The Fibrinogen-like Globe of Tenascin-C Mediates Its Interactions with Neurocan and Phosphacan/Protein-tyrosine Phosphatase-β

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Two nervous tissue-specific chondroitin sulfate proteoglycans, neurocan, and phosphacan (the extracellular domain of protein-tyrosine phosphatase-β), are high-affinity ligands of tenascin-C. Using portions of tenascin-C expressed as recombinant proteins in human fibrosarcoma cells, we have demonstrated both by direct radioligand binding assays and inhibition studies that phosphacan binding is retained in all deletion variants except those lacking the fibrinogen-like globe and that phosphacan binds to this single domain with nearly the same affinity (Kd ~ 12 nM) as to native or recombinant tenascin-C. However, maximum binding of neurocan requires both the fibrinogen globe and some of the adjacent fibronectin type III repeats. Binding of phosphacan and neurocan to intact tenascin-C, and of phosphacan to the fibrinogen globe, is significantly increased in the presence of calcium. Chondroitinase treatment of the proteoglycans did not affect their binding to either native tenascin-C or to any of the recombinant proteins, demonstrating that these interactions are mediated by the proteoglycan core proteins rather than through the glycosaminoglycan chains. These results are also consistent with rotary shadowing electron micrographs that show phosphacan as a rod terminated at one end by a globular domain that is frequently seen apposed to the fibrinogen globe in mixtures of phosphacan and tenascin-C. C6 glioma cells adhere to and spread on deletion variants of tenascin-C containing only the epidermal growth factor-like domains or the fibronectin type III repeats and the fibrinogen globe. In both cases cell adhesion was inhibited by similar concentrations of phosphacan, demonstrating that the fibrinogen globe is not necessary for this effect, which is apparently mediated by a direct action of phosphacan on the cells rather than by its interaction with the proteoglycan binding site on tenascin-C.

We have previously reported that neurocan and phosphacan/protein-tyrosine phosphatase-β, two major nervous tissue-specific chondroitin sulfate proteoglycans, are high affinity ligands of tenascin-C (apparent Kd ~ 3 nM) and that phosphacan inhibits the adhesion of C6 glioma cells to tenascin-C (1). Neurocan is a multidomain proteoglycan with a 136-kDa core protein (2) and together with versican and brevican is a member of the aggrecan family of hyaluronan-binding proteoglycans (3). It contains N-terminal immunoglobulin-like and hyaluronan-binding domains, a C-terminal domain consisting of EGF-, lectin-, and complement regulatory protein-like sequences, and a nonhomologous central domain of 593 amino acids, which contains the attachment sites for the three chondroitin sulfate chains and a large number of O-glycosidic oligosaccharides. In contrast, phosphacan (4, 5), which contains a 173-kDa core protein and three chondroitin sulfate chains, is an mRNA splicing product that represents the entire extracellular domain of a receptor-type protein-tyrosine phosphatase that also occurs as a chondroitin sulfate proteoglycan in brain (3). Phosphacan and protein-tyrosine phosphatase β have an N-terminal carboxylic anhydride-like domain followed by a fibronectin type III sequence. The phosphatase has, in addition to two cytoplasmic catalytic domains, an extracellular juxtamembrane sequence of 860 amino acids that may be deleted by alternative splicing (6) and appears to contain most of the chondroitin sulfate attachment sites that are actually utilized (7). The binding of phosphacan/protein-tyrosine phosphatase β to tenascin-C is mediated at least in part by N-linked oligosaccharides on the proteoglycan (8).

