Tissue-specific Heterogeneity in α-Dystroglycan Sialglycosylation

SKELETAL MUSCLE α-DYSTROGLYCAN IS A LATENT RECEPTOR FOR VICIA VILLOSA AGGLUTININ B4 MASKED BY SIALIC ACID MODIFICATION

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Because the polypeptide core of α-dystroglycan is encoded by a single gene, the difference in apparent molecular mass between α-dystroglycans expressed in various tissues is presumably due to differential glycosylation. However, little is presently known about the tissue-specific differences in α-dystroglycan glycosylation and whether these modifications may confer functional variability to α-dystroglycan. We recently observed that laminin-1 binding to skeletal muscle α-dystroglycan was dramatically inhibited by heparin, whereas the binding of commercial merosin to skeletal muscle α-dystroglycan was only marginally inhibited (Pall, E. A., Bolton, K. M., and Ervasti, J. M. (1996) J. Biol. Chem. 271, 3817–3821). In contrast to 156-kDa skeletal muscle α-dystroglycan, both laminin-1 and merosin binding to 120-kDa brain α-dystroglycan were sensitive to heparin. We have now examined the laminin binding properties of 140-kDa α-dystroglycan purified from cardiac muscle and observed that like skeletal muscle α-dystroglycan, heparin inhibited cardiac α-dystroglycan binding to laminin-1, but not to merosin. On the other hand, cardiac and brain α-dystroglycans could be distinguished from skeletal muscle α-dystroglycan by their reactivity with the terminal GalNAc-specific lectin Vicia villosa agglutinin. Interestingly, skeletal muscle α-dystroglycan became reactive with V. villosa agglutinin upon digestion with sialidase from Clostridium perfringens, Arthrobacter neurofaciens, or Streptococcus, but not Vibrio cholerae or Newcastle disease virus sialidase. While none of the sialidase treatments affected the laminin binding properties of α-dystroglycan, the sum of our results suggests that skeletal muscle α-dystroglycan contains a novel sialic acid residue linked α2–6 to GalNAc. These properties are also consistent with the cellular characteristics of a GalNAc-terminated glycoconjugate recently implicated in neuromuscular synaptogenesis. Thus, variations in α-dystroglycan sialglycosylation may prove as useful markers to further elucidate the role of α-dystroglycan glycoforms in different tissues and perhaps within a single cell type.

α-Dystroglycan is a membrane-associated, extracellular glycoprotein (1) originally identified as a subunit of the dystrophin-glycoprotein complex (2) that is missing or abnormal in a number of muscular dystrophies (3, 4). In skeletal muscle, the dystrophin-glycoprotein complex is distributed throughout the sarcolemmal membrane (5) and is believed to serve as a transmembrane link between the cortical cytoskeleton and the extracellular matrix, based on the interaction of dystrophin with F-actin (6) and the binding of α-dystroglycan to the laminin family of proteins (7, 8). While these data certainly support a structural role for the dystrophin-glycoprotein complex in maintaining sarcolemmal membrane integrity (9–12), α-dystroglycan has also been implicated in more dynamic developmental processes in other tissues (13, 14) and even in muscle cells. For example, a highly similar complex of proteins (including α-dystroglycan) is associated with utrophin (15), an autosomal homologue of dystrophin (16). In contrast to the dystrophin-glycoprotein complex, the utrophin-glycoprotein complex is specifically localized to the neuromuscular junction (15, 17), suggesting that it may play a role in neuromuscular synapse formation. In support of this possibility, α-dystroglycan co-clusters with acetylcholine receptors in a heterologous expression system (18). α-Dystroglycan also binds agrins (19–22), a group of widely expressed and alternatively spliced basal lamina proteins that can induce acetylcholine receptor aggregation at the motor endplate (23, 24). However, α-dystroglycan antibodies that block agrin binding in vitro have yielded equivocal results in experiments testing their ability to perturb the clustering activity of agrin in cell culture (20–22). Furthermore, truncated agrins that fail to bind α-dystroglycan retain acetylcholine receptor clustering activity (25–27). Finally, Yancopoulos and co-workers (28, 29) have recently demonstrated that agrin acts via a novel receptor tyrosine kinase complex. Thus, a specific role for α-dystroglycan in neuromuscular synapse formation remains to be elucidated.

