Comparison of the Specificities of Laminin, Thrombospondin, and von Willebrand Factor for Binding to Sulfated Glycolipids

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The adhesive glycoproteins laminin, thrombospondin, and von Willebrand factor bind specifically and with high affinity to sulfated glycolipids. These three glycoproteins differ, however, in their sensitivity to inhibition of binding by sulfated monosaccharides and polysaccharides. Heparin strongly inhibits binding of thrombospondin but only weakly inhibits binding of laminin and von Willebrand factor. Fucoidan strongly inhibits binding of both laminin and thrombospondin but not of von Willebrand factor. Laminin shows significant specificity for inhibition by monosaccharides, whereas thrombospondin does not. Thus, specific spatial orientations of sulfate esters may be primary determinants for the three proteins.

Laminin, thrombospondin, and von Willebrand factor also differ in their relative binding affinities for purified sulfated glycosphingolipids. The three proteins strongly prefer terminal-sulfated lipids and bind only weakly to sulfated gangliotriaosyl ceramide with a sulfated buton the penultimate galactose. Thrombospondin binds with highest affinity to galactosyl sulfatide but only weakly to more complex sulfatides, whereas von Willebrand factor prefers galactosyl sulfatide but binds with moderate affinity to various sulfated glycolipids. Laminin is also less selective than thrombospondin but is less sensitive for detection of low sulfatide concentrations. Galactosyl sulfatide at 1–5 pmol can be detected by staining of lipids separated on high performance TLC with 125I-thrombospondin or 125I-von Willebrand factor. 125I-von Willebrand factor was examined as a reagent for detecting sulfated glycolipids in tissue extracts. Rat kidney lipids contain 5 characterized sulfated glycolipids: galactosyl ceramide P-sulfate, lactosyl ceramide II-sulfate, gangliotriaosyl ceramide II-sulfate, and bis-sulfated gangliotriaosyl and gangliotetraosyl ceramides. von Willebrand factor detects all of these sulfatides as well as several additional minor sulfated lipids. Complex monosulfated lipids are detected in several human tissues including kidney, erythrocytes, and platelets by this technique.

The three adhesive proteins laminin, thrombospondin, and von Willebrand factor bind specifically to sulfatides but not to other anionic glycolipids, phospholipids, or cholesterol 3-sulfate (1–3). Based on these data, binding to sulfatides is not a simple ionic interaction. It is not clear, however, what determinants are recognized for binding by each protein. The binding of thrombospondin and von Willebrand factor to sulfatides differ in their sensitivity to inhibition by polysaccharides (2, 3). Heparin and fucoidan are the best inhibitors of thrombospondin binding, whereas a high molecular weight dextran sulfate is the best inhibitor of von Willebrand factor binding. In contrast, little specificity was observed by comparing inhibition of thrombospondin binding by monosaccharide sulfates, phosphates, a uronic acid, and simple inorganic anions (2).

To clarify the basis for specificity of binding to sulfatides, two approaches were taken. Polysaccharides and monosaccharides were tested as inhibitors of laminin binding to sulfatides to allow a comparison of inhibitory activities among the three proteins, and direct binding to several glycolipids differing in oligosaccharide structure and position of sulfation was measured using all three proteins. von Willebrand factor was also examined as a reagent for detecting sulfated lipids in tissue extracts because of its high sensitivity and lower relative specificity for oligosaccharide structure. Staining of thin layer chromatograms with 125I-von Willebrand factor detects the known sulfatides and, in some tissues, reveals previously undetected sulfated lipids of unknown structure.