In addition to their high-affinity binding to tenascin-C, neurocan and phosphacan also interact with several immunoglobulin superfamily neural cell adhesion molecules including Ng-CAM/L1, N-CAM, TAG-1/axonin-1, Nr-CAM, and contactin (9–12). Most of these interactions have apparent dissociation constants in the subnanomolar range, and they are affected in different ways by the removal of N-linked oligosaccharides or chondroitin sulfate chains (8, 11). Immunocytochemical studies of embryonic and early postnatal nervous tissue showed an overlapping localization of the proteoglycans with all of their identified ligands, further supporting the biological significance of their ability to interact in vitro. In addition to their demonstrated high-affinity interactions with neural cell adhesion and extracellular matrix proteins, neurocan and phosphacan are also potent inhibitors of cell adhesion and inhibit or stimulate neurite outgrowth depending on the cell type and other factors (1, 9, 10, 12, 13). Our data therefore suggest that these two chondroitin sulfate proteoglycans are components of a multidimensional mechanism for the regulation of cell-cell and cell-matrix interactions at different sites and periods during nervous tissue histogenesis and that the multiplicity of ligands with differing affinities and properties could provide a means for the fine tuning of various regulatory processes.

To better understand the molecular basis for these multiple but differentially regulated interactions, and perhaps to eventually design agents that will affect the binding process, we
FIG. 1. Characterization of the tenascin-C variants. A, models of one subunit of each tenascin-C variant are depicted as a linear array from the N to C terminus, showing the central domain (segment of circle), heptad repeats (wavy line), EGF-like repeats (diamonds), FN III repeats (rectangles), and the fibronectin globule (circle), and the designation of each protein variant is indicated. Above the chick embryo fibroblast tenascin-C (CEF TN) two further splicing variants are drawn. B, purified CEF TN (1), TN 260 (2), TN 190 (3), TN EGF (4), TN FN (5), TN FB (6), TN FF (7), TN EFN1–5 (8), and TN EFN2 (9) were electrophoresed on a 6.75% SDS-polyacrylamide gel after reduction and stained with Coomasie Blue. Positions of the molecular size markers (in kilodaltons) are indicated to the right of the gel. C, rotary shadowing electron micrographs of CEF TN, TN 260, and TN EFN1–5.

have begun studies aimed at identifying the functionally active regions of these large multidomain proteins. In the present report we demonstrate the critical role of the fibrinogen globule at the C terminus of tenascin-C for its interactions with both neurocan and phosphacan/protein-tyrosine phosphatase-\(\beta\). We also show that some of the adjacent fibronectin type III repeats are involved in interactions of tenascin-C with neurocan and that the inhibitory effect of phosphacan on the adhesion of rat C6 glioma cells is attributable to a direct action of phosphacan on the cells rather than by blocking adhesion sites on tenascin-C.

MATERIALS AND METHODS

Production of Tenascin-C Variants—Native tenascin-C from chick embryo fibroblasts (CEF TN)\(^1\) was isolated as described (14). The recombinant chick tenascin-C variants were expressed in stably transfected HT1080 cells (American Type Culture Collection). The construction and purification of the smallest naturally occurring tenascin-C splicing variant TN 190, the mutants missing either the fibrinogen globule in TN FB (15), the EGF-like repeats in TN EGF\(^-\), the fibronectin type III repeats in TN FN\(^-\), the fibronectin type III repeats as well as the fibrinogen globule in TN FF\(^-\), or the EGF-like repeats and the fibronectin type III repeats in TN EFN\(^-\), respectively, have been described previously. TN 260, containing all of the 14 known fibronectin type III repeats of chick tenascin-C, was produced by inserting the central region of Petri dishes that had been coated with proteins. After 90 min, unbound cells were removed by a gentle wash using PBS. Bound cells were fixed with 1% glutaraldehyde, stained with cresyl violet, and counted in five fields of 2.2 mm\(^2\) corresponding to 15% of the protein-coated area.

RESULTS

Recombinant Tenascin—CEF TN and the recombinant chick tenascin-C variants were isolated as described under “Materials and Methods.” Models of all tenascin-C proteins used in this study are shown in Fig. 1A. Since CEF TN consists of a mixture of three major splicing variants (21), their alternative structures are indicated. TN 260 is the largest possible tenascin variant containing all existing extra repeats (22), whereas TN 190 is the smallest one consisting of constant repeats only. TN EGF\(^-\) is missing the EGF-like repeat, TN FN\(^-\) all fibronectin type III repeats, and TN FB\(^-\) the fibrinogen globule. Even larger deletions were made in TN FF\(^-\), which is missing the fibronectin type III repeats as well as the fibrino-
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The fibrinogen globe and the fibronectin type III repeats for 6–8 (TN EFN1–5), whereas there was a variant containing the fibrinogen globe and fibronectin repeats (S.E.) recombinant Tenascins—

From the data summarized in Fig. 2, it can be seen that phosphacan bound well (11–17% of input counts/min bound minus cpm bound to wells coated with BSA) measured at equilibrium (12 h) using binding buffer containing 150 mM NaCl, and all values are means of duplicate determinations ± S.E.