Although α-dystroglycan is the product of a single gene (30), its apparent molecular mass varies dramatically in different tissues (7, 8, 19, 31, 32) presumably due to differences in glycosylation (7, 8), which is essential for its binding to laminin-1 (8) and agrin (20–22). Detailed carbohydrate analysis has been severely hampered by the exceedingly low abundance of α-dystroglycan in native tissues. None the less, characterization of α-dystroglycans purified from diverse tissues and species (1, 8, 19, 33–38) has identified many common structural features as well as potential tissue-specific differences in glycosylation (see Table I). Relevant to a putative role in neuromuscular synaptogenesis, neural forms of α-dystroglycan express terminal GalNAc residues (35, 36), whereas GalNAc-terminated glycoconjugate(s) have been implicated in acetylcholine receptor clustering (39–41). However, the GalNAc-terminated molecule(s) mediating acetylcholine receptor clustering are muscle cell-specific (40, 41), yet adult skeletal muscle and cultured myotubes apparently express α-dystroglycan lacking terminal GalNAc residues (33, 41). Of further concern is that all previously identified (35, 36) tissue-specific...
variations in α-dystroglycan glycosylation have relied solely on comparisons across species as well as tissues (see Table 1). Since species-specific differences in glycosylation are prevalent in nature, but are not generally crucial to basic glycprotein function (42), it is important to characterize the tissue-specific differences in α-dystroglycan glycosylation and to determine whether these modifications may confer functional variability to α-dystroglycan.

We recently observed (43) a significant difference in the heparin inhibition of skeletal muscle α-dystroglycan binding to laminin-1 versus merosin (a mixture of laminin-2 and -4), which may provide a basis for merosin-specific stabilization of cultured myotubes (44) and explain why laminin-1 up-regulation fails to compensate for merosin deficiency in some forms of muscular dystrophy (45, 46). Consistent with our findings with different laminins, alternatively spliced forms of agrin also bind α-dystroglycan in either a heparin-sensitive or -insensitive manner, dependent on the presence of a four-amino acid sequence at the A/Y splice site (27, 47). In contrast to our findings with 156-kDa skeletal muscle α-dystroglycan, we further observed that both laminin-1 and merosin binding to 120-kDa brain α-dystroglycan were sensitive to heparin (43), suggesting that tissue-specific differences in α-dystroglycan post-translational modification may influence its interactions with extracellular ligands. Cardiac muscle α-dystroglycan exhibits an electrophoretic mobility intermediate (140 kDa) to that of skeletal and brain α-dystroglycans (7, 8, 48), suggesting that it could exhibit laminin binding properties more similar to skeletal muscle α-dystroglycan, brain α-dystroglycan, or somewhere in between. Therefore, we have now examined the effect of heparin on the binding of purified cardiac muscle α-dystroglycan to laminin-1 and merosin. Our results suggest that cardiac and skeletal muscle α-dystroglycans are functionally related but distinct from nervous tissue α-dystroglycans with regard to merosin binding in the presence of heparin. However, we further demonstrate that cardiac muscle and brain α-dystroglycans appear to be similarly modified with terminal GalNAc residues, which are also present on skeletal muscle α-dystroglycan, but masked by a novel sialic acid modification. While sialic acid modification does not appear to be important for the laminin binding properties of α-dystroglycan, we discuss situations in which differences in sialoglycosylation may reflect α-dystroglycan glycoforms with unique or varied function, even in the same cell.

