Complex-type Asparagine-linked Oligosaccharides on Phosphacan and Protein-tyrosine Phosphatase-ζ/β Mediate Their Binding to Neural Cell Adhesion Molecules and Tenascin*

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Phosphacan, a soluble nervous tissue-specific chondroitin sulfate proteoglycan, is an alternative splicing product representing the entire extracellular domain of a transmembrane receptor-type protein-tyrosine phosphatase (RPTPζ/β) that also occurs as a chondroitin sulfate proteoglycan in brain. We have previously demonstrated that phosphacan binds with high affinity to neural cell adhesion molecules (Ng-CAM/L1 and N-CAM) and to the extracellular matrix protein tenasin and that it is a potent inhibitor of cell adhesion and neurite outgrowth. Tryptic digestions of [125]I-labeled phosphacan contain two glycopeptides that bind to Ng-CAM/L1, N-CAM, and tenasin. The larger of these (17 kDa) begins at Gin-209 near the end of the carboxy anhydrate-like domain of phosphacan/RPTPζ/β, whereas a 13-kDa glycopeptide begins at His-361 located in the middle of the fibronectin type III-like domain. Treatment of phosphacan with peptide N-glycosidase under non-denaturing conditions reduced its binding to the neural cell adhesion molecules and tenasin by 65–75% whereas endo-β-N-acetylglucosaminidase H had no effect, and peptide N-glycosidase treatment both decreased the molecular sizes of the tryptic peptides to ~11 kDa and abolished their binding. Based on the amino acid sequence of phosphacan, it can be concluded that each of the tryptic peptides contains one potential N-glycosylation site (at Asn-232 and Asn-381), and analyses of the isolated glycopeptides demonstrated the presence of sialylated complex-type oligosaccharides. Our results therefore indicate that the interactions of phosphacan/RPTPζ/β with neural cell adhesion molecules and tenasin are mediated by asparagine-linked oligosaccharides present in their carbonic anhydrase- and fibronectin type III-like domains.

We have previously described the isolation and biochemical properties of phosphacan, a nervous tissue-specific chondroitin sulfate proteoglycan that is synthesized by astrocytes (Rauch et al., 1991). Cloning of phosphacan (Maurel et al., 1994) demonstrated that it is an alternative splicing product representing the entire extracellular domain of a receptor-type protein-tyrosine phosphatase (RPTPζ/β) named RPTPζ/β (Krueger and Saito, 1992; Levy et al., 1993; Maurel et al., 1995). A keratan sulfate-containing glycoform (phosphacan-KS) also occurs in postnatal brain (Rauch et al., 1991; Maurel et al., 1994; Meyer-Puttlitz et al., 1995). Phosphacan binds reversibly and with high affinity to the neural cell adhesion molecules Ng-CAM/L1 and N-CAM (Kd ~0.1 nM) and to the extracellular matrix protein tenasin (Kd = 3 nM), but not to over a dozen other cell membrane and extracellular matrix proteins tested (Milev et al., 1994; Grumet et al., 1994). These studies also demonstrated that phosphacan is a potent inhibitor of neuronal and glial adhesion and of neurite outgrowth. Because of its potential importance both as a developmentally regulated extracellular matrix proteoglycan of nervous tissue and as the ligand-binding domain of a transmembrane protein-tyrosine phosphatase (which also occurs in the form of a chondroitin sulfate proteoglycan in brain; Shitara et al., 1994), we have attempted to identify the region of phosphacan/RPTPζ/β that participates in its interactions with nervous tissue proteins, using the glycosylated proteoglycan that is synthesized by rat brain in vivo. Phosphacan contains an N-terminal carbonic anhydrase-like domain followed by a single fibronectin type III sequence and a glycosaminoglycan linkage region to which are attached three to four chondroitin sulfate chains and (in the case of phosphacan-KS) a similar or greater number of keratan sulfate chains which vary in length and fine structure (Rauch et al., 1991; Maurel et al., 1994). We have found that two tryptic glycopeptides derived from the carbonic anhydrase- and fibronectin type III-like domains of phosphacan/RPTPζ/β bind to the neural cell adhesion molecules and tenasin and that their interactions are mediated by asparagine-linked oligosaccharides.

