectins (>30% in pairwise comparisons) (2, 8, 9).

Each I-type lectin binds to carbohydrate structures bearing a nonreducing terminal sialic acid (1, 6, 10). Sialic acids are a common nonreducing terminus of vertebrate glycoconjugates and appear to play uniquely important roles in recognition phenomena. Because sialic acids may be linked to Gal, GalNAc, or other sialic acid residues at various positions and because they may carry different substituents on their 9-carbon base structure, the sialic acids represent a diverse family of carbohydrate determinants (11). In certain sialic acid-dependent recognition systems, determinant stringency is low. For example, selectins bind to oligosaccharides bearing truncated sialic acids (12) or appropriately placed anionic groups (sulfates, carboxylic acids) otherwise unrelated to the sialic acid structure (13–16). In contrast, sialoadhesins appear to have more stringent sialic acid specificities (see “Discussion”) (9). In this study, we used cells expressing different sialoadhesins to explore and compare the fine structural preferences of their binding to target sialylated glycoconjugates.

EXPERIMENTAL PROCEDURES

Gangliosides—The ganglioside structures used in this study are shown schematically in Fig. 3. Purified bovine brain GM2, GM3, GQ1b, GM1, GM2, and GQ1b were from EY Laboratories (San Mateo, CA) or Matreya, Inc. (Pleasant Gap, PA), and GQ1b was from Accurate Chemical & Sciences (Pleasant Gap, PA). 1 The abbreviations used are: MAG, myelin-associated glycoprotein; SMP, Schwann cell myelin protein; mAb, monoclonal antibody; GQ1b, GM2, GM1, GM4, GD1a, GD1b, GD3, GM1, GM2, and GQ1b were from EY Laboratories (San Mateo, CA) or Matreya, Inc. (Pleasant Gap, PA), and GQ1b was from Accurate Chemical & Sciences (Pleasant Gap, PA). 1 The abbreviations used are: MAG, myelin-associated glycoprotein; SMP, Schwann cell myelin protein; mAb, monoclonal antibody; GQ1b, GM2, GM1, GM4, GD1a, GD1b, GD3, GM1, GM2, and GQ1b were from EY Laboratories (San Mateo, CA) or Matreya, Inc. (Pleasant Gap, PA), and GQ1b was from Accurate Chemical & Sciences (Pleasant Gap, PA). 1 The abbreviations used are: MAG, myelin-associated glycoprotein; SMP, Schwann cell myelin protein; mAb, monoclonal antibody; GQ1b, GM2, GM1, GM3, GM4, GD1a, GD1b, GD3, GM1, GM2, and GQ1b were from EY Laboratories (San Mateo, CA) or Matreya, Inc. (Pleasant Gap, PA), and GQ1b was from Accurate Chemical & Sciences (Pleasant Gap, PA).
the freshly collected cells. Quantitation of cell adhesion was via an
treatment, viability ranged from 81 to 85%, essentially unchanged from
trypan blue exclusion on representative transfected cells. Prior to pre-
ing 1.5 mg/ml bovine serum albumin. Cell viability was determined by
cells/ml in Hepes-buffered Dulbecco’s modified Eagle’s medium contain-
at 37 °C with end-over-end mixing. Cells were collected by centrifuga-
dase (Calbiochem) were added. Suspensions were incubated for 1.5–2 h
513 (MAG/SMP cross-reactive) (4, 7), SER-4 (sialoadhesin) (26), and
immunocytochemistry using the following monoclonal antibodies: mAb
ceed Ig-like domain variant, CD22β) (23, 24).
Plasmids were propagated in Escherichia coli MC1061/pβ and puri-
ified by polyethylene glycol precipitation. COS-1 cells, routinely main-
tained in 10% fetal calf serum in Dulbecco’s modified Eagle’s medium at
37 °C in a humidified atmosphere of 90% air and 10% CO2, were
transiently transfected with lectin-expressing plasmids via a high effi-
