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Characterization of Dystroglycan–Laminin Interaction in Peripheral Nerve

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Abstract: Dystroglycan is encoded by a single gene and cleaved into two proteins, α- and β-dystroglycan, by posttranslational processing. The 120-kDa peripheral nerve isoform of α-dystroglycan binds laminin-2 comprised of the $\alpha 2$, $\beta 1$, and $\gamma 1$ chains. In congenital muscular dystrophy and *dy* mice deficient in laminin $\alpha 2$ chain, peripheral myelination is disturbed, suggesting a role for the dystroglycan–laminin interaction in peripheral myelinogenesis. To begin to test this hypothesis, we have characterized the dystroglycan–laminin interaction in peripheral nerve. We demonstrate that (1) α-dystroglycan is an extracellular peripheral membrane glycoprotein that links β-dystroglycan in the Schwann cell outer membrane with laminin-2 in the endoneurial basal lamina, and (2) dystrophin homologues Dp116 and utrophin are cytoskeletal proteins of the Schwann cell cytoplasm. We also present data that suggest a role for glycosylation of α-dystroglycan in the interaction with laminin. **Key Words:** Dystroglycan—Laminin—Schwann cell—Myelination—Basal lamina.
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Dystroglycan is encoded by a single gene and cleaved into two proteins, α- and β-dystroglycan, by posttranslational processing (Ibraghimov-Beskrovnaya et al., 1992). In skeletal muscle, α-dystroglycan with a molecular mass of 156 kDa binds the basal lamina component, laminin (Ibraghimov-Beskrovnaya et al., 1992; Ervasti and Campbell, 1993), and β-dystroglycan binds to cytoskeletal proteins dystrophin and utrophin (see Tinsley et al., 1994). Laminin is a heterotrimer made up of three chains of classes α, β, and γ, and exists in numerous trimeric isoforms in different tissues (see Burgeson et al., 1994). Laminin α chains have globular (G) domain repeats at the C-terminus, and laminin-1, comprised of the $\alpha 1$, $\beta 1$, and $\gamma 1$ chains, binds to α-dystroglycan via the G repeats in the $\alpha 1$ chain (Gee et al., 1993). In peripheral nerve, laminin-2, comprised of the $\alpha 2$, $\beta 1$, and $\gamma 1$ chains, is expressed in the endoneurium (Leivo and Engvall, 1988; Sanes et al., 1990). α-Dystroglycan with a molecular mass of 120 kDa is expressed surrounding peripheral nerve fibers in vivo and binds both laminin-1 and 2 in vitro (Yamada et al., 1994). Recently, laminin $\alpha 2$ chain was shown to be deficient in congenital muscular dystrophy and *dy* mice, which have muscular dystrophy and peripheral dysmyelination (Sunada et al., 1994; Tomé et al., 1994; Xu et al., 1994). Furthermore, the expression of laminin in the basal lamina is associated with Schwann cell development (Leivo and Engvall, 1988; Obremski and Bunge, 1995). These findings suggest a role for the interaction of dystroglycan with laminin in peripheral myelinogenesis. To begin to test this hypothesis, we have characterized the dystroglycan–laminin interaction in peripheral nerve.

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Abbreviations used: AAL, *Aleuria aurantia* lectin; BSA, bovine serum albumin; cLSM, confocal laser scanning microscope; Con A, concanavalin A; DSA, *Datura stramonium* agglutinin; G, globular; GlcNAc, *N*-acetylglucosamine; GNA, *Galanthus nivalis* agglutinin; Jacalin, jackfruit agglutinin; LCA, *Lens culinalis* lectin; Lotus, *Lotus tetragonobulus* lectin; PHA-E_4, *Phaseolus vulgaris* E_4; PMSF, phenylmethylsulfonyl fluoride; PNA, peanut agglutinin; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SNA, *Sambucus nivalis* agglutinin; UEA-1, *Ules europaeus* agglutinin 1; VVA-B_4, *Vicia villosa* agglutinin isolectin B_4; WGA, wheat germ agglutinin.

MATERIALS AND METHODS

Immunochemical analysis

Immunostaining of cryosections (7 μm) from rabbit sciatic nerve was performed as previously described (Matsumura et al., 1993; Yamada et al., 1994). Specimens were observed and fluorescent images were obtained on a Zeiss confocal laser scanning microscope (cLSM) model LSM 310, using an argon ion laser (λ = 488 nm) (Yamada et al., 1994). Three to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting, and laminin-blot overlay were performed as described previously (Matsumura et al., 1993; Yamada et al., 1994).

