Self-association of N-Syndecan (Syndecan-3) Core Protein Is Mediated by a Novel Structural Motif in the Transmembrane Domain and Ectodomain Flanking Region*

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We expressed domains of the core protein of the transmembrane heparan sulfate proteoglycan N-syndecan (syndecan-3) either individually or as maltose-binding protein fusion proteins. Biochemical characterization of the purified proteins revealed that some of them were capable of self-association and formed stable, noncovalent multimeric complexes. The formation of N-syndecan core protein complexes was also demonstrated in mammalian cells by in situ cross-linking. Identification of structural motifs in the core protein of N-syndecan responsible for the formation of these complexes was accomplished by analyzing a series of constructs comprising different regions of the protein as well as site-directed mutants. Self-association was assayed by SDS-polyacrylamide gel electrophoresis, glutaraldehyde cross-linking, and size-exclusion high pressure liquid chromatography. Our results indicated that (i) the transmembrane domain of the N-syndecan core protein was required but not sufficient for the formation of stable complexes; (ii) the minimal amino acid sequence that conferred the ability of the N-syndecan core protein to form multimeric complexes included the last four amino acids (ERKE) of the extracellular domain plus the transmembrane domain; (iii) point mutations that changed the basic residues in this sequence to alanine residues either partially or completely abolished the ability of the N-syndecan core protein to form complexes; and (iv) replacement of conserved glycine residues in the transmembrane domain with leucine abolished complex formation. This property is similar to the oligomerization activity of other transmembrane receptors and suggests that regulated self-association may be important for the biological activity of transmembrane proteoglycans.

The syndecans are a gene family of transmembrane cell-surface heparan sulfate proteoglycans (HSPGs) that are expressed in highly regulated cell type- and development-specific patterns (1, 2). Four syndecan core proteins, designated syndecan-1–4 (also called syndecan, fibroglycan, N-syndecan, and amphiglycan or ryudocan, respectively), have been identified by molecular cloning from mammalian cells (reviewed in Ref. 3). The syndecans have structurally distinct extracellular domains, but highly conserved transmembrane and cytoplasmic domains. Although the functions of the syndecan family of HSPGs are not known in detail, they are believed to play important roles in morphogenesis and differentiation by binding to a variety of extracellular ligands, including matrix adhesive proteins such as fibronectin and collagens, and certain polypeptide growth factors (e.g. basic fibroblast growth factor) (4–12).

The highly conserved nature of the primary structures of the short cytoplasmic domains of the syndecans strongly suggests that they may, in addition, play a role in transducing stimuli provided by extracellular ligand binding into cytoplasmic signals. This could occur either by binding to and organizing cytoskeletal proteins or by participating in the generation of intracellular signals. Syndecan-1, association of the core protein with actin filaments has been demonstrated (13, 14). Syndecan-4, but not other members of the family, has been reported to be localized to focal adhesions in cultured fibroblasts (15). These are specialized sites of tight binding of the cell membrane to the substrate as well as sites of membrane attachment of intracellular actin filaments. Several regulatory signaling molecules are concentrated at focal adhesion sites, including some integrin receptors and a tyrosine kinase called pp125FAK (16).

A common feature of signaling mechanisms mediated by cell-surface receptor proteins that contain a single transmembrane domain is the noncovalent dimerization or oligomerization of the proteins in response to ligand binding (17, 18). Where this phenomenon has been studied in detail, oligomerization appears to be an essential part of the receptor activation process. Oligomerization has been demonstrated both for receptors with cytoplasmic tyrosine kinase domains (e.g. the epidermal growth factor receptor) and for receptors with short noncatalytic cytoplasmic domains. In the latter case, oligomerization may result in the binding and activation of soluble kinases. One consequence of this mechanism of activation is that these receptors can be activated by antibody-mediated cross-linking (19, 20), which induces oligomerization in the absence of ligand. In addition, it has been shown that some truncated forms of these receptors oligomerize in the absence of ligand binding and are constitutively activated. This suggests that specific domains of these proteins can mediate oligomerization and that other domains inhibit oligomerization until the receptor is activated by ligand binding, presumably as a result of a change in receptor conformation. It is the transmembrane domains of these receptors that are principally involved in mediating oligomerization.

