10E4 Antigen of Scrapie Lesions Contains an Unusual Nonsulfated Heparan Motif

The carbohydrate antigen on heparan sulfate recognized by monoclonal antibody 10E4 is uniquely codistributed with the abnormal prion protein, PrPSc, even in the earliest detectable brain lesions of scrapie-infected mice. Determining the chemical structure of 10E4 antigen is, therefore, an important aspect of structure elucidation of scrapie lesions, and a prerequisite for designing experiments to understand its role in scrapie pathogenesis. Toward this aim, we have examined preparations of heparan sulfate, with differing sulfate contents, for binding by 10E4 antibody. The highest antigenicity was observed in a preparation (HS-1) with the lowest sulfate content. HS-1 was partially depolymerized with heparin lyase III, and oligosaccharide fragments examined for 10E4 antigen expression by the neoglycolipid technology. An antigen-positive and two antigen-negative tetrasaccharides were isolated and examined by electrospray mass spectrometry. The antigen-positive tetrasaccharide sequence on heparan sulfate was thus deduced to contain a unique unsulfated motif that includes an N- unsubstituted glucosamine in the sequence, UA-GlcN-UA-GlcNAc. Antibody binding experiments with neoglycolipids prepared from a series of heparan/heparan sulfate disaccharides, and the trisaccharide derived from the antigen-positive tetrasaccharide after removal of the terminal hexuronic acid, show that both the penultimate glucosamine and the outer nonsulfated hexuronic acid are important for 10E4 antigenicity.

Scrapie is the prototype of an unusual group of transmissible neurodegenerative diseases characterized by deposition of an alternatively folded, abnormally protease-resistant, isoform of the host prion protein, PrPSc, which is proposed to constitute the infective agent (1). There is considerable progress in knowledge of the molecular and cellular biology of prions, but precise details of the mechanism of conversion to the disease-associated form, PrPSc, remain to be elucidated (2). Much interest has been stimulated in the interactions of PrP with the glycosaminoglycans heparan sulfate and heparin. This has arisen as a result of the finding (3) that amyloid plaques, which occur in the clinically manifest stage of experimental scrapie (as in the human transmissible encephalopathies and Alzheimer’s disease) are rich in heparan sulfate proteoglycans (HSPGs).1 The effects of administering heparin and heparan sulfate and other highly acidic materials have been investigated on the course of experimental scrapie infections (4, 5). It has been shown that such acidic compounds can “cure” infected cells or lengthen the survival of scrapie-infected animals. It is not yet known, however, whether these effects are mediated by direct binding to PrPSc and PrPSc, or by indirect mechanisms. Nor is it known whether endogenous glycosaminoglycans or proteoglycans are involved in the normal physiological interactions with prion protein or in pathological interactions as has been suggested (5). It will be important to elucidate at the molecular level any such interactions as a lead to rational therapeutic designs.

Heparan sulfates are major components of the proteoglycans in nervous tissue occurring at the surface of cells and in the extracellular matrix (6–8). The carbohydrate chains are negatively charged polymers containing disaccharide units of hexuronic acid and N-acetylated or N-sulfated glucosamine. There are diverse patterns of sulfation along the oligosaccharide chains, there being less sulfation overall in heparan sulfates relative to heparin (9). Evidence has been forthcoming for the possible involvement of a specific carbohydrate sequence of heparan sulfate in scrapie pathogenesis. In an immunohistochemical study aimed at examining the temporal relationship between HSPGs and the known sites of PrP pathology, a mouse model of scrapie was selected, which is characterized by both fibrillar amyloid and nonamyloid PrPSc (10). Six monoclonal antibodies to HSPGs were investigated of which three were directed to core proteins and three to the carbohydrate chains. The presence of one of the carbohydrate antigens, detected with 10E4 antibody (but not with the other antibodies), correlated strikingly with the areas of abnormal PrP deposition, not only in the amyloid plaques, but also in the earliest detectable lesions in the brain of the infected mice. In particular, the presence of the 10E4 antigen in proximity to the diffuse forms of PrPSc in neuro-anatomically defined target sites, suggests a

1 The abbreviations used are: HSPGs, heparan sulfate proteoglycans; ADHP, N-acetylglucosaminyl(N-9-anthracenylmethyl)-1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine; CID, collision-induced dissociation; ES-MS, electrospray-mass spectrometry; Gla, glucuronic acid; GlcN, glucosamine; GlcNAc, N-acetylglucosamine; GlcNS, N-sulfated glucosamine; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; HS, heparan sulfate; NGL, neoglycolipid; PBS, phosphate-buffered saline; PrPSc, normal cellular prion protein; PrPSc, abnormal scrapie prion protein; SAX, strong anion exchange; TBS, Tris-buffered saline; UA, hexuronic acid; âUA, 4,5-unsubstituted hexuronic acid.

