Identification of the Core Protein Carrying the Tn Antigen in Mouse Brain: Specific Expression on Syndecan-3

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ABSTRACT. We isolated glycoproteins carrying the Tn antigen, which was expressed spatiotemporally in the developing mouse brain. The Tn antigen was expressed on two molecular species with a molecular weight from 200 to 350 kDa and 110 to 160 kDa, as judged on SDS-PAGE. Although the two glycoproteins showed different susceptibilities to heparitinase I and solubilities in a salt solution, after treatment with V8 protease they showed the same mobility corresponding to a molecular weight of 90 kDa on SDS-PAGE, suggesting that these two molecules shared a common core protein. Partial N-terminal sequences of the glycoproteins were determined, i.e. AQRXRNENFERPV and ALAAPXAPAMLP, which were identified as the sequences of the N-terminal and central portions of syndecan-3, respectively. Both glycoproteins were reactive to anti-mouse syndecan-3 antibody. These results suggest that one is a soluble syndecan-3 cleaved between mucin-like domain and transmembrane domain, and the other is a membrane-bound syndecan-3 lacking N-terminal glycosaminoglycan attachment sites, and that both glycoproteins have a mucin-like domain characteristic of syndecan-3, in which the Tn antigen may be expressed.

Key words: syndecan-3/Tn antigen/mucin/brain development

Developing nervous tissues contain various proteoglycans, the expression of which is regulated spatiotemporally. It has been reported that these proteoglycans play important roles in nervous morphogenetic processes. Most of their functional roles seem to be derived from their binding properties to cell adhesion molecules, extracellular matrix components, growth factors and neurotrophic factors (Margolis and Margolis 1993; Oohira et al., 1994). In addition to glycosaminoglycans, many proteoglycans have other types of carbohydrate chains such as N- and O-glycans. Not only glycosaminoglycans but also N- and O-glycans and core proteins seem to be involved in a variety of biological interactions (Gould et al., 1992; Milev et al., 1998; Herndon et al., 1999; Oohira et al., 2000). Syndecan-3 is known to be a heparan sulfate proteoglycan present in the developing brain. This proteoglycan has a unique mucin-like domain unlike other syndecans (Carey, 1996). We reported previously that the Tn antigen, which is one of the cancer-associated carbohydrate antigens, was expressed in developing mouse central nervous tissues, in particular, in the developing cerebral cortex and cerebellum (Akita et al., 2001). To characterize the core protein, we purified glycoproteins carrying the Tn antigen from early postnatal mouse brains. Two molecular species expressed the Tn antigen. The major glycoprotein, with a molecular weight ranging from 200 to 350 kDa, exhibited similar susceptibility to glycosidases including heparitinase I to that of syndecan-3 (Chernousov and Carey, 1993; Watanabe et al., 1996). Furthermore, a partial N-terminal sequence of the major glycoprotein was revealed to coincide with that of syndecan-3 (Kung et al., accession No. U52826). Another glycoprotein was demonstrated to be syndecan-3 devoid of the N-terminal portion.

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Abbreviations: CHAPS, 3-[[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate; E, embryonic day; HRP, horseradish peroxidase; NEM, N-ethylmaleimide; P, postnatal day; PMSF, phenylmethanesulfonyl fluoride.
Materials and Methods

Animals and antibodies

ICR strain mice (SLC Inc., Shizuoka, Japan) were used for the purification of glycoproteins carrying the Tn antigen. A murine anti-Tn monoclonal antibody, designated as MLS 128, was prepared as described previously (Numata et al., 1990). MLS 128 recognizes a glycopeptide including a cluster of N-acetylgalactosamine (GalNAc) α1-Ser/Thr residues (Nakada et al., 1991, 1993; Inoue et al., 1994). Rabbit anti-mouse syndecan-3 core protein antibody was raised against the recombinant polypeptide of syndecan-3 according to Kusano et al. (2000). Monoclonal antibody 3G10, which is directed to the desaturated uronate residues produced from heparan sulfate on heparitinase treatment, was purchased from Seikagaku Kogyo (Tokyo, Japan).

