The Proteoglycan Lectin Domain Binds Sulfated Cell Surface Glycolipids and Promotes Cell Adhesion*

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The lecticans are a group of chondroitin sulfate proteoglycans characterized by the presence of C-type lectin domains. Despite the suggestion that their lectin domains interact with carbohydrate ligands, the identity of such ligands has not been elucidated. We previously showed that brevican, a nervous system-specific lectican, binds the surface of B28 glial cells (Yamada, H., Fredette, B., Shitara, K., Hagihara, K., Miura, R., Ranisch, B., Stallec, W. B., and Yamaguchi, Y. (1997) J. Neurosci. 17, 7784–7795). In this paper, we demonstrate that two classes of sulfated glycolipids, sulfatides and HNK-1-reactive sulfoglucuronylglycolipids (SGGLs), act as cell surface receptors for brevican. The lectin domain of brevican binds sulfatides and SGGLs in a calcium-dependent manner as expected of a C-type lectin domain. Intact, full-length brevican also binds both sulfatides and SGGLs. The lectin domain immobilized as a substrate supports adhesion of cells expressing SGGLs or sulfatides, which was inhibited by monoclonal antibodies against these glycolipids or by treatment of the substrate with SGGLs or sulfatides. Our findings demonstrate that the interaction between the lectin domains of lecticans and sulfated glycolipids comprises a novel cell substrate recognition system, and suggest that lecticans in extracellular matrices serve as substrate for adhesion and migration of cells expressing these glycolipids in vivo.

The lecticans are a family of chondroitin sulfate proteoglycans (CSPGs) characterized by the presence of a C-type lectin domain in their core proteins (1, 2). The C-terminal globular domains of lecticans, or “proteoglycan lectin domain” (PLD), consist of one or two epidermal growth factor (EGF)-like domains, a C-type lectin domain, and a complement regulatory protein (CRP) domain. This arrangement of domains is similar to that of selectins, suggesting that lecticans are also involved in the recognition of carbohydrate ligands.

Lecticans are the most abundantly expressed family of proteoglycans in the nervous system. The lectican family includes aggrecan (3), versican (4), neurocan (5), and brevican (6), all of which are expressed in the nervous system at certain stages of development (1, 7). Although aggrecan and versican were initially characterized as connective tissue proteoglycans, their expression in the nervous system has been demonstrated in a number of reports (8–12). Brevican and neurocan are specifically expressed in the nervous system (6, 13–15). Structural similarities with selectins and the abundant expression in the nervous system suggest that lecticans play major roles in carbohydrate recognition in the nervous system.

The identity of the ligand to PLDs has been a focus of our interest. We previously showed that the PLD of versican binds tenascin-R, an extracellular matrix (ECM) protein predominantly expressed in the nervous system (16). More recently, we demonstrated that the PLDs of all lecticans bind tenascin-R, and that brevican and tenascin-R indeed form a complex in adult rat brain extracts (17). These results suggest that the lectican-tenascin-R interactions, especially the brevican-tenascin-R interaction, are relevant to the assembly of the adult brain ECM. However, these interactions are not carbohydrate-protein interactions expected of C-type lectin domains; they are protein-protein interactions between the PLDs and fibronectin type III domains (FNIII) 3–5 of tenasin-R (17). Regarding carbohydrate interactions by PLDs, several studies have demonstrated that PLDs can bind simple sugars and heparin/heparan sulfate in vitro (18–20). However, these studies failed to identify the nature of any physiological carbohydrate ligands for PLDs. Thus the ability of PLDs to behave as C-type lectins in vivo and the identity of physiological carbohydrate ligands for PLDs are issues that have not been addressed.

We have previously shown that purified brevican binds to the surface of primary astrocytes as well as of several immortalized rat neural cell lines (21). Binding studies with B28 glial cells demonstrated that the binding is mediated by the C-terminal 50-kDa fragment of the brevican core protein which includes the PLD (21). It was initially suspected that tenascin-R deposited to the surface of these cells may act as the “receptor” for brevican. However, we have found that these cells do not have any tenascin-R on their surface nor did they secrete any tenascin-R into culture supernatants. Furthermore, a number of assays failed to identify any cell surface protein that specifically binds brevican PLD. Since it has been reported that the lectin domains of P- and L-selectins bind sulfated glycolipids, such as sulfatides (22) and HNK-1-reactive sulfoglucuronylglycolipids (SGGLs) (23), we examined the possibility that the cell surface brevican receptor is a glycolipid
rather than a glycoprotein. In this paper, we report that the PLDs of lecticans bind these sulfated glycolipids. We also show that the interaction between brevican and sulfated glycolipids supports adhesion of cells expressing these glycolipids on their surfaces. These observations suggest that the PLD-sulfated glycolipid interactions are a novel cell substrate recognition system.