Gen globe, TN EFN1–5 that lacks both the EGF-like repeats and fibronectin type III repeats 1–5. The proteins were analyzed by gel electrophoresis after reduction and found to migrate as single bands (except for CEF TN, which consists of three bands corresponding to the three splicing variants) at the expected positions according to their subunit molecular weight (Fig. 1B). All proteins assemble into hexameric molecules as the authentic tenascin-C isolated from chick embryo fibroblasts. Examples are shown on the electron micrographs in Fig. 1C. Clearly the TN 260 has much longer arms than CEF TN, whereas TN EFN1–5 consists of hexamers with very short arms composed of three fibronectin type III repeats and terminating in the fibrinogen globe. The hexameric structures of the other tenascin-C variants have been shown previously (15, 16).

Binding of Neurocan and Phosphacan to Native and Recombinant Tenascins—From the data summarized in Fig. 2, it can be seen that phosphacan bound well (11–17% of input counts/min) to native chick tenasin-C as well as to recombinant TN 260 (containing the six extra fibronectin type III repeats present in a tenasin-C splice variant), to deletion variants lacking either all of the EGF or fibronectin type III repeats, and to TN 190 (which serves as a control for the deletion variants). Approximately the same degree of binding was observed to the fibrinogen globe alone (TN EFN1–5) and to a recombinant fragment containing the fibrinogen globe and fibronectin repeats 6–8 (TN EFN1–5), whereas there was <3% binding to deletion variants lacking the fibrinogen globe (TN FB1–5) or the fibrinogen globe and the fibronectin repeats (TN PF1–5).

In relation to a TN 190 control, neurocan binding was reduced by 20% in the deletion variant lacking only the fibronectin type III repeats (TN FN1–5), by 50% in variants lacking the fibrinogen globe (TN FB1–5), and by 80% in the deletion variant (TN FF1–5) lacking both domains (Fig. 2). The importance of both the fibrinogen globe and the fibronectin type III repeats for neurocan interactions with tenasin-C was further supported by experiments using the complementary proteins, insofar as there was only 5% binding to the fibrinogen globe, but binding increased to 12% if fibronectin repeats 6–8 were also present (Fig. 2). However, binding to TN EFN1–5 was still 30% less than to TN 190, suggesting that fibronectin repeats 1–5 may also enhance neurocan interactions with tenasin-C, and TN 260, which contains six extra FN III repeats, bound neurocan somewhat better than TN 190.

Binding of neurocan and phosphacan to native tenasin-C and to the recombinant tenascins was not affected by chondroitinase treatment of the proteoglycans (Ref. 1 and data not shown). These results demonstrate that although the fibrinogen globe also contains the major heparin binding site of tenasin-C (15), its interactions with neurocan and phosphacan are mediated by the proteoglycan core (glyco)proteins rather than through the glycosaminoglycan chains.

Because binding of neurocan and phosphacan to the deletion variant lacking the EGF repeats was in both cases ~44% greater than to TN 190 (Fig. 2), it would appear that the EGF domain may inhibit the interactions of both proteoglycans. The relative amounts of neurocan or phosphacan bound to the various proteins was generally similar in experiments using an 8-fold range of concentrations (1.6–12.5 nM) for coating the plastic wells, although the percent binding increased as a function of increasing protein concentration (data not shown). These results, together with the direct demonstration of binding to the fibrinogen globe (with or without the three adjacent FN III repeats), indicate that the major differences in binding to the tenasin deletion variants do not merely reflect different substrate coating efficiencies. Binding of both proteoglycans reached equilibrium after 9–12 h and was fully reversible after incubation with an excess of unlabeled ligand (data not shown).