Purification of glycoproteins carrying the Tn antigen from early postnatal mouse brains

The brains of postnatal mice (P5-7) were used and all procedures were carried out at 4°C or on ice unless otherwise stated. The brains (wet weight: 140 g) were homogenized in 3.5 volume of an extraction solution comprising 1% TritonX-100, 25 mM Tris-HCl buffer, pH 7.5, 0.15 M NaCl, 10 mM EDTA, 1 mM PMSF, 0.5 mM NEM, pepstatin-A (1 μg/ml), and leupeptin (1 μg/ml) with a Polytron homogenizer, extracted for 15 h, and then centrifuged at 25,000 x g for 10 min, the resulting supernatant being centrifuged at 105,000 x g for 1 h. The pellet was re-homogenized in an equal volume of the extraction solution and then centrifuged as described above. The combined supernatants were fractionated by ammonium sulfate precipitation. After discarding the precipitates at 40% saturation, the glycoproteins were precipitated by further addition of solid ammonium sulfate to 85% saturation. After centrifugation at 20,000 x g for 30 min, the precipitate was dissolved in a solution of 0.5% TritonX-100, 25 mM Tris-HCl buffer, pH 7.5, 0.1 M NaCl, and 0.5 mM EDTA, and then dialyzed against the same solution. The dialyzed material was applied to a column of Q-Sepharose Fast Flow column (3 x 15 cm) equilibrated with a solution of 0.5% TritonX-100, 25 mM Tris-HCl buffer, pH 7.5, 0.1 M NaCl, and 0.5 mM EDTA. After washing the column with 25 mM Tris-HCl buffer, pH 7.5, containing 0.25 M NaCl and 0.1% CHAPS, the glycoproteins carrying the Tn antigen were eluted with a solution of 0.1% CHAPS, 25 mM Tris-HCl buffer, pH 7.5, and 1.5 M NaCl. After dialysis of the eluate against 25 mM Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl, 2 mM EDTA and 0.1% CHAPS, excessive amounts of MLS 128 and protein A-Sepharose were added, followed by mixing overnight. The immune complex was poured into a column. After extensively washing of the column with the above solution, the immune complex was eluted with 0.1 M citrate-NaOH buffer, pH 3.8, containing 0.15 M NaCl and 0.5% CHAPS. The eluate was dialyzed against 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM NEM, 5 mM EDTA, 0.5% CHAPS and 4 M guanidine-HCl. After adding solid cesium chloride to a density of 1.34 mg/ml, the eluate was centrifuged at 230,000 x g for 72 h and then fractionated with a density gradient fractionator, DGF-U (Hitachi, Tokyo, Japan). The fractions in which the antigen was detected were collected. The glycoproteins carrying the Tn antigen were finally purified by gel filtration on a TskgelG4000SWXL column (0.78 x 30 cm) (Tosoh Corporation, Tokyo, Japan). In some cases, the immune complex prepared as described above was directly subjected to SDS-PAGE. Two glycoproteins corresponding to molecular weights of 200-350 kDa and 110-160 kDa were extracted from the gel.

Enzymatic treatments with glycosidases and proteases

The glycoproteins (0.1 μg protein) were treated with various glycosidases and proteases. The glycoproteins were treated with 0.2 units/ml of heparitinase I or chondroitinase ABC (Seikagaku Kogyo Co., Tokyo, Japan) in 30 mM Tris-acetate buffer, pH 7.5, containing 10 mM EDTA, 10 mM NEM, 1 mM PMSF and pepstatin-A (0.25 mg/ml) at 37°C for 1 h according to Kato et al. (1985). Sialidase (Nacalai Tesque, Kyoto, Japan) treatment (0.06 units/ml) was performed in 30 mM sodium acetate buffer, pH 5.0, containing 5 mM EDTA, 5 mM NEM, 1 mM PMSF and pepstatin-A (0.07 mg/ml) at 37°C for 20 h. N-Glycosidase F (Takara Shuzo, Ohtsu, Japan) treatment (0.04 units/ml) was performed at 37°C for 20 h according to manufacturer’s instructions. Digestion with 0.1 units/ml of α-N-acetylgalactosaminidase (Sigma, St. Louis, MO) was carried out at 37°C for 20 h in 30 mM sodium acetate buffer, pH 4.0, containing 5 mM EDTA, 5 mM NEM, 1 mM PMSF and pepstatin-A (0.07 mg/ml). O-Sialoglycoprotein endopeptidase (Laboratories Limited, Hornby, Ontario, Canada) digestion was performed in 50 mM Hepes buffer, pH 7.2, at 37°C for 20 h, and digestion with V8 protease (Roche Molecular Biochemicals, Mannheim, Germany) was performed in 50 mM ammonium bicarbonate buffer, pH 7.8, at 37°C for 20 h. All protease digestions were carried out with a substrate/enzyme ratio of 100 : 3.