There is indirect evidence to suggest that syndecan family proteoglycans are activated by ligand-mediated cross-linking. In cultured Schwann cells transfected to express syndecan-1,
association of the proteoglycan with actin filaments can be induced by antibody-mediated cross-linking of the proteoglycan core protein (14). This actin association appears to be mediated by the cytoplasmic domain of the proteoglycan since a mutant form of syndecan-1 lacking most of the cytoplasmic domain fails to associate with actin filaments under these conditions. N-Syndecan (syndecan-3) is a transmembrane HSPG that is expressed in a fairly restricted pattern, most prominently in the developing nervous system (21). This proteoglycan has been shown to bind basic fibroblast growth factor with high affinity (K D = 0.5 nM) (12) and may function as a “co-receptor” for this or related growth factors during nervous system development (10). N-Syndecan also binds with high affinity to heparin-binding growth-associated molecule (HB-GAM), an extracellular heparin-binding protein with potent neurite outgrowth-promoting activity (22).

In this paper, we demonstrate that recombinant N-syndecan core protein self-associates to form stable, noncovalent multimeric complexes in vitro. This property requires the presence of the transmembrane domain as well as a short extracellular domain sequence that is conserved in syndecan core protein sequences.

MATERIALS AND METHODS

Bacterial Expression of MBP-N-Syndecan Fusion Proteins—We have described previously the molecular cloning of a cDNA coding for the N-syndecan (syndecan-3) core protein from neonatal rat Schwann cells and its expression and purification as a β-galactosidase fusion protein (21). Specific domains of the protein were subcloned for expression as MBP fusion proteins. The cDNA segments representing the domains of interest were amplified by polymerase chain reaction (PCR) using cDNA-specific primers. In addition to the cDNA sequence, the sense primers contained an in-frame EcoRI recognition sequence at the 5’-end and the antisense primers contained an in-frame translational stop codon (TAG) followed by a PstI recognition sequence to facilitate direct subcloning of the PCR products. Plasmid DNA containing the full-length N-syndecan cDNA was used as a template in the PCRs (30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 4 min) in a DNA thermal cycler (Perkin-Elmer). The products of the PCR were digested with EcoRI and PstI and subcloned into EcoRI- and PstI-digested pHM, pMALC, or pMALC2 (New England Biolabs Inc., Beverly, MA) bacterial expression plasmids. Following transformation of these plasmids into Escherichia coli strain M109 competent cells, recombinant clones were analyzed by restriction digestion and nucleotide sequence analysis to confirm the presence of the N-syndecan cDNA in the correct reading frame.

Site-directed mutations were generated by PCR amplification. Sense orientation primers containing the desired base changes were synthesized with an in-frame EcoRI recognition site at the 5’-end and used for PCR amplification of wild-type cDNA templates with an antisense primer that contained an in-frame stop codon and a PstI recognition site. The products were subcloned as described above. Introduction of the mutations was confirmed by DNA sequence analysis of the plasmids.

Expression of the MBP-N-syndecan fusion proteins was induced by the addition of isopropyl-β-D-thiogalactopyranoside to the bacterial cultures (final concentration of 0.3 mM) and incubation at 37°C for 2 h. The cells were lysed by lysozyme treatment and sonication, and the fusion proteins were purified by affinity chromatography on an amylose column as described by the manufacturer (New England Biolabs Inc.). The fusion proteins were further characterized by Western blot analysis with either anti-MBP antibodies (New England Biolabs Inc.) or anti-N-syndecan antibodies.