ciency procedure (using 40 μg/ml DEAE-dextran) (25). Transfected cells
were returned to culture for 40–50 h to allow lectin expression to
proceed and then were detached from plates for adhesion experiments (see
below). Lectin expression was confirmed by flow cytometry and/or
immunocytochemistry using the following monoclonal antibodies: mAb
Chemicon 2112 (CD22; Chemicon International, Inc., Temecula, CA).
Microplate Cell Adhesion to Adsorbed Glycolipids—Adhesion was
performed as reported previously (20, 22, 27). Aliquots (50 μl) of etha-
nol/water (1:1) containing phosphatidylcholine (0.5 μm), cholesteryl (2.0 μm),
and gangliosides (concentrations as indicated) were added to mi-
crowells (96-well Serocluster, Costar Corp., Cambridge, MA). Plates
were incubated for 90 min uncovered at ambient temperature to allow
partial evaporation and lipid adsorption (28, 29), after which the wells
were washed with water. Wells were preblocked by addition of 100 μl/well Heps-buffered Dulbecco’s modified Eagle’s medium contain-
ing 1.5 mg/ml bovine serum albumin. Plates were covered and incubated for 10 min at 37 °C prior to cell addition (see below).
Transfected COS cells were harvested using hypertonic Ca2+/Mg2+-
free phosphate-buffered saline containing 1 mM EDTA as described
(22), collected by centrifugation, and resuspended at 107 cells/ml in
Dulbecco’s phosphate-buffered saline containing 2 mg/ml bovine serum
albumin. Transfected cells were pretreated with neuraminidase, which
enhances cell adhesion without changing carbohydrate binding speci-
dicity (20), as follows. Aliquots of cells (500 μl) were placed in 1.5-ml
microcentrifuge tubes, and 10 milliunits of Vibrio cholerae
neuraminidase (Calbiochem) were added. Suspensions were incubated for 1.5–2 h
at 37 °C with end-over-end mixing. Cells were collected by centrifuga-
tion, washed twice with Dulbecco’s phosphate-buffered saline contain-
ing 2 mg/ml bovine serum albumin, and resuspended at 250,000 cells/
well in Heps-buffered Dulbecco’s modified Eagle’s medium contain-
ing 1.5 mg/ml bovine serum albumin. Cell viability was determined by
trypsin blue exclusion on representative transfected cells. Prior to pre-
treatment, cells were 84% viable. After neuraminidase or control pre-
treatment, viability ranged from 81 to 85%, essentially unchanged from
the freshly collected cells. Quantitation of cell adhesion was via an
enzyme assay (see below) that measured only viable cells.
Aliquots of the cell suspension (200 μl) were added to preblocked,
lipid-adsorbed microwells and incubated at 4 °C for 10 min to allow the
cells to settle and then at 37 °C for 45 min. To gently remove nonad-
erent cells after the incubations, plates were immersed in phosphate-
buffered saline, inverted, and placed in an immersed P1000 Mix box that
was sealed with a gasket to exclude air (27). The inverted plate in its
fluid-filled chamber was placed in a centrifuge carrier and centrifuged
at 110 g for 6 min. The box was again immersed in phosphate-buffered saline;
the plate was removed and righted (while immersed); and excess sur-
face buffer was removed by aspiration, leaving 300 μl/well. Adherent
cells were lysed by addition of 20 μl of 10% Triton X-100 to each well, and
80 μl were removed to a fresh 96-well plate for quantitation. Cell
adhesion was quantitated by measuring lactate dehydrogenase activity in
the cell lysate after addition of 120 μl of 1% Triton-X-100, 10 mM NADH, 10
μM ADP, 10 mM MgCl2, 10 mM KCl, and 20 mM imidazole, pH 7.4, and
7.5 mM glucose.