EXPERIMENTAL PROCEDURES

Purification of α-Dystroglycans—α-Dystroglycan was solubilized from rabbit skeletal muscle membranes (49) or cardiac membranes (7) by extraction with 8 M urea and purified by sequential WGA-Sepharose, DEAE-cellulose chromatography, and CaCl2 gradient centrifugation (43). α-Dystroglycan was also prepared from low ionic strength extracts of frozen rabbit brain, bovine brain, and rabbit sciatic nerve (Pel-Freez Biologicals) by laminin-1 affinity chromatography (51) followed by CaCl2 gradient centrifugation as described previously (43). Purified α-dystroglycans were dialyzed exhaustively against double distilled H2O and quantitated by A280 using E280 = 0.83 cm2/mg, calculated from the predicted amino acid sequence of αβ-dystroglycan precursor (7) with the proteolytic cleavage site located between Gly-653 and Ser-654 (35).

SDS-Polyacrylamide Gel Electrophoresis and Blotting Assays—All samples were electrophoretically separated on 3–12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes as described previously (1). Molecular mass standards were purchased from Sigma. α-Dystroglycan was identified on nitrocellulose membranes with 1 μg/ml specific polyclonal antibodies affinity-purified (1) from the yolk immunoglobulin fraction of White Leghorn chickens (50) immunized with the dystrophin-glycoprotein complex (51). Primary antibody labeling of α-dystroglycan was detected with a peroxidase-conjugated goat anti-chicken secondary antibody (Callbiochem) by chemiluminescence using SuperSignal CL-HRP (Pierce) as substrate.

To evaluate lectin binding to α-dystroglycan, nitrocellulose membranes were blocked for 1 h in phosphate-buffered saline (8 mM monobasic sodium phosphate, 42 mM dibasic sodium phosphate, pH 7.5, and 0.15 M NaCl) containing 0.05% Tween 20. Blocked membranes were incubated for 1 h in phosphate-buffered saline containing 0.05% Tween 20 and 10 μg/ml peroxidase-conjugated Vicia villosa agglutinin isolectin B4 (VVA-B4), Psophocarpus tetragonolobus agglutinin (PTA), Dolichos biflorus agglutinin (DBA), WGA, peanut agglutinin (PNA) (all from Sigma), or lima bean agglutinin (LBA) (EY Laboratories, San Mateo, CA). After two 10-min washes with phosphate-buffered saline, lectin staining was detected by chemiluminescence.

To assess laminin and merosin binding to α-dystroglycan, nitrocellulose membranes were blocked in phosphate-buffered saline containing 5% nonfat dry milk for 1 h at room temperature. Blocked membranes were rinsed briefly with TBS (50 mM Tris-HCl, pH 7.4, and 150 mM NaCl) and incubated for 2 h at room temperature in TBS containing 3% bovine serum albumin, 1 mM CaCl2, 1 mM MgCl2, and 1 μg/ml native laminin (Upstate Biotechnology, Inc., Lake Placid, NY) or merosin (Life Technologies, Inc.) in the absence or presence of 1 mg/ml porcine muscle merosin (Sigma). Laminin/merosin-binding proteins were detected with affinity-purified polyclonal laminin antibodies (Sigma) by chemiluminescence (43).

Solid-phase Binding Assay—A solid-phase microtiter assay using iodinated cardiac α-dystroglycan was performed as described previously (43). Briefly, Immulon 1 removable microtiter wells (Dynatech Laboratories Inc., Chantilly, VA) were coated with laminin or merosin in TBS; aspirated, and blocked with TBS containing 3% bovine serum albumin, 1 mM CaCl2, and 1 mM MgCl2. Triplicate wells were incubated for 2 h at room temperature with 1 nM 125I-labeled cardiac α-dystroglycan in 0.1 ml of TBS containing 3% bovine serum albumin, 1 mM CaCl2, and 1 mM MgCl2 in the absence or presence of the indicated concentration of heparin. After two brief washes with TBS containing 3% bovine serum albumin, 1 mM CaCl2, and 1 mM MgCl2, the wells were counted in a Packard 1650 γ-counter.