Saturation and Binding Affinity of Neurocan and Phosphacan to Recombinant Tenascins—Phosphacan bound to recombinant TN 190 with the same affinity (Kd ~ 2 nM) that we previously found for native tenasin-C (1), whereas binding to the fibrinogen globe alone (TN EFN1–5) showed a somewhat lower affinity (Kd ~ 12 nM; Fig. 3). The <3% residual binding of phosphacan to the deletion variant lacking only the fibrinogen globe (TN FB1–5) was probably nonspecific, since it did not yield a meaningful saturation curve (data not shown). Neurocan also demonstrated high affinity binding to both TN EFN1–5—

![Fig. 2. Binding of ^125I-labeled neurocan and phosphacan to native and recombinant tenascins. All tenascins were coated in removable wells at a concentration of 4 nM, and labeled proteoglycans were used at ~100,000 cpm/well. The percent bound represents specific binding (total counts/min bound minus cpm bound to wells coated with BSA) measured at equilibrium (12 h) using binding buffer containing 150 mM NaCl, and all values are means of duplicate determinations ± S.E.](image)

![Phosphacan/TN 190](image)

![Phosphacan/TN EFN1–5](image)

![Fig. 3. Saturation curves and Scatchard plots for the binding of ^125I-labeled phosphacan to recombinant TN 190 and the fibrinogen globe of tenasin-C (TN EFN1–5). Binding values represent specific binding as defined in the legend to Fig. 2. Points in the saturation curves are averages of duplicate determinations ± S.E.](image)
and TNEF₂, with apparent dissociation constants of ~10 nM (Fig. 4).

**Inhibition by Recombinant Tenascins of Neurocan and Phosphacan Binding to Tenascin-C—**Binding of phosphacan to chick tenascin-C was inhibited to the extent of 55–65% by tenascin-C, TN 190, TN FN, TN EFN, and TN EFN₁⁻⁵, but there was no inhibition by deletion variants that do not contain the fibrinogen globe (i.e. TN FB₂ and TN FF₂; Fig. 5). These results are in excellent agreement with those from direct binding studies of phosphacan to the various recombinant tenascins described above (Fig. 2), in which it was determined that only the fibrinogen globe is required.

Neurocan binding was inhibited to a lesser extent (25–35%) and was seen only with proteins containing fibronectin type III repeats (i.e. tenascin-C, TN 190, and TN FB₂), whereas the smaller recombinant fragments and deletion variants that were lacking fibronectin type III repeats actually enhanced binding of neurocan to tenascin-C (data not shown). The explanation for the enhanced binding is not clear, although neurocan may interact with small soluble tenascin fragments or deletion variants to form a complex that is capable of binding to immobilized tenascin-C. However, these results do support the conclusion from the direct binding studies that some of the fibronectin type III repeats are necessary for maximal interaction of neurocan and tenascin-C.

**Effects of Calcium and Reduction of Disulfide Bonds in Tenascin-C on Its Interactions with Neurocan and Phosphacan—**We previously obtained evidence suggesting that calcium may play a role in the interaction of phosphacan with human tenascin-C (8). More detailed studies have now shown that calcium is required for maximal interaction of phosphacan with native or recombinant tenascin-C and with smaller tenascin variants containing the fibrinogen globe, as demonstrated by the ~75% decrease in binding observed in calcium- and magnesium-free buffers containing EDTA (Fig. 6). Magnesium can partially substitute for calcium in phosphacan binding to the full-length tenascins but has relatively little effect on its interactions with the fibrinogen globe alone, and divalent cations also had some effect on the small residual binding of phosphacan to the tenascin-C deletion variant (TN FF) in which the fibrinogen globe and the fibronectin type III repeats are lacking. There was a similar calcium requirement for neurocan binding to full-length native and recombinant tenascin-C, whereas the absence of calcium and magnesium had only minor and variable effects on neurocan interactions with the tenascin-C deletion variants tested (data not shown).