A

B

C

D

E

F

G

H

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K

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Y

Z

FIG. 1. Adhesion of COS cells expressing sialoadhesins to ad-
sorbed gangliosides. COS cells transiently transfected to express
MAG (A), SMP (B), or sialoadhesin (C) were collected from tissue
dishes; pretreated with neuraminidase to enhance adhesion; and
plated in polyethylene glycol-pretreated microtiter plate wells. Plates
were coated with various carbohydrates and gangliosides. After
incubation, nonadherent cells were removed by aspiration, and adherent
cells were quantitated enzymatically (see “Experimental Procedures”).
Adhesion is expressed relative to the total number of cells added to each well
and represents the mean ± S.E. of 3–103 replicate determinations. Back-
ground adhesion, represented by a horizontal line in each panel, was
determined on wells adsorbed with phosphatidylcholine and cholesteryl
without gangliosides (7.5 ± 0.6% for MAG; 11.0 ± 0.8% for SMP, and
5.9 ± 0.6% for sialoadhesin). ○, GDb1a; □, GDb1b; ▽, GM3; △, GQ1b;
□, Gdb1a; △, GQ1b; ○, GM3.

determinations. Where indicated, the statistical significance of adhe-
sion to ganglioside-adsorbed surfaces compared with control surfaces
(adsorbed with phosphatidylcholine and cholesteryl, but no ganglioside)
was determined using a two-tailed Student’s t test.

RESULTS

Ganglioside Binding Specificities of Sialoadhesins—MAG, SMP,
and sialoadhesin-transfected COS cells bound specifically
to ganglioside-adsorbed surfaces (Figs. 1–3). Adhesion to the
most potent target gangliosides was typically very high
(>80% of the cells added), whereas background adhesion
to surfaces adsorbed with phosphatidylcholine and cholesteryl
without ganglioside was low. COS cells transfected with CD22
failed to adhere to any ganglioside tested (GDb1a, GDb1b, GDb2,
GDb1b, GQ1b, and GQ1b). COS cells transfected with either of the

Scientific Corp. (Westbury, NY). GDb1a (NeuAc form) was from Sigma.
GDb1b, GDb1b, GDb1a, and its derivatives, and GDb2 derivatives were
synthesized de novo using previously described methods (17–20). GDb1a,
gangliosides bearing sialic acids with truncated glycerol side chains
(7/8- aldehydes) were prepared by mild periodate oxidation followed (as
indicated) by sodium borohydride reduction to form the 7/8-alcohols
(20). GDb2, gangliosides bearing sialic acid ethyl esters, 1-amides, and
1-alcohols were prepared as described (20). Products were analyzed by
thin-layer chromatography and fast atom bombardment mass spec-
trometry at the Middle Atlantic Mass Spectrometry Laboratory (21).

F-type Lectin Transfection of COS Cells—Full-length F-type lectin
CD22 was cloned into the eukaryotic expression vector pCDM8 (sialoadhesin only) or pCDM8. The lectins used in this study included mouse sialoadhesin (8), both the long (L-MAG) (22) and short (S-MAG) splice variants of rat MAG, quail SMP (4), and human CD22 (7/8-
glike domain variant, CD22β) (23, 24).

Glycolipid added (pmol/well)

Cell adhesion (% of cells added)
two splice variants of MAG (L-MAG and S-MAG) demonstrated the same extent and specificity of adhesion to a representative set of ganglioside-adsorbed surfaces (GM1, GD1a, GD1b, GT1b, and GQ1b) (data not shown). Therefore, L-MAG-transfected COS cells were used in subsequent experiments, and all data presented on MAG-mediated adhesion refer to the long splice variant.