EXPERIMENTAL PROCEDURES

Materials—Laminin was purified from 0.5 m NaCl extracts of mouse Engelbreth Holm Swarn tumor by DEAE-cellulose chromatography (4) and 4 m NaCl precipitation. Calcium-replete thrombospondin was purified from the supernatant of thrombin-activated human platelets (5). von Willebrand factor was purified from human cryoprecipitate by chromatography on Sepharose 4B (6). Thrombospondin and protein A (Pharmacia) were labeled with Na125I (ICN) by the IODO-GEN method (2, 7) to specific activities of 10 and 50 μCi/μg, respectively. von Willebrand factor was iodinated using immobilized lactoperoxidase (6, 8, 9). Bovine serum albumin (A7030, fatty acid and globulin free), dextran sulfates, fucoidan, colominic acid (Escherichia coli), glucose 6-sulfate, ascorbate 2-sulfate, cholesterol (grade 1, 99%), cholesterol 3-sulfate, and most monosaccharide phosphates were from Sigma. Heparin (160 units/mg) was from The Upjohn Co. Hyaluronic acid (bovine vitreous humor) was obtained from Worthington. Monosulfates of galactose and glucose were generously provided by Dr. Alexander Roy (The Australian National University, Canberra City, Australia). Methyl-α-D-glucosamine 2N,3O-bis-sulfate and methyl-α-D-glucosamine 3-sulfate was provided by Dr. Irwin Leder (National Institutes of Health). The latter compound was N-acetylated and purified by gel filtration on Bio-Gel P-2. All anionic sugars were converted to sodium or potassium salts for use in inhibition studies.

Glycolipids—Acidic lipid fractions from sheep and human erythrocytes and human platelets were prepared as previously described (1, 2). Acidic lipid fractions from hog gastric mucosa were prepared as described (10) except that drying with acetone was omitted. A fresh hog stomach was obtained from the National institutes of...
Health Animal Farm (Foolsville, MD). Acidic lipid fractions and purified sulfated glycolipids were prepared from rat kidneys (Sprague-Dawley, Pel-Freez) according to Tadano and Iahizuka (11-13). Identities of the purified lipids were confirmed by comigration on thin layer chromatography in neutral and acidic solvents with the authentic lipids generously provided by Dr. I. Iahizuka (Teikyo University School of Medicine, Japan). Acidic lipid fractions from human meconium and mouse intestine were generously supplied by Dr. J. Magnani (National Institutes of Health) and Dr. G. Hansson (University of Göteborg, Sweden), respectively. Triglyceroalkylacylglycerol III-sulfate was prepared by Dr. B. Slomiany (New York Medical College). Lactosyl ceramide I'-sulfate was isolated from human kidney obtained at autopsy as described by Martensson (14). Galactosyl ceramide I'-sulfate was prepared by direct sulfation of galactosyl cerebroside (15, 16). Galactosyl cerebroside (Sigma, 50 mg) in 0.75 ml of dry pyridine and 10 mg of sulfur trioxide pyridine complex (Aldrich) were stirred at 37 °C for 1 h. The reaction mixture was precipitated with acetone and purified by chromatography on Bio-Sil HA with a gradient from chloroform/methanol, 96:4, to chloroform/methanol, 2:1. Fractions free of contaminating 2-, 3-, or 4-monosulfates and bis-sulfated lipid were combined. Lack of contaminating 3-sulfate in the purified lipid was confirmed by quantitative cleavage on periodate oxidation (17). Concentrations of galactosyl ceramide I'-sulfate and galactosyl ceramide I'-sulfate (bovine brain, Supelco) were determined by dry weight. Other sulfated lipids were determined by the dye-binding assay of Kean (18) as modified by Tadano-Aritomi and Iahizuka (19).

Methods—Binding of laminin, thrombospondin, and von Willebrand factor to lipids separated on thin layer chromatograms was performed as previously described (1-3). The ionic strength of the buffers was 0.15 for thrombospondin and von Willebrand factor and 0.22 for laminin. To quantitate binding to reference lipids, autoradiograms were prepared at various exposures, and binding was determined by densitometry (QuickScan, Helena Laboratories) in the linear range. Galactosyl ceramide I'-sulfate at two concentrations was included as an internal standard on each chromatogram.