In some experiments, the fusion proteins were incubated with Factor Xa, a recombinant specific protease recognized by site-directed antibody antibodies against bacterially expressed N-syndecan core protein have been reported previously (21). We also prepared anti-peptide antibodies directed against the COOH-terminal cytoplasmic domain of N-syndecan. A synthetic peptide corresponding to the terminal peptide KQEEFYA was synthesized and covalently coupled to maleimide-activated keyhole limpet hemocyanin (Pierce). The conjugate was injected into rabbits along with a synthetic adjuvant mixture (RIBI Immunochemicals) as described previously (21). Anti-core protein antibodies were purified by affinity chromatography on a column containing immobilized recombinant core protein coupled to Sepharose beads as described previously. The antibodies reacted specifically with bacterially expressed N-syndecan core protein (data not shown) and N-syndecan expressed in mammalian cells (see below) on immunoblots.

-size-exclusion Chromatography—Purified MBP-N-syndecan fusion proteins were injected onto a 7.5 mm (inner diameter) × 30-cm SEC4000 gel filtration column (Beckman Instruments) pre-equilibrated with 50 mM sodium phosphate, pH 7.5, 0.1% CHAPS, 0.2 M NaCl. The column was eluted with the same buffer at a flow rate of 1 mL/min. Elution profiles of the proteins from the chromatogram were documented by absorbance at 280 nm using an on-line UV monitor. Column fractions (0.5 mL) were collected and analyzed by SDS-polyacrylamide gel electrophoresis. The column was calibrated using commercial preparations of purified globular proteins (Sigma).

Expression in 293 Cells—cDNA coding for full-length N-syndecan core protein was subcloned into the mammalian expression vector pCMV5neo (23). Embryonic human kidney 293 cells were transiently transfected by calcium phosphate coprecipitation as described previously (13). For cross-linking studies, the cells were incubated in medium containing 10 mM dimethyl 3,3'-dithiobispropionimidate2HCl (Pierce), a homobifunctional reducible cross-linker, for 30 min at room temperature. The cells were extracted with 50 mM Tris-HCl, pH 7.5, 0.5% deoxycholate, 0.5% Nonidet P-40, 0.1% SDS. Aliquots of the cell extracts were diluted in SDS gel sample buffer either lacking or containing 5% 2-mercaptoethanol, subjected to electrophoresis on 7.5% SDS-polyacrylamide gels, and transferred to Immobilon-P membranes. The core proteins were detected by incubating the membranes with anti-N-syndecan antibodies that were visualized by enhanced chemiluminescence (Amersham Corp.).

RESULTS

N-Syndecan Core Protein Forms SDS-resistant Dimers—When we expressed various domains of the core protein of the transmembrane HSPG N-syndecan in bacteria, we observed that some of these formed unusually stable dimers and higher order multimeric complexes. Fig. 1 shows the results obtained with a polypeptide containing the transmembrane domain (24 amino acids), the cytoplasmic domain (34 amino acids), and a truncated extracellular domain (67 amino acids), expressed in fusion with MBP. The fusion protein migrated on an SDS-polyacrylamide gel as two major bands, one migrating at the predicted monomeric molecular mass of 58 kDa and the other migrating at exactly twice the predicted monomeric molecular mass, i.e., as a dimer. The identification of these bands as MBP-N-syndecan fusion proteins was confirmed by immunoblot analysis with anti-MBP and anti-N-syndecan antibodies (data not shown). Several lines of evidence suggested that dimerization was an inherent property of the proteoglycan core protein. Similar results were obtained when the same core protein fragment was expressed as a fusion protein with β-galactosidase or when the entire coding sequence of the mature core protein was expressed as an MBP fusion protein (data not shown). When the core protein was released from MBP by cleavage with Factor Xa, the core protein polypeptide migrated on an SDS-polyacrylamide gel as an apparent dimer (Fig. 1). Similar results were obtained when the full mature core protein coding sequence was expressed (see below), indicating that
this was not solely a property of truncated core proteins. The formation of dimers by N-syndecan polypeptides was not a result of nonreducible disulfide bonds since the N-syndecan core protein does not contain cysteine residues. Thus, the ability to form stable dimers is a property of the N-syndecan core protein, and the structural features that confer this property reside within the COOH-terminal 125 amino acids.