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eluting with 0.2 M NH4Cl (pH 3.5) at a flow rate of 15 ml/h. The eluate fractionated on a gel filtration column of Bio-Gel P-6 (1.6 x 36 cm) eluted with water at a flow rate of 20 ml/h, lyophilized, and the digestion mixture was desalted on a Sephadex G-10 column (1.6 x 36 cm). The inset shows the binding signals elicited with antibody 10E4 (10 μg/ml) when tested with the HS preparations, HS-1, -2, -3, -4 and with chondroitin sulfate A, which had been added at the indicated concentrations to the plastic microwells, as described under “Experimental Procedures.”

close relationship between this determinant on heparan sulfate and the formation of the scrapie lesions.

We are investigating the carbohydrate sequence of the 10E4 determinant by the neoglycolipid (NGL) technology (11) in conjunction with electrospray mass spectrometry (ES-MS). A recent advance involving the generation of oligosaccharide probes in the form of fluorescent NGLs (12) has enhanced the power of the technology in pinpointing and isolating active oligosaccharides among highly heterogeneous populations. Here we describe the identification by this means and the assignment of an unusual carbohydrate sequence in the 10E4 antigen.

EXPERIMENTAL PROCEDURES

Materials—Mouse monoclonal antibody 10E4, IgM (13) was from Sekagaku (Japan). Rabbit anti-mouse IgG-peroxidase was from Dako (Denmark). Protein LA-peroxidase was from Actigen (Cambridge, UK). FAST™ 3,3'-diaminobenzidine and 2,2'-azino-bis(3-ethylbenzthiazoline-sulfonic acid) were from Sigma (Poole, UK). Heparan sulfate fractions, HS-1, -2, -3, -4 and with chondroitin sulfate A, which had been added at the indicated concentrations to the plastic microwells, as described under “Experimental Procedures.” The immunostained bands a and b in panel B correspond to peaks a and b, respectively, in Fig. 3B.

Depolymerization of HS by Heparin Lyase III and Fractionation of the Fragments—HS-1 was partially depolymerized by limited digestion with heparin lyase III essentially as described (15), and the reaction mixture was kept at room temperature for 30 min and immediately lyophilized. Preparative TLC (11) was performed to isolate the major ADHP was essentially as described above, but the incubation time was limited to 24 h. Preparative TLC (11) was performed to isolate the major NGL products from the two reaction mixtures, and to remove the Hg reagent salts, excess lipid and side reaction products. NGL products from the two reaction mixtures, and to remove the Hg reagent salts, excess lipid and side reaction products.

Chromatogram binding experiments with antibody 10E4 and NGLs prepared from depolymerized HS-1 fragments. Fluorescent NGLs (200 pmol/lane) prepared from HS-1 fractions 3-6 (panel A′), and subfractions 3-1, -2, -3, -5 and -8 (panels B′/B″) were chromatographed by HPTLC in chloroform/methanol/water, 60:35:8, by volume. Panels A and B are photographs taken under UV light at 366 nm, and panels A′ and B′ are the same plates overlaid with antibody 10E4 (10 μg/ml); antibody binding was detected as described under “Experimental Procedures.” The immunostained bands a and b in panel B′ correspond to peaks a and b, respectively, in Fig. 3B.

Recent NGLs were prepared and resolved by high performance (HP) TLC essentially as described previously (12). In brief, the following were added to lyophilized HS fragments, typically 50 nmol: 3 μl of water, 50 μl of N-aminooctyl-N-(9-anthracenylmethyl)-1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (ADHP) stock solution (7 nmol/μl CHCl3/MeOH 13, v/v) and 10 μl of freshly prepared tetrabutylammonium cyanoborohydride solution (20 μg/μl of methanol). The mixture was incubated at 60 °C for 90 h. Reaction mixtures were resolved by HPTLC using a solvent mixture of chloroform/methanol/water, 60:35:8. The fluorescent NGLs were visualized under UV light at 366 nm.