Enzyme Treatments—Purified α-dystroglycans in 1% SDS were incubated at 100 °C for 5 min; diluted 10-fold into 0.5 mM acetate, pH 5.5, and 1% Triton X-100; and incubated for 24 h at 37 °C in the absence or presence of sialidase (10 milliunits/μg α-dystroglycan) from Vibrio cholerae, Clostridium perfringens, Archaeobacter neurofaciens (all from Boehringer Mannheim), Newcastle disease virus (Oxford GlycoSystems Inc., Rosedale, NY), or nonpathogenic Streptococcus strain 6646K (Seikagaku America Inc., Ijamsville, MD). Purified brain α-dystroglycan was digested with jack bean β-N-acetylgalactosaminidase, chicken liver α-N-acetylgalactosaminidase, or Echerichia coli β-galactosidase (all from Sigma) as described previously (40). Control and enzyme-digested samples were analyzed by SDS-polyacrylamide gel electrophoresis and the blotting assays described above.

RESULTS

The laminin-1 and merosin binding properties of cardiac muscle α-dystroglycan were first qualitatively compared in the blot overlay assay (Fig. 1A). As previously observed for 156-kDa skeletal muscle α-dystroglycan (43), laminin-1 and merosin binding to 140-kDa cardiac muscle α-dystroglycan were similarly inhibited by 10 mM EDTA or 0.5 M NaCl (data not shown). However, the addition of 1 mg/ml heparin significantly inhibited laminin-1 binding to skeletal and cardiac muscle α-dystroglycans, but had negligible effects on merosin binding to either α-dystroglycan (Fig. 1A). Cardiac muscle α-dystroglycan binding to laminin and merosin was further compared by examining the concentration dependence of heparin inhibition (0–2 mg/ml) using a solid-phase microtiter assay (Fig. 1B). 125I-Labeled cardiac α-dystroglycan binding to laminin-1 was significantly more sensitive to heparin over the range of 0.1–2 mg/ml in comparison with merosin, which was notably insensitive to heparin at all concentrations tested (Fig. 1B). Thus, cardiac and skeletal muscle α-dystroglycans share the capacity to bind merosin in a heparin-insensitive manner. In contrast,
the binding of laminin-1 and merosin to 120-kDa α-dystroglycans purified from peripheral nerve (32) or brain (43) was significantly inhibited by heparin. The sum of these results supports our hypothesis that tissue-specific post-translational modifications alter the capacity of some α-dystroglycans to discriminate between different forms of laminin.

Because α-dystroglycan is extensively glycosylated (1, 8, 19, 33, 35–37), carbohydrate modifications seemed most likely to account for the differences in laminin binding properties observed between muscle and neural α-dystroglycans. The lectin reactivities reported for α-dystroglycans prepared from different tissues and species (Table I) suggested the presence of terminal GalNAc residues on neural forms of α-dystroglycans expressed in other tissues. Alternative, subterminal GalNAc-containing oligosaccharides on skeletal muscle α-dystroglycan might be blocked from interacting with VVA-B₄ by further modification with terminal sialic acid (41, 42). To address this possibility, we tested the ability of various sialidases to expose cryptic VVA-B₄-binding sites on skeletal muscle α-dystroglycan. Digestion of the skeletal muscle dystrophin-glycoprotein complex with sialidase from V. cholerae results in an increased electrophoretic mobility of α-dystroglycan with concomitant loss of staining by WGA and Maackia amurensis agglutinin (specific for Neu5Acα2–3Gal) and exposure of latent binding sites for PNA (1), but no loss of laminin binding activity (8). We confirmed these results with purified skeletal muscle α-dystroglycan, yet found that it exhibited no reactivity with VVA-B₄ upon digestion with V. cholerae sialidase (Fig. 4). Consistent with results obtained using V. cholerae sialidase, skeletal muscle α-dystroglycan digested with C. perfringens sialidase exhibited an increased electrophoretic mobility with concomitant loss of staining by WGA and exposure of latent binding sites for PNA (Fig. 5). However, α-dystroglycan digested with C. perfringens sialidase also became strongly reactive with VVA-B₄ (Fig. 5) and DBA, but not with LBA or PTA (data not shown). Skeletal muscle α-dystroglycan also stained strongly with VVA-B₄ after digestion with sialidases from A. neurofaciens and Streptococcus strain 6646K, but not with Newcastle disease virus sialidase (Table II). Thus, skeletal muscle α-dystroglycan contains sialic acid moieties resistant to V. cholerae and Newcastle disease virus siali-
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**TABLE I**