Inhibition studies of laminin binding to sulfatides were done by solid phase assays on 96-well flexible microtiter plates (Falcon 3912) as previously described (1). Wells were coated with 75 ng of bovine brain sulfatide and 30 ng of cholesterol by drying from methanol. All inhibitors were dissolved in isotonic Tris-BSA(1), and the pH was adjusted to 7.8 where necessary by addition of NaOH. Binding of laminin (10 μg/ml) in the presence of inhibitors was determined in triplicate both on sulfatide-coated wells and on uncoated wells. Specific binding (typically 20-35% of added radioactivity in the absence of inhibitors) was calculated by subtraction of nonspecific binding (2-5% of added radioactivity) determined at each inhibitor concentration.

RESULTS

Inhibition of Laminin Binding to Sulfatides by Carbohydrates—The inhibition of laminin binding to immobilized sulfatides by polysaccharides and monosaccharides was examined using the solid phase microtiter plate assay. The activities of various anionic polysaccharides as inhibitors of sulfatide binding are summarized in Table I. For comparison, inhibition data for thrombospondin (2) and von Willebrand factor (3) are also presented. The sulfated fucan, fucoidan, is a good inhibitor, although the enhancement of nonspecific binding obtained in the presence of this inhibitor limits the reliability of the I<sub>50</sub> value. Other sulfated polysaccharides inhibit weakly or are inactive. Hyaluronate and colominic acid, an α2-8-linked polymer of sialic acid, are also inactive.

Data for inhibition of binding by simple anions and by

![image](image-url)

TABLE I

| Inhibition by anionic polysaccharides of the binding of laminin, thrombospondin, and von Willebrand factor to sulfatides |
|---------------------------------------------------------------|
| **Inhibitor** | **Protein** | **I<sub>50</sub> mg/ml** |
|----------------|-------------|--------------------------|
|                | Laminin     | TSP                      | vWF                      |
| Fucoidan       | 4           | 0.3                      | 160                      |
| Dextran sulfate (M, 500,000) | 300 | 2.2                      | >1000                    |
| Dextran sulfate (M, 5000)       | 120         | 28                       | >1000                    |
| Heparin        | 600         | 10                       | >1000                    |
| Keratan sulfate C4     | 300         | >1000                    | 700                      |
| Keratan sulfate 2A     | >1000       | ND                       | >1000                    |
| Chondroitin sulfate   | >1000       | >1000                    | >1000                    |
| Hyaluronate         | >1000       | 140                      | >1000                    |
| Colominic acid      | >1000       | ND                       | ND                       |

* Concentration giving 50% inhibition of protein binding to 75, 200, or 250 ng of sulfatide/well for laminin, thrombospondin (TSP), and von Willebrand factor (vWF), respectively. Most data for thrombospondin and von Willebrand factor are taken from Refs. 2 and 3.

** Non-specific binding of laminin to albumin-coated wells was enhanced by high molecular weight dextran sulfate. This value may be falsely low.

* ND, not determined.

TABLE II

| Inhibition of the binding of laminin to sulfatides |
|--------------------------------------------------|
| **Inhibitor** | **I<sub>50</sub> mM** |
|----------------|-----------------------|
| Monosaccharide sulfates | | |
| D-Gal-2-SO<sub>4</sub> | 25 |
| D-Gal-3-SC| 27 |
| D-Gal-4-SC | 22 |
| D-Gal-6-SC | 31 |
| D-Glc-2-SC | 18 |
| D-Glc-3-SC | 31 |
| D-Glc-4-SC | 31 |
| D-Glc-6-SC | 28 |
| Methyl-a-D-GlcNAc-3-SC | 8 |
| Methyl-a-D-GlcNac-2,3-bis-SC | 13 |
| L-Ascorbate-2-SC | 10 |
| Other anions and sugars | | |
| CI<sup>-</sup> | 120 |
| SO<sup>-</sup> | 27 |
| Cyclohexylsulfamate | 30 |
| D-Glc-1-P | 14 |
| D-Glc-6-P | 44 |
| D-Man-6-P | 16 |
| D-Fru-1-P | 23 |
| D-Fru-6-P | 22 |
| D-Galacturonate | 24 |
| D-Gal | >200 |
| D-Glc | >200 |