**EXPERIMENTAL PROCEDURES**

**Materials**—Mixed bovine brain gangliosides, purified bovine brain sulfatides, and gangliosylceramides were purchased from Matreya (Pleasant Gap, PA). The HNK-1 monoclonal antibody was purchased from Becton Dickinson (Bedford, MA). Mouse monoclonal anti-tenascin-R antibody (clone 596) (24) and monoclonal anti-sulfatide antibody were gifts from Drs. Melitta Schachner (University of Hamburg, Hamburg, Germany) and Yoshio Hirabayashi (RIKEN, Wako, Japan), respectively. The Fc fusion protein of L-selectin (L-selectin Ig chimera) and a HeLa cell line transfected with cDNAs for HNK-1 sulfotransferase, glucuronyltransferase, and N-CAM were obtained from Dr. Minoru Fukuda (Burnham Institute, La Jolla, CA).

**Recombinant Proteins**—A chimeric protein of brevican PLD fused with Fc region of human IgG (brevican PLD chimera) and biotinylated recombinant fragment proteins (rCLDs) of lecticans were prepared as described previously (17). Brevican PLD chimera consisted of a short segment of the central domain, an EGF domain, a lectin domain, and a CDR domain of rat brevican. rCLDs consist only of the lectin domains.

**Preparation of Authentic, Full-length Brevican**—For preparation of recombinant brevican with no fusion partner, CHO cell were stably transfected with an expression vector pcDNA-3-BV, which has a full-length rat brevican cDNA (3.0-kilobase pair EcoRI fragment) (25) inserted into a unique EcoRI site of pcDNAI_amp (Invitrogen, San Diego, CA). For the preparation of radiolabeled probe for TLC-ligand binding assay, cultures of a cloned CHO transfectant were metabolically labeled with 100 μCi/ml Tran-35S-Label (ICN, Costa Mesa, CA). 35S-Labeled or unlabeled brevican was purified from culture supernatants by affinity chromatography on the FNI13–5 fragment of tenascin-R as follows. The glutathione S-transferase fusion protein of the FNI13–5 fragment (17) was bound to glutathione-agarose and covalently mixed with dimethylpimelimidate according to Gersten and Marchalonis (26). Culture supernatants of CHO transfectants were incubated overnight at 4 °C with the FNI13–5 affinity resin prequilliated with TBS containing 5 mM CaCl2. After extensive washing, the labeled brevican was eluted from the affinity resin with 20 mM EDTA.

**Flow Cytometry, Immunoblotting, and Ligand Overlay Assays**—For flow cytometry, cells were dissociated with trypsin-EDTA (Irvine Scientific, Irvine, CA), suspended in 10% fetal calf serum in Dulbecco’s modified Eagle’s medium, and incubated in the medium for 2 h at 37 °C. Cell were then washed three times with PBS containing 0.1% sodium azide, and incubated with PBS containing 1% BSA and 0.1% sodium azide for 30 min on ice. Cells were then incubated with brevican PLD chimera or primary antibodies for 30 min on ice. After washing three times with PBS containing 0.1% sodium azide, the cells were incubated with fluorescein-conjugated goat antibodies to human IgG (Sigma) or to mouse IgG1-IgM (BioSource) for 20 min on ice, washed again, and resuspended in 0.5–1.0 ml of PBS containing 0.1% sodium azide. The cells were then examined on a FACSort (Becton Dickinson, Oxford, CA). Cell surface biotinylation was performed with sulfo-NHS-biotin (Pierce) according to the manufacturer’s instruction. Biotinylated molecules were collected by streptavidin-agarose. Ligand overlay and immunoblotting assays were performed as described previously (17).

**Preparation of Glycolipids**—For preparation of total glycolipid fraction, tissues or cells were extracted by sonication with chloroform/methanol/water (2:1:0.8). After removal of the remaining precipitates by centrifugation, the supernatant was dried under N2 stream. The resulting residue was dissolved in chloroform/methanol (2:1), and then 1/10 volume of 2 M KOH was added. The solution was incubated at 37 °C for 1.5 h, followed by the visualization of reactive bands with chemiluminescence in the case of 35S-labeled brevican, the dried plates were directly exposed to Kodak BioMax film for 3 days.

