Eukaryotic Expression of Recombinant Biglycan

POST-TRANSLATIONAL PROCESSING AND THE IMPORTANCE OF SECONDARY STRUCTURE FOR BIOLOGICAL ACTIVITY*

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Biglycan is a small chondroitin sulfate proteoglycan found in many tissues and is structurally related to decorin, fibromodulin, and lumican. The biological function of biglycan is poorly understood, although several studies have indicated interaction with other extracellular matrix components. We have initiated studies of structural and functional domains of biglycan by transient eukaryotic expression using the vaccinia virus/T7 bacteriophage expression system. A recombinant vaccinia virus, vBGN4 encoding the mature biglycan core protein as a polyhistidine fusion protein under control of the T7 phage promoter was expressed in HT-1080 cells and UMR106 cells. The structure of the recombinant biglycan secreted by these cells was defined by analyzing molecules labeled in the presence of [35S]sulfate, [3H]glucosamine, and [35S]methionine. Glycoforms of biglycan were separated by imidazole gradient elution, under non-denaturing conditions, and comprised: a large proteoglycan form substituted with two chondroitin sulfate chains of molecular mass ~34 kDa (HT-1080 cells) or ~40 kDa (UMR106 cells); a small proteoglycan form substituted with two chondroitin sulfate chains with a median molecular mass ~28 kDa; and a core protein form secreted devoid of glycosaminoglycan chains. All the glycoforms were substituted with two N-linked oligosaccharides, and the disaccharide composition of the two glycosaminoglycan populations were identical. Approximately 70% of the recombinant biglycan secreted by HT-1080 cells was substituted with chondroitin sulfate chains, whereas about 50% of the biglycan expressed by UMR106 cells was substituted with chondroitin sulfate chains. Infection with vBGN4 in both HT-1080 and UMR106 cells resulted in the production of approximately 10 mg of biglycan/10^9 cells per 24 h. The native recombinant biglycan was shown to bind to collagen type V and the complement protein, C1q. However, when the secondary structure of recombinant biglycan was disrupted by exposure to 4 M guanidine hydrochloride, the affinity for collagen type V was dramatically reduced. These data demonstrate the importance of secondary structure to the function of this small proteoglycan.

Biglycan belongs to a family of small proteoglycans that are characterized by a core protein that consists of a leucine-rich repeat (LRR) domain. Other members of this family include decorin, fibromodulin, lumican, and chicken PG lb. The LRR is a consensus sequence of 20–29 residues containing leucine in conserved positions, and is often present as a tandem repeat. The LRR domain of human biglycan consists of 8 repeats of 24 residues. The small proteoglycans belong to a subset of the LRR protein superfamily due to the presence of four similarly spaced cysteine residues at the amino-terminal of the LRR domain. The LRR has been identified in an increasing number of proteins derived from yeast, plant, insect, and mammalian species (reviewed in Ref. 1) and is likely to play a role in protein-protein interactions (2, 3).

Biglycan consists of a core protein of about 42 kDa subjected to extensive post-translational modifications including substitution with two glycosaminoglycan chains. Biglycan extracted from bone or cartilage is devoid of signal and propeptide sequences. Glycosylation of the core protein involves addition of glycosaminoglycan chains and N-linked oligosaccharides. The composition of the glycosaminoglycan polymer is tissue specific; in skin and cartilage, biglycan is substituted with dermatan sulfate chains, however, in bone it is substituted with chondroitin sulfate chains. Biglycan core protein contains two asparagine consensus tripeptides for substitution with N-linked oligosaccharides, which are conserved in the core protein of the structurally related decorin.

Numerous studies investigating the interactions of the small chondroitin sulfate proteoglycans, biglycan and decorin, with other matrix components have commonly used proteoglycan extracted from tissue. These reports include descriptions of binding to collagen type I and II (4, 5), collagen type V (6), collagen type VI (7), collagen type XIV (8), transforming growth factor-β (9, 10), complement component C1q (11), and divalent cation-dependent self-association (12). The biological significance of these interactions remains unresolved since efficient extraction of biglycan and decorin from cartilage and bone requires the use of chaotropic solvents and detergents which can result in the denaturation of these proteins.