* Concentration giving 50% inhibition of laminin binding (10 μg/ml) to 75 mg of sulfatide in the solid phase radioassay (1).

neutral and anionic monosaccharides are summarized in Table II. Increasing the ionic strength by addition of chloride inhibits binding by 50% at 120 mM above isotonic conditions. Sulfate is 4.4-fold more potent than chloride. Whereas galactose and glucose are not inhibitory, most anionic sugars are more active than expected from their contribution to the ionic strength. The most potent inhibitor of those compounds tested is methyl-a-D-GlcNAc 3-sulfate which is 16-fold more potent than chloride anion.

Binding of Laminin, Thrombospondin, and von Willebrand Factor to Sulfated Glycolipids—In previous studies, laminin, thrombospondin, and von Willebrand factor bound with higher affinity to galactosyl ceramide I'-sulfate than to cholesterol 3-sulfate. Many other sulfated glycolipids have been reported in higher animals (10-14), several of which bind 125I-thrombospondin using the chromatogram binding assay (Fig.
Thrombospondin binds to gangliotetraosyl ceramide 1'-sulfate, gangliotriaosyl ceramide II',III'-bis-sulfate, and gangliotetraosyl ceramide II',IV'-bis-sulfate isolated from rat kidney, although higher concentrations of these lipids are required than of galactosyl ceramide 1'-sulfate to detect binding. Staining of a mixture of sulfated lipids from mouse intestine (Fig. 1) revealed a slow migrating lipid recently identified as gangliotetraosyl ceramide IV'-sulfate (20) as well as mono- and dihexosyl sulfatides. In contrast, triglucosyl alkylacylglycerol III'-sulfate is not stained, although contaminating monohexosyl sulfatide in this preparation is labeled (Fig. 1). Thus, thrombospondin binds to some but not all sulfated glycolipids, and the avidity of binding may depend on oligosaccharide structure.

The binding of thrombospondin to increasing concentrations of naturally occurring sulfated glycolipids and synthetic galactosyl ceramide 1'-sulfate was quantified by densitometric analysis of autoradiograms (Fig. 2). Relative affinities derived from these experiments and from analogous experiments using laminin and von Willebrand factor are presented in Table III. All three proteins bound with highest affinity to galactosyl ceramide 1'-sulfate. Thrombospondin was most specific for the monohexosyl sulfatides as lactosyl ceramide 1'-sulfate was 10-fold less active and higher sulfated lipids were even weaker. The other proteins showed similar but less extreme decreases in affinity with increasing oligosaccharide size. In contrast, thrombospondin was least specific for sulfation on the 3-position of galactose. Synthetic galactosyl ceramide 1'-sulfate was applied for galactosyl ceramide 1'-sulfate quantification by densitometric analysis of autoradiograms exposed in the linear range. Integrated peak areas are plotted as a function of the amount of lipid applied for galactosyl ceramide 1'-sulfate (●), lactosyl ceramide-1'-sulfate (○), gangliotriaosyl ceramide II'-sulfate (■), a mixture of gangliotetraosyl ceramide-II',III'-bis-sulfate and gangliotetraosyl ceramide-II',IV'-bis-sulfate (□) and galactosyl ceramide-1'-sulfate (▲).