EXPERIMENTAL PROCEDURES

Isolation of Phosphacan and Enzyme Digestions—Phosphacan was isolated from PBS extracts of 7-day and adult rat brain by DEAE-cellulose ion exchange chromatography and Sepharose CL-6B gel filtration, followed by immunoreactivity chromatography using the 3F8 monoclonal antibody (Rauch et al., 1991). The proteoglycan was labeled to a specific activity of ~106 cpm/mol with 125I by the lactoperoxidase/glucose oxidase method using Enzymobeads (Bio-Rad). Typically, 25 μg of protein were labeled per reaction, and free iodine was removed by gel filtration on a PD-10 column (Pharmacia Biotech Inc.).

Trypsin and chondroitinase ABC digestions were performed at 37 °C in 100 mM Tris-HCl buffer, pH 8.0, containing 30 mM sodium acetate. A range of trypsin to phosphacan ratios was tested, from which it was found that the optimal conditions for generation of peptides capable of binding to Ng-CAM were 2-h digestion at an enzyme-substrate ratio of 1 to 20 (w/w) or 18 h at a ratio of 1 to 200–400. L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin was obtained from Worthington, and following digestion the enzyme was inactivated using 0.5 mM N-tosyl-L-lysine chloromethyl ketone (Sigma).

For removal of asparagine-linked oligosaccharides, phosphacan was treated for 6 h at 37 °C with either recombinant peptide N-glycosidase

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1 The abbreviations used are: RPTP, receptor-type protein-tyrosine phosphatase; PBS, phosphate-buffered saline; Endo, endo-β-N-acetylglucosaminidase; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis.
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(125I)$^{125}$I-labeled phosphacan, and tenascin gave a pattern identical to that seen in lane D (not shown). Peptide N-glycosidase treatment of 125I-labeled phosphacan isolated from 7-day postnatal (lanes E and F) or adult (lanes G and H) rat brain results in the disappearance of the 17- and 13-kDa tryptic glycopeptides and the appearance of a new band at ~11 kDa (lanes F and H).

RESULTS

Tryptic (glyco)peptides prepared from 125I-labeled phosphacan gave a complex pattern on an SDS-PAGE gradient gel (Fig. 1A). When this mixture was added to plastic wells coated with chick Ng-CAM, its rat homolog L1/NILE, or rat or chick N-CAM and tenascin gave a pattern identical to that seen in lane D (not shown). Peptide N-glycosidase treatment of 125I-labeled phosphacan isolated from 7-day postnatal (lanes E and F) or adult (lanes G and H) rat brain results in the disappearance of the 17- and 13-kDa tryptic glycopeptides and the appearance of a new band at ~11 kDa (lanes F and H).

To determine the origin of these peptides within the phosphacan sequence, unlabeled proteoglycan was mixed with iodinated material, digested with trypsin, fractionated by SDS-PAGE, and transferred to a ProBlott membrane. The positions of the 13- and 17-kDa bands were identified by autoradiography after elution from tenascin-coated wells.

In view of the fact that two peptides were observed to bind to the neural cell adhesion molecules and tenasin and that both peptides contained potential N-glycosylation sites (at Asn-232 and Asn-381), we considered the possibility that oligosaccharides might be involved in these interactions. Moreover, there was a blank at Asn-381 in our amino acid sequence of the phosphacan-tenascin interactions (Grunmet et al., 1994; Milev et al., 1994). However, the 13- and 17-kDa peptides could also be identified by SDS-PAGE and autoradiography after elution from tenascin-coated wells.