Monoclonal antibodies IIH6 against α-dystroglycan, 43DAG/8D5 against the C-terminus of β-dystroglycan, $VIA4_2$ against the cysteine-rich/C-terminal domains of dystrophin, and 2D9 against the G domain of human laminin α2 chain were characterized previously (Ervasti and Campbell, 1993; Cullen et al., 1994; Hori et al., 1994). Affinity-purified sheep antibody against dystroglycan fusion protein D (anti-FPD) and affinity-purified rabbit antibody against the C-terminus of utrophin (anti-DRP) were characterized previously (Ibraghimov-Beskrovnaya et al., 1992; Matsumura et al., 1993). Monoclonal antibodies DYS2 against the C-terminus of dystrophin and DRP1 against the C-terminus of utrophin were purchased from Novocastra. Monoclonal antibodies 5H2 against human laminin α2, 11D5 against human laminin α1, and 4E10 against human laminin β1 chains were purchased from GibcoBRL. Monoclonal antibody 15E2E2 against bovine S-100 protein was purchased from Chemicon. Monoclonal antibodies against sialyl Tn and sialyl Lewis A were purchased from Ohtsuka Phamaceutical. Monoclonal antibody anti-HNK-1 and monoclonal antibody against Lewis X were purchased from Becton Dickinson. Monoclonal antibody against sialyl Lewis X was kindly provided by Dr. Nobuo Hanai (Kyowa Hakko Kogyo). Monoclonal antibodies against heparan sulfate and keratan sulfate were purchased from Seikagaku Corporation.

Lectin staining of the nitrocellulose transfers was performed using the following lectins: Peroxidase-labeled wheat germ agglutinin (WGA), concanavalin A (Con A), and *Vicia villosa* agglutinin isolectin B_4 ($VVA-B_4$) were purchased from Sigma. Peroxidase-labeled jackfruit agglutinin (Jacalin) was purchased from E-Y Laboratories. Biotin-labeled *Phaseolus vulgaris* E_4 ($PHA-E_4$), *Lens culinalis* lectin (LCA), *Aleuria aurantia* lectin (AAL), *Lotus tetragonobulus* lectin (Lotus), and *Ules europaeus* agglutinin 1 (UEA-1) were purchased from Seikagaku Corporation. The DIG Glycan Differentiation Kit was purchased from Boehringer-Mannheim.

Alkaline extraction of crude peripheral nerve membranes

Crude bovine peripheral nerve membranes were suspended at a protein concentration of 2.5 mg/ml in 50 m*M* Tris-HCl, pH 7.4, containing a cocktail of protease inhibitors, i.e., benzamidine (0.75 m*M*), phenylmethylsulfonyl fluoride (PMSF) (0.1 m*M*), pepstatin A (0.7 μ*M*), aprotinin (76.8 n*M*), and leupeptin (1.1 μ*M*) (buffer A). The suspension was titrated to pH 11, extracted for 1 h at room temperature, and centrifuged at 140,000 *g* for 30 min at 25°C. The supernatants were titrated to pH 7.4. The pellets were suspended in the original volume of buffer A. The suspension was titrated to pH 12, extracted for 1 h at room temperature, and centrifuged at 140,000 *g* for 30 min at 25°C. The supernatants were titrated to pH 7.4. The pellets were suspended in the original volume of buffer A.

Isolation of α-dystroglycan and its associated proteins from crude peripheral nerve membranes

Crude bovine peripheral nerve membranes were extracted at a protein concentration of 5 mg/ml in 50 m*M* Tris-HCl, pH 7.4, containing 1% digitonin, 0.5 *M* sucrose, 0.5 *M* NaCl, and the aforementioned cocktail of protease inhibitors for 2 h at 4°C. Digitonin extracts (60 ml) were circulated over 10 ml of WGA-Sepharose (Pharmacia) at 4°C overnight. After extensive wash, the WGA-Sepharose was eluted with buffer B (50 m*M* Tris-HCl, pH 7.4, 0.1% digitonin, 0.75 m*M* benzamidine, and 0.1 m*M* PMSF) containing 0.3 *M* *N*-acetylglucosamine (GlcNAc) and 0.5 *M* sucrose. The eluates were circulated over 1 ml of laminin-Sepharose (Yamada et al., 1994) at 4°C overnight in the presence of 1 m*M* $CaCl_2$ and 1 m*M* $MgCl_2$. After extensive wash, the laminin-Sepharose was eluted with buffer B containing 10 m*M* EDTA.

Isolation of α-dystroglycan from crude peripheral nerve membranes

Crude bovine peripheral nerve membranes were suspended at a protein concentration of 5 mg/ml in buffer A containing 0.5 *M* NaCl. The suspension was titrated to pH 12, extracted for 1 h at room temperature, and centrifuged at 140,000 *g* for 30 min at 25°C. The supernatants were titrated to pH 7.4 and centrifuged at 140,000 *g* for 30 min at 4°C. WGA- and laminin-affinity chromatographies of the supernatants were performed as described above.