**Cell Adhesion Assay**—For preparation of substrates, solutions of brevican PLD chimera or human IgG (100 μg/ml) in calcium- and magnesium-free Hank’s balanced salt solution (CMF/HBSS) were applied on nitrocellulose-coated plastic (21), and incubated at 37 °C for 2 h. After washing three times with CMF/HBSS, uncoated surfaces were blocked with incubating with CMF/HBSS containing 2% heat-inactivated BSA (HBSS/BSA) for 2 h at 37 °C. After washing three times with HBSS/BSA, cells suspended in Opti-MEM (Life Technologies, Inc.) containing 0.1% heat-inactivated BSA were plated at a density of 5 × 105 (B28 cells), or 1 × 106 (MDCK cells) per ml, and incubated for 1 h at 37 °C. After gentle washing, attached cells were fixed with 4% paraformaldehyde in PBS and counted under microscope at 200× magnification. For perturbation with antibodies, cells were preincubated with 100 μg/ml HNK-1 or anti-sulfatide monoclonal antibodies diluted in CMF/HBSS/BSA for 30 min on ice prior to the plating of the cells. For perturbation by glycolipids, the substrate of brevican PLD chimera was preincubated with SGLC (sulfate, or galactosylceramide at concentration of 5 μM) in CMF/HBSS/BSA for 1 h at 37 °C before plating of the cells.

**RESULTS**

**Brevican Binds to Cell Surfaces through Non-tenascin-R Binding Sites**—We have shown that the PLD-containing C-terminal 80-kDa fragment of brevican core protein binds to primary astrocytes and B28 cells, which is an immortalized glial cell line. Binding studies with cell monolayers ruled out hyaluronan, heparan sulfate, and chondroitin sulfate as cell surface “receptors” for the brevican C-terminal fragment (21).

To facilitate the identification of the putative brevican receptor, a fusion protein of brevican PLD and Fc region of human IgG (brevican PLD chimera) and a biotin-labeled recombinant lectin domain of brevican (brevican rCLD) were produced. We first examined the binding of brevican PLD chimera to a series of immortalized neural cell lines derived from BDIX rats (30) in flow cytometric assay. Among 16 cell lines tested, brevican PLD chimera bound to B28 cells (Fig. 1A) and four other glial cell lines, namely B9, B15, B49, and B92. The chimera showed no binding to other cell lines with fibroblastic or neuronal phenotypes, including B23 (Fig. 1B), B19, B27, B35, B50, B65, B82, B103, B104, B108, or B111 cells (data not shown). These results suggest that the cell surface binding of the 80-kDa fragment is mediated by the PLD of the brevican core protein, and that the brevican receptor is expressed in various neural cell lines.

The lectin domain of brevican binds tenascin-R by a protein-protein interaction (17). Although tenasin-R is a secreted protein, it is possible that tenasin-R is present on the surface of B28 cells through interaction with cell surface tenascin-R binding proteins (e.g., contactin) or by nonspecific aggregation, thereby acting as an apparent brevican receptor. However, as described previously (22), we demonstrated that the receptors of the surface of B28 cells either by flow cytometric assay or by immunocytochemistry (data not shown). Furthermore, the ligand overlay assay with brevican PLD chimera, which we used to identify tenasin-R as a protein ligand to lectican PLDs in adult rat brain extracts (17), did not detect tenasin-R in surface-biotinylated proteins from B28 cells (Fig. 1C).
Proteoglycan Lectin Domains Bind Sulfated Glycolipids

Having ruled out tenasin-R as the putative receptor for brevican PLD, we next examined the possibility that cell surface glycoproteins carrying specific carbohydrates would be the receptor for brevican PLD, as is the case with selectins. However, the ligand overlay experiment demonstrated that B28 cells lacks not only tenasin-R but also any cell surface proteins that specifically interact with brevican PLD chimera (Fig. 1C), suggesting that there are no glycoprotein ligands for the brevican PLD in B28 cells. We further searched for cell surface brevican-binding proteins in B28 cells by immunoprecipitation of surface-labeled materials and affinity chromatography on a column bearing the 80-kDa brevican fragment that includes PLD. None of these experiments could identify glycoproteins that would specifically bind brevican PLD (data not shown).