![Fig. 1. Binding of 125I-thrombospondin to sulfated glycolipids separated on thin-layer chromatograms. Lipids were chromatographed on aluminum-backed silica gel high performance TLC plates (E. Merck) in chloroform/methanol/0.2% aqueous CaCl₂, 60:35:7. The chromatograms were air dried, soaked for 1 min in 0.1% polysobutylmethacrylate in hexane (Polysciences, Inc.), dried, sprayed with phosphate-buffered saline, and immersed in Tris-BSA (60 μl/cm²) and incubated in a covered Petri dish for 3 h at 4 °C. The chromatogram was washed by dipping in 5 changes of cold phosphate-buffered saline at 1-min intervals, dried, and exposed to x-ray film (XAR-5, Eastman Kodak) for 8–24 h. Glycolipids on duplicate plates were visualized by spraying with orcinol-H₂SO₄. Left panel, staining of sulfated lipids with 125I-thrombospondin (10 μCi/μg) in Tris-BSA (60 μl/cm²) and incubated in a covered Petri dish for 3 h at 4 °C. The chromatogram was washed by dipping in 5 changes of cold phosphate-buffered saline at 1-min intervals, dried, and exposed to x-ray film (XAR-5, Eastman Kodak) for 8–24 h. Glycolipids on duplicate plates were visualized by spraying with orcinol-H₂SO₄, and stained with orcinol-H₂SO₄. Left panel, staining of sulfated lipids with 125I-thrombospondin (2 μCi/μg) in Tris-BSA (60 μl/cm²) and incubated in a covered Petri dish for 3 h at 4 °C. The chromatogram was washed by dipping in 5 changes of cold phosphate-buffered saline at 1-min intervals, dried, and exposed to x-ray film (XAR-5, Eastman Kodak) for 8–24 h. Glycolipids on duplicate plates were visualized by spraying with orcinol-H₂SO₄.)

![Fig. 2. Binding of 125I-thrombospondin to purified sulfated glycolipids. Lipids were chromatographed on silica gel high performance TLC plates. The dried chromatograms were stained with 125I-thrombospondin as described in Fig. 1. Binding was quantified by densitometry of autoradiograms exposed in the linear range. Integrated peak areas are plotted as a function of the amount of lipid applied for galactosyl ceramide 1'-sulfate (●), lactosyl ceramide-1'-sulfate (○), gangliotriaosyl ceramide II'-sulfate (■), a mixture of gangliotetraosyl ceramide-II',III'-bis-sulfate and gangliotetraosyl ceramide-II',IV'-bis-sulfate (□) and galactosyl ceramide-1'-sulfate (▲).]

| Protein          | Lipid             | Relative affinity* |
|------------------|-------------------|--------------------|
| Thrombospondin   | GalCer-1'-SO₄     | 1.00 (at 30 pmol)  |
|                  | GalCer-1'-SO₄     | 0.92               |
|                  | LacCer-1'-SO₄     | 0.10               |
|                  | GgOse₃Cer-1'-SO₄  | ≤0.009             |
|                  | GgOse₃Cer-1',1',1'-SO₄ | 0.043          |
|                  | GgOse₃Cer-1',1',1'-SO₄ | 0.13          |
| von Willebrand factor | GalCer-1'-SO₄ | 1.00 (at 100 pmol) |
|                  | GalCer-1'-SO₄     | 0.17               |
|                  | LacCer-1'-SO₄     | 0.20               |
|                  | GgOse₃Cer-1',1'-SO₄ | 0.03          |
|                  | GgOse₃Cer-1',1',1'-SO₄ | 0.13          |
| Laminin          | GalCer-1'-SO₄     | 1.00 (at 100 pmol) |
|                  | GalCer-1'-SO₄     | 0.29               |
|                  | LacCer-1'-SO₄     | 0.12               |
|                  | GgOse₃Cer-1',1'-SO₄ | 0.06          |
|                  | GgOse₃Cer-1',1',1'-SO₄ | 0.06          |

* Binding of each protein to galactosyl ceramide 1'-sulfate was assigned a value of 1.00 at the indicated concentrations of lipid. Relative affinities for other lipids were calculated from the number of moles of test lipid required to give identical binding as to the indicated quantity of galactosyl ceramide 1'-sulfate.

was 52% as active as the natural 3'-sulfate for thrombospondin binding, but only 17 and 29% as active for von Willebrand factor and laminin binding, respectively.