Enzymatic deglycosylation of peripheral nerve α-dystroglycan

Enzymatic deglycosylation of peripheral nerve α-dystroglycan was performed using neuraminidase from *Arthrobacter ureafaciens* (Nacalai Tesque), *N*-glycosidase F from *Flavobacterium meningosepticum* (Boehringer-Mannheim), *O*-glycosidase from *Diplococcus pneumoniae* (Boehringer-Mannheim), chondroitinase ABC from *Proteus vulgaris* (Seikagaku), heparinase from *Flavobacterium heparinum* (Seikagaku), heparitinase from *Flavobacterium heparinum* (Seikagaku), and keratanase from *Pseudomonas* species (Seikagaku). For neuraminidase treatment, α-dystroglycan was first made 1% in SDS and incubated at 100°C for 5 min, then diluted 10-fold with concentrated buffer and distilled water to final concentrations of 0.5 *M* acetate buffer, pH 5.5, 1% Triton X-100, and 0.1% SDS, and incubated with neuraminidase (10 mU/μg of substrate) at 37°C for 24 h. For control, samples were treated identically in the absence of neuraminidase. For treatment with *O*-glycosidase, samples were treated identically, but *O*-glycosidase (0.25 mU/μg of substrate) was added to the incubation buffer. For *N*-glycosidase F treatment, α-dystroglycan was first made 1% in SDS and incubated at 100°C for 5 min, then diluted 10-fold with concentrated buffer and distilled water to final concentrations of 50 m*M* Tris-HCl, pH 7.4, 1% Triton X-100, and 0.1% SDS, and incubated with *N*-glycosidase F (0.12 U/μg of substrate) at 37°C for 24 h. For treatment with glycosaminoglycan chain lyases, α-dystroglycan was incubated with enzymes in the following conditions: chondroitinase ABC (50 mU/μg of substrate) in 100 m*M* Tris-HCl, 50 m*M* acetate buffer, pH 8.0, at 37°C for 2 h; heparinase (1 mU/μg of substrate) in 10 m*M* phosphate buffer,

pH 6.8, containing 1 m*M* $CaCl_2$ at 35°C for 5 h and then at 37°C for 3 h; heparitinase (1 mU/μg of substrate) in 10 m*M* phosphate buffer, pH 6.8, containing 10 μM $CaCl_2$ at 43°C for 3 h; and keratanase (2 mU/μg of substrate) in 50 m*M* Tris-HCl, pH 7.4, at 37°C for 2 h. Deglycosylation reaction was stopped by adding SDS sample buffer to the incubation buffer and boiling at 100°C for 5 min.

Neuraminidase treatment of nitrocellulose transfers was performed as follows: Peripheral nerve α-dystroglycan was separated by 3–12% SDS-PAGE and transferred to nitrocellulose membranes. The nitrocellulose transfers were incubated, with or without *A. ureafaciens* neuraminidase (1 U/ml), in 0.5 *M* acetate buffer, pH 5.5, containing 1% Triton X-100 and 0.1% SDS at 37°C for 24 h.

Quantification of laminin-binding activity by ELISA

Peripheral nerve α-dystroglycan (1 μg) was coated onto microtiter wells. After rinsing with 10 m*M* triethanolamine, pH 7.6, 140 m*M* NaCl, 1 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$ (LBB), the wells were blocked with LBB containing 3% bovine serum albumin (3% BSA-LBB). Biotinylated laminin-1 (50 μl) (2 n*M* in 3% BSA-LBB) was added to the wells and incubated overnight at room temperature. After rinsing with LBB, the binding of laminin-1 was detected using the ABC kit (Vector). *o*-Phenylenediamine dihydrochloride was used as substrate and absorbance was measured at 492 nm.

RESULTS

Characterization of dystroglycan complex in peripheral nerve

The results of cLSM analysis of rabbit sciatic nerve are shown in Fig. 1. Immunoreactivities for α- and β-dystroglycan and laminin-2 were localized surrounding the outermost layer of myelin sheath of nerve fibers (Fig. 1). On the other hand, immunoreactivities for utrophin and Dp116, a dystrophin gene product specific to peripheral nerve (Byers et al., 1993), resembled that for Schwann cell marker S-100 and appeared to be localized in the Schwann cell cytoplasm (Fig. 1).