The Proteoglycan Lectin Domain Binds Sulfatides and HNK-1-reactive SGGLs—It has been reported that sulfated cell surface glycolipids, sulfatides and HNK-1-reactive SGGLs, bind to the lectin domains of P- and L-selectin (22, 23). Therefore, we investigated the possibility that glycolipids would act as cell surface receptors for brevican PLD. To test this, we prepared glycolipids from the adult rat cerebellum and probed with brevican PLD chimera in the TLC-ligand overlay assay. As shown in Fig. 2A, brevican PLD chimera reacted with a single band (lane 2) among a number of glycolipid species extracted from the cerebellum (lane 1). The reactive band migrated at the same position as purified sulfatides (compare panel A, lane 2 with panel B, lane 1), suggesting that the band represents sulfatides. To further identify this reactive band, standard glycolipids were examined by TLC-ligand overlay assay (Fig. 2, B–F). The brevican PLD chimera bound to purified sulfatides (C, lane 1), but not to either the neutral glycosphingolipids (lane 2) or gangliosides (lane 3). Binding to sulfatides was abolished in the presence of EDTA (D, lane 1), as expected of a C-type lectin interaction. The brevican PLD chimera did not bind galactosylceramide, an unsulfated precursor of sulfatides (indicated by asterisk in B, lane 2), suggesting that a sulfate group is necessary for binding. L-selectin Ig chimera also bound to sulfatides, but not to other glycolipids (E), consistent with previous reports (23). Human IgG did not bind any glycolipids (F).

The brevican PLD chimera contains not only the C-type lectin domain but also EGF and CRP domains flanking the lectin domain (17). To examine the location of the sulfatide binding site, the binding of biotinylated recombinant protein consisting of only the lectin domain (brevican rCLD) was examined. Like the PLD chimera, brevican rCLD specifically bound to sulfatides (Fig. 3E, lane 1), but not to neutral glycolipids, including galactosylceramide, or to gangliosides (Fig. 3E, lane 2). Moreover, rCLDs of aggrecan, neurocan, and versican also showed specific binding to sulfatide (Fig. 3, B–D). All of these interactions were completely suppressed in the presence of EDTA (data not shown). These results show that the C-type lectin domain of all four lecticans bind sulfatides in a divalent cation-dependent manner.

Considering that L- and P-selectins bind another class of sulfated glycolipid, HNK-1-reactive SGGLs (23), we tested the binding of PLDs to the HNK-1-reactive SGGLs. A sample of dog sciatic nerve-derived SGGLs consisting of roughly equal amounts of SO$_3$-GlcU-nLc-4-Cer and SO$_3$-GlcU-nLc-6-Cer was tested for binding to brevican PLD chimera (Fig. 4). The brevican PLD chimera bound to both SO$_3$-GlcU-nLc-4-Cer and SO$_3$-GlcU-nLc-6-Cer (lane 3). The binding was EDTA-sensitive (lane 4). Consistent with previous reports, L-selectin Ig chimera bound SGGLs (lane 5), but human IgG did not (lane 6). These results demonstrate that PLD binds HNK-1-reactive SGGLs.

HNK-1-reactive carbohydrates are present on several glycoproteins as well as on glycolipids (31, 32). Thus, there remained a possibility that glycoproteins carrying HNK-1-reactive glycans could bind PLDs and act as PLD receptors. To address this question, we performed two experiments. First, we prepared extracts containing large amounts of HNK-1-positive glycoproteins from E19 mouse brain. These brain extracts indeed contained several protein bands intensely reactive with the HNK-1 antibody in immunoblotting (Fig. 5A, lane 1). We probed this sample with brevican PLD chimera in ligand overlay assay. Brevican PLD chimera, while it efficiently bound to tenasin-R in adult brain extracts (see Fig. 1C, lane 4), did not bind any of these HNK-1-positive glycoproteins (Fig. 5A, lane 2). Second, we examined by flow cytometry the binding of brevican PLD chimera to a HeLa-derived cell line that expresses HNK-1 carbohydrates only on glycoproteins, not on glycolipids. This cell line has been transfected with cDNAs for N-CAM, a glucu-
The glucuronyltransferase acts only on glycoprotein glycans and not on glycolipids (33). Because the parental HeLa cells express no HNK-1-reactive SGGLs, all of the HNK-1 carbohydrates expressed by these cells are contained on glycoproteins, mainly on N-CAM.2 Flow cytometric analysis confirmed that these cells express high levels of cell surface HNK-1 carbohydrates (Fig. 5B, a). Despite this, these cells do not bind brevican PLD chimera at all (Fig. 5B, b). In contrast, B28 cells, which express HNK-1-reactive SGGL (see below), show strong binding of brevican PLD chimera (see Fig. 1). Although these results do not entirely rule out the possibility that HNK-1 carbohydrates carried by glycoproteins could be recognized by PLD under some conditions, they demonstrate that HNK-1 carbohydrates attached to glycoproteins are not recognized by PLDs as efficiently as HNK-1-reactive SGGLs.