In some experiments, higher order SDS-resistant complexes were observed following SDS gel electrophoresis of the MBP-N-syndecan fusion protein. These migrated at 4 times the predicted monomeric molecular mass and presumably represented stable tetramers (data not shown).

The existence of these complexes in the presence of SDS suggested that they were very stable and that larger complexes may be formed in the absence of denaturing detergent. To detect such complexes, we carried out glutaraldehyde cross-linking in a solution that contained the nonionic detergent Nonidet P-40. As shown in Fig. 1, the protein was cross-linked by glutaraldehyde to high molecular mass complexes that failed to enter the polyacrylamide gel. This suggested that the core proteins were present in large complexes containing at least 8 monomeric units.

Core Protein Complexes in Mammalian Cells—To rule out the possibility that the self-association was an artifact of bacterial expression or protein behavior in solution, we analyzed the oligomerization state of N-syndecan core proteins in a human cell line (293) that was transfected to express rat N-syndecan. These cells do not contain endogenous proteins that interact in a solution that contained the nonionic detergent. The existence of these complexes in the presence of 2-mercaptoethanol, which resulted in the separation of the cross-linker functional groups, the high molecular mass complexes were not observed. Under these conditions, the dimer and monomer core protein bands were visible, with the former being more prominent. The prevalence of the dimeric form may be explained by the presence of disulfide bonds within the cross-linker that were resistant to reduction. The presence of SDS-resistant dimers and cross-linked high molecular mass complexes was identical to the behavior of bacterially expressed core proteins described above.

The Transmembrane Domain Is Essential but Not Sufficient for Complex Formation—We used glutaraldehyde cross-linking of bacterially expressed core proteins to identify specific structural motifs in the core protein that conferred the ability to form these complexes. Polypeptides that contained the truncated extracellular domain, the transmembrane domain, or the cytoplasmic domain individually fused to MBP failed to produce cross-linked high molecular mass complexes (Fig. 3, con-
Serum albumin (68 kDa), and ovalbumin (45 kDa). The migration of the molecular mass markers myosin (200 kDa), bovine transmembrane domain.

Arrows indicate the positions of migration of the molecular mass markers myosin (200 kDa), bovine serum albumin (68 kDa), and ovalbumin (45 kDa).

The extracellular domains of syndecan family core proteins are not highly conserved in amino acid sequence. An exception is a short extracellular domain sequence just preceding the membrane-spanning domain that consists of one or two basic residues flanked by acidic residues. A polypeptide that contained this sequence, corresponding to the last four amino acids of the extracellular domain of N-syndecan (ERKE), in tandem with the transmembrane domain produced glutaraldehyde-cross-linked high molecular mass complexes (Fig. 3, construct 4).

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The cross-linking and gel permeation chromatography data polypeptides. These data were consistent with the results of glutaraldehyde cross-linking and suggested that these polypeptides existed as monomers. When the polypeptide containing the truncated ectodomain in tandem with the transmembrane domain was analyzed by gel permeation chromatography, two major peaks were detected (Fig. 4). One of these eluted at 10.3 min and corresponded to the predicted monomeric size of the fusion protein. The other peak eluted at 5.6 min (column void volume of 5.0 min), which corresponded to an $M_r > 1 \times 10^6$, when the column was calibrated with globular molecular weight standards. The trailing shoulder of this high molecular weight peak was broad, indicating the presence of complexes of intermediate size. Very similar results were obtained when the polypeptide containing the extracellular, transmembrane, and cytoplasmic domains in tandem was analyzed and when the polypeptide containing the ERKE sequence in tandem with the transmembrane domain was analyzed (Fig. 4). The formation of high molecular weight complexes was not affected by the ionic strength of the column buffer, up to 1 M NaCl (data not shown). These results were consistent with the results of glutaraldehyde cross-linking and indicated that the transmembrane domain was required but not sufficient for the formation of multimeric complexes and that the addition of the four flanking extracellular amino acids was sufficient to confer this property.
Self-association of N-Syndecan Core Protein