Because a potential calcium-binding site in the fibrinogen globe of tenascin-C might involve disulfide bonds, we also evaluated the effects of reducing the recombinant tenascins with dithiothreitol. This treatment decreased phosphacan binding to full-length tenascin-C by >70% but had less effect on binding to
the fibrinogen globe alone, and interactions of neurocan were either unaffected (with TN 190) or actually enhanced in the case of several of the deletion variants (Fig. 7).

**Rotary Shadowing Electron Microscopy of Phosphacan Complexes with Tenascin-C**—In electron micrographs shown in Fig. 8A phosphacan appeared as a rod with kinks, a globular domain at one end, and several fine thread-like lateral projections. The globular region can be assumed to correspond to the N-terminal carbonic anhydrase-like domain and the fibronectin type III repeat of phosphacan, which we have shown previously to be involved in binding to tenascin-C (8), and the long rod to the remainder of the core protein, which is known to contain numerous O-glycosidic oligosaccharides and chondroitin sulfate side chains visible as the fine lateral projections. In mixtures of phosphacan with tenascin-C the two molecules are often seen in association with each other (Fig. 8, B and C).

The globular region of phosphacan was frequently seen next to the fibrinogen globe of tenascin-C, confirming the results from our study of deletion variants; both types of data indicate that the fibrinogen globe of tenascin-C is the phosphacan binding domain.

**Effects of Phosphacan on Adhesion and Spreading of C6 Glioma Cells on Recombinant Tenascins**—We have reported previously that phosphacan (but not neurocan) inhibits the adhesion of rat C6 glioma cells to tenascin-C, whereas there is no effect of either proteoglycan on adhesion to laminin (1). We have now analyzed this effect in more detail using deletion variants and recombinant fragments of tenascin-C. C6 cells showed good adhesion and spreading only on native chick tenascin-C and on deletion variants lacking the EGF-like repeats (TN EGF⁻) or both the fibronectin type III repeats and the fibrinogen globe (TN FF⁻) when coated on the bacteriological dishes employed in our previous studies (Fig. 9), whereas there was good adhesion and varying degrees of spreading on all recombinant tenascins when applied to tissue culture plastic (data not shown). The adhesion and spreading on the variant lacking the EGF-like repeats are consistent with other reports that this domain has antiadhesive properties for various cell types (23, 24), but the EGF-like domain itself is evidently not antiadhesive for C6 cells inssofar as TN FF⁻ containing only the EGF-like domain and the central N-terminal oligomerization region, supported adhesion and spreading almost as well as TN EGF⁻. However, adhesion and spreading on the EGF-like domains (TN FF⁻) were significantly more sensitive to inhibition by phosphacan, as demonstrated by our finding that concentrations as low as 1 µg/ml have a noticeable effect on the number of adherent cells and on process length (Fig. 9E), and very few processes were seen at a phosphacan concentration of 5 µg/ml (Fig. 9F), whereas there was good spreading of C6 cells on substrates coated with TN EGF⁻ and 5 µg/ml of phosphacan (Fig. 9B). Concentrations of phosphacan approaching 20 µg/ml were required to completely inhibit process extension on TN EGF⁻ (Fig. 9C).

While the TN FB⁻ deletion variant did not support adhesion on bacteriological plates (data not shown), the fibrinogen globe alone (TN EFN⁻) or together with fibronectin type III repeats...
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6–8 (TN EFN1–5) allowed good adhesion and cell spreading (Fig. 10, A/B and D/E). Process extension on both substrates was completely inhibited by phosphacan at a concentration of 10 μg/ml (Fig. 10, C and F). However, the fibrinogen globe is not an essential adhesion domain for C6 cells, because deletion of both the fibronectin type III repeats and the fibrinogen globe was inhibited by phosphacan in a concentration-dependent manner, reaching 90% inhibition at a phosphacan coating concentration of 20 μg/ml (Fig. 11). These results indicate both that the tenascin-C domains involved in C6 cell adhesion are not confined to the fibrinogen globe and that the antiadhesive effect of phosphacan is exerted directly on the cells independent of its ability to bind to tenascin-C (and potentially block cell adhesion domains).