**Intact Brevican Also Binds Sulfated Glycolipids—**We next examined whether intact, full-length brevican, not just the recombinant PLD fragment, binds these sulfated glycolipids. To test this, we isolated 35S-labeled brevican from culture supernatants of CHO cells transfected with full-length rat brevican cDNA, and used it as a probe in the TLC-ligand overlay assay. As shown in Fig. 6, native brevican bound to both sulfatides and SGGLs (lane 1). No binding to neutral glycosphingolipids or gangliosides was found (lane 2). The glycolipid binding by brevican was inhibited with EDTA (lane 3), as was the case with the PLD chimera and the rCLDs. These results demonstrate that not only recombinant PLD fragment

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2 M. Fukuda, personal communication.
but also intact brevican binds sulfated glycolipids.

**Binding of Brevican PLD Chimera to B28 Cells Is Mediated by HNK-1-reactive Glycolipids**—To determine if the cell surface binding of brevican observed with the B28 cells (see Fig. 1A) (21) is indeed mediated by sulfated glycolipids, we examined whether these cells contain sulfatides and/or SGGLs. Analysis of an acidic glycolipid fraction from B28 cells demonstrated that B28 cells express HNK-1-reactive SGGLs but not sulfatides (Fig. 7A, lanes 2 and 3). Brevican PLD chimera binds B28 cell-derived SGGLs (lane 4). B, binding of brevican PLD chimera to B28 cells is inhibited by HNK-1 antibody. B28 cells that had been preincubated with or without the HNK-1 antibody, were incubated with brevican PLD chimera, followed by staining with fluorescein-conjugated anti-human IgG and analysis by flow cytometry. Solid line, binding of brevican PLD chimera to untreated B28 cells; broken line, binding of brevican PLD chimera to B28 cells pretreated with the HNK-1 antibody; dotted line, staining with fluorescein-conjugated secondary antibody alone. Note that the HNK-1 monoclonal antibody abolished the binding of brevican PLD chimera to B28 cells (broken line).
shown to express sulfatides (35), on substrates of brevican PLD. The expression of sulfatides by MDCK cells was confirmed by flow cytometric assay and TLC-immunoblotting with anti-sulfatide monoclonal antibody (data not shown). The brevican PLD chimera was adsorbed to nitrocellulose-coated dishes, and cell adhesion to the substrate was quantitatively analyzed. Both the B28 and MDCK cells adhered to the brevican PLD substrate (Figs. 8, panels A1 and B1) but not to a human IgG substrate (panels A5 and B5). Pretreatment of cells with the HNK-1 antibody (panel A2) or anti-sulfatide antibody (panel B2) significantly reduced the number of cells that adhered to the brevican PLD substrate. This adhesion was also inhibited by pretreatment of the brevican PLD substrate with purified SGGLs (panel A3) or sulfatides (panel B3) before plating of cells. Pretreatment of the substrates with galactosylceramides did not have any effect on the adhesion of either cell type (panels A4 and B4). Quantitative analysis of these results is shown in Fig. 8 (C and D). These results demonstrate that the PLD-glycolipid interactions can support cell adhesion, and suggest that brevican present in the brain ECM may serve as an adhesive substrate for neural cells expressing HNK-1-reactive SGGLs or sulfatides.

FIG. 8. Brevican PLD-glycolipid interactions support cell adhesion. A, adhesion of B28 cells to the substrate of brevican PLD chimera. Cells were plated on the substrate of brevican PLD chimera (panels 1–4) or human IgG (panel 5). In panel 2, cells were preincubated with 100 µg/ml HNK-1 monoclonal antibody before plating. In panels 3 and 4, substrates were preincubated with 5 µg/ml SGGLs or 5 µg/ml galactosylceramide, respectively, before plating. Photographs were taken 1 h after plating. B, adhesion of MDCK cells to the substrate of brevican PLD chimera. The arrangement of the experiment was the same as in A, except that cells were preincubated with anti-sulfatide monoclonal antibody instead of HNK-1 monoclonal antibody in panel 2, and with sulfatide instead of SGGLs in panel 3. C and D, quantitation of cell adhesion. Cell adhesion assays were performed as described above. Number of adhered cells per field was counted under 200× magnification. Results represent the mean ± S.D. of adhered cells from experiments in quadruplicate. Experimental arrangement in columns 1–5 corresponds to those of panels 1–5 in A and B. Bre, brevican PLD chimera; hlgG, human IgG; HNK-1, HNK-1 monoclonal antibody; αSulf, anti-sulfatide monoclonal antibody; Sulf, sulfatides; GalC, galactosylceramide.