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Monosaccharide analyses of the two tryptic glycopeptides obtained from glycosidase-treated phosphacan (Fig. 1, lanes F and H). As expected, there were only traces of radioactivity bound to Ng-CAM and N-CAM using tryptic peptides prepared from peptide N-glycosidase-treated phosphacan (data not shown). Because there are developmental changes in the glycosylation of phosphacan (Rauch et al., 1991), we also tested binding and the effects of N-deglycosylation using proteoglycan isolated from both 7-day postnatal and adult brain, but no age-related differences were found (Figs. 1 and 3).

Electrophoresis of glycosidase-treated phosphacan showed no evidence of protease activity in the enzyme preparation, and control experiments showed that residual glycosidase present in the diluted proteoglycan used for the binding assay did not affect binding by acting on the CAM or tenasin during the binding incubation period. In fact, treatment of Ng-CAM with peptide N-glycosidase increased the binding of phosphacan by nearly 100%, but peptide N-glycosidase treatment of N-CAM and tenasin had no effect on phosphacan binding (data not shown). Moreover, glycosidase treatment of Ng-CAM had no detectable effect on the binding of phosphacan tryptic glycopeptides, suggesting that N-linked oligosaccharides on Ng-CAM may modulate its interactions with phosphacan by affecting Ng-CAM conformation or through steric factors.

Monosaccharide analyses of the two tryptic glycopeptides showed in both cases the presence of glucosamine, mannose, galactose, and sialic acid in molar ratios characteristic of triantennary oligosaccharides (Table I). A large portion of the oligosaccharides on the 17-kDa glycopeptide also contain fucose. These results are consistent with the finding that treatment of phosphacan with Endo H, which releases high-mannose and hybrid oligosaccharides, had no effect on its binding to Ng-CAM, N-CAM, or tenasin (data not shown). We have previously found that phosphacan contains oligosaccharides with 3-sulfated residues that are recognized by the HNK-1 monoclonal antibody (Rauch et al., 1991), as well as Lewis^b^ oligosaccharides (Allendoerfer et al., 1995) that are not present on other soluble chondroitin sulfate proteoglycans of rat brain. However, staining of immunoblots demonstrated that neither of the tryptic glycopeptides that bind to neural cell adhesion molecules and tenasin show HNK-1 or Lewis^b^ reactivity, indicating that these structures are not involved in the interactions we have described.

The only amino acid sequence similarity between the two tryptic glycopeptides resides in the octapeptides ILQNLIPN (beginning at residue 215) and ILNLLPN (beginning at residue 374), which have 75% identity (Fig. 2A). A synthetic peptide containing the ILNLLPN sequence and corresponding to a lysine followed by the first 21 amino acids of the 13-kDa glycopeptide produced up to 60% inhibition of phosphacan binding to Ng-CAM. However, there was even greater inhibition by a scrambled control peptide having the same amino acid composition, and binding inhibition studies using the first or last ten amino acids of this peptide sequence as well as a number of unrelated peptides also failed to support the involvement of these octapeptide sequences in phosphacan binding (Fig. 4). The highly variable inhibitory effects of the tested peptides probably reflects their ability to interact nonspecifically with the oligosaccharides involved in phosphacan binding.

**DISCUSSION**

Although there has been considerable interest in the function of glycans in molecular recognition, protein sorting, and developmental processes, the dramatic decrease in binding of phosphacan/RPTPζ/β to Ng-CAM/L1, N-CAM, and tenasin fol-

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2. P. Milev, B. Meyer-Puttlitz, R. K. Margolis, and R. U. Margolis, unpublished results.
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Table 1: Composition of oligosaccharides on tryptic glycopeptides

| Oligosaccharide               | Molar ratio 13-kDa glycopeptide/17-kDa glycopeptide |
|-------------------------------|---------------------------------------------------|
| Mannose                      | 1.0 / 1.0                                         |
| N-Acetylglucosamine           | 1.5 / 1.4                                         |
| Galactose                     | 1.2 / 1.0                                         |
| Fucose                        | Trace / 0.2                                       |
| N-Acetylgalactosamine         | Present / Present                                 |

* Because the samples for hydrolysis and high performance liquid chromatographic analysis of neutral and amino sugars were from different gel lanes and bands (see “Experimental Procedures”) than those used for determination of sialic acid, it is not possible to reliably calculate the molar ratio of N-acetylgalactosamine in relation to that of the other monosaccharides, but significant amounts were detected in both glycopeptides.