Membrane-associated cytoskeletal and peripheral membrane proteins, but not integral membrane proteins, are known to be extracted from the membranes by alkaline treatment (Ervasti and Campbell, 1991). Based on this, we performed alkaline extraction of the crude bovine peripheral nerve membranes. α-Dystroglycan of 120 kDa was extracted at pH 11, but β-dystroglycan of 43 kDa was not extracted at pH 11 or 12 (Fig. 2). Together with the fact that α-dystroglycan corresponds to the N-terminal half of the dystroglycan precursor protein containing the signal peptide but no transmembrane domain and that β-dystroglycan corresponds to the C-terminal half containing a transmembrane domain (Ibraghimov-Beskrovnaya et al., 1992), these results suggested that peripheral nerve α-dystroglycan was a peripheral membrane protein, whereas peripheral nerve β-dystroglycan was an integral membrane protein. The extracellular matrix protein laminin

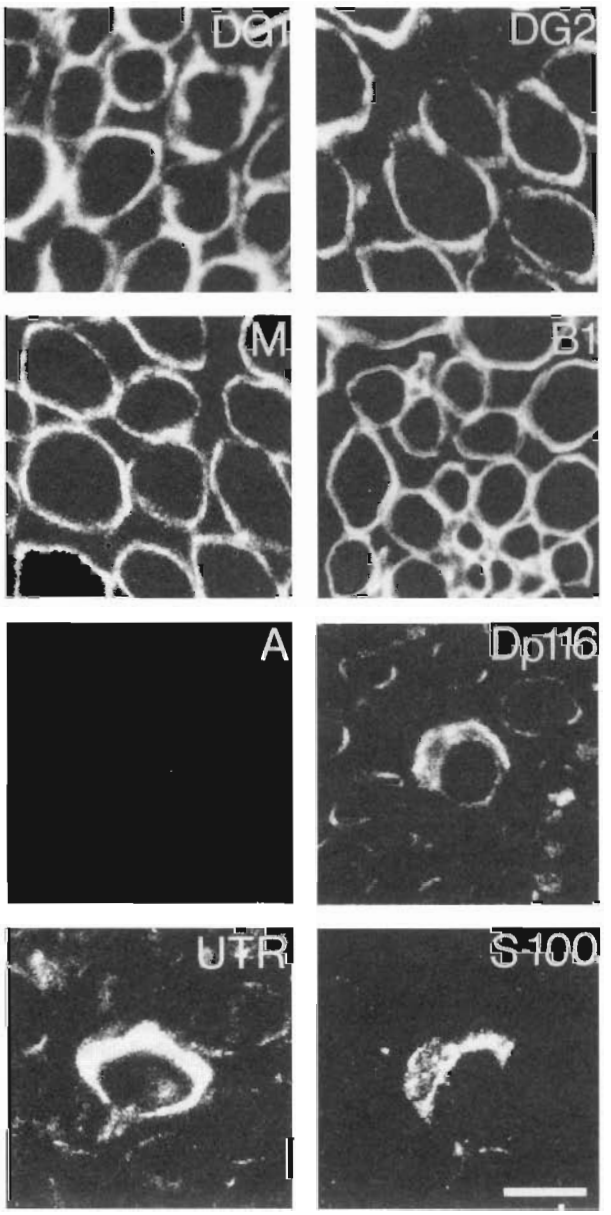

FIG. 1. cLSM analysis of rabbit sciatic nerve. Shown are the cLSM fluorescent images of rabbit sciatic nerve. α-Dystroglycan (DG1), β-dystroglycan (DG2), laminin α2 chain (M), laminin β1 chain (B1), laminin α1 chain (A), Dp116, utrophin (UTR), and S-100 protein were detected by antibodies anti-FPD, 43DAG/8D5, 5H2, 4E10, 11D5, $VIA4_2$, DRP1, and 15E2E2, respectively. Bar, 10 μm.

α2 chain of 320 kDa was partially extracted at pH 11 and almost completely extracted at pH 12 (Fig. 2), together with the β1 and γ1 chains (not shown). Dp116 and utrophin, of 116 and 395 kDa, respectively, were both extracted at pH 11 (Fig. 2), in good agreement with the prediction from the primary structure that they are cytoskeletal proteins (Tinsley et al., 1994; Byers et al., 1993).