Immunoblotting

SDS-polyacrylamide gel electrophoresis on a 2–15% gradient gel was performed according to Laemmli (Laemmli, 1970). After electrophoresis, the separated proteins were transferred to a Zeta-probe membrane (Bio-Rad, Hercules, CA) at 20 V for 15 h. The membrane was incubated with 50 mM Tris-HCl buffer, pH 7.5, containing 5% BSA and 0.15 M NaCl to block nonspecific staining, followed by successive incubation with anti Tn antibody (MLS 128) or anti syndecan-3 core protein antibody and HRP-protein G (Zymed, South San Francisco, CA). The membrane was visualized with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Buckinghamshire, England).

N-Terminal sequencing of glycoproteins carrying the Tn antigen

The N-terminal sequences of the glycoproteins were determined with an Applied Biosystems sequencer model 492.
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Immunohistochemistry

Immunohistochemical staining with MLS 128 was carried out as described previously (Akita et al., 2001). The tissues were fixed overnight in Bouin’s fixative at 4°C and embedded in paraffin. Paraffin sections, 4 μm thick, were obtained with a rotary microtome. After deparaffinization, the sections were treated with 0.3% hydrogen peroxide in absolute methanol for 15 min to block endogenous peroxidase activity, and then with rabbit normal serum. After incubation with MLS 128 (10 μg/ml) at room temperature for 2 h and subsequent rinsing with PBS, the sections were incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG immunoglobulin (Zymed) at a dilution of 1:200 in 1% bovine serum albumin (BSA)-PBS for 1 h, washed with PBS, and then visualized with 0.03% 3,3′-diaminobenzidine tetrahydrochloride (DAB), 0.003% hydrogen peroxide and 10 mM imidazole in 50 mM Tris-HCl buffer, pH 7.6. To stain cell nuclei, sections were treated with hematoxylin. Control experiments were performed similarly using normal murine IgG (Zymed) instead of MLS 128. Immunohistochemical staining for the syndecan-3 core protein was carried out using Histofine SAB rabbit kit (Nichirei, Tokyo, Japan).

Results

Isolation of glycoproteins carrying the Tn antigen from early postnatal mouse brains

In a previous paper, we demonstrated immunohistochemically that the Tn antigen was expressed in the developing mouse brain, and that a glycoprotein with a molecular weight of about 250 kDa was immunoprecipitated from a mouse brain extract with MLS 128. The glycoprotein could be extracted with a solution without any detergent as described previously (Akita et al., 2001). When extracted with a solution containing 1% TritonX-100, as described under Materials and Methods, another minor glycoprotein corresponding to 140 kDa was found in addition to the major glycoprotein, as shown in Fig. 1A. To examine the developmental changes of the glycoproteins, expression of the glycoproteins was followed immunochemically in extracts of whole mouse brains from E14 to P56. The two glycoproteins showed similar expression patterns and peaked at an early postnatal stage (Fig. 1B, C). Based on these results, early postnatal brains were used as the starting material for purification of the glycoproteins. A crude extract was fractionated by ammonium sulfate precipitation and then subjected to Q-Sepharose Fast Flow anion exchange chromatography. The glycoproteins were eluted with a high salt solution containing 1.5 M NaCl, and the eluate, after dialysis, was mixed with MLS 128 and protein A-Sepharose. The immune complex was poured into a column. After extensive washing, the complex was eluted with 0.1 M citrate-NaOH buffer, pH 3.8, and then fractionated by cesium chloride density gradient centrifugation in the presence of 4 M guanidine-HCl. An aliquot of each fraction was subjected to SDS-PAGE followed by western blotting, as shown in Fig. 2. The Tn antigen was detected with MLS 128 as described under Materials and Methods. An aliquot of each fraction was subjected to SDS-PAGE, followed by western blotting. The Tn antigen was detected with MLS 128 as described under Materials and Methods. The immunoprecipitates were subjected to SDS-PAGE, followed by western blotting. The Tn antigen was detected with MLS 128 as described under Materials and Methods.
lar size from 110 to 160 kDa was detected in lighter fractions. Since these molecules were not stained on silver or CBB staining, aliquots obtained at each step were biotinylated and subjected to SDS-PAGE followed by western blotting. The bands were detected by means of the streptavidin-peroxidase reaction. The finally purified glycoproteins carrying the Tn antigen gave a major smeared band and a minor one, which were clearly detected on immunochemical staining (Fig. 3B). No other contaminating proteins were detected (Fig. 3A, lane 4). From 140 g of early postnatal brain, 3.2 μg of the glycoproteins carrying the Tn antigen was obtained. In some cases, the immune complex was directly subjected to SDS-PAGE and the two glycoproteins were separated by extraction from the gel as described under Materials and Methods.

