Schwann Cells Secrete a Novel Collagen-like Adhesive Protein That Binds N-Syndecan*

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Schwann cells secrete an apparently novel collagen-like adhesive protein that interacts with cells through cell surface heparan sulfate proteoglycans. A heparin-binding glycoprotein was purified from conditioned medium of cultured rat Schwann cells. The protein, p200, which has an apparent molecular mass of approximately 200 kDa, was identified by its ability to bind the cell surface heparan sulfate proteoglycan N-syndecan (syndecan-3) in a membrane overlay assay. Soluble heparin but not chondroitin sulfate inhibited the binding, suggesting the involvement of heparan sulfate chains of proteoglycan in the interaction. Purified p200 promoted the attachment and spreading of Schwann cells. Adhesion to p200 was blocked by heparin, suggesting that heparan sulfate proteoglycans are cell surface receptors for p200. The tissue distribution of p200 was determined by immunoblot analysis with anti-p200 antibodies. Among neonatal rat tissues examined p200 was detected only in sciatic nerve and, at lower levels, in skeletal muscle. p200 expression in sciatic nerve was detectable only during the first 2–3 weeks of postnatal development and was not detected in adult rats. Immunofluorescent staining of rat sciatic nerve showed that p200 was localized in the extracellular matrix surrounding individual Schwann cells-axon units. Two tryptic peptides from p200 were purified and sequenced. These contained multiple GXX collagen-like repeats. Bacterial collagenase digestion of p200 produced a product with an apparent molecular mass of approximately 90 kDa. These data suggest that Schwann cells secrete an apparently novel collagen-like adhesive protein that interacts with cells through cell surface heparan sulfate proteoglycans.

During peripheral nerve development Schwann cells deposit basal lamina on the outer surface of the individual Schwann cell-axon units. Using primary co-cultures of Schwann cells and nerve cells, it was observed that under culture conditions in which Schwann cells do not assemble basal lamina (for instance in culture medium lacking ascorbate), the myelination and ensheathment of axons does not occur (1). The addition of exogenous basal lamina-like matrix such as Matrigel or certain basal lamina components restores the Schwann cell’s ability to myelineate axons (2, 3). These findings demonstrate that Schwann cell contact with extracellular matrix (ECM) is required for normal ensheathment and myelination of axons.

In vivo and in vitro studies have identified a number of Schwann cell integrins, which are thought to mediate interaction of these cells with ECM (4–6). The repertoire of Schwann cell integrins generally corresponds to the molecular composition of basal lamina. For example, Schwann cells express α6β1 (4) and α5β1 (6) integrin, which have been shown to function as receptors for laminin and/or collagen, both of which are secreted by Schwann cells and are present in the peripheral nerve ECM. In addition to integrins, Schwann cells possess cell surface proteoglycans that might also be involved in the interactions of the cells with the ECM, including basal lamina. It has been shown that glypicans, a lipid-anchored cell surface proteoglycan of Schwann cells, was able to bind laminin (7). It is likely, therefore, that this proteoglycan can mediate the interaction of Schwann cells with laminin by acting cooperatively with one of the laminin-binding integrins. Schwann cells also express at least one member of the syndecan family of transmembrane proteoglycans, N-syndecan (syndecan-3) (8). The extracellular ligands for N-syndecan in the peripheral nervous system are unknown. Previously, we found that N-syndecan purified from neonatal rat brain binds to basic fibroblast growth factor (9) and to heparin-binding growth-associated molecule, a secreted adhesive protein that is expressed abundantly in late embryonic and neonatal nervous tissues (10–12). In contrast, rat brain N-syndecan failed to bind a number of potential ECM ligands, including components of basal lamina such as collagen type IV and laminin (9).

To obtain additional information on the nature of Schwann cell interactions with the extracellular environment, we have attempted to identify potential extracellular ligand(s) for N-syndecan in conditioned medium of cultured Schwann cells. This source was chosen because, as was found previously, cultured Schwann cells synthesize and secrete the proteins found in the peripheral nerve ECM in vivo, including several types of collagen and laminin (13). Here we report the purification and initial biochemical characterization of a new collagen-like adhesive glycoprotein, p200, from Schwann cell conditioned medium. p200 binds to N-syndecan, probably through the heparan sulfate chains of proteoglycan. The temporal and tissue-specific patterns of p200 expression strongly suggest that the protein plays an important role in Schwann cell development.