The fluorescent NGLs were fractionated on an amide column (TSKamide 50, 4.6 x 250 mm) using a gradient of 10 to 70% B in solvent A (soybean oil/PEG/methanol/water 130:70:9 and solvent B: chloroform/methanol/water 10:20:8, both containing 0.1% formic acid) in 30 min and a flow rate of 0.5 ml/min. The eluates were monitored with a fluorescence detector at 256 nm (excitation) and 405 nm (emission).

Removal of ΔUA from Tetrasaccharides—The 4,5-unsaturated hexuronic residue (ΔUA) at the nonreducing terminus of the HS-1 tetrasaccharides was removed by oxymercuration (16). In brief, to a solution of 25 nmol of tetrasaccharide in 18 μl H2O was added 2 μl of Hg reagent (20 mM mercuret acid in 130 mM sodium acetate, pH 5.0). The reaction mixture was kept at room temperature for 30 min and immediately lyophilized. Conjugation of the lyophilized reaction product to ADHP was essentially as described above, but the incubation time was limited to 24 h. Preparative TLC (11) was performed to isolate the major NGL products from the two reaction mixtures, and to remove the Hg reagent salts, excess lipid and side reaction products.

Chromatogram Binding Assays—NGLs were resolved by HPTLC using as solvent chloroform/methanol/water, 60:35:8, by volume. Binding of monoclonal antibody 10E4 to NGLs chromatographed on aluminum-backed HPTLC plates (Merck) was assayed essentially as described previously (17) except that Flexigum was not used. In brief, after blocking nonspecific binding sites with 1% casein (Pierce) in Tris-buffered saline, 10 mM Tris-HCl buffer, 150 mM NaCl, pH 8.0 (TBS), the chromatogram was overlaid with 10E4 antibody (10 μg/ml) in 1% casein; antibody binding was detected using protein-LA-peroxidase (1/500 of stock) in 1% casein followed by FAST™ 3,3-diaminobenzidine peroxidase substrate. For comparing intensities of antibody binding, immunostained bands were scanned, and the derived peak areas scored using a Shimadzu CS-9000 scanner (Shimadzu Corporation, Kyoto, Japan).
TABLE I

**Determination by ES-MS analysis of the monosaccharide contents and substitutions with acetyl and sulfate groups in oligosaccharides within the pooled HS-1 oligosaccharide fractions 1–6**

| Fraction | Monosaccharide content | Acetyl | Sulfate | Observed mass | Calculated mass |
|----------|------------------------|--------|---------|--------------|----------------|
| 1        | Di-                    | 0      | 1       | 417.2        | 417.1          |
| 2        | Di-                    | 1      | 0       | 379.2        | 379.1          |
| 3        | Tetra-                 | 1      | 1       | 796.4        | 796.2          |
|          |                        | 2      | 2       | 876.4        | 876.1          |
|          |                        | 1      | 0       | 716.4        | 716.2          |
| 4        | Tetra-                 | 2      | 0       | 758.5        | 758.2          |
| 5        | Hexa-                  | 2      | 2       | 1175.6       | 1175.3         |
|          |                        | 0      | 0       | 1255.6       | 1255.3         |
| 6        | Octa-                  | 2      | 4       | 1085.5       | 1095.3         |
|          |                        | 2      | 2       | 1752.5       | 1752.3         |
|          |                        | 1      | 3       | 1630.5       | 1630.3         |
|          |                        | 1      | 4       | 1710.6       | 1710.2         |
|          | Hexa-                  | 3      | 0       | 1137.4       | 1137.3         |

**RESULTS**

Initial binding experiments revealed that the preparation of heparan sulfate, HS-1, that had the lowest sulfate content elicited the greatest binding signal with 10E4 antibody (Fig. 1, inset). No binding signals were detected with chondroitin sulfate A, which has a sulfate content similar to that of HS-1. The HS-1 preparation was selected for further investigation. Partial depolymerization of HS-1 was carried out using heparin lyase III, and this was followed by gel filtration (Fig. 1). Pooled fractions containing the smaller fragments, fractions 1 to 6, were subjected to ES-MS analysis (Table I), and thereby found to be di- to octasaccharides with various N-acetyl and sulfate contents.