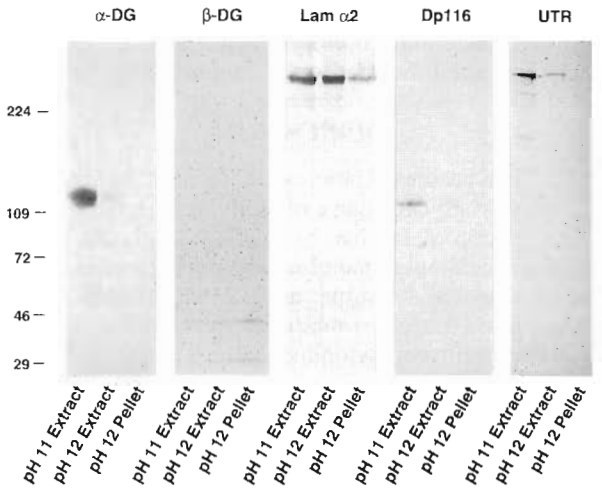

FIG. 2. Alkaline extraction of the crude bovine peripheral nerve membranes. pH 11 extracts (120 μl) (first lane), the pH 12 extracts of the pH 11 pellets (second lane), and the pH 12 pellets (third lane) of the crude bovine peripheral nerve membranes were separated by 3–12% SDS-PAGE and transferred to nitrocellulose membranes. α-Dystroglycan (α-DG), β-dystroglycan (β-DG), laminin α2 chain (Lam α2), Dp116, and utrophin (UTR) were detected by antibodies IIH6, 43DAG/8D5, 2D9, DYS2, and anti-DRP, respectively. The 30-kDa band detected by 43DAG/8D5 is presumed to be a proteolytic fragment or the unglycosylated form of β-dystroglycan. Molecular weight standards ($\times 10^{-3}$) are shown on the left.

We isolated, using WGA- and laminin-affinity chromatographies, α-dystroglycan and its associated proteins from the digitonin extracts of the crude bovine peripheral nerve membranes. As expected, laminin-binding α-dystroglycan was isolated by these procedures (Fig. 3). β-Dystroglycan, which did not bind laminin in blot overlay, was also isolated (Fig. 3), suggesting that it was associated with α-dystroglycan, which bound to laminin-Sepharose. On the other hand, Dp116 and utrophin were not isolated by these procedures (Fig. 3).

Role of carbohydrate chains of peripheral nerve α-dystroglycan in laminin binding

We isolated, using WGA- and laminin-affinity chromatographies, α-dystroglycan from the alkaline extracts of the crude bovine peripheral nerve membranes. We tested its reactivity with monoclonal antibodies specific for carbohydrate chains. α-Dystroglycan was stained intensely with monoclonal antibody anti-HNK-1, indicating that it had 3-sulfated glucuronyl-substituted oligosaccharide (Fig. 4a) (Chou et al., 1986). On the other hand, it was not stained with monoclonal antibodies against heparan sulfate, keratan sulfate, sialyl Tn, Lewis X, sialyl Lewis X (Fig. 4a), or sialyl Lewis A (not shown).

The lectin-binding properties of peripheral nerve α-dystroglycan are shown in Fig. 4b. Peripheral nerve α-dystroglycan was stained positive with WGA, which recognizes clusters of sialic acids and/or bisecting GlcNAc (Fig. 4b) but was not stained with PHA-E_4, a lectin specific for bisecting GlcNAc (not shown), indicating that it contained clusters of sialic acids. It was stained positive with both *Maackia amurensis* agglutinin, a lectin specific for sialic acid–linked α2–3 to galactose, and peanut agglutinin (PNA), a lectin specific for unsubstituted Ser/Thr-linked disaccharide Galβ1-3GalNAc unit (Fig. 4b). It was also stained positive with Jacalin, which also recognizes Galβ1-3GalNAc (Fig. 4b). It was not stained with *Sambucus nivalis* agglutinin (SNA), which recognizes sialic acid linked α2–6 to galactose (Fig. 4b). These results indicate that peripheral nerve α-dystroglycan is sialylated α2–3 to galactose in O-glycans. Peripheral nerve α-dystroglycan was stained positive with VVA-B_4 (Fig. 4b), indicating that it contained nonreducing terminal β-GalNAc. It was stained positive with both Con A and *Galanthus nivalis* agglutinin (GNA) (Fig. 4b), indicating that it contained N-linked mannose residues. It was not stained with *Datura stramonium* agglutinin (DSA), which recognizes Galβ1-4GlcNAc in complex and hybrid N-glycans, in O-glycans, and GlcNAc in O-glycans (Fig. 4b). The results of staining with Con A, GNA, PHA-E4, and DSA, when combined, suggest that the N-glycan(s) of peripheral nerve α-dystroglycan could be high-mannose type. Peripheral nerve α-dystroglycan was not stained with lectins specific for various types of fucose residues, AAL, UEA-1 (Fig. 4b), LCA, or Lotus (not shown).

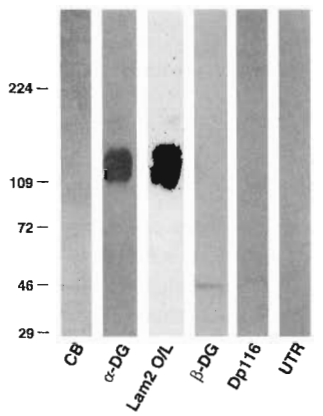

FIG. 3. Characterization of peripheral nerve α-dystroglycan and its associated proteins. α-Dystroglycan and its associated proteins were isolated from the digitonin extracts of the crude bovine peripheral nerve membranes by WGA- and laminin-affinity chromatographies. EDTA eluates (120 μl) of the laminin-Sepharose were separated by 3–12% SDS-PAGE and transferred to nitrocellulose membranes. α-Dystroglycan (α-DG), β-dystroglycan (β-DG), Dp116, and utrophin (UTR) were detected by antibodies IIH6, 43DAG/8D5, DYS2, and anti-DRP, respectively. Lam2 O/L indicates the identical nitrocellulose transfer overlaid with laminin-2. Heavily glycosylated α-dystroglycan was not identified clearly on the SDS gel stained with Coomassie Blue (CB) (Yamada et al., 1994). Molecular weight standards ($\times 10^{-3}$) are shown on the left.