MATERIALS AND METHODS

Schwann Cell Cultures—Schwann cells were cultured from neonatal rat sciatic nerves as described previously. The cells were grown in DMEM containing 10% fetal calf serum plus 2 μM forskolin on poly-L-lysine coated culture dishes. Conditioned medium was collected every 2–3 days and stored at −70 °C until used. Heparin-Agarose Chromatography and Overlay Assay for N-Syndecan Binding Proteins—Conditioned medium from cultured rat Schwann cells was subjected to affinity chromatography on a heparin-agarose column. Conditioned medium was loaded onto the column, and the column was washed extensively with buffer containing 25 mM Tris-HCl, 50 mM NaCl, 1 mM PMSF, 4 mM benzamidine HCl, pH 7.6. Bound
material was eluted with a linear gradient of 50 mM to 2 M NaCl in 50 mM Tris-HCl, pH 7.6, plus protease inhibitors. Aliquots of column fractions were analyzed by SDS gel electrophoresis on 4–15% polyacrylamide gradient gels. Two identical sets of gels were run. One of the gels was stained with Coomassie Brilliant Blue. The other gel was electro-transferred to a nitrocellulose membrane at 80 V for 3–4 h, followed by blocking with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 2 h. The membranes were incubated at 4°C overnight with 125I-labeled rat brain N-syndecan, prepared as described previously (9). After four rinses with PBS, the membranes were dried and subjected to autoradiography.

Production of Anti-p200 Antibodies—Antibodies to p200 were produced as previously described (14). Cultures were incubated with 1% N-syndecan, 1% mannosyl N-syndecan-binding protein, 0.1 M N-syndecan-binding protein, 0.1 M N-syndecan-7-methyl mannoside, 0.1 M N-syndecan, 1 mM PMSF, 4 mM benzamidine HCland incubated overnight at 4°C on a shaker. The mixture was then packed into a column, and the eluate was collected in 0.2 M NaCl fractions. The bound material was eluted with 0.1 M NaCl (for purification of p200) followed by 1 M NaCl (for purification of p200) and bound proteins were eluted with a linear gradient of 0.2 M to 0.8 M NaCl in 50 mM Tris-HCl, pH 7.6. The elution of p200 was monitored by immunoblot analysis. p200 was eluted as a broad peak at approx. 0.2 M NaCl. The supernatants were removed and the pellets were extracted with 1% Triton X-100 in PBS plus PMSF followed by centrifugation. The final pellets were solubilized in gel sample buffer containing 2% SDS and 5% 2-mercaptoethanol. Aliquots of the PBS, Triton X-100, and SDS solubilized material containing equal quantities of total protein (determined by a turbidity assay (17)) were loaded onto SDS-polyacrylamide gels. p200 was detected by immunoblot analysis as described above.

Immunofluorescence microscopy of cryostat sections of rat sciatic nerve were carried out as described previously (18). Peptide Sequencing of p200—Internal peptides derived from p200 were obtained by in-gel digestion of the protein with trypsin, essentially as described previously (19), followed by purification of the resulting peptides on an AminoQuant C18 reverse phase column (Hewlett Packard).

For digestion of p200 immobilized on a heparin-agarose column, an aliquot of partially purified p200 was dialyzed against 50 mM Tris-HCl, pH 7.6, 0.5 M NaCl, 0.5 mM PMSF, and used for overlay assay. p200-containing samples were separated on 4–15% polyacrylamide gradient gels, transferred to nitrocellulose, and blocked with 1% BSA as described above for overlay with 125I-N-syndecan. The membranes were then incubated overnight at 4°C in a solution containing N-syndecan-enriched fractions prepared from Schwann cell conditioned medium as described above plus 1% BSA (1:1). After four washes with 50 mM Tris-HCl, pH 7.6, 100 mM NaCl bound N-syndecan was detected with affinity purified anti-N-syndecan antibodies essentially as described previously for immunoblot analysis with these antibodies (8), except that an ECL detection system was used. As a control, the N-syndecan incubation step was omitted. In some experiments partially purified rat brain N-syndecan was used instead of Schwann cell N-syndecan, and the binding was also detected with anti-N-syndecan antibodies as described above.