**10E4 Antibody Binding to HS-1 Fragments**

HS-1 oligosaccharide fractions 3 to 6 were converted to fluorescent NGLs, resolved by HPTLC, and examined for 10E4 antibody binding (Fig. 2, A and A'). Although little or no inhibitory activity could be detected in the free oligosaccharides at the highest concentrations tested (3 mg/mL, results not shown), antigen-positive components were detected among the NGLs of fractions 3, 5, and 6, but not in fraction 4. Those in fractions 5 and 6 were clearly minor components as they were barely detectable by fluorescence. Fraction 3, which contained tetrasaccharides (Table I) and in which the antigen-positive components appeared relatively more abundant (Fig. 2A), was selected for further study. The nonderivatized fraction 3 oligosaccharides were further resolved by SAX HPLC (Fig. 3A) and the five most abundant subfractions, 3-1, -2, -3, -5, and -8, were converted into fluorescent NGLs and examined for 10E4 antibody binding (Fig. 2, B and B'). A duplex of strongly immunoreactive components was revealed in fraction 3-1, but no immunoreactivity was detected in the four other fractions tested. The mixture of NGLs in fraction 3-1 was resolved by HPLC (Fig. 3B), and peaks designated a and b were found by chromatogram binding (not shown) to correspond to the respective immunoreactive components a and b in Fig. 2, B and B'.

**Sequence Determination of Antigen-positive and Antigen-negative Tetrasaccharides by Mass Spectrometry**

**Antigen-positive Tetrasaccharide**—The ES mass spectrum of the NGL component a established the molecular mass as being 1610.8 Da ([M−2H]2+: m/z 804.4 and [M−3H]3+: m/z 535.9) (results not shown). This corresponds to the NGL of a nonsulfated tetrasaccharide containing a single acetyl group, as was detected also among the nonderivatized oligosaccharides in fraction 3 (Table I). The second immunoreactive component b was deduced to be a dehydrated form of component a (molecular mass of 1592.6 Da). The dehydration, a common occurrence in the derivatization of oligosaccharides having N-acetylgalactosamine (GlcNAc) at the reducing end (11)2 indicates that the tetrasaccharide contains a GlcNAc residue at the reducing end. CID MS/MS of the above-mentioned, doubly charged ion at m/z 804.4 from component a showed, in the low mass region (Fig. 4A), C- and B-type fragment ions (20) arising from the nonreducing terminus of the lipid-linked oligosaccharide (Fig. 5). Thus the sequence of the tetrasaccharide could be deduced as follows. The C-type fragment ion at m/z 175 clearly identifies a ΔUA residue at the nonreducing end. The fragment ion at m/z 336 represents a mass increment of 161, corresponding to an N-unsubstituted glucosamine (GlcN) residue. The B-type fragment ion at m/z 494 represents a mass increment of 158, corresponding to a hexuronic acid (UA) and serves to identify unambiguously the sequence ΔUA-GlcN-UA. The remaining mass of 1115 Da (1609 minus 494 Da) is in accord with the

2 M. S. Stoll and W. Chai, unpublished observation.
presence of a GlcNAc residue linked to the lipid moiety. Thus, the sequence of the antigen-positive NGL, α, is

\[ \text{ΔUA-GlcN-UA-GlcNAc-ADHP}. \]

Knowing that the predominant cleavage site of the highly purified recombinant heparin lyase III is at a glucuronic acid (GlcA) residue (21–23), the antigen-positive oligosaccharide in the native HS-1 is most likely GlcA-GlcN-UA-GlcNAc.