Due to the extensive post-translational modifications of the small chondroitin sulfate proteoglycans, an expression system capable of these complex modifications, including correct fold-
ing of the core protein, would be advantageous. Prokaryotic expression systems are capable of generating high yields of protein, however, these proteins lack the post-translational modifications that may regulate folding, solubility, and biological activity. In Escherichia coli expression systems, biglycan and decorin are synthesized devoid of carbohydrate addition and the recombinant core protein is often insoluble requiring the use of denaturing solvents for efficient extraction from inclusion bodies (14–16). The baculoviral expression system is capable of high level production of processed protein, but it is unlikely that insect cells have the post-translational machinery required for effective processing of a proteoglycan, despite the recent report of Drosophila heparan sulfate (17). Recombinant proteoglycans expressed in stably transfected mammalian cells are likely to be folded and glycosylated correctly, however, effective purification of the recombinant proteoglycan often involves the use of denaturing solvents.

Using the vaccinia/T7 bacteriophage expression system (18), in conjunction with a polyhistidine fusion vector, we have successfully expressed and purified native recombinant human biglycan (present study) and decorin (36). We have purified chemical amounts of recombinant human biglycan under mild, non-denaturing conditions using metal chelating chromatography. The recombinant biglycan is expressed as two glycoforms: proteoglycan and core protein devoid of glycosaminoglycan chains. The proteoglycan form is substituted with two chondroitin sulfate chains and 2 \( N \)-linked oligosaccharides. Recombinant biglycan was shown to bind to collagen type V and the complement protein, C1q. The secondary structure of recombinant biglycan is shown to be important for mediating the interaction with collagen type V.

**EXPERIMENTAL PROCEDURES**

**Materials**— Dulbecco’s modified Eagle’s medium (DMEM), \( L \)-methionine- and \( L \)-cysteine-free DMEM, and Lipofectin transfection reagent were obtained from Life Technologies, Inc. Fetal bovine serum was obtained from Interagen. \( N \)-Glycosidase F and the detergents CHAPS and Nonidet P-40 were obtained from Boehringer Mannheim. Factor Xa protease was from Pierce. Chondroitinase ABC (Proteus vulgaris) and chondroitinase ACII (Arthrobacter aurescens) was acquired from Seikagaku. \( L^{-}\)\( [35S] \)Sulfate (5 mCi/ml) was from Amersham. Trans\( ^{35S} \)-label (comprising a mixture of \( L^{-}\)\( [35S] \) methionine and \( L^{-}\)\( [35S] \) cysteine, 10 mCi/ml) was obtained from ICN Biomedicals. \( \beta \)-(1\( \rightarrow \)3)\( \beta \)GlcA polysaccharide hydrochloride (1 mCi/ml) was obtained from American Radiolabeled Chemicals. All restriction enzymes were acquired from Boehringer Mannheim; Sequence version 2.0, sequencing kit was obtained from U. S. Biochemical Corp.; \( \alpha \)-dinitrophenylidex primers were synthesized on an Applied Biosystems DNA synthesizer. Imidodiacetic acid immobilized on Sepharose 6B Fast Flow and C1q were obtained from Sigma. Sephadex G-50 and prepacked Superose 6 were from Pharmacia LKB Biotech. Human collagen types I, III, V, VI, and bovine collagen type II were obtained from Southern Biotechnology Associates, Inc., Birmingham, AL. Centrifor-10 ultrafiltration devices were obtained from Amicon. All other chemicals were commercially available and of analytical grade. Cell lines used for virus propagation, titration, and expression were obtained from the American Type Culture Collection (VT-1, ATCC-CCL70; HeLa, ATCC-CCL2; 143B (Hu TK(+) + 2 extra amino acids (Glu-Ser) between the polyhistidine sequence and the Factor Xa site; and two extra residues (Leu-Glu) between the Factor Xa site and the start of the mature core protein sequence of biglycan.

**Generation of Recombinant Vaccinia Virus**— A recombinant vaccinia virus expressing the polyhistidine biglycan fusion, BG4, was generated as described by Mackett et al. (19). The presence of thymidine kinase sequences flanking the expression cassette of pBG4 permitted homologous recombination when the plasmid was transfected into cells that had been infected with wild-type vaccinia virus. Recombinant virus (thymidine kinase – phenotype) was selected by propagation in 1343 cells in medium containing 5-bromodeoxyuridine.