Characterization of α-dystroglycan glycosylation by lectin staining and glycosidase sensitivity

| Lectin/enzyme       | Carbohydrate specificity | Rabbit skeletal (1, 8) | Rabbit skeletal (33) | Oxine brain (35, 36) | Bovine sciatic nerve (36) | Torpedo electric organ (19, 37) |
|---------------------|--------------------------|------------------------|----------------------|----------------------|--------------------------|-------------------------------|
| ConA                | Mannose                  | +                      | +                    | +                    | +                        | +                             |
| PNA                 | Galβ1-3GalNAc            | +                      | +                    | +                    | +                        | +                             |
| Jacalin             | Galβ1-3GalNAc            | +                      | +                    | +                    | +                        | +                             |
| WGA                 | GlcNAc, Neu5Ac           | +                      | +                    | +                    | +                        | +                             |
| MAA                 | Neu5Ac2-3Gal            | +                      | +                    | +                    | +                        | +                             |
| SNA                 | Neu5Ac2-6Gal            | +                      | +                    | +                    | +                        | +                             |
| Lotus               | Fucose                   | –                      | –                    | –                    | –                        | –                             |
| UEA-I               | Fucose                   | –                      | –                    | –                    | –                        | –                             |
| DBA                 | Terminal GalNAc         | ND                     | –                    | +                    | ND                       | +                             |
| VVA-B4              | Terminal GalNAc         | ND                     | ND                   | +                    | ND                       | ND                            |
| WFA                 | GalNAc                  | ND                     | ND                   | +                    | ND                       | ND                            |
| N-Glycosidase F     | N-Linked glycans         | +                      | ND                   | +                    | +                        | +                             |
| Neuraminidase       | Sialic acid             | +                      | ND                   | +                    | +                        | +                             |
| O-Glycosidase       | O-Linked glycans         | +                      | ND                   | +                    | +                        | +                             |
| Hepase              | HS                      | –                      | –                    | –                    | –                        | –                             |
| Nitrous acid        | HS                      | –                      | –                    | –                    | ND                       | ND                            |
| Chondroitinase      | CS                      | –                      | –                    | ND                   | –                        | –                             |
| Keratanase          | KS                      | –                      | –                    | –                    | –                        | –                             |

Notes:
- References are given in parentheses.
- ConA, concanavalin A; MAA, M. amurensis agglutinin; UEA-I, Ulex europaeus agglutinin I; WFA, Wisteria floribunda agglutinin; HS, heparan sulfate; CS, chondroitin sulfate; KS, keratan sulfate; ND, not determined.
- PNA reactivity is evident only after neuraminidase digestion.

**FIG. 2.** *V. villosa* agglutinin reacts with cardiac, brain, and sciatic nerve α-dystroglycans, but not with skeletal muscle α-dystroglycan. Shown are identical nitrocellulose transfers of SDS-polyacrylamide gels loaded with α-dystroglycan purified from rabbit skeletal muscle (SKEL), cardiac muscle (CARD), brain, or sciatic nerve and stained with affinity-purified chicken polyclonal antibodies to α-dystroglycan (α-DG Ab; upper left panel) or stained with 1 μg/ml peroxidase-conjugated VVA-B4, LBA, PTA, or DBA. The reactivity of each lectin for different anomers of N-acetyl-d-galactosamine is indicated by a plus or minus sign.

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α-DG Ab

VVA-B4

VVA-B4 + GalNAc

**FIG. 3.** *V. villosa* agglutinin appears to recognize terminal β-linked GalNAc residues on brain α-dystroglycan. Shown are identical nitrocellulose transfers of SDS-polyacrylamide gels containing electrophoretically separated α-dystroglycan purified from rabbit brain and stained with 1 μg/ml peroxidase-conjugated VVA-B4, LBA, PTA, or DBA. The reactivity of each lectin for different anomers of N-acetyl-d-galactosamine is indicated by a plus or minus sign.