DISCUSSION

This study has utilized a new approach to identify the regions of tenascin-C that are involved in its interactions with two nervous tissue-specific chondroitin sulfate proteoglycans, neurocan and phosphacan. Rather than expressing and studying only isolated domains, whose properties may not reflect those seen when they are present in the context of the entire molecule, we have employed deletion variants of tenascin-C that lack one or more homology domains and compared their effects in molecular and cell interactions with those obtained using native or full-length recombinant tenascin-C. All tenascin-C variants assembled correctly to hexameric molecules of the expected characteristics as determined by gel electrophoresis, their reactivity with monoclonal antibodies, and their molecular dimensions as revealed by electron microscopy. The expression of these tenascin-C variants by mammalian cells also increases the probability that they will be properly folded and glycosylated. Our studies demonstrated both by direct radioligand binding assays and by inhibition experiments that phosphacan binding is retained in all deletion variants except those lacking the fibrinogen globe and that phosphacan binds to this single domain with nearly the same high affinity as to native or recombinant tenascin-C. However, maximum binding of neurocan and inhibition of its interactions with tenascin-C require both the fibrinogen globe and some of the adjacent fibronectin type III repeats.

The C-terminal globular region of tenascin-C is homologous to the globular domain of the β and γ chains of fibrinogen and contains a sequence that is similar to the calcium-binding site identified in the fibrinogen γ chain (21, 25). This site has been defined as the EF hand (consisting of an α-helix, a calcium binding loop, and another α-helix) based on a structural analysis of parvalbumin (26). It has been shown that 45Ca binds to tenascin-C immobilized on nitrocellulose (27) and that the binding of cytotactin-binding proteoglycan to tenascin-C in coaggregation assays is decreased in the presence of EDTA (28). Based on this information, it has been proposed that the fibrinogen globe contains an EF hand calcium binding site and that binding of calcium to this region may determine a specific conformation that is important for its function (29).

It was therefore of interest to examine the effects of divalent cations on the interactions of phosphacan and neurocan with tenascin-C. Our results indicate that calcium is required for maximal binding of phosphacan to both native and full-length recombinant tenascin-C as well as to the fibrinogen globe alone (−75% decrease in binding in the absence of calcium; Fig. 6), whereas in the case of neurocan interactions this requirement for calcium was seen only with respect to the complete tenascin-C molecule. Local changes in the concentration of extracellular calcium could play a regulatory role in tenascin-proteoglycan interactions, both directly by affecting the conformation of the proteoglycan binding site in the fibrinogen globe of tenascin-C, and because calcium may serve as a major counterion for the carboxyl and sulfate groups on the chondroitin sulfate

Fig. 9. Effects of phosphacan on the adhesion and spreading of C6 cells on recombinant substrates containing either the fibronectin type III repeats and the fibrinogen globe (TN EGF) or the EGF-like domains (TN FF) of tenascin-C. A, TN EGF (10 μg/ml); B, TN EGF and phosphacan (1 μg/ml); C, TN EGF and phosphacan (20 μg/ml); D, TN FF (10 μg/ml); E, TN FF and phosphacan (1 μg/ml); F, TN FF and phosphacan (5 μg/ml). Bar, 100 μm.
chains of neurocan and phosphacan. However, it must also be recognized that the calcium-binding site in the fibrinogen globe of tenascin-C is only putative and that other regions of the molecule may be affected, as would appear to be the case for neurocan-tenascin interactions.

We also examined the effects of reducing disulfide bonds in tenascin-C to evaluate their importance for its binding properties. Treatment of tenascin-C with dithiothreitol would be expected to convert the tenascin hexabrachions to trimers and possibly to monomers, since the trimers are thought to be formed by a triple coiled-coil region in the molecule that is stabilized by disulfide bonds, and hexamers are formed by disulfide linkage of two trimers. Reduction should also alter the conformation of the disulfide-bonded EGF-like repeats and the fibrinogen globe (21). Our data indicate that the integrity of disulfide-stabilized structures in tenascin-C contributes significantly to its interaction with phosphacan, because treatment of TN 190 with dithiothreitol reduced binding by 70%, and reduction had a lesser but still significant effect on binding to the fibrinogen globe (Fig. 7). In contrast, interactions with neurocan were not affected by reduction of TN 190, and in the case of some deletion variants binding of neurocan was actually increased. These results support other evidence that the fibronectin type III repeats have an ancillary role in neurocan-tenascin interactions, since they do not contain cysteine residues and would therefore not be expected to be directly affected by reducing agents.