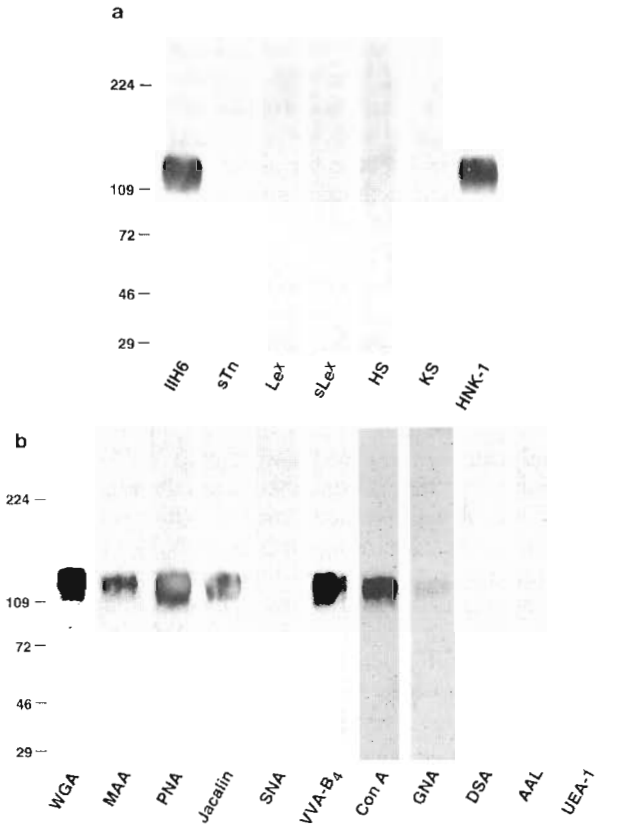

FIG. 4. Characterization of carbohydrate chains of peripheral nerve α-dystroglycan. **a:** α-Dystroglycan (3 μg) was separated by 3–12% SDS-PAGE, transferred to nitrocellulose membranes, and then reacted with monoclonal antibodies against α-dystroglycan (IIH6), sialyl Tn (sTn), Lewis X (Le^x), sialyl Lewis X (sLe^x), heparan sulfate (HS), keratan sulfate (KS), or monoclonal antibody anti-HNK-1. **b:** The identical nitrocellulose transfers as shown in (A) were reacted with various lectins. Molecular weight standards ($\times 10^{-3}$) are shown on the left.

To see if carbohydrate residues of α-dystroglycan are involved in laminin binding, we tested, by blot-overlay assay, the binding of laminin to peripheral nerve α-dystroglycan treated with deglycosylation enzymes. Treatment of α-dystroglycan with both *A. ureafaciens* neuraminidase (Fig. 5) and *V. cholerae* neuraminidase (not shown) greatly reduced the binding of laminin. Treatment with *O*-glycosidase, together with neuraminidase, did not have further effects on the binding of laminin (Fig. 5). The binding of laminin was not affected by treatment with *N*-glycosidase F, chondroitinase ABC, heparinase, heparitinase, or keratanase (Fig. 5). When nitrocellulose transfers of α-dystroglycan were treated with neuraminidase, the binding of laminin to α-dystroglycan was reduced (Fig. 6a). The presence of 0.1 *M* sialic acid, but not 0.1 *M* GlcNAc, in the blot-overlay medium inhibited the binding of laminin to α-dystroglycan (Fig. 6b). Inhibition of laminin-binding by sialic acid was quantified by ELISA. Two forms of sialic acid, *N*-acetyl- and *N*-glycolylneuraminic acids, and colominic acid, polymer of sialic acids, inhibited the binding of laminin (Fig. 6c).

DISCUSSION

The data presented here, taken together, indicate that (1) α-dystroglycan is an extracellular peripheral membrane glycoprotein that links β-dystroglycan in the Schwann cell outer membrane with laminin-2 in the endoneurial basal lamina, and (2) dystrophin homologues Dp116 and utrophin are cytoskeletal proteins of the Schwann cell cytoplasm (Fig. 7), although more precise localization of these proteins would require analysis by immunoelectron microscopy. It also remains to be determined if a fraction of Dp116 and utrophin is complexed with β-dystroglycan beneath the Schwann cell outer membrane.