Schwann Cell Adhesion Assay—The procedure described previously was followed (16), with some modifications. Briefly, culture dishes were coated with nitrocellulose as described (16), and protein samples were applied to the surface of the dishes in 10–12-μl droplets. Adhesive proteins tested were mouse tumor laminin (20 μg/ml), human plasma fibronectin (50 μg/ml), and purified p200 (4 μg/ml). The droplets were incubated for 1 h at 37°C in a humid atmosphere. Due to the low concentrations of p200, fractions showing positive signals applied 1–2 times onto the same spot to achieve a higher coating concentration. The droplets were removed by aspiration, and the plates were blocked with 1% BSA in 50 mM Tris-HCl, pH 7.5, 10 mM NaCl for 1 h at 37°C, followed by two brief washes with Hank’s balanced salt solution. Schwann cells to be used in this assay were detached by brief incubation with 0.05% trypsin. The trypsin was inhibited by the addition of soybean trypsin inhibitor and 10% fetal calf serum in DMEM. The cells were pelleted by centrifugation and resuspended in DMEM without serum. Aliquots of the cell suspension were added to the coated plates and incubated at 37°C for 2 h. For antibody inhibition experiments, droplets of diluted antibodies or antisera were applied onto the same spots after aspiration of the adhesive protein droplets and incubated for 1 h at 37°C. The incubation was followed by blocking with 1% BSA in 50 mM Tris-HCl, pH 7.5, 10 mM NaCl. For fibronectin antibodies a 50 μg/ml solution of affinity purified antibodies in the same buffer was used. Droplets containing buffer only were used as controls. For the heparin inhibition experiments heparin was added to the cell suspensions at a final concentration of 10 μg/ml immediately before the addition of the cells to the coated plates. At the end of the incubation the nonadherent cells were removed, the plates were washed gently with DMEM twice and examined by phase contrast microscopy.

Identification of p200 in Tissue Extracts by Immunoblot Analysis—Neonatal rat tissues were homogenized in PBS with PMSF on ice. The homogenates were centrifuged at 200,000 g at 4°C for 1 h at 37°C, the supernatants were removed, and the pellets were extracted with 1% Triton X-100 in PBS plus PMSF followed by centrifugation. The final pellets were solubilized in gel sample buffer containing 2% SDS and 5% 2-mercaptoethanol. Aliquots of the PBS, Triton X-100, and SDS solubilized material containing equal quantities of total protein (determined by a turbidity assay (17)) were loaded onto SDS-polyacrylamide gels. p200 was detected by immunoblot analysis as described above.

Immunofluorescence microscopy of cryostat sections of rat sciatic nerve were carried out as described above (18).
tions. Control samples were treated identically, except that the enzyme was omitted.

Heparitinase Digestion—Schwann cell N-syndecan was digested with heparitinase (Seikagaku Kogyo Co.) as described previously (9) for 15 h at 38 °C. At the end of the incubation EDTA was added to a final concentration of 5 mM, and digested N-syndecan was dialyzed exhaustively against Tris buffer containing 1 mM PMSF. A control sample was treated identically in buffer lacking enzyme. Undigested or digested N-syndecan was used in overlay assays with p200 as described above, and the binding was detected with anti-N-syndecan antibodies.

RESULTS

Identification of an N-Syndecan-binding Protein in Schwann Cell Conditioned Medium—Conditioned medium from cultured Schwann cells was chromatographed on heparin-agarose followed by elution of bound proteins with a linear NaCl gradient. Aliquots of column fractions eluted by the salt gradient were analyzed by electrophoresis in 4–15% SDS-polyacrylamide gradient gels under nonreducing conditions; identical gels were either stained with Coomassie Brilliant Blue (A) or transferred to nitrocellulose and overlaid with 125I-N-syndecan (B). Numbers and arrows on the left indicate positions of migration of molecular mass standards (in kDa).

![Image](schwann_cell_collagen-like_adhesive_protein)

**Fig. 1. Identification of an N-syndecan-binding protein in Schwann cell conditioned medium.** Conditioned medium from cultured Schwann cells was chromatographed on heparin-agarose followed by elution of bound proteins with a linear NaCl gradient. Aliquots of column fractions eluted by the salt gradient were analyzed by electrophoresis in 4–15% SDS-polyacrylamide gradient gels under nonreducing conditions; identical gels were either stained with Coomassie Brilliant Blue (A) or transferred to nitrocellulose and overlaid with 125I-N-syndecan (B). Numbers and arrows on the left indicate positions of migration of molecular mass standards (in kDa).