Fig. 5. Concentration dependence of N-syndecan self-association. The fusion protein containing the 12-kDa COOH-terminal ectodomain fragment and the transmembrane and cytoplasmic domains (shown in Fig. 1) was subjected to gel permeation chromatography. Before injection into the column, the protein concentration was adjusted as follows: A, 1 mg/ml; B, 0.1 mg/ml; C, 0.01 mg/ml. The ratio of multimer to monomer changed from 2:1 to 0.75:1, with a decrease in concentration. The column was eluted as described in the legend to Fig. 4.

indicated that the size of the stable complexes was very large. The presence of intermediate-sized complexes detected by gel permeation chromatography and the presence of SDS-resistant dimers detected by gel electrophoresis suggested that these high molecular mass complexes were in equilibrium with intermediates of smaller size. Consistent with this, the extent of complex formation as determined by gel permeation chromatography was dependent on the concentration of the protein. As shown in Fig. 5, when the concentration of protein injected onto the column was decreased, the fraction of the total protein that eluted as complexes decreased. The relative amounts of the large- and intermediate-sized complexes were also shifted in favor of the latter. The ratio of multimer to monomer decreased from 2:1 to 0.75:1 with a decrease in concentration. A rough estimate of the dissociation constant for the complexes was determined by nonlinear regression analysis of the areas under the monomer and complex peaks. The $K_d$ was calculated to be $5 \times 10^{-9} M$.

Identification of Essential Extracellular Domain Residues—Three out of four of the mammalian syndecans contain a dibasic sequence preceding the transmembrane domain. To determine whether these residues were required for self-association of the N-syndecan core protein, we analyzed mutant polypeptides containing the extracellular tetrapeptide in tandem with the transmembrane domain. In the mutants, either the Arg or the Lys residue of the ERK sequence was changed to an Ala residue. As shown in Fig. 3, when complex formation was assayed by gel permeation chromatography, the EAKE mutant formed complexes, but the extent of complex formation was reduced, as indicated by an increase in the relative amount of products of intermediate size as well as monomers (Fig. 4). Consistent with the cross-linking data, complex formation by the EAAE mutant was completely inhibited. These results suggested that the conserved basic residues contributed to complex formation and that the Lys residue was essential.

Identification of Essential Transmembrane Domain Residues—Inspection of the rat syndecan family transmembrane domain sequences reveals the presence of a regular pattern of glycine residues (see “Discussion”) near the central portion of the domain. Since glycine residues are infrequent in transmembrane domains (18), we examined the effect of replacement of two of these glycine residues with leucines on the ability of the protein to self-associate. As shown in Fig. 6, the core protein with the wild-type transmembrane domain sequence (lane 2) formed SDS-stable dimers that could be visualized on polyacrylamide gels. In contrast, the core protein with the Gly → Leu substitutions (lane 1) failed to form these complexes. The association state of the purified core proteins was also assessed by gel permeation chromatography. As shown in Fig. 6, a large fraction of the mutant core protein molecules were present in the monomer peak. Some self-association was evident, but to a significantly lesser extent compared with the wild-type core protein molecules (the multimer/monomer ratio decreased from 2:1 for the wild-type construct to 0.3:1 for the Gly → Leu mutant). These results were consistent with the inability of the mutant proteins to form SDS-resistant dimers (Fig. 6) and strongly suggested that specific structural features of the transmembrane domain were responsible for core protein self-association.