**Antigen-negative Tetrasaccharides**—The main components in the antigen-negative NGL fractions 3-3 and 3-5 were similarly analyzed by ES-MS (spectra not shown). The main component in fraction 3-3, molecular mass of 1652.8 Da ([M–2H]2+: m/z 825.4 and [M–3H]3+: m/z 549.9), is deduced to contain a nonsulfated tetrasaccharide with two acetyl groups. Here also, the carbohydrate sequence could be assigned from the fragment ions produced by CID MS/MS of the doubly charged ion m/z 825.4 (Fig. 4B). The fragment ion at m/z 175 indicated a ΔUA residue at the nonreducing end. The fragment ion at m/z 378 (a mass increment of 203) corresponds to a GlcNAc residue linked to the ΔUA and that at m/z 536 (a mass increment of 158) corresponds to an additional UA. The three fragment ions identify the sequence ΔUA-GlcNAc-UA. The remaining mass (1115 Da) indicates a GlcNAc residue at the reducing terminal linked to the lipid. From these results, the sequence of this antigen-negative tetrasaccharide is deduced to be:

\[ \text{ΔUA-GlcNAc-UA-GlcNAc}. \]

The main component in fraction 3-5, molecular mass 1690.9 Da ([M–2H]2+: m/z 844.4 and [M–3H]3+: m/z 562.6) is deduced to be a tetrasaccharide with one sulfate and one acetyl group. The sequence \( \text{ΔUA-GlcNS-UA-} \) can be assigned from the fragment ions produced by CID MS/MS of ion m/z 844.4 (Fig. 4C): C-type fragment ions at m/z 175 (ΔUA), m/z 416, a mass increment of 241 corresponding to GlcNS and the B-type ion m/z 574 a mass increment of 158 corresponding to UA. The remaining mass of 1115 Da, again, indicates a GlcNAc residue linked to the lipid moiety. The location of sulfate in 3-5 can be assigned to the GlcN residue, as the fragment at m/z 416 (Fig. 4C) corresponds to a mass increment of 80 from m/z 336. Thus, sequence of this antigen-negative tetrasaccharide is deduced to be:

\[ \text{ΔUA-GlcNS-UA-GlcNAc}. \]

**10E4 Antibody Binding to Disaccharides from Heparin and Heparan Sulfate**

There was immunoreactivity in regions of lanes 3, 5, and 6 corresponding to NGLs of disaccharides. As these were minor components, we examined for 10E4 immunoreactivity the NGLs of a series of twelve structurally defined heparin/heparan sulfate disaccharides (Table II and Fig. 6). Among these, the NGL derived from ΔUA-GlcN gave by far the strongest immunoreactivity.
FIG. 5. Proposed sequence and calculated molecular mass of the antigen-positive tetrasaccharide and of the derived fluorescent NGL. The sequence of the tetrasaccharide was deduced from the CID MS/MS ions (Fig. 4A) that are from fragmentation of the NGL. The second immunoreactive neoglycolipid, with a measured molecular mass of 1592.6 Da, was deduced to be a dehydrated analog in which the elements of water are lost from the reducing end GlcNAc or lipid tail (11) during neoglycolipid formation.

| Designation | Sequence | Relative binding intensity |
|-------------|----------|---------------------------|
| I-A         | ΔUA(2S)-GlcNAc(6S) | — |
| II-A        | ΔUA-GlcNAc(6S) | 10.4 |
| III-A       | ΔUA(2S)-GlcNAc | — |
| IV-A        | ΔUA-GlcNAc | — |
| I-S         | ΔUA(2S)-GlcNS(6S) | — |
| II-S        | ΔUA-GlcNS(6S) | — |
| III-S       | ΔUA(2S)-GlcNS | 12.3 |
| IV-S        | ΔUA-GlcNS | — |
| I-H         | ΔUA(2S)-GlcN(6S) | — |
| II-H        | ΔUA-GlcN(6S) | 16.5 |
| III-H       | ΔUA(2S)-GlcN | 100 |
| IV-H        | ΔUA-GlcN | — |

Antibody binding intensity was scored by scanning the immunostained NGL bands at 460 nm; intensities are expressed as peak areas relative to that for IV-H taken as 100%; —, little or no binding detected.

Investigation of the Requirement of the Nonreducing Terminal Hexuronic Acid in 10E4 Antigenicity

The major NGL product obtained after oxymercuration treatment to remove the terminal unsaturated hexuronic acid in the 10E4 antigen-positive tetrasaccharide fraction 3-1 (Fig. 6A, lane 4) gave, by TLC-liquid secondary ion-MS, a [M−H]− ion from m/z 1451 and two fragment ions at m/z 1290 and m/z 1114 (not shown), corresponding to the expected sequence, GlocNAc-UA-GlcNAc-ADHP. The lipid secondary ion mass spectrum of the major NGL product of the similarly treated antigen-negative tetrasaccharide fraction 3-3 (Fig. 7A, lane 3) corresponded to that for GlcNAc-UA-GlcNAc-ADHP, ([M−H]− ion at m/z 1493 and fragment ions at m/z 1290 and m/z 1114).