Characterization of carbohydrate moieties of glycoproteins carrying the Tn antigen

The smeared band of the major glycoprotein on SDS-PAGE suggests that it may be highly glycosylated by O-glycans and other carbohydrate chains. To characterize the carbohydrate moieties, we treated the glycoproteins with various glycosidases. On digestion with heparitinase I, the major glycoprotein gave a less broad band corresponding to a molecular mass of 160 kDa, whereas the minor one did not show any detectable change on SDS-PAGE (Fig. 4A, lanes 4 and 5). Chondroitinase digestion did not affect the electrophoretic mobility of the major glycoprotein at all (Fig. 4A, lane 6). Monoclonal antibody 3G10, which reacts with desaturated uronate residues produced from heparan sulfate on treatment with heparitinase, recognized the major glycoprotein that had been treated with heparitinase I (data not shown). These results indicate that the major glycoprotein, but not the minor one, is a heparan sulfate proteoglycan. After heparitinase treatment, the major glycoprotein was digested with sialidase (Fig. 4A, lane 7) or N-glycanase (Fig. 4A, lane 8). Sialidase treatment increased its mobility slightly, but N-glycanase digestion did not cause any detectable change in its mobility. In addition, we demonstrated previously that the major glycoprotein was reactive to Peanut agglutinin and Agaricus bisporus agglutinin-1, but not to wheat germ agglutinin or Concanavalin A (Akita et al., 2001). These results indicate that the major glycoprotein possesses heparan sulfate glycosaminoglycans and O-glycans. The Tn antigenicity was further confirmed by the fact that the antigenicity was lost on treatment with α-N-acetylgalactosaminidase (Fig. 4A, lanes 9 and 10).

Characterization of the core protein

The two glycoproteins were treated with O-sialoglycoprotein endopeptidase, which is a neutral metalloprotease and has the ability to degrade sialylated mucin-type glycoproteins. Expectedly, the Tn antigenicity was abolished upon treatment with this enzyme (Fig. 4B, lanes 3 and 4), indicating that both glycoproteins have a mucin-like domain. It should be noted that after treatment with V8 protease, these glycoproteins showed the same mobility corresponding to a molecular weight of 90 kDa (Fig. 4B, lanes 5 and 6), suggesting that they share a common core protein. After treatment with V8 protease, the major glycoprotein was not susceptible to heparitinase I, probably due to the cleavage of glycosaminoglycan attachment region (Fig. 4B, lanes 7 and 8). To further characterize the two glycoproteins, the N-terminal amino acid sequences of the glycoproteins were analyzed. The N-terminal sequence of the major glycoprotein was revealed to be AQRXRNENFERPV, which coincided with the sequence from amino acid 45 to 57 of mouse syndecan-3. Since the sequence from the N-terminus to amino acid 44 is postulated to be a signal sequence (Kung et al., accession No. U52826), cleavage of the signal peptide may produce the N-terminus of the major glycoprotein. The N-terminal sequence of the minor glycoprotein was ALAAPXAPAML.
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Fig. 4. Immunoreactivity of the glycoproteins treated with glycosidases (A) and proteases (B). The purified glycoproteins were digested with various glycosidases and proteases, followed by SDS-PAGE. After blotting onto a membrane, the glycoproteins were detected as described in Fig. 1. A. Lane 1: purified glycoproteins; lane 2: major glycoprotein extracted from the gel; lane 3: minor glycoprotein extracted from the gel; lane 4: major glycoprotein treated with heparitinase I; lane 5: minor glycoprotein treated with heparitinase I; lane 6: major glycoprotein treated with chondroitinase ABC; lane 7: major glycoprotein treated with heparitinase I and sialidase successively; lane 8: major glycoprotein treated with heparitinase I and N-glycanase successively; lane 9: major glycoprotein treated with N-acetylgalactosaminidase; lane 10: minor glycoprotein treated with N-acetylgalactosaminidase. B. Lane 1: major glycoprotein extracted from the gel; lane 2: minor glycoprotein extracted from the gel; lane 3: major glycoprotein treated with O-sialoglycoprotein endopeptidase; lane 4: minor glycoprotein treated with O-sialoglycoprotein endopeptidase; lane 5: major glycoprotein treated with V8 protease; lane 6: minor glycoprotein treated with V8 protease; lane 7: major glycoprotein treated with V8 protease and heparitinase I successively; lane 8: minor glycoprotein treated with V8 protease and heparitinase I successively.