**Vaccinia Virus**— The recombinant vaccinia virus, VTF7-3 encoding bacteriophage T7 RNA polymerase has been described (18). Recombinant vaccinia virus was propagated and crude stocks prepared from HeLa cells using standard procedures (19).

**Co-infection Procedure**— Clonal monolayers of HT-1080 or UM-1 tumor cells were co-infected with VTF7-3 (10 plaque forming units/cell) and vBG4 (10 plaque forming units/cell). Virus was allowed to adsorb for 2 h at 37°C. At 8 h post-infection, the virus inoculum was replaced with serum-free DMEM or serum-free DMEM containing radiolabels. Medium was harvested at 30 h post-infection.

**Purification of Recombinant Biglycan**— Culture medium was harvested and the macromolecular fraction of the medium separated from unincorporated radioactive precursors by chromatography on Sephadex G-50. The 5 mCi/ml samples were equilibrated and eluted with 5 mCi/ml sodium iodoacetate, 0.5 mCi/ml NaCl, 20 mCi/ml Tris-HCl, pH 8.0. The eluted macromolecular fraction was applied to a 2-ml column of iodoacetamide immobilized on Sepharose 6B that had been equilibrated with nickel chloride (20). After sample application, the column was washed with stepwise increases in iodacetamide concentration (see above) and bound material eluted with a continuous imidazole gradient of 60–400 mM (total volume, 40 ml). Fractions (1 ml) were collected at a flow rate of 0.33 ml/min and analyzed for radioactivity.

**Purification of Recombinant Biglycan in the Presence of \( 4 \mu \)M Guanidine Hydrochloride**— The macromolecular fraction was separated from unincorporated radioactive precursors by chromatography on Sephadex G-50. The 5 mCi/ml samples were equilibrated and eluted with 5 mCi/ml sodium iodoacetate, 4 mCi/ml guanidine hydrochloride, 20 mCi/ml Tris-HCl, pH 8.0, 0.5% Triton X-100 and then applied to a 2-ml nickel column. The column was washed and bound material eluted as described above, except that 4 mCi guanidine hydrochloride was included in all solvents. Fractions (1 ml) were collected at a flow rate of 0.33 ml/min and analyzed for radioactivity.

**Analytical Column Chromatography**— A Superose 6 column (1.0 cm \( \times \) 30 cm) was eluted with 4 mCi guanidine hydrochloride, 50 mCi sodium acetate, pH 6.0, 0.5% CHAPS at a flow rate of 0.4 ml/min. Calibration was with radiolabeled protein standards, with the plot of \( M_r \) vs. \( r \), linear in the range M, 25,000–200,000. Molecular size estimates for glycosaminoglycan chains are based on the data of Wastenson (22).

**Enzymatic Digestions**— Chondroitinase ABC (100 munits) or chondroitinase ACII (100 munits) were used directly on aliquots of culture medium (50–100 \( \mu \)l) at 37°C for 2 h. \( N \)-Glycosidase F digestion was
Fig. 1. Construction of polyhistidine fusion vector, BGN4. The cDNA for human biglycan (P16) encodes a signal sequence, a propeptide, and the mature core protein (see text for details).

done by treating aliquots of culture medium with 0.5% SDS, 0.1 M β-mercaptoethanol at 95 °C for 5 min, cooled rapidly on ice, and an equal volume of PBS, 0.1% Nonidet P-40 added to the sample; the sample was then digested with N-glycosidase F (25 units) at 37 °C for 16 h.

Protein Sequencing—Samples were subjected to SDS-PAGE and electrotransferred to polyvinylidene difluoride membrane (Millipore). Recombinant protein was visualized by Coomassie Blue staining, the third peak within the 60–400 mM imidazole linear gradient, pool III; and the remainder of the bound material eluted as a third peak within the 60–400 mM imidazole linear gradient, pool III.