In this study, we have characterized the carbohydrate moieties of peripheral nerve α-dystroglycan by using carbohydrate-specific antibodies and lectins. Although complete structural analysis of carbohydrate moieties awaits future research, our results suggest a role for sialylation of α-dystroglycan in the binding of laminin. The results thus far indicate that α-dystroglycan is a mucin-type glycoprotein in brain (Smalheiser and Kim, 1995), cardiac muscle (Brancaccio et al., 1995), and peripheral nerve (this study). A mucin-like motif is detectable in the central region of α-dystroglycan and, indeed, cardiac muscle α-dystroglycan was shown to consist of two globular domains

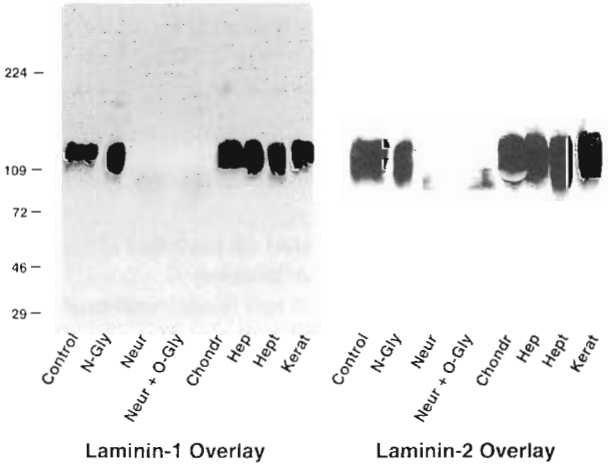

FIG. 5. Binding of laminin to deglycosylated peripheral nerve α-dystroglycan. α-Dystroglycan was treated with *N*-glycosidase F (N-Gly), neuraminidase (Neur), neuraminidase and *O*-glycosidase (Neur + O-Gly), chondroitinase ABC (Chondr), heparinase (Hep), heparitinase (Hept), or keratanase (Kerat). Control indicates α-dystroglycan treated identically but in the absence of neuraminidase. Deglycosylated α-dystroglycan (3 μg) was separated by 3–12% SDS-PAGE, transferred to nitrocellulose membranes, and then overlaid with laminin-1 (**left**) or 2 (**right**). Molecular weight standards ($\times 10^{-3}$) are shown on the left.

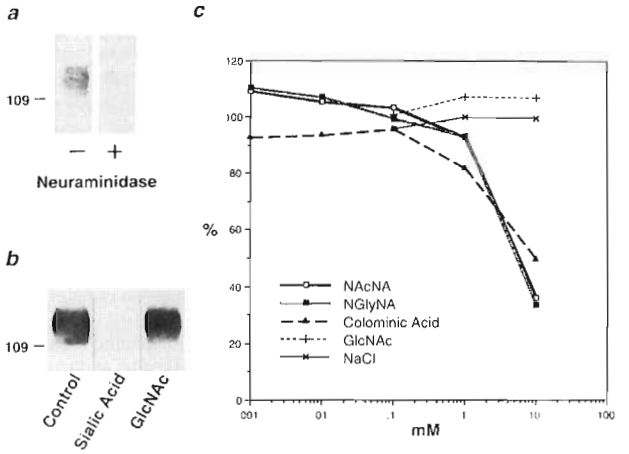

FIG. 6. **a:** Effect of neuraminidase treatment on the binding of laminin to peripheral nerve α-dystroglycan. α-Dystroglycan (3 μg) was separated by 3–12% SDS-PAGE and transferred to nitrocellulose membranes. The nitrocellulose transfers were incubated with or without neuraminidase (+ and −, respectively) and then overlaid with laminin-1. Molecular weight standard (10^{-3}) is shown on the left. **b:** Effect of sialic acid on the binding of laminin to peripheral nerve α-dystroglycan (blot overlay). α-Dystroglycan (3 μg) was separated by 3–12% SDS-PAGE, transferred to nitrocellulose membranes, and then overlaid with laminin-1 in the presence of 0.1 *M* sialic acid, 0.1 *M* GlcNAc, or in the absence of these sugars (Control). Molecular weight standard ($\times 10^{-3}$) is shown on the left. **c:** Effect of sialic acid on the binding of laminin to peripheral nerve α-dystroglycan (ELISA). In the presence of various concentrations of *N*-acetylneuraminic acid (NAcNA), *N*-glycolylneuraminic acid (NGlyNA), colominic acid, GlcNAc, or NaCl, laminin-1 was incubated with α-dystroglycan (1 μg) coated onto microtiter wells. Bound laminin-1 was quantified by ELISA. Points are the percentage values compared with the values of the wells incubated in the absence of inhibitors. Points represent the mean values of triplicate wells in one experiment representative of three similar experiments.

connected by a rod-shaped segment (Brancaccio et al., 1995), consistent with the prediction that a mucin domain is extended into a rigid rod. The densely clustered array of O-glycans in the mucin domain may present terminal sialic acids in a polyvalent manner for the interaction with laminin (Fig. 7c).