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arin-agarose affinity, gel filtration, and lectin affinity chromatography as described under “Materials and Methods.” The p200-containing fractions were identified by immunoblotting with anti-p200 antiserum and pooled for further purification. Aliquots of the starting material (lanes 1) as well as pooled fractions eluted from Q-Sepharose (lanes 2), heparin-agarose (lanes 3), Sepharose CL-6B (lanes 4), and Pisum sativum agglutinin-Sepharose (lanes 5) were subjected to SDS gel electrophoresis followed by Coomasie staining (A, lanes 1 and 2) or silver staining (A, lanes 3–5), transfer to Immobilon and immunostaining with anti-p200 antiserum (B), or transfer to nitrocellulose and overlay with 125I-N-syndecan (C). D and E, specificity of anti-p200 antiserum. Aliquots of 10% fetal calf serum in DMEM (lanes 1), Schwann cell conditioned medium (lanes 2), or conditioned medium of mixed primary culture from neonatal rat brain cortex (lanes 3) were analyzed by SDS gel electrophoresis followed by Coomasie staining (D) or transfer to Immobilon and staining with anti-p200 antiserum (E).

**Fig. 2. Purification of p200.** A–C, p200 was purified from Schwann cell conditioned medium using a combination of anion-exchange chromatography, affinity chromatography, and gel filtration as described under “Materials and Methods.” The p200-containing fractions were identified by immunoblotting with anti-p200 antiserum and pooled for further purification. Aliquots of the starting material (lanes 1) as well as pooled fractions eluted from Q-Sepharose (lanes 2), heparin-agarose (lanes 3), Sepharose CL-6B (lanes 4), and Pisum sativum agglutinin-Sepharose (lanes 5) were subjected to SDS gel electrophoresis followed by Coomasie staining (A, lanes 1 and 2) or silver staining (A, lanes 3–5), transfer to Immobilon and immunostaining with anti-p200 antiserum (B), or transfer to nitrocellulose and overlay with 125I-N-syndecan (C). D and E, specificity of anti-p200 antiserum. Aliquots of 10% fetal calf serum in DMEM (lanes 1), Schwann cell conditioned medium (lanes 2), or conditioned medium of mixed primary culture from neonatal rat brain cortex (lanes 3) were analyzed by SDS gel electrophoresis followed by Coomasie staining (D) or transfer to Immobilon and staining with anti-p200 antiserum (E).

**p200 Is Adhesive for Schwann Cells—**We tested the ability of purified p200 to promote attachment and spreading of Schwann cells. Laminin was used as a positive control because it was shown previously that this protein is adhesive for Schwann cells (7). When p200 was used to coat the surfaces of tissue culture dishes, it strongly promoted the attachment and spreading of Schwann cells (Fig. 3a). The activity was blocked by anti-p200 antiserum but not by anti-laminin antiserum (Fig. 3, c and d). Adhesion to laminin was inhibited with anti-laminin antiserum. Anti-p200 antiserum was ineffective in inhibiting Schwann cell adhesion to laminin (Fig. 3, d and f). Similar
results were obtained using another adhesive protein, fibronectin. Anti-p200 antiserum did not inhibit adhesion of Schwann cells to fibronectin. Anti-fibronectin polyclonal antibodies strongly inhibited Schwann cell adhesion to fibronectin but not to p200 (data not shown).

We also tested the effect of heparin on Schwann cell adhesion to different substrata. Heparin was moderately inhibitory for Schwann cell adhesion to laminin. Fewer cells attached to laminin in the presence of heparin, and processes extended by the cells were shorter compared with controls (Fig. 3, b and h). This result was consistent with previous observations (7). The inhibitory effect of heparin on Schwann cell adhesion to p200 was essentially complete. Very few cells attached to p200 in the presence of heparin. The few cells that attached extended very short, if any, processes (Fig. 3g). This result strongly suggests a direct involvement of cell surface heparan sulfate proteoglycans in the interaction of Schwann cells with p200.

Expression of p200 in Vivo—A panel of neonatal rat tissues was analyzed to determine the distribution of p200 in vivo. The tissues were extracted sequentially with PBS, Triton X-100, and SDS. The extracts were subjected to immunoblot analysis with anti-p200 antiserum. The results are shown in Fig. 4.
Strong p200 immunoreactivity was detected in sciatic nerve PBS extracts. Skeletal muscle extracts produced a weaker but detectable signal. The remainder of the tissues tested did not contain any detectable immunoreactive proteins. Analysis of Triton X-100 and SDS extracts showed that in the case of both sciatic nerve and skeletal muscle, the amount of p200 was lower than what was observed in the PBS extracts (and barely detectable in SDS extracts). None of the other tissues produced any additional signals (data not shown). Analysis of sciatic nerve extracts under nonreducing conditions showed that the mobility of the p200-immunoreactive band did not change significantly, indicating that the protein does not form disulfide-bonded multimers in vivo (data not shown).