We have demonstrated previously that two tryptic glycopeptides derived from the N-terminal carbonic anhydrase-like and fibronectin type III domains of phosphacan bind to tenascin-C. This interaction is mediated at least in part by sialylated complex-type oligosaccharides occupying the single N-glycosylation site on each glycopeptide, insofar as their binding is abolished following treatment of phosphacan with peptide N-glycosidase (8). It has also recently been reported that a 35-kDa human serum protein with a fibrinogen domain has C-type lectin activity that is thought to be mediated by this domain (30). Rotary shadowing electron micrographs of phosphacan show a rod that is terminated at one end by a globular domain that interacts with the fibrinogen globe of tenascin-C (Fig. 8). The results of our deglycosylation studies indicate that the globular domain represents the N-terminal portion of phosphacan core protein. From the concentration and monosaccharide composition of glycoprotein-
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The three potential chondroitin sulfate attachment sites that are most likely to be utilized are all located in the C-terminal portion of the phosphacan core protein, although additional attachment sites may be present more N-terminally at Ser-595 and Ser-645 (3, 4). In electron micrographs one sees fine thread-like structures (Fig. 8) that extend laterally from the core protein and are consistent in appearance with that of glycosaminoglycan chains visualized in other proteoglycans (35, 36). Although these appear to arise from sites throughout the core protein, some of those seen near the globular region may in fact represent chondroitin sulfate chains with attachment sites in the C-terminal half of the proteoglycan that follow the core protein before reaching out into the surrounding space (as has been observed previously in the case of aggrecan (35)).

Recent investigations have shown a complex pattern of tenascin-C effects on cell adhesion and the promotion of neurite outgrowth, in which the concerted action of several domains leads to the diverse cellular responses observed (16). The effects of phosphacan on C6 cell adhesion to tenascin-C are similar to the previously observed inhibition of neuronal adhesion to the neural cell adhesion molecule Ng-CAM/L1. It was concluded from these studies that inhibition of neuronal adhesion by both neurocan and phosphacan (which bind to Ng-CAM/L1) is mediated by direct effects on the cells rather than via binding to the substrate, since the proteoglycans also inhibited adhesion to a substrate consisting of anti-Ng-CAM antibodies, to which the proteoglycans do not bind (9, 10). Phosphacan also inhibited the adhesion of C6 cells to Ng-CAM (7) but not to laminin (1). The results obtained using an Ng-CAM substrate are probably also mediated by a direct inhibitory effect of phosphacan on the cells, although this question has not yet been addressed experimentally, whereas the lack of an inhibitory effect on a laminin substrate could be due to the involvement of different cell receptors as well as to stronger cell-matrix interactions that are not susceptible to inhibition by phosphacan (at least at the concentrations tested).

Our studies provide the first direct biochemical and electron microscopic evidence for specific, high-affinity binding of proteins to the fibrinogen globe of tenascin-C. The terminal globular domain of fibrinogen is involved in protein-protein interactions in the process of fibrin polymerization, binding of fibrinogen to bacteria, and to receptors on platelets (37). Thrombospondin also binds to distinct sites on the distal parts of the β and α chains of fibrinogen (38), and by analogy with fibrinogen it has been suggested that the fibrinogen globe of tenascin-C may play a role in its association with other proteins (39). The fibrinogen globe of tenascin-C has been indirectly implicated in binding to β-integrins (40) and has been shown by electron microscopy to be attached to collagen fibrils in chicken vitreous humor (41). Because of its location at the C-terminal tips of the hexabrachion arms, the fibrinogen globe is ideally situated to mediate the interactions of tenascin-C with cells and with other proteins. Phosphacan and neurocan bound to this domain may serve as a bridge between tenascin-C and neuronal cell adhesion molecules to which these proteoglycans also bind with high affinity (3, 9–11) or in other ways modulate its biological properties.

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