The two neural sialoadhesins, MAG and SMP, had similar ganglioside binding specificities (Figs. 1–3). The abundant brain gangliosides GD1a (at $12.5 \text{ pmol/well}$) and GT1b (at $25 \text{ pmol/well}$) supported highly significant adhesion $(p < 0.0002)$ of both MAG- and SMP-transfected COS cells (Fig. 1, A and B). Other gangliosides including GM3 and GM4 also supported significant adhesion of both lectins, although only at $10$-fold higher ganglioside concentrations compared with GM1a. Other gangliosides including GM3a and GQ1b also supported significant adhesion of both lectins, although only at $10$-fold higher ganglioside concentrations compared with GM1a. In contrast, neither MAG nor SMP bound to GM3. GD1b or GQ3 indicating that both lectins require a terminal a2,3-linked sialic acid. All gangliosides that supported statistically significant adhesion of SMP contained the NeuAc a2,3Gal terminal structure (see Fig. 3), whereas all nonsupportive gangliosides lacked this terminal structure. MAG supported adhesion to the same gangliosides, although typically with higher efficiency (greater number of adherent cells). This may be due to more efficient transfection with the MAG plasmid, higher expression of the transfected MAG, and/or more effective ganglioside binding by MAG. Flow cytometry using a MAG/SMP cross-reactive antibody (mAb 513) indicated that more MAG-transfected cells (48.2%) expressed the highest level of lectin compared with SMP-transfected cells (28.3%). Within these highest expressing populations, the mean fluorescence intensities were similar (496 and 441 relative units for MAG and SMP, respectively).

In addition to gangliosides bearing the NeuAc-a2,3Gal terminal, GQ1b (which bears only a2,8-linked sialic acid termini) supported a low amount of adhesion by MAG-transfected cells. This preparation of GQ1b, however, was contaminated with a small amount of contaminating GT1b (20). NeuAc-NeuAc linkages are all a2,8; other NeuAc linkages are as noted in the key.

In contrast to MAG and SMP, sialoadhesin had a distinctly broader binding specificity. Several gangliosides with terminal NeuAc-a2,3Gal structures (GD1a, GT1b, GM3, and GQ1b) as well as GD1b (which bears only a terminal NeuAc-a2,8NeuAc structure) supported nearly equivalent sialoadhesin-mediated adhesion (Fig. 1C). GD3 and GQ1b, which also bear only NeuAc-a2,8NeuAc

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**Table 1.** Potency of gangliosides supporting MAG-, SMP-, and Sialoadhesin-mediated cell adhesion

| Ganglioside | MAG | SMP | Sialoadhesin |
|-------------|-----|-----|--------------|
| GM1a | $+$ | $+$- | $+$ |
| GD1a | $+$ | $+$- | $+$ |
| GT1b | $+$- | $+$ | $+$ |
| GM3 | $-$ | $-$ | $-$ |
| GM4 | $-$ | $-$ | $-$ |

**Key:**
- Glc
- Gal
- 2,3-NeuAc
- 2,6-NeuAc

**Fig. 2.** Adhesion of COS cells expressing sialoadhesins to adsorbed α and β series gangliosides. Adhesion of COS cells transiently transfected to express MAG (A), SMP (B), or sialoadhesin (C) to microwells adsorbed with phosphatidylcholine, cholesterol, and the indicated gangliosides was determined as described under "Experimental Procedures" and in the legend to Fig. 1.

**Fig. 3.** Structure-function studies of MAG-, SMP-, and sialoadhesin-mediated cell adhesion to gangliosides. Binding data are summarized from Figs. 1 and 2. Potency (concentration supporting approximately half-maximal adhesion) is indicated in the following ranges: $+++$, $<10 \text{ pmol/well}$; $++$, $10–100 \text{ pmol/well}$; $+{,}+$, very low but statistically significant adhesion over background; and $-$, no adhesion over background at any concentration tested. Statistically significant adhesion above background (two-tailed Student’s t test) is indicated as follows: *, $p < 0.001$; and ‡, $p < 0.01$. §, this preparation of GQ1b contains a small amount of contaminating GT1b (20). NeuAc-NeuAc linkages are all a2,8; other NeuAc linkages are as noted in the key.