To examine the time course of p200 expression during peripheral nerve development, PBS extracts of sciatic nerve from rats of different postnatal ages as well as adult rats were subjected to immunoblot analysis with anti-p200 antiserum. Extracts from 2- and 14-day-old rats contained similar amounts of p200. There was a sharp decrease in the p200 level in 21-day-old rats. p200 was not detected in adult rat sciatic nerve extracts (Fig. 5). Analysis of Triton X-100 and SDS extracts of adult sciatic nerve did not show any immunoreactivity (not shown). Thus, the data shown in Fig. 5 do not reflect a change in p200 solubility during maturation.

Immunofluorescence Microscopy—Indirect Immunofluorescent staining of cryostat sections was used to determine the distribution of p200 within the sciatic nerve. Anti-p200 staining of sciatic nerves from 10-day-old rats was confined to the outer surfaces of individual axon-Schwann cell units (Fig. 6b). This conclusion was supported by the finding that the anti-p200 staining pattern was very similar to what was observed with anti-laminin antibodies (Fig. 6a). These results strongly suggest an association of p200 with the peripheral nerve ECM. When sciatic nerves from adult rats were stained with anti-laminin antibodies, a similar staining pattern was observed (Fig. 6c). This was in contrast to results obtained with anti-p200 antibodies, which did not stain adult rat nerve (Fig. 6d). This result is consistent with results obtained by immunoblot analysis and demonstrates a lack of p200 expression in adult peripheral nerve. In control experiments immunofluorescent staining of sections from 10-day-old rat cortex was done using both anti-laminin and anti-p200 antibodies. Anti-laminin antibodies stained small blood vessels within the cortex and the pial covering of the cortex (Fig. 6e). Anti-p200 antibodies failed to stain cortex (Fig. 6f), consistent with immunoblotting data.

Schwann Cell N-Syndecan Binds p200—The results described above show that p200 has a very restricted tissue distribution and may be expressed exclusively in the peripheral nervous system during early postnatal development. The N-syndecan used for the overlay assays described above was purified from neonatal rat brain. It has been reported that heparan sulfate structural variations are observed with syndecan-1 isolated from different cell types, which can result in different ligand binding properties (20, 21). This prompted us to repeat the overlay assays using N-syndecan from Schwann cells. A modified overlay assay that used anti-N-syndecan antibodies for detection of bound N-syndecan was used (see "Materials and Methods"). When analyzed by immunoblotting with anti-N-syndecan antibodies Schwann cell N-syndecan migrated as a broad smear with a molecular mass of >250 kDa (Fig. 7A, lane 7). Schwann cell N-syndecan had an average apparent molecular mass larger than that of brain N-syndecan (Fig. 7A, lane 8), suggesting that some molecular heterogeneity does exist.

Schwann cell N-syndecan, like its counterpart from rat brain, bound to p200 in the membrane overlay assay (Fig. 7A, lanes 2 and 5). When the N-syndecan incubation step was omitted, no positive signal was detected (Fig. 7A, lane 6). The addition of heparin to the incubation medium containing N-syndecan blocked the binding, whereas chondroitin sulfate had no effect (Fig. 7A, lanes 3 and 4). Heparin did not interfere with the detection of N-syndecan with anti-N-syndecan antibodies on immunoblots (Fig. 7A, lane 9). When heparan sulfate chains of N-syndecan were removed by heparitinase treatment of the proteoglycan and the deglycosylated core protein was used for overlay with p200, no binding was detected (Fig. 7B, lane 4).

p200 Contains a Collagen-like Domain—To further characterize p200 we attempted to determine amino acid sequences of internal peptides. p200 that had been purified by Q-Sepharose and heparin-agarose chromatography was subjected to preparative SDS gel electrophoresis. The p200 band was excised from the gel and subjected to in-gel digestion with trypsin. The resulting peptide mixture was fractionated by reverse phase high pressure liquid chromatography, and several of the purified peptides were sequenced by automated Edman degrada-
tion. Sequence data were obtained for two peptides (Fig. 8). Each of these sequences contained the repeating amino acid sequence motif GXX (where X is frequently proline) characteristic of collagens. A computer search of current EMBL and GenBank sequence data bases did not produce an exact match with either of these sequences.