Besides α-dystroglycan, laminin binds a variety of glycolipids and proteins (see Kennedy et al., 1983; Gee et al., 1993). At present, it is unclear if sialylation alone is responsible for the binding of α-dystroglycan with laminin, because laminin was suggested to recognize highly charged oligosaccharides on proteins rather than monosaccharides (Kennedy et al., 1983). In this respect, it is of interest that, similar to brain α-dystroglycan (Smalheiser and Kim, 1995), peripheral nerve α-dystroglycan is HNK-1 positive. Furthermore, the HNK-1 carbohydrate epitope, found in a number of neural cell adhesion molecules, plays important roles in cell adhesion and has been implicated in the binding to laminin (Künemund et al., 1988; Hall et al., 1993). It would be thus interesting to see if this carbohydrate

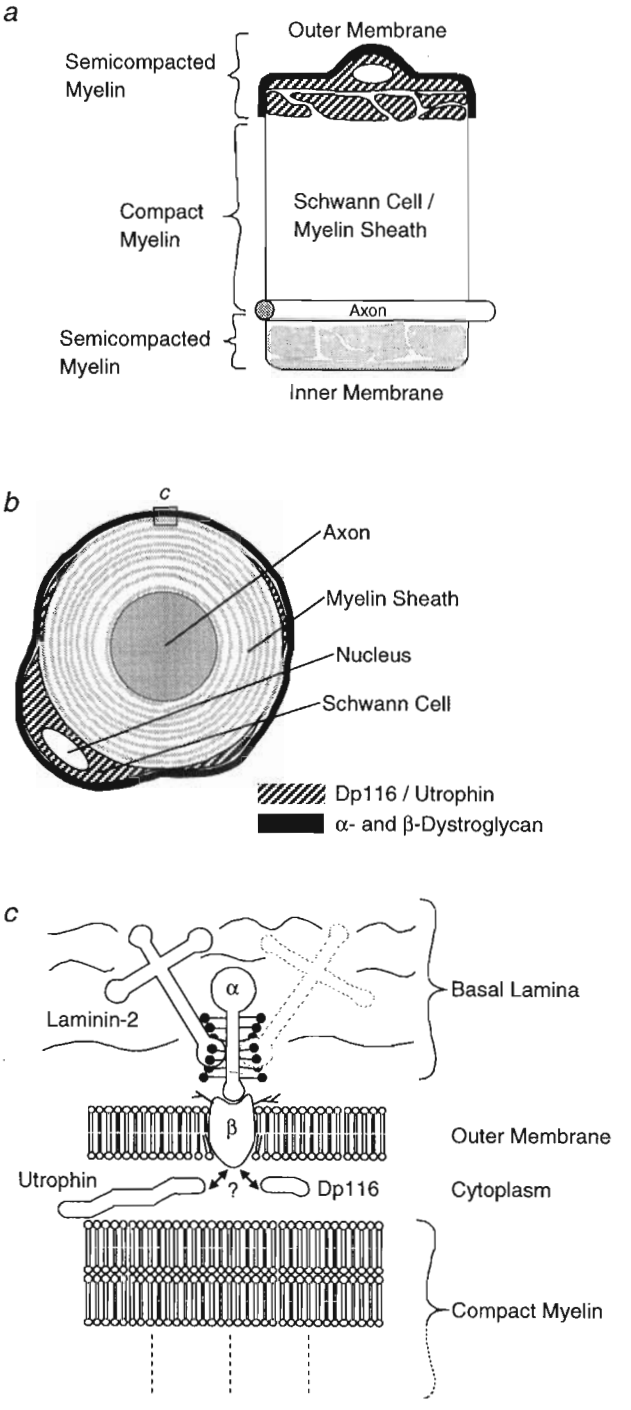

FIG. 7. Hypothetical model of dystroglycan–laminin interaction in peripheral nerve. **a** and **b:** α- and β-dystroglycan are localized in the Schwann cell outer membrane, whereas Dp116 and utrophin are localized in the Schwann cell cytoplasm. **c:** α-Dystroglycan (α), which is an extracellular peripheral membrane glycoprotein, links β-dystroglycan (β) in the Schwann cell outer membrane with laminin-2 in the endoneurial basal lamina. The mucin domain of α-dystroglycan is extended into a rigid rod. Clusters of sialic acid residues in O-glycans of α-dystroglycan are presumed to be involved in the interaction with the G domain of laminin α2 chain.

structure is also involved in the interaction of α-dystroglycan with laminin.

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