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**FIG. 2.** Adhesion of COS cells expressing sialoadhesins to adsorbed α and β series gangliosides. Adhesion of COS cells transiently transfected to express MAG (A), SMP (B), or sialoadhesin (C) to microwells adsorbed with phosphatidylcholine, cholesterol, and the indicated gangliosides was determined as described under "Experimental Procedures" and in the legend to Fig. 1. 

**FIG. 3.** Structure-function studies of MAG-, SMP-, and sialoadhesin-mediated cell adhesion to gangliosides. Binding data are summarized from Figs. 1 and 2. Potency (concentration supporting approximately half-maximal adhesion) is indicated in the following ranges: $+++$, $<10 \text{ pmol/well}$; $++$, $10–100 \text{ pmol/well}$; $+{,}+$, very low but statistically significant adhesion over background; and $-$, no adhesion over background at any concentration tested. Statistically significant adhesion above background (two-tailed Student’s t test) is indicated as follows: *, $p < 0.001$; and ‡, $p < 0.01$. §, this preparation of GQ1b contains a small amount of contaminating GT1b (20). NeuAc-NeuAc linkages are all a2,8; other NeuAc linkages are as noted in the key.

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**Sialic Acid Binding Specificities of I-type Lectins**
The MAG/SMP cross-reactive antibody mAb 513 (4, 7), shown previously to block MAG binding to neurons (31) and gangliosides (22), demonstrated the carbohydrate-binding site structural similarity between MAG and SMP and their difference from sialoadhesin. As shown in Fig. 4, mAb 513 eliminated or markedly reduced binding of MAG and SMP to G_{T1b}, whereas binding of sialoadhesin was unaffected. The anti-sialoadhesin blocking mAb 3D6 (32) inhibited binding of sialoadhesin to G_{T1b} (data not shown).

Sialic Acid Substructure Binding Specificities of I-type Lectins—Sialic acid is a complex monosaccharide, with a carboxylic acid, an N-acetyl group, and a glycerol side chain within its structure (see Fig. 7). Chemically modified and synthetic gangliosides were used to determine which sialic acid substituent groups are required for binding by sialoadhesin family members.

Since G_{D1a} supports highly significant adhesion of MAG, SMP, and sialoadhesin (Fig. 1), it was used as a basis for testing sialic acid chemical modifications. G_{D1a} was selectively oxidized with periodate under conditions that cleave exclusively between C-7–C-8 and C-8–C-9 on the sialic acid glycerol side chain. Mass spectrometry indicated equal conversion of GD1a sialic acids to their corresponding 7- and 8-carbon aldehydes (data not shown). A portion of the resulting GD1a aldehydes was reduced with sodium borohydride, resulting in conversion to the corresponding 7- and 8-carbon alcohols. As shown in Fig. 5, neither the 7/8-aldehyde nor 7/8-alcohol sialic acid derivatives of GD1a supported binding of any of the I-type lectins tested. Similarly, modifications of the carboxylic acids on GD1a abrogated binding. Conversion of both sialic acids on GD1a to the corresponding 1-ethyl esters, 1-amides, or 1-alcohols completely eliminated binding of MAG, SMP, and sialoadhesin-transfected COS cells (Fig. 5). The structures of all
Sialoadhesins (1, 8, 9) are a functionally and structurally related subfamily of carbohydrate-binding immunoglobulin superfamily members (1-type lectins) (2). The sialoadhesin family consists of the eponymous member (sialoadhesin), MAG, SMP, CD22, and CD33. MAG and SMP are expressed on myelinating cells in the nervous system, sialoadhesin on a subset of macrophages, CD22 on certain B lymphocytes, and CD33 on cells of myelomonocytic lineage (9). Sialoadhesins mediate cell-cell interactions by binding to target sialylated glycoconjugates (1, 6, 10, 32). They share the same general polypeptide domain structure: an amino-terminal V-set Ig-like domain followed by one or more C2-set Ig-like domains, a transmembrane domain, and a short cytoplasmic tail. The ligand recognition site has been localized to the amino-terminal V-set domain (sialoadhesin) (34) or the V-set domain with contributions from the adjacent C2-set domain (CD22) (34, 35). Additionally, sialoadhesins have extensive sequence similarity. The first two amino-terminal Ig-like domains of MAG and SMP are 56% identical (72% similar, including conservative amino acid replacements), and other sialoadhesins range from 32 to 43% sequence similarity in pairwise comparisons. Site-directed mutagenesis (36, 37) indicates that sialoadhesin and CD22 bind to sialylated glycoconjugates via amino acids on one surface of the V-set domain. This is consistent with sialoadhesin’s sialic acid substituent group binding specificity (see below), whereas MAG’s specificity indicates a more complex binding site.