To determine whether we could obtain additional evidence for the presence of collagen-like structure in p200, we digested the protein with purified bacterial collagenase. The resulting product was separated on 4–15% polyacrylamide gradient gels and analyzed by silver staining, immunoblotting with anti-p200 antiserum, and overlay assay with N-syndecan. Collagenase treatment of purified p200 caused the complete disappearance of the 200-kDa band on silver-stained gels (Fig. 9A, lane 2). Collagenase digestion produced a band with an apparent molecular mass of approximately 90 kDa that was detected by immunoblot analysis with anti-p200 antiserum (Fig. 9B, lane 2). When a more concentrated, partially purified sample of p200 was subjected to collagenase digestion, a band with identical mobility could also be visualized on silver stained gels (Fig. 10A, lane 2). The electrophoretic mobility of the other bands on the gel did not change after collagenase treatment, indicating that p200 cleavage was not a result of contaminating proteolytic activity (Fig. 10A, lanes 1 and 2). These data strongly suggest that p200 is a hybrid molecule that consists of a large collagen-like domain plus a noncollagenous domain of approximately 90 kDa that is resistant to bacterial collagenase digestion.

As can be seen in Figs. 9C (lane 2) and 10C (lane 2), collagenase digestion resulted in a loss of N-syndecan binding activity of the resulting product as determined by overlay assay. To investigate this further we carried out collagenase digestion of p200 adsorbed onto heparin-agarose. p200 was applied to a small heparin-agarose column and subjected to collagenase digestion. After the incubation the column was washed, and the bound material was eluted with high salt buffer. Aliquots of these fractions were analyzed by immunoblotting with anti-p200 antibodies. No immunoreactive products were detected in either the collagenase-released material or in the washes (data not shown); in contrast, an immunoreactive band of approximately 90 kDa was detected in the high salt elution (Fig. 10B, lane 4). The electrophoretic mobility of this band was identical to that of the product obtained by collagenase digestion of p200 in solution (compare lanes 2 and 4 of Fig. 10B). However, the material eluted from heparin-agarose after collagenase digestion had no detectable N-syndecan binding activity when tested using the overlay assay (Fig. 10C, lane 4). These data indicate that although the collagenase-resistant fragment of p200 re-

**Fig. 6. Immunofluorescent staining of nerve tissue sections.** Cryostat sections of 10-day-old (a and b) or adult (c and d) rat sciatic nerves or 10-day-old rat cortex (e and f) were stained with anti-laminin antibodies (a, c, and e) or anti-p200 antibodies (b, d, and f). Bar, 100 μm.
tained heparin binding activity, its N-syndecan binding activity was abolished.

Biochemical Characterization of p200—The observation that p200 displayed lectin binding activity suggested the presence of N-linked oligosaccharides. To test this possibility p200 was treated with N-glycosidase-F followed by SDS gel electrophoresis and silver staining, immunoblot analysis with anti-p200 antiserum, and overlay assay with N-syndecan. The electrophoretic mobility of p200 was also compared under nonreducing and reducing conditions. Under nonreducing conditions the electrophoretic mobility of p200 was changed only slightly, and the protein retained full N-syndecan binding activity (Fig. 11, lane 1, see also Fig. 1B). N-Glycosidase F treatment caused a small shift in mobility that was detected both by silver staining and immunoblotting (Fig. 11, lanes 3 and 4). The deglycosylated protein retained N-syndecan binding activity, indicating that N-linked oligosaccharides were not required for the interaction of p200 with the proteoglycan.