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α-GalNAc

β-GalNAc

O-linked GalNAc

skeletal muscle (Fig. 6), cardiac muscle (data not shown), sciatic nerve (data not shown), or brain (Fig. 6) α-dystroglycan, either in the presence or absence of heparin. Similar results were obtained using sialidases from *A. neurofaciens*, *V. cholerae*, *Streptococcus* strain 664K, and Newcastle disease virus (Table II) and α-dystroglycan purified from bovine brain (data not shown). In the study reporting a loss of laminin binding upon digestion with sialidase (36), it was possible that the extreme alkaline conditions used to solubilize α-dystroglycan from sciatic nerve membranes may have degraded O-acetylated sialic acid derivatives (52, 53) on α-dystroglycan that are otherwise resistant to sialidase digestion. However, the laminin binding activity of saponified rabbit or bovine brain α-dystroglycan was also not diminished after sialidase digestion (data not shown). Thus, the sum of our results indicates that the sialic acid moieties of α-dystroglycan that are labile to a variety of sialidases do not appear to be necessary for α-dystroglycan binding to laminin-1 or merosin.

**DISCUSSION**

Previous studies have demonstrated that most, if not all, α-dystroglycans express Galβ1-3GalNAc-Ser/Thr moieties variably modified by α2–3-sialic acid in a tissue-specific fashion.
V. cholerae neuraminidase-digested (−) skeletal α-dystroglycan stained with affinity-purified chicken polyclonal antibodies to α-dystroglycan (α-DG Ab), peroxidase-conjugated VVA-B4, or WGA or overlaid with laminin-1 (LAM).

(1, 35, 36). Ovine brain α-dystroglycan uniquely expresses an α2–6-sialic acid associated with an Asn-linked oligosaccharide based on its reactivity with Sambucus nigra agglutinin (SNA) that is removed by digestion with N-glycosidase F (35). More recently, the structure Siaα2–3Galβ1–4GlcNAcβ1–2Man-Ser/Thr was shown to compose two-thirds of the sialylated, Ser/Thr-linked oligosaccharides of bovine peripheral nerve α-dystroglycan (38). Based on our present results, we propose a novel sialic acid modification of skeletal muscle α-dystroglycan, Siaα2–6GalNAc, using the nomenclature suggested by Varki (53) to indicate a sialic acid with an unidentified substitution. We have shown that digestion of skeletal muscle α-dystroglycan with any of three sialidases that hydrolyze α2–3-, α2–6-, or α2–8-sialic acid linkages unmasks a terminal GalNAc residue that is normally exposed in α-dystroglycans prepared from a variety of other tissues (Table II). Newcastle disease virus sialidase, which cleaves α2–3- and α2–8-linkages, but not α2–6-linkages, failed to expose the terminal GalNAc residue, suggesting an α2–6-linkage. While both V. cholerae sialidase and the lectin SNA would be expected to react with Siaα2–6GalNAc, neither is reactive on substrates in which the carbohydrate group in the sialic acid moiety is derivatized (54, 55). Thus, the failure of SNA to recognize skeletal muscle α-dystroglycan (Table I) and of V. cholerae sialidase to expose the terminal GalNAc residue (Fig. 4 and Table II) can be best explained by the presence of one or more Siaα2–6GalNAc moieties on skeletal muscle α-dystroglycan. Similar structures may also be present on other tissue forms of α-dystroglycan, as we have observed that VVA-B4 staining of α-dystroglycans purified from rabbit cardiac muscle, sciatic nerve, and brain tissue with sialidase digestion. However, only skeletal muscle α-dystroglycan displays a complete lack of VVA-B4 reactivity prior to digestion with appropriate sialidases.