Target ligands for the sialoadhesins are glycoconjugates in which a terminal sialic acid is essential for binding (1, 2, 6, 9, 10, 38). In previous studies, CD22 bound only to α2,6-linked sialic acids (1, 39), whereas MAG and CD33 bound only to α2,3-linked sialic acids (1, 10, 22). Sialoadhesin binding predominantly to terminal α2,3-linked sialic acids (1), although weaker binding to α2,8-linked sialic acids was demonstrated (32). Among structures with α2,3-linked sialic acids, MAG bound preferentially to “3-O” structures (NeuAcα2,3Galβ1,3GalNAc), which are common termini on gangliosides (the major sialoglycoconjugates of the nervous system) (40) and O-linked glycoproteins (1). Sialoadhesin and CD33 bound similarly to “3-O” and “3-N” (NeuAcα2,3Galβ1,4GlcNAc) structures (1, 10).

Our prior studies demonstrated that (i) MAG bound to gangliosides with the specificity G_{D1a} > G_{D1b} = G_{D1b} = G_{D3} ≫ G_{G1} = G_{G1} = G_{G1b}, the latter of which did not support adhesion; and (ii) modification of the glycerol side chain, carboxylic acid, or N-acyl group abrogated MAG-mediated adhesion (20, 22). This study confirms and extends those findings. MAG-medi-
ated adhesion was repeated to the above gangliosides as well as to G_{M4}, KDN-G_{M4}, six synthetic derivatives of G_{M4}, and various gangliosides with α2,6-sialic acids linked to the GalNAc(II) of the gangliotetraose core. These new data were compared directly with adhesion of COS cells expressing CD22, sialoadhesin, SMP, and the short isofrom of MAG.

CD22 failed to bind to α2,3- or α2,8-linked sialic acid termini on gangliosides (data not shown). In contrast (Figs. 1 and 2), sialoadhesin bound to most of the gangliosides tested, including many with terminal α2,3- or α2,8-sialic acids. Nine of 12 gangliosides tested supported sialoadhesin binding within the same ~10-fold concentration range, indicating that sialoadhesin does not markedly distinguish the sialic acid linkage (α2,3 versus α2,8) or the neutral core (e.g. compare G_{D1b} and G_{M4} in Fig. 3). A previous study of detergent-solubilized 125I-sialoadhesin binding to gangliosides using thin-layer chromatography plate overlay (32) also reported a broad specificity.

Consistent with their extensive sequence similarity, the two neural sialoadhesins, SMP and MAG, were remarkably similar in their ganglioside binding (Figs. 1–3). Both bound only to structures bearing terminal α2,3-linked sialic acids (e.g. G_{D1b} and G_{D1a}), and failed to bind to those terminated with α2,8-linked structures (e.g. G_{D1b} and G_{D2b}). Among glycoconjugates with α2,3-linked sialic acid termini, SMP and MAG distinguished sialic acid linkage patterns and neutral core variations. In contrast to sialoadhesin, di- and trisialogangliosides with the gangliotetraose core (G_{D1a} and G_{D2b}) supported adhesion of SMP and MAG ~10-fold better than did monosialogangliosides (G_{M3} and G_{M4}). Furthermore, the Chol-1 ganglioside, G_{D1b}, was 10-fold more potent than any other ganglioside tested (Figs. 1–3). Chol-1 gangliosides are quantitatively similar structures that are expressed exclusively on cholinergic neurons (30, 41). The functional significance of their preferential binding to the neural sialoadhesins is not known. Although the terminal tetrasaccharide on G_{D1b} is also found on O-linked glycoproteins (42, 43), polyclonal antibodies against Chol-1 gangliosides do not cross-react with any glycoprotein (44), suggesting that the oligosaccharide on G_{D1b} adopts a unique conformation that fits particularly well in the SMP and MAG binding pockets. In addition to having similar carbohydrate recognition specificities, the observation that both SMP- and MAG-mediated adhesion to gangliosides is inhibited by the same conformationally restricted monoclonal antibody (mAb 513) (45) confirms the similarity of their binding sites.