**DISCUSSION**

Schwann cells undergo a multistep process of development including an initial association with axons, proliferation, deposition of basal lamina, and, finally, myelination and ensheathment of nerve fibers (see Ref. 13 for review). In vitro studies have shown that if Schwann cells are prevented from forming basal lamina, then normal myelination and ensheathment do not occur (1, 22). These findings demonstrate that basal lamina assembly and interactions of Schwann cells with basal lamina components are critical steps in Schwann cells development. A number of Schwann cell basal lamina components have...
To understand better the mechanisms through which Schwann cells interact with the extracellular environment, we attempted to identify new molecule(s) that might be involved in this process. We purified a high molecular weight heparin-binding glycoprotein, p200, from conditioned medium of cultured Schwann cells. A surprising property of p200 was that among the dozens of heparin-binding proteins present in Schwann cell conditioned medium, p200 was the only one that displayed detectable N-syndecan binding activity in a membrane overlay assay. Using this overlay assay, it was shown that N-syndecan isolated from rat brain or from Schwann cells binds to p200. Heparin but not chondroitin sulfate completely inhibited binding, and deglycosylated N-syndecan core protein failed to bind to p200, strongly suggesting that the heparan sulfate chains of the proteoglycan were primarily involved in the interaction. We also found that p200 was adhesive for Schwann cells. This activity was also inhibited by heparin, consistent with a role for cell surface heparan sulfate proteoglycans in Schwann cell-p200 interactions.

The tissue distribution of p200 was examined by immunoblot analysis. We found high levels of p200 expression in neonatal sciatic nerve and lower but detectable levels in neonatal skeletal muscle. Surprisingly, other tissues examined were negative for p200 immunoreactivity. In the case of skeletal muscle, it is possible that the p200 that is detected is from peripheral nerves innervating the muscle rather than from the muscle itself. Analysis of the time course of p200 expression in peripheral nerve showed that p200 had a restricted time frame of expression. It was detected in sciatic nerve only during the first 2–3 weeks after birth and was not detected in adult nerves. Finally, analysis of sequential extractions of neonatal sciatic nerve with PBS, nonionic detergent, and SDS revealed that most of the p200 was extracted with PBS, suggesting a loose association of the protein with the tissue.

Immunofluorescent staining of sciatic nerve sections using affinity purified anti-p200 antibodies was also carried out. In sciatic nerve obtained from 10-day-old rats, the antibodies stained the area surrounding individual Schwann cell-axon units, similar to the pattern that was observed with anti-laminin antibodies. It cannot be determined, however, based solely on light microscopy data, with what particular extracellular structures p200 was associated (i.e. basal lamina, collagen fibrils, etc.). To do this, more detailed studies at the electron microscope level will need to be done. In adult sciatic nerve sections anti-p200 staining was not observed, consistent with the results obtained by immunoblot analysis.

Amino acid sequencing of internal peptides produced from p200 yielded unique sequences that were not found in current sequence data bases. These peptides sequences had the repeating GXG sequence motif characteristic of collagens. Bacterial collagenase treatment of p200 caused a reduction in the apparent molecular mass to approximately 90 kDa, suggesting the presence of collagen-like sequences linked to a large noncollagenous domain. The collagenase digestion product retained full heparin binding activity but lost N-syndecan binding activity. This result was unexpected, because heparin inhibited binding of N-syndecan to p200. Further studies will be needed to resolve this issue.

The existence of a collagenous domain within a protein structure is generally considered to be strong evidence that this domain participates in triple helix formation. For many collagens and collagen-like proteins this process is followed by interchain disulfide cross-linking, providing structural stability to the molecule. We were not able to find any evidence that p200 participates in the formation of disulfide-bonded multimeric complexes.
The mechanisms underlying Schwann cell interaction with p200 remain unknown. As was mentioned above, data reported here strongly suggest that cell surface heparan sulfate proteoglycans are receptors for p200 in Schwann cells. We have shown in vitro evidence for binding of N-syndecan to p200. The binding of Schwann cell glycan to p200 has not been tested. We cannot exclude the possibility that additional Schwann cell receptors, such as an integrin, might also be involved in the interaction with p200.

The function of p200 in the nervous system also remains elusive. The fact that p200 expression in sciatic nerve is restricted to the first 2–3 weeks of neonatal development, together with localization of p200 in the ECM surrounding individual axon-Schwann cell units, strongly suggests that this protein plays an important role in peripheral nerve development. It is interesting that the time frame of p200 expression generally coincides with that of N-syndecan, the putative receptor, 2 again suggesting a direct association between these two proteins in vivo.

It should be pointed out that N-syndecan is also expressed at high levels in neonatal rat brain and spinal cord, tissues that are apparently devoid of p200. This suggests that in the central nervous system N-syndecan binds to ligands other than p200. Thus N-syndecan might have different functions in the central and peripheral nervous systems. Further studies are necessary to prove this hypothesis, as well as to determine the exact functions of both proteins in the developing nervous system.

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