**Binding of 125I-von Willebrand Factor to Tissue Sulfatides**—Based on its lower relative specificity for oligosaccharide structure and high sensitivity for detection of sulfatides (3), von Willebrand factor was examined as a reagent for detection.
of sulfated glycolipids in tissue extracts. \(^{125}\)I-von Willebrand factor was shown previously to label only sulfatides on chromatograms of lipid extracts from sheep and human erythrocytes and human platelets (3). Autoradiograms detecting binding to acidic lipids from these and other tissues are presented in Fig. 3. Sulfated lipids detected in sheep and human erythrocytes (lanes 1, 2, 5, and 6) have been discussed previously (3). Four sulfated lipids have been isolated and characterized from rat kidney (11-13). Staining of a monosulfated fraction of alkali-stable rat kidney lipids (lanes 9 and 11) reveals high concentrations of galactosyl sulfatide, some dihexosyl sulfatide, faint staining of gangliotriaosyl ceramide II'-sulfate, and staining of one and possibly a second characterized lipid migrating slower than NeuAca2-3Galp1-4GlcCer. Both known bis-sulfated lipids are stained by von Willebrand factor (lane 10) and orcinol (lane 12). Another uncharacterized lipid of low mobility is stained with von Willebrand factor (lane 10). The fastest migrating bands faintly stained in lane 10 are not glycolipids based on yellow to brown colors given with the orcinol reagent.

Whereas monosulfated lipids were detected in many tissues, the bis-sulfated lipids of rat kidney were not detected in any of the other tissues examined (results not shown). Human kidney contained three labeled bands (lane 13) probably corresponding to mono-, di-, and trihexosyl sulfatides. Even at high loading (lane 14) no other lipids were detected. Galactosyl sulfatide and lactosyl ceramide II'-sulfate have been characterized from human kidney (14). The structure of the trihexosyl lipid is unknown. Lipids of similar mobility to the trihexosyl sulfatide of kidney are also stained in human platelets at high loading (lane 16) and in human meconium (lane 4) suggesting that complex sulfated lipids are present in many human tissues. Two slow-migrating lipids are also stained in hog gastric mucosa (lane 17). These may correspond to the lactotriaosyl and ceramide III'-sulfate and lactoneotetraosyl ceramide III'-sulfate identified in this tissue by Slomiany and co-workers (10, 21).

**DISCUSSION**

Comparison of laminin, thrombospondin, and von Willebrand factor using quantitative inhibition and glycolipid binding assays indicates that the three sulfatide-binding proteins differ in their specificities. Each protein exhibits characteristic sensitivities to inhibitors and relative binding avidities for different sulfated glycolipids. In no case can we conclude, however, that the galactose 3-sulfate residue on the sulfatide molecule is specifically recognized.

A surprising result is that galactosyl ceramide I'-sulfate is the best glycolipid ligand for all three proteins (Table III). The specificity relative to the synthetic galactosyl ceramide-I'-sulfate is moderate, ranging from 2-fold for thrombospondin to almost 6-fold for von Willebrand factor. In contrast, the presence of additional sugar between galactose 3-sulfate and the ceramide as in lactosyl sulfatide decreases binding from 5- to 10-fold. The longer chain lipids examined, even those with two sulfate esters, are even weaker. Furthermore, addition of a terminal N-acetylgalactosamine to lactosyl sulfatide reduced binding from 2- to 11-fold. Thrombospondin...
also did not bind to the sulfated triglucosylglycerolipid which has terminal 6-sulfate (22) (Fig. 1). Thus, the three proteins prefer sulfated glycolipids with sulfate esters on terminal nonreducing residues over lipids with internal sulfate esters. Proximity of the sulfate ester to the ceramide is also strongly preferred, even though the accessibility of the sulfate esters to the proteins probably increases with increasing distance from the lipid. Preference for galactosyl sulfatide could be due to specificity for a conformation of the sugar which occurs only in the simple sulfatide or could indicate specific interactions between the proteins and the ceramide head groups as has been proposed for antibodies to seminolipid (23). Since interactions including hydrogen bonding between galactose and the head group of the ceramide may stabilize the active conformation of the sugar (24), these two possibilities will be difficult to distinguish.