Sialic acids are unusual among monosaccharides in their complexity and diversity (11). They carry a carboxylic acid (C-1), an N-acetyl group attached to C-5, and a glycerol side chain attached to C-6 (Fig. 7), each of which is involved in molecular recognition by certain sialoadhesins. Blocking the carboxylic acid abrogates binding (Fig. 5), as does replacement of the acetamido group with a hydroxyl (compare GM4 with KDN-GM4 in Fig. 6) or truncation of the glycerol side chain (Fig. 5). These data are consistent with prior studies on the sensitivity of sialoadhesin and CD22 binding to modifications of the sialic acid residue (6, 33, 46–48) and contrast with studies on selectins, in which extensive modifications of sialic acids have no effect (12, 14, 49). In fact, substitution of the entire sialic acid (e.g. on sialyl-LeX or sialyl-Le^X) with a sulfate ester results in retention of ligand binding by all selectins (13, 14), but abrogates binding by CD22 (47).

The sialic acid substructural binding specificities of sialoadhesin and MAG have implications for ligand docking on the proteins. For sialoadhesin, modification of the C-8 or C-9 hydroxyl, the acetamido nitrogen or methyl group (33), or the C-1 carboxylic acid eliminated binding (Figs. 5 and 6), whereas removal of the C-4 or C-7 hydroxyl was without effect. This pattern is consistent with binding primarily to a single face of the sialic acid (top face in Fig. 7). Sialic acid binding to sialoadhesin can be compared with x-ray crystallography of sialic acid binding to the influenza virus hemagglutinin (50), in which a carboxylate oxygen, the acetamido nitrogen, and the 8- and 9-hydroxyls extend in a depression on the hemagglutinin surface, whereas the 7-hydroxyl faces the solvent. This model is consistent with Ig-domain studies and site-directed mutagenesis (34, 37), which place the ligand-binding site of sialoadhesin on a contiguous cluster of residues on the surface of the GFCC Cβ-sheet of the V-set Ig-like domain.

Sialic acid modifications that block sialoadhesin binding also block MAG binding. In addition, removal of either the 4- or 7-hydroxyl inhibits MAG binding (Fig. 6). Since the 7-hydroxyl and 8/9-hydroxyl extend in opposite directions (Fig. 7), a more complex model of MAG binding is implicated. One possibility is that the MAG binding site consists of a deep pocket or apposing polypeptide sheets. Alternatively, the 7-hydroxyl group may stabilize a conformation of the oligosaccharide that is preferentially bound by MAG at a single protein surface. To date, no direct evidence addresses whether one or more than one protein surface on MAG is responsible for sialic acid binding, although biophysical and electron microscopic studies suggest that MAG may have a bent rod configuration with apposed Ig-like domains (51, 52). Studies using chimeric molecules indicate that the first three Ig domains of MAG are necessary and sufficient for binding to neurons (45) and sialylglycoconjugates (1), although the sialic acid substructure specificities of truncated forms of MAG have not been reported. Further protein structural and functional studies will be needed to establish the sialylglycoconjugate-binding site on MAG (and on SMP) and to determine the precise role each sialic acid hydroxyl group plays in protein binding.

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