The functional role(s) of tissue-specific differences in α-dystroglycan sialoglycosylation remain to be elucidated. However, it should be noted that unusual terminating carbohydrate structures (most notably derivatives of sialic acid and GalNAc) are often critical tissue-specific determinants in the function of biologically important glycoconjugates (42, 56). Sanes (39, 40) and co-workers demonstrated that the adult neuromuscular junction is specifically stained with lectins that recognize terminal GalNAc residues. The GalNAc-specific lectin VVA-B4 was further shown to modestly induce acetylcholine receptor clustering by itself and significantly potentiate the clustering activity of agrin in cultured myotubes (41). However, the GalNAc-terminated molecule(s) mediating these effects remained to be identified since VVA-B4 did not appear to bind directly to agrin, acetylcholine receptors, or myotube α-dystroglycan (41). Interestingly, digestion of myotubes with C. perfringens neuraminidase was shown to dramatically expose latent VVA-B4-binding sites distributed throughout the sarcolemma and to stimulate acetylcholine receptor clustering in the absence of agrin (41). The sum of these data leads us to hypothesize the coexpression of two α-dystroglycan glycoforms in skeletal muscle: 1) an abundant extrasynaptic form containing a terminal β-linked GalNAc residue that is blocked from interacting with VVA-B4 by further modification with sialic acid and 2) a low abundance motor endplate-specific form with an exposed GalNAc residue involved in neuromuscular synaptogenesis. Because the motor endplate makes up only 0.1% of the total sarcolemmal membrane area, this hypothesis may be exceedingly difficult to test further in skeletal muscle using biochemical methods. The strong staining of cardiac muscle α-dystroglycan with VVA-B4 also raises the question of whether VVA-B4-reactive α-dystroglycan could play a more general role in establishing and/or maintaining the necessary postsynaptic organization required for appropriate nerve-muscle communication. The heart is extensively innervated by both sympathetic and parasympathetic neurons with numerous varicosities that appear to overlie a significantly greater fraction of cardiac sarcolemmal surface area than is the case with motor neurons in skeletal muscle (57–59). On the other hand, it is of some concern that VVA-B4 failed to label the surface of Schwann cells (40), which express a VVA-B4-reactive form of α-dystroglycan (36). It is possible that the binding of native ligands to Schwann cell α-dystroglycan in tissue sections hinders its binding to VVA-B4. Alternatively, VVA-B4-reactive α-dystroglycan may be one of several GalNAc-terminated glycoconjugates concentrated at the neuromuscular junction (40), but too diffusely distributed in the Schwann cell membrane to be detected with VVA-B4 by histochemical analysis. All of these issues will be more directly addressed with the availability of probes specific for different glycoforms of α-dystroglycan.

With regard to the carbohydrate structures important for α-dystroglycan binding to laminin, Matsumura and co-workers (36) recently reported that prolonged sialidase digestion of bovine sciatic nerve α-dystroglycan diminished its laminin binding activity. It was further observed that 10 mM sialic acid or 3′-sialyl-N-acetyllactosamine inhibited the binding of nanomolar concentrations of laminin to bovine sciatic nerve α-dystroglycan by 65% (36) or 33% (38), respectively. These results were interpreted to indicate that Siaα2–3Galβ1–4GlcNAc is an essential determinant for laminin binding by α-dystroglycan. Unfortunately, the inhibitor studies of laminin binding (36, 38) did not evaluate negatively charged control sugars or measure...
the effect of the control sugars used at any concentration higher than that reportedly effective for sialic acid inhibition. The examination of negatively charged control sugars such as glucose 6-phosphate or glucose 6-sulfate is relevant because these monosaccharides effectively block sulfatide binding to the laminin E3 domain (60, 61), which is also the site for α-dystroglycan binding (31, 34). We observed that sialidase digestion had no effect on the laminin binding activity of α-dystroglycan purified from a variety of tissues, regardless of enzyme source (Table II). Our results are consistent with all other previous studies that have evaluated sialidase digestion for an effect on α-dystroglycan binding to extracellular ligands (1, 19, 33, 34). The glycan sialoglycosylation may prove as useful markers to further elucidate the role of α-dystroglycan glycoforms in different tissues and perhaps within a single cell type.

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