Comparison of polysaccharide inhibition for the three proteins also reveals differences in specificity (Table I). Laminin and thrombospondin are similar in that fucoidan is the best inhibitor and the order of activities of most inhibitors is the same. Heparin, however, is a potent inhibitor of thrombospondin but not of laminin binding. Heparin is also a potent inhibitor of hemagglutination by thrombospondin, and monoclonal antibodies to the amino-terminal heparin-binding domain block hemagglutination and binding of thrombospondin to sulfatides (12, 29). In contrast, studies with proteolytic fragments of laminin suggest that the heparin-binding domain of laminin is distinct from the sulfatide-binding domains (1, 26). von Willebrand factor behaves differently from the other proteins. Fucoidan is relatively weak and heparin is inactive as an inhibitor of binding to sulfatides (3). Instead, high molecular weight dextran sulfate is the only potent inhibitor for von Willebrand factor binding of those tested.

Monosaccharide and anion inhibition of thrombospondin binding has been reported previously (2). The best inhibitor, methyl-α-D-GlcNAc 3-sulfate is only 3.7-fold more active than Cl−. Most sulfated sugars were no more potent than expected based on their contribution to the ionic strength. The lack of specificity among galactose sulfates is consistent with the present finding that thrombospondin does not strongly prefer galactosyl ceramide 1′-sulfate over the 6-sulfate isomer. Laminin may be somewhat more specific since monosaccharides are up to 15-fold better inhibitors than Cl−. The structures of the most active inhibitors, however, seem quite unrelated, and galactose 3-sulfate is not one of the best inhibitors. Galactose 6-sulfate is weaker than galactose 3-sulfate by a factor of 1:1, whereas the respective glycolipid isomers differ 3.4-fold in activity. Again, the conformation of the ceramide-linked sugars may be different and could account for the greater binding specificity obtained with glycolipids.

Some sugar phosphate esters as well as sulfate esters inhibit laminin binding to sulfatides. Mannose 6-phosphate and glucose 1-phosphate are more potent than any of the hexose sulfates examined. This result may be relevant to reports that both mannose 6-phosphate and fucoidan inhibit binding of lymphocytes to high endothelial venules (27). Although it is unlikely that laminin is involved in this interaction, these results demonstrate that hapten inhibition can be misleading when used to characterize receptors to which proteins bind primarily by ionic interactions.

Laminin promotes attachment and spreading of several cell lines (4, 28, 29). It is not known whether thrombospondin functions in cell adhesion other than in platelets. Thrombospondin is secreted, however, by many cell types (30-33) and is localized by antibodies (31, 34) and uptake of labeled protein (35) in the extracellular matrix. The ability of fucoidan to reverse spreading of bovine aortic endothelial cells (36) and to inhibit binding of laminin and thrombospondin to sulfatides suggests that fucoidan may act by disrupting binding of sulfated glycoconjugates on endothelial cells to laminin or thrombospondin in the extracellular matrix. Bovine aortic endothelial cells also secrete thrombospondin (30). The effects of fucoidan on this and other cell adhesive interactions (36-41) suggest that sulfated glycoconjugates may be receptors for cell-cell or cell-matrix adhesion. Defining the specificities of adhesive proteins for these glycoconjugates provides a basis for using the specific inhibitory polysaccharides to examine the role of sulfatide binding in their biological activities.

Although the three proteins examined bind best to galactosyl ceramide 1′-sulfate, the specificity of von Willebrand factor is broad enough to use it to detect many sulfated lipids in extracts of tissues (Fig. 3). This method can be used to detect new sulfated lipids and to detect simple sulfatides in tissues where they are present at very low concentrations. Where the relative binding avidity is known, the assay can be used for quantitative assay of the tissue distribution of sulfated glycolipids and changes in their concentration during development.

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