**DISCUSSION**

The data presented here indicate that the core protein of the transmembrane HSPG N-syndecan self-associates to form stable dimers, tetramers, and higher order complexes. The presence of these complexes was demonstrated using several different methods, including SDS gel electrophoresis, covalent cross-linking, and gel permeation chromatography. This property required the presence of the transmembrane domain of the core protein as well as additional sequence from the ectodomain. A stretch of sequence as short as four amino acids of the ectodomain contiguous with the transmembrane domain was sufficient to confer complex formation. Within this four-amino acid segment, basic residues that are conserved among syndecan family core protein sequences appeared to be required. Furthermore, bacterial constructs containing the entire transmembrane and cytoplasmic domains of N-syndecan were also capable of forming high molecular mass complexes.2

Based on the structural requirements in the core protein necessary for complex formation, the association appeared to be mediated by a combination of noncovalent hydrophobic and nonhydrophobic interactions. The basic unit of these complexes appeared to be dimers. On polyacrylamide gels, SDS-resistant dimers and tetramers, but not trimers or hexamers, were observed for polypeptides that were capable of complex formation. Results of glutaraldehyde cross-linking and gel permeation chromatography indicated that in the absence of SDS (but in the presence of either nonionic or zwitterionic detergent), the complexes consisted of a mixture of units of discrete size. The extent of complex formation was dependent on the protein concentration, suggesting an equilibrium between the monomeric and associated forms. Complex formation was also observed with recombinant core protein expressed in a human cell line. This indicated that the ability to form complexes was not a result of bacterial expression of the proteins. Self-association did not involve interchain disulfide bonds since it was not influenced by the presence of 2-mercaptoethanol or dithiothreitol (data not shown) and was observed for constructs that did not contain cysteine residues. The ability of the core proteins to self-associate was not due to nonspecific aggregation of the hydrophobic domain proteins since constructs that contained this domain without added ectodomain sequence behaved as monomers under all conditions examined. Furthermore, substitution of conserved glycine residues within the transmembrane domain with leucines, which would increase the hydrophobicity of the protein, reduced self-association. This also indicated that specific structural features of the transmembrane domain were important for this property.

A tentative model for the dimerization of the N-syndecan core protein is shown in Fig. 7. A comparison of the rat syndecan family core protein transmembrane domains reveals a regular pattern of small and bulky side chain residues within the NH2-terminal half of the domain. Moreover, this portion of the transmembrane domain is predicted to assume an extended conformation based on the high frequency of glycine residues, which are unfavorable for a-helical structures and favor extended structures, especially in the NH2-terminal direction (24). Contributing to the maintenance of an extended structure within this region are dipeptide sequences that are highly favorable for this type of structure. In contrast, the predicted conformation of the COOH-terminal half of the transmembrane domain is a-helical. The spacing of the side chains on neighboring polypeptides could allow for interdigitation of the alternating small and bulky side chain residues and tight packing within the membrane. This arrangement would be stabilized by electrostatic interactions between charged residues at the extracellular side of the plasma membrane. Lower right, this pattern of small and bulky side chain residues is also found in other single transmembrane domain proteins that are activated by ligand-induced dimerization (e.g., platelet-derived growth factor receptor (PDGFR-B)), activated by antibody-induced clustering (μ	extsubscript{2} integrin), or known to form stable noncovalent dimers (glycoporphin). Sequences are from Refs. 17 and 18. LFA, lymphocyte function-associated antigen.