Fig. 5. Alignments of N-terminal amino acid sequences obtained from the two glycoproteins bearing the Tn antigen with deduced amino acid sequence of mouse syndecan-3. Aligned residues identical to mouse syndecan-3 are indicated by colons.

Expression of the Tn antigen in brain and cartilage primordium

So far, expression of syndecan-3 has been investigated in the rat brain using antibody against the core protein of syndecan-3 and in situ hybridization of mRNA (Watanabe et al., 1996; Carey et al., 1997; Hsueh and Sheng, 1999). It has been reported that syndecan-3 is present at low levels in the late embryonic brain, and that the level increases during the early postnatal period. In the present biochemical study, we showed that the Tn antigen was carried on syndecan-3 specifically. Expectedly, distribution of the Tn antigen in the postnatal mouse brain was similar to that of syndecan-3 as shown in Fig. 7C and reported by Watanabe et al. (1996) and Carey et al. (1997). The antigen was detected throughout the cerebral cortex layers and localized in the molecular layer of the cerebellar cortex as shown in Fig. 7A, B, E, F. Since syndecan-3 is also expressed during the formation of the cartilage (Gould et al., 1995), we examined expression of the Tn antigen in the mouse cartilage primordium. As shown in Fig. 7G, H, the Tn antigen was detected in the mesenchymal condensed areas surrounding the mouse cartilage.

Discussion

The Tn antigen is well known as one of the tumor-associated carbohydrate antigens (Springer et al., 1974). MLS 128, a monoclonal antibody recognizing the Tn antigen, was established by immunizing mice with a human colorectal cancer cell line, LS 180 cells (Numata et al., 1990). Using gly-
coproteins reactive to MLS 128, we elucidated the epitopic structure to consist of three consecutive residues of GalNAcα1-Ser/Thr as a minimum antigenic site (Nakada et al., 1991; 1993; Inoue et al., 1994). It is generally agreed that tumorigenically transformed state has some similarities to that of the embryonic stages. We demonstrated previously that the Tn antigen was expressed in embryonic and early postnatal mouse brain, in particular, in developing cerebral cortex and cerebellum (Akita et al., 2001). The antigen, which was expressed throughout the embryonic stages, peaked around postnatal day 3 and decreased gradually with age as shown in Fig. 1. Expression of the antigen was also restricted temporally. In the present study, we isolated the glycoproteins carrying the Tn antigen from early postnatal mouse brains. Both the two glycoproteins corresponding to molecular weights ranging from 200 to 350 kDa and from 110 to 160 kDa were isolated and identified as syndecan-3 by amino acid sequencing and immunochemical study, these findings being consistent with the report that syndecan-3 has a mucin-like domain different from other syndecans (Carey, 1996). In fact, the mucin like-domain on syndecan-3 contains some possible Tn-antigenic sites in the form of consecutive Ser/Thr residues, which could express the Tn antigenicity if the residues Ser/Thr are substituted only by GalNAc residues. The Tn antigen is not expressed exclusively on syndecan-3 in other tissues or cancer cell lines. Among cancer cell lines we examined, the Tn antigen was carried on CD44 in a rat pancreatic cancer cell line, BSp73ASML (Nakada et al., unpublished data) and CD43 in a T-lymphoid cell line, Jurkat (Inoue et al., 1994), in addition to mucins in epithelial cancer cell lines. These glycoproteins have consecutive GalNAcα1-Ser/Thr commonly. CD44 has a similar structural feature as that of syndecan-3 in respect to the fact that glycosaminoglycans and O-glycans are attached to the N-terminal and central regions of the molecule, respectively. It is reported that the O-glycans carried on CD44 play an important role in

Fig. 6. Immunoreactivity of the glycoproteins with the anti-mouse syndecan-3 antibody. The major and minor glycoproteins were subjected to SDS-PAGE, blotted onto a Zeta-probe membrane, and then detected with the anti syndecan-3 antibody (lanes 1 and 2) and MLS 128 (lanes 3 and 4).