FIG. 4. Effects of synthetic peptides on binding of 125I-phosphacan to Ng-CAM. The peptides used were: KHEFLTDGYQDLGAILNNLIPN (●, from amyloid precursor-like protein 1; α2–6 linkage); YGGFMTEKSTOPTLVLTALKNAIKHAKKKQ (◆, from the amyloid precursor protein); and CPVNHGSQGEEKHRH (○, from glypcan). Points represent the average of duplicate determinations.

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REFERENCES

Allendoerfer, K. L., Margolis, R. K., Magnani, J. L., and Patterson, P. H. (1995) Soc. Neurosci. Abstr. 21, 276

Friedlander, D., Milew, P., Karthikeyan, L., Margolis, R. K., Margolis, R. U., and Grumet, M. (1994) J. Biol. Chem. 269, 119–120

Milew, P., Karthikeyan, L., Margolis, R. K., and Grumet, M. (1994) J. Biol. Chem. 269, 12142–12146

Hirani, S., Bernasconi, R. J., and Rasmussen, J. R. (1987) Anal. Biochem. 162, 485–492

Horstkorte, R., Schachner, M., Magyar, J. P., Vorherr, T., and Schmitz, B. (1993) J. Cell Biol. 121, 1409–1421

Knuepfer, N. X., and Saito, H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7417–7421

Leach, B. S., Collawn, J. F., Jr., and Fish, W. W. (1980) Biochemistry 19, 5734–5741

Lee, Y. C., and Lee, R. T. (1994) Neoglyconjugates: Preparations and Applications, Academic Press, San Diego, CA

Levy, J. B., Canoli, P. D., Silvennoinen, O., Barnea, G., Morse, B., Honegger, A. M., Haung, J. T., Cannizzaro, L. A., Park, S.-H., Druck, T., Huebner, K., Sap, J., Ehrlich, M., Musacchio, J. M., and Schlessinger, J. (1993) J. Biol. Chem. 268, 10573–10581

Margolis, R. U., and Margolis, R. K. (1994) Methods Enzymol. 245, 105–126

Maurel, P., Meyer-Puttlitz, B., Flad, M., Margolis, R. U., and Margolis, R. K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2522–2526

Milew, P., Meyer-Puttlitz, B., Flad, M., Margolis, R. U., and Margolis, R. K. (1995) DNA Sequence 5, 323–328

Meyer-Puttlitz, B., Milew, P., Unker, E., Zimmer, I., Margolis, R. U., and Margolis, R. K. (1995) J. Neurochem. 65, 2327–2337

Milew, P., Friedlander, D. R., Sakurai, T., Karthikeyan, L., Flad, M., Margolis, R. K., Grumet, M., and Margolis, R. U. (1994) J. Cell Biol. 127, 1703–1715

Nuck, R., Zimmermann, M., Sauvageot, D., Josic, D., and Reutter, W. (1990) Glycoconjugate 7, 279–286

Parekh, B. R., Tse, A. G. D., Dwek, R. A., Williams, A. F., and Rademacher, T. W. (1987) EMBO J. 6, 1233–1244

Parekh, B. R., Dwek, R. A., Thomas, J. R., Odenakker, G., Rademacher, T. W., Wittwer, A. J., Howard, S. C., Nelson, R., Siegel, N. R., Jennings, M. G., Harakas, N. K., and Feder, J. (1989) Biochemistry 28, 7844–7862

Pele, P., Nativ, M., Campbell, P. L., Sakurai, T., Martinez, R. L., Leavy, S. D., Oschi, J., Barnea, G., Ploerman, G. D., Grumet, M., and Schlessinger, J. (1995) Cell 82, 251–260

Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S., and Varki, A. (1995) J. Biol. Chem. 270, 7523–7532

Rauch, U., Gao, P., Janetzko, A., Flaccus, A., Hilgenberg, L., Tekotte, H., Margolis, R. K., and Margolis, R. U. (1991) J. Biol. Chem. 266, 14785–14801

Rauch, U., Karthikeyian, L., Maurel, P., Margolis, R. U., and Margolis, R. K. (1992) J. Biol. Chem. 267, 19536–19547

Segrest, J. P., and Jackson, R. L. (1972) Methods Enzymol. 28, 54–63

Shitara, K., Yamada, H., Watanabe, K., Shimono, M., and Yamaguchi, Y. (1994) J. Biol. Chem. 269, 20189–20193

Tarentino, A. L., Gomez, C. M., and Plummer, T. H. (1985) Biochemistry 24, 4655–4671

Weitzhandler, M., Kadlecok, D., Avdalovic, N., Forte, J. G., Chow, D., and Townsend, R. R. (1993) J. Biol. Chem. 268, 5121–5130

Other oligosaccharides may also participate in the binding process in addition to those on the two identified tryptic glycopeptides. The affinity of phosphacan/RPTP β for neural cell adhesion molecules and tenascin is several orders of magnitude greater than that for most lectin interactions with monovalent oligosaccharide ligands, which are usually in the micromolar range (Lee and Lee, 1994), and is therefore consistent with multiple interactions. A more detailed structural characterization of the phosphacan/RPTP β glycans is clearly of interest, both because this information may be useful for the design of agents that affect interactions of a receptor-type protein-tyrosine phosphatase with its ligands, and insofar as the asparagine-linked oligosaccharides involved in the binding of phosphacan to neural cell adhesion molecules and tenascin are also likely to mediate its potent inhibitory effects on cell adhesion and neurite outgrowth that we reported previously (Milew et al., 1994).

Flowing removal of N-linked oligosaccharides represents one of the still relatively few instances in which they have been demonstrated to play a role in protein interactions in vertebrate tissues. Because glycosylation is tissue- and cell type-specific (Parekh et al., 1987, 1989), we chose to utilize for our studies phosphacan purified from brain, since recombinant forms may not address the function of the native proteoglycan, and this is presumably the reason why carbonic anhydrase and fibronectin type III domains of phosphacan/RPTP β expressed as Fc fusion proteins in COS7 or 293 cells were not capable of binding to Ng-CAM, N-CAM, or tenascin (Peles et al., 1995). The lack of binding of a recombinant protein containing the phosphacan/RPTP β amino acid sequence up to Leu-415 provides additional evidence for the role of specific oligosaccharides in the binding process.

Our results suggest that the neural cell adhesion molecules and tenascin, all of which are involved in cell interactions, contain a lectin sequence that binds the phosphacan/RPTP β oligosaccharides, and like certain other lectins, the binding of phosphacan by tenascin requires the presence of divalent cations.2 The phosphacan binding site may be located in the Ig-like domains of Ng-CAM and N-CAM, since it has previously been reported that the fourth Ig-like domain of N-CAM contains a recognition site for high-mannose oligosaccharides on L1 (Horstkorte et al., 1993) and that a soluble immunoglobulin fusion construct of CD22, a B cell-specific receptor of the Ig-superfamily, binds to the trisaccharide Sia(α2–6)Gal(β1-4)GlcNAc that is present in N- and O-linked oligosaccharides (Powell et al., 1995).

Because the samples for hydrolysis and high performance liquid chromatographic analysis of neutral and amino sugars were from different gel lanes and bands (see “Experimental Procedures”) than those used for determination of sialic acid, it is not possible to reliably calculate the molar ratio of N-acetylgalactosamine in relation to that of the other monosaccharides, but significant amounts were detected in both glycopeptides.
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