Separate cultures of HT-1080 and UMR106 cells were also incubated with Trans35S-label (Fig. 2, c and f). Fractions from Trans35S-labeled pools were analyzed by 7.5% SDS-PAGE and processed for fluorography (Fig. 2, c and f). Pool I from both cell lines contained a heterogeneous population of 35S-labeled proteins (Fig. 2c, fraction no. 26), with no immunoreactive biglycan. Pool II resolved into a major proteoglycan signal (square bracket) and a less intense core protein band (Fig. 2, c and f), fractions 40 and 41, respectively. Pool III contained a mixture of proteoglycan and core protein (fraction 59), with later eluting fractions clearly enriched in core protein (Fig. 2, c and f), fractions 62 and 61, respectively. The proteoglycan from pool II migrated as a heterogeneous smear centered at ~100 kDa, whereas the proteoglycan in pool III was somewhat smaller at ~90 kDa. The recombinant fusion core protein migrated as a single band, molecular mass ~ 49 kDa. Semi-quantitative analysis of the fluorograph by direct counting of bands excised from the gel indicated that about 70% of the recombinant biglycan secreted by the HT-1080 cells was substituted with glycosaminoglycan chains, in contrast, only 50% of the core protein secreted by UMR106 cells was fully glycosylated. The proteoglycan and core protein in pool II and pool III from both cell lines could be immunoprecipitated with a polyclonal antiserum (LF112) gen-

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Fig. 2. Purification of recombinant biglycan from HT-1080 cells and UMR106 cells. Cells were co-infected with vBGN4 and vTF7–3 and incubated for 20 h in serum-free medium containing radionuclide. The medium was harvested and applied to a nickel chelating column. Fractions eluted from the column were monitored for radioactivity. a, infected HT1080 cells incubated in serum-free medium containing [3H]glucosamine (●) and [35S]sulfate (○). b, infected HT1080 cells incubated in serum-free medium containing Trans35S-label (●). c, Imidazole gradient is indicated by a dashed line. c, aliquots from selected fractions from panel b were analyzed by 7.5% SDS-PAGE and visualized by fluorography. d, infected UMR106 cells incubated in serum-free medium containing [3H]glucosamine (●) and [35S]sulfate (○). e, infected UMR106 cells incubated in serum-free medium containing Trans35S-label (●). f, Aliquots from selected fractions from panel e were analyzed by 7.5% SDS-PAGE and visualized by fluorography. Square bracket shows position of intact biglycan proteoglycan, a line indicates the position of the core protein; molecular weight standards are indicated.

Pool II; and the remainder of the bound material eluted as a third peak within the 60–400 mM imidazole linear gradient, pool III.

Separate cultures of HT-1080 and UMR106 cells were also incubated with Trans35S-label to radiolabel the core protein. Culture medium was applied to a nicked column and three 35S-labeled peaks were eluted (Fig. 2, b and e) in positions co-incident with those observed for the glycoconjugate labeling (Fig. 2, a and d). Fractions from Trans35S-labeled pools were analyzed by 7.5% SDS-PAGE and processed for fluorography (Fig. 2, c and f). Pool I from both cell lines contained a heterogeneous population of 35S-labeled proteins (Fig. 2c, fraction no. 26), with no immunoreactive biglycan. Pool II resolved into a major proteoglycan signal (square bracket) and a less intense core protein band (Fig. 2, c and f), fractions 40 and 41, respectively. Pool III contained a mixture of proteoglycan and core protein (fraction 59), with later eluting fractions clearly enriched in core protein (Fig. 2, c and f), fractions 62 and 61, respectively. The proteoglycan from pool II migrated as a heterogeneous smear centered at ~100 kDa, whereas the proteoglycan in pool III was somewhat smaller at ~90 kDa. The recombinant fusion core protein migrated as a single band, molecular mass ~ 49 kDa. Semi-quantitative analysis of the fluorograph by direct counting of bands excised from the gel indicated that about 70% of the recombinant biglycan secreted by the HT-1080 cells was substituted with glycosaminoglycan chains, in contrast, only 50% of the core protein secreted by UMR106 cells was fully glycosylated. The proteoglycan and core protein in pool II and pool III from both cell lines could be immunoprecipitated with a polyclonal antiserum (LF112) gen-

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Expression and Purification of Recombinant Biglycan under Non-denaturing Conditions—We used the construct shown in Fig. 1 which includes downstream of the T7 promoter an encephalomyocarditis virus untranslated region that facilitates cap-independent ribosome binding and hence increases translation efficiency up to 10-fold (26). The expression vector pBGN4 comprises cDNA sequence encoding the mature core protein of human biglycan ligated to a polyhistidine fusion cassette under the control of the T7 promoter. The fusion cassette encodes the canine insulin signal sequence (INS), six consecutive histidine residues (POLYHIS), and the factor Xa recognition site (Xa). A recombinant vaccinia virus, vBGN4, encoding the T7-regulated BGN4 construct, was generated by a homologous recombination event between wild-type vaccinia virus and thymidine kinase flanking sequences in the plasmid, pBGN4.