A common characteristic of deglycosylated syndecan family core proteins is that they migrate on SDS-polyacrylamide gels at positions corresponding to apparent molecular masses that are significantly higher than the molecular masses predicted from cDNA sequence analysis (3). This appears to be due to a structural feature of the core proteins that causes them to resist denaturation or to bind SDS poorly. Anomalous migration on SDS gels is also observed for N-syndecan core protein. The mature core protein has a predicted molecular mass of ~45 kDa based on the cDNA sequence. The migration on SDS-polyacrylamide gels of the full-length nonglycosylated core pro-

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tein is consistent with an apparent molecular mass of ~95 kDa. The anomalous migration of the N-syndecan core protein is due largely to the extracellular domain, especially the proline- and threonine-rich central spacer domain. This domain, with a predicted molecular mass of ~39 kDa, migrates on SDS-polyacrylamide gels at an apparent molecular mass of 70 kDa.\(^2\)

Heparitinase- or nitrous acid-digested native or recombinant N-syndecan that has been glycanated migrates as a broad band at a molecular mass of 110–120 kDa. This discrepancy of 15–25 kDa between this form and the nonglycosylated core protein is apparently due to the presence of carbohydrates that resist heparitinase or nitrous acid digestion.\(^2\) In most of the experiments reported here, we utilized core protein fragments that lacked the extracellular spacer domain and carbohydrates. These proteins in their monomeric forms migrated on SDS-polyacrylamide gels and eluted from gel permeation columns in a manner that was very close to the behavior predicted from their cDNA sequences. Thus, the shifts in migration or elution that we report here and attribute to protein self-association are not simply the result of anomalous behavior of monomeric polypeptides.

The extent to which the ability to self-associate is shared by other core proteins of the syndecan family has not been addressed systematically. The structural motifs we identified that confer self-association upon the N-syndecan core protein are conserved among the known members of the syndecan gene family. Deglycosylated syndecan-2 (fibroglycan), with a predicted \(M_r\) of 23,000, migrates on SDS gels as bands of \(M_r\) ~ 45,000 and 90,000 (25), a finding that is most easily explained by the formation of SDS-resistant dimers of the core protein, similar to what we have reported here for N-syndecan. The fibroglycan core protein sequence lacks a basic residue at position -2 (relative to the membrane-spanning domain) of the ectodomain. Mutation of the lysine residue at this position in the N-syndecan core protein abolished complex formation. We have been unable to observe oligomerization of syndecan-1 core protein under conditions in which they can be observed for N-syndecan (data not shown). The formation of complexes was seen, however, when a chimera consisting of the syndecan-1 extracellular domain and the N-syndecan transmembrane domain was analyzed (data not shown).

An important unresolved question concerns the effect of glycosaminoglycan chains on core protein self-association. Preliminary results of experiments using purified native N-syndecan from neonatal rat brain (12) indicated that this form of the proteoglycan cannot be cross-linked to high molecular mass aggregates.\(^2\) However, these N-syndecan molecules may contain core proteins truncated at the COOH-terminal ends as a result of membrane "shedding." This has been demonstrated to occur in cell culture for all syndecans (1) and appears to result from a proteolytic cleavage of the protein core in the extracellular domain near the membrane attachment site. Supporting this idea is the finding that the purified brain N-syndecan molecules fail to react with an antibody directed against the cytoplasmic domain.\(^2\)

The functional consequences of this syndecan "activation" are unclear, although some data indicate that cytoskeletal coupling may be involved. During the spreading of Schwann cells, stably expressed syndecan-1 on the cell surface transiently co-localizes with nascent actin filaments polymerizing around the cell edges (13). This apparent association of syndecan-1 with cytoskeletal filaments is lost when spreading is completed. Antibody-mediated cross-linking of syndecan-1 on the surface of spread cells restores co-localization of the proteoglycan with actin filaments (14). The syndecan-1 microfilament association appears to be functional since antibody-mediated aggregation of syndecan-1 in spreading cells results in a redistribution of the actin filaments. In these experiments, antibodies directed against the proteoglycan core protein may be mimicking the self-association mediated by binding an extracellular ligand. The nature of the activation is not known, but self-association of the core proteins within the membrane could result in the formation of new structures that can regulate cytoplasmic activities such as nucleation of actin polymerization. Consistent with this, we found that actin filament association requires the presence on the core protein of the cytoplasmic domain.

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