Fig. 7. Immunohistochemical staining of mouse brain and cartilage primordium. On P4 mouse brain, the immunoreactivity was observed through the cerebral cortex layers (A) and the molecular layer (ML) of the cerebellar cortex (E). Similar staining on P4 mouse cerebral cortex was detected with anti-mouse syndecan-3 core protein antibody as well (C). The Tn antigen was also detected in the E12 mouse cartilage primordium of vertebra (V) and limb bud (LB) (G, H). Control experiments performed using normal mouse IgG (B, F) or preimmune rabbit IgG (D) did not show any immunoreactivity. I-IV, cortical layers. Scale bar: A-D, 50 μm; E and F, 200 μm; G, 500 μm; H, 100 μm.
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modulating of the binding to hyaluronan (Bennett et al., 1995). Gould et al. (1992) postulated that the multiple O-linked oligosaccharides of syndecan-3 might interact with the lectin domain of versican, since these interactions are involved in cartilage and nerve development. Thus, in the developing brain some characteristic O-glycans on syndecan-3 may modulate a biological function or interact with certain lectins. Since consecutive GalNAc α1-Ser/Thr is synthesized by a highly specific UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase (GalNAc-transferase) (Inoue et al., 2001), the appearance of the Tn antigen in the developing mouse brain should be dependent on the expression of both syndecan-3 and the specific GalNAc-transferase. Disappearance of the Tn antigen may be caused by elongation of the carbohydrate chain. It would be interesting to see if the biological functions of the syndecan-3 change when some sugars are added to the Tn antigen. It has been reported that the deduced amino acid sequence of syndecan-3 is compatible with a transmembrane proteoglycan, but syndecan-3 purified from rat brain fails to react with an antibody directed against its cytoplasmic domain (Bernfield et al., 1993), indicating that most of syndecan-3 in the brain is cleaved and shed from the membrane through endoproteolytic cleavage. In fact, the major processed syndecan-3 could be extracted from mouse brains with an extraction solution not containing any detergent. The processed syndecan-3 could also be obtained from mouse brains, which had been boiled for 10 min immediately after removal from the mouse (data not shown). On the other hand, a detergent was necessary to extract the minor syndecan-3. Therefore, the minor syndecan-3 should contain its cytoplasmic domain and can be considered as an immature uncleaved form of syndecan-3 (Carey, 1997; Hsueh and Sheng, 1999). The N-terminal sequence of the minor syndecan-3, however, coincided with that on the N-terminal side of the mucin-like domain, indicating that this form was generated through endoproteolytic cleavage at the C-terminal side of a methionine residue (amino acid 101), which is located on the N-terminal side of the mucin-like domain. The minor syndecan-3 could also be obtained from mouse brains boiled immediately after removal from the mice (data not shown). The possibility that syndecan-3 had been degraded by some proteases derived from other subcellular compartments such as the lysosome during the isolation procedure seems to be ruled out, being consistent with the report by Hsueh and Sheng (1999). Although it is generally agreed that syndecans are primary signaling cell surface receptors (Carey, 1997), syndecan-3 seems to be processed rapidly after its synthesis. Fig. 8 shows the two processed syndecan-3 schematically based on our results and deduced amino acid sequences (Kung et al., accession No. U52826). Thus, two syndecan-3 ectodomains containing N-terminal glycosaminoglycan attachment sites can be shed from the membrane. One is the major syndecan-3 described above, and the other is a syndecan-3 fragment cleaved on the N-terminal side of the mucin-like domain, the residual portion of which is the minor syndecan-3 bound to the membrane. These soluble fragments of syndecan-3 may contribute to the structure of the extracellular matrix, and/or retention and storage of heparin-binding factors via their heparan sulfate glycosaminoglycans.

Acknowledgments. This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas, No. 10178102, from the Ministry of Education, Science and Culture of Japan, by the Foundation for Bio-venture Research Center from the Ministry of Education, Science and Culture of Japan, and by the Fugaku Trust for Medical Research.

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Fig. 8. Schematic structure of processed mouse syndecan-3. The major and minor forms are devoid of the transmembrane and cytoplasmic domain, and N-terminal glycosaminoglycan attachment domain, respectively.
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(Received for publication, July 3, 2001 and in revised form, September 11, 2001)