Several cell lines were screened for effective expression of recombinant biglycan, with optimal expression in HT-1080 and UMR106 cells. HT-1080 cells and a human cell line derived from a fibrosarcoma with an "epithelial-like" phenotype. UMR106 cells originated from a rat osteosarcoma and express an osteoblast phenotype (29). Neither cell line synthesizes biglycan (Refs. 30 and 31, and data not shown). Confluent monolayers of HT-1080 and UMR106 cells were co-infected with vTF7–3 and vBGN4, and incubated in medium containing [3H]glucosamine and [35S]sulfate. After 30 h, culture medium was harvested and applied to an nickel chelating column. Portions of the bound material eluted as a third peak within the 60–400 mM imidazole linear gradient, pool III.
A proportion of the proteoglycan smear resisted chondroitinase ABC digestion (Fig. 3, lanes 2 and 5). Further treatment with N-glycosidase F (Fig. 3, lanes 3 and 6) yielded multiple bands suggesting the presence of glycoprotein(s). This glycoprotein was also observed when digested samples were purified by immunoprecipitation but not purified from control cultures infected with VTF7-3 alone. Furthermore, this glycoprotein did not co-purify with recombinant decorin (36). When biglycan was purified by the nickel column in denaturing solvents containing 4 M guanidine hydrochloride, this protein contaminant was not detected. These data indicate a direct interaction between recombinant biglycan and this unknown glycoprotein in non-denaturing solvents.

Characterization of Proteoglycan Forms of Recombinant Biglycan—HT-1080 recombinant biglycan (pools II and III) purified by nickel chelating chromatography (Fig. 2a) were chromatographed on a Superose 6 column (Fig. 4, a and b). Pool II eluted as a single peak at $K_d$ 0.32, corresponding to an apparent molecular mass $\sim$ 125 kDa; and for pool III, a single peak was eluted at $K_d$ 0.38, corresponding to an apparent molecular mass $\sim$ 100 kDa. This value represents a more accurate molecular size for the intact proteoglycan than that obtained by SDS-PAGE (above).

The contribution of the glycosaminoglycan chains to the size of the two pools of proteoglycan was investigated. The chondroitin sulfate chains of both proteoglycan pools were released by $\beta$-elimination and then chromatographed on a Superose 6 column (Fig. 4, c and d). Pool II yielded a broad monodisperse peak with a median $K_d$ 0.46, which corresponds to an apparent molecular mass $\sim$ 34 kDa. Since the core protein preparation derived from chondroitinase ABC digestion migrated on SDS-PAGE with a molecular mass $\sim$ 49 kDa (see Fig. 3), and the overall hydrodynamic size of the recombinant biglycan is $\sim$ 130 kDa, there are two chondroitin sulfate chains per core protein, i.e. both Ser-Gly attachment sites are utilized. The chondroitin sulfate chains from pool III eluted as a broad monodisperse peak with a median $K_d$ 0.51 corresponding to $\sim$ 28 kDa. The estimation of the size of the chondroitin sulfate chains from pool III also indicates that both glycosaminoglycan attachment sites are occupied. The minor peak (Fig. 4, c and d, solid bar) that eluted with a median $K_d$ 0.85 comprised exclusively $^3$H label and represents the N-glycopeptides released by the alkaline treatment.

Pool II and III isolated from UMR106 cells by nickel affinity chromatography (Fig. 2d) also represent two subpopulations of proteoglycans substituted with chondroitin sulfate chains of different molecular size. The chromatographic analysis is summarized in Table I. Pool II comprised a single broad peak at $K_d$ 0.3 (molecular mass $\sim$ 130 kDa). Pool III was resolved into two distinct peaks, an early eluting proteoglycan peak with the $^{35}$S and $^3$H coincident at $K_d$ 0.36 (molecular mass $\sim$ 105 kDa), and a second sharp peak at $K_d$ 0.52 (molecular mass $\sim$ 56 kDa) consisting exclusively of $^3$H label. This second peak represented core protein with the $^3$H label incorporated into the N-linked oligosaccharides. This peak was masked in the equivalent HT-1080 pool due to the higher proportion of proteoglycan relative to core protein. The chondroitin sulfate chains from pool II eluted at $K_d$ 0.42 (molecular mass $\sim$ 40 kDa) and those isolated from pool III were significantly smaller at $K_d$ 0.5 (molecular mass $\sim$ 29 kDa).