The lipid-linked trisaccharides derived from the antigen-positive and antigen-negative tetrasaccharides, together with the “parent” tetrasaccharides were examined for 10E4 antigenicity by TLC overlay (Fig. 7B). Immunoreactivity was detected, as before, in the 3-1 tetrasaccharide but not in the trisaccharides. Thus the nonreducing end hexuronic acid in the 3-1 tetrasaccharide is required for 10E4 antigenicity.

DISCUSSION

The 10E4 antigen-active tetrasaccharide is, to our knowledge, the first carbohydrate antigen sequence to have been assigned on heparan sulfate. Unexpectedly, the sequence lacks sulfate and constitutes a unique motif with an internal N-unsubstituted glucosamine. This residue is important for antigenicity of the tetrasaccharide as shown by the lack of antibody binding to the two tetrasaccharide analogs investigated, one of which has a N-acetylated, and the other a N-sulfated glucosamine at this position. The importance of N-unsubstituted glucosamine for eliciting strong 10E4 antibody binding is corroborated by the binding experiments with the series of NGLs derived from heparin/heparan sulfate disaccharides; only the disaccharide IV-H, containing the N-unsubstituted glucosamine shows strong binding. The weak 10E4 binding to the NGLs of the three disaccharides, III-H, IV-S, and II-A, raises the possibility that, in the heparan sulfate polysaccharide, certain of the monosulfated glucosamine and N-acetylgalcosamine residues may contribute to antigenicity. From the binding data with the tri- and disaccharides, a further conclusion is drawn, namely that a hexuronic acid on the nonreducing side of the glucosamine is essential for 10E4 antigenicity and that it must be nonsulfated. Collectively our results are in
Arguably the strongest evidence for the natural occurrence of N-unsubstituted glucosamine is that it has been identified as being a dominant part of the antigens recognized by two monoclonal antibodies, 10E4 and JM-403, each of which immunostains fresh frozen tissues with a distinct pattern (10, 13, 24, 29). Analyses of embryonic and adult tissues revealed that the expression of 10E4 antigen varies markedly during organogenesis and development (13). Thus 10E4 antigen, like a number of monoclonal antibody-defined carbohydrate antigens, is a differentiation antigen, which by analogy with those of the blood group Lewis\textsuperscript{a} and Lewis\textsuperscript{b} series (30–33) can be predicted to have biological functions. There is strong precedence for assignments of roles to heparan sulfates, for example, as ligands and modulators of the activities of growth factors (34). Just as exogenously added polyanines can deplete PrP\textsuperscript{Sc} from certain scrapie-infected cells (35), we propose that the 10E4 determinant, with its content of a free amine (and in multivalent display on heparan sulfate), is a candidate endogenous ligand for a protein or proteins in the pathway of PrP\textsuperscript{Sc} to PrP\textsuperscript{Sc} conversion.

Thus it will be important to determine the size and complete sequence of the 10E4 antigen, and investigate its role in the normal and pathological behavior of prion protein. In particular, the immunoreactive oligosaccharide(s) visualized as minor components in our chromatogram binding experiments with hexasaccharide fragments of HS-1 should be characterized, as the initial studies of David et al. (13) indicated the 10E4 antigen contains glucosamine that is N-sulfated. It can be predicted, therefore, that the complete 10E4 antigen encompasses the tetrasaccharide sequence we have identified, flanked on one or other side by additional monosaccharide residues including N-sulfated glucosamine. The sulfation issue in regard to 10E4 antigen sequence will be important to clarify, as it has been suggested (5) that certain endogenous glycosaminoglycans or proteoglycans influence the in vivo behavior of PrP. Moreover, cell biological experiments have indicated that endocytosis of PrP is a prerequisite for the formation of PrP\textsuperscript{Sc} (2) and that negatively charged polysaccharides increase the rate of endocytosis of PrP\textsuperscript{Sc} (36). Further work is under way to characterize hexasaccharides and longer oligosaccharides for 10E4 antigen activity.

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