DISCUSSION

In this paper, we demonstrate that the PLDs of the lectican family proteoglycans bind two types of sulfated cell surface glycolipids, sulfatides and HNK-1-reactive SGGLs, in a divalent cation-dependent manner. Not only the Ig chimera of the brevican PLD but also the intact brevican core protein possesses the same binding activity. These interactions support the adhesion of sulfatide- or SGGL-expressing cells.

Previous reports on the carbohydrate interaction of PLDs have demonstrated that PLDs are capable of binding simple carbohydrates, including fucose, galactose, and N-acetylgalcosamine (16, 18, 19), and heparin/heparan sulfate (20). However, these studies failed to provide data indicating biological significance of the carbohydrate interactions or to identify physiological ligands carrying these carbohydrate determinants. The present study for the first time identifies sulfatides and SGGLs as physiologically relevant carbohydrate ligands for PLDs.

The PLDs of lecticans share several binding properties with selectins. Like PLDs, P- and L-selectins have been shown to bind sulfatides and HNK-1-reactive SGGLs (22, 23, 36). Both the PLD-sulfated glycolipid and the selectin-sulfated glycolipid interactions are divalent cation-dependent. The presence of sulfate residues on terminal sugars is required for binding (22, 23, 36). On the other hand, selectins and PLDs bind differently to gangliosides, another class of acidic glycolipids containing sialic acids. P- and L-selectins have been shown to bind not only sulfated glycolipids but also gangliosides (37, 38), whereas we showed that brevican PLD does not bind gangliosides. This suggests that the mere presence of negative charges is not sufficient for the binding of brevican PLD to sulfated glycolipids.

A noteworthy property of PLDs is that they do not recognize glycoproteins carrying HNK-1 carbohydrates (see Fig. 5), whereas they do bind to HNK-1 carbohydrates carried by a glycolipid. There are two possible explanations for this selective binding. PLDs may recognize not only the terminal sialoglycosylactosamine, the structure that is shared by HNK-1-reactive glycolipids and glycoprotein glycans, but also additional sugar residues of glycolipid chains. In this case, it would be necessary for the PLD to recognize more than four sugar residues to distinguish between HNK-1-reactive glycolipids and glycoproteins. This seems unlikely, because functional groups of carbohydrate determinants interacting with selectins are restricted to the three terminal sugar residues (39, 40). It seems more likely that the low density of HNK-1 carbohydrates on neural glycoproteins limits their ability to
bind PLDs. In fact, this situation would be similar to what has been encountered with selectins. Ordinary glycoproteins, even if they carry a selectin-binding carbohydrate determinant such as sialyl Lewisx, are weak selectin binders; the presence of clusters of the carbohydrate determinant on a polypeptide backbone is required for efficient selectin binding (41, 42). As a result, essentially all known glycoprotein ligands for selectins are mucin-type glycoproteins that contain clusters of O-linked glycans (43–46). On the other hand, neural glycoproteins that carry HKN-1 carbohydrates are not mucin-type glycoproteins, and therefore presumably are incapable of sustaining high affinity interactions. Although it is possible for PLDs to bind to HNK-1-reactive oligosaccharides when presented at a high density, it is unlikely that neural glycoproteins carrying HKN-1 carbohydrates are not mucin-type glycoproteins, though this situation would be similar to what has been shown that brevican can inhibit neurite outgrowth from these cells (56). Despite these observations, the identity of the SGGL/sulfatide-binding moiety is responsible for this activity (6). However, it should be noted that lecticans do not always carry chondroitin sulfates. One of the versican splicing variants expressed mainly in the brain lacks domains that contain glycosaminoglycan attachment sites (64). Brevican is a so-called part-time proteoglycan and roughly 20–50% of brevican molecules isolated from whole brain are devoid of chondroitin sulfates (6, 7). Thus, our present findings suggest the possibility that brevican may act as a bifunctional substrate for cell adhesion, depending on whether it contains chondroitin sulfate chains. Such a putative dual effect on cell adhesion suggests that the regulation of chondroitin sulfate chain synthesis could serve as a biological switch determining whether a CSPG is inhibitory or promoting to cell adhesion.

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