Proteoglycans in pool II and III from both cell lines were incubated with chondroitinase ABC and applied to the Superose 6 column. The chromatograms for the two pools of proteoglycan synthesized by HT-1080 cells were similar (Fig. 4, e and f). A minor peak was eluted at $K_d$ 0.52 (molecular mass $\sim$ 56 kDa), however the majority of the $^3$H and $^{35}$S label shifted to the Vo, of the column. The minor peak, consisting exclusively of $^3$H label, represents core protein devoid of glycosaminoglycan chains (Fig. 4, e and f, asterisk). Similar profiles were observed for pool II and III purified from UMR106 cells (not shown).

The disaccharide composition of the large and small proteoglycans (pools II and III, respectively) purified from HT-1080 cells were essentially identical, with a typical distribution of
about 75% 4-sulfated, 18% 6-sulfated and the remainder unsulfated-chondroitin disaccharides (Table I). There was no significant difference in the sulfation pattern of the disaccharides between the two cell lines. The characterization of the two pools of proteoglycan synthesized by the HT-1080 and UMR106 cells is summarized in Table I.

Chemical amounts of recombinant biglycan were generated by co-infection of HT-1080 cells with vTF7–3 and vBGN4, and purification was done under both native and denaturing conditions (see “Experimental Procedures”). The recovery of recombinant biglycan was significantly improved (~50%) when purified in the presence of 4 M guanidine hydrochloride, and allowed an estimate of biglycan production based on specific activity and theoretical extinction coefficient; HT-1080 cells secrete about 10 mg of biglycan/10^9 cells per 24 h, which is about 3-fold less than that observed with similar experiments for decorin expression (36).

**Biglycan Binds Collagen Type V and C1q—** Recombinant Trans^{35}S-labeled biglycan proteoglycan (>1 × 10^5 cpm/μg) and core protein (>1 × 10^5 cpm/μg) from HT-1080 cells were purified by nickel chelating chromatography in non-denaturing conditions. Aliquots of each fraction were analyzed by 7.5% SDS-PAGE and purity verified by Coomassie Blue staining. A minor contaminant of core protein (less than 5%) was present in the proteoglycan fraction (data not shown). The other fraction contained >95% core protein. ^{35}S-Labeled proteoglycan or core protein were incubated in microtiter wells coated with collagen types I, II, III, V, VI, C1q, and BSA (core protein was not incubated with collagen type VI). Wells were washed and the amount of ^{35}S-biglycan that bound under saturating conditions was determined (Fig. 5A). The proteoglycan form of recombinant biglycan bound to collagen type V, 8-fold in excess of the BSA control. This interaction appears to be mediated by the core protein since it also bound collagen type V, 5-fold above background. The complement protein C1q bound dramatically to both glycoforms (proteoglycan and core protein), ~14-fold in excess of the BSA control.

The effect of chaotropic solvents on the binding of biglycan was investigated. Recombinant ^{35}S-labeled proteoglycan and core protein were purified from HT-1080 cells in the presence of 4 M guanidine hydrochloride. Proteoglycan and core protein were separated by anion exchange chromatography, exchanged into PBS^+, and incubated in microtiter wells coated with collagen types I, II, III, V, VI, C1q, and BSA for 3 h at room temperature. The amount of ^{35}S-labeled biglycan bound was determined (Fig. 5B). The binding of guanidine hydrochloride-treated biglycan to collagen types III, V, and VI was markedly reduced (Fig. 5B, hatched bars) when compared with biglycan purified under native conditions (Fig. 5B, solid bars). Binding to collagen type V was decreased by about 80%. However, the binding of biglycan to C1q was unaffected by purification of recombinant biglycan in the presence of chaotropic solvents.

**DISCUSSION**

The limitations imposed by traditional recombinant protein expression systems which do not properly glycosylate proteo-

![Figure 4](https://example.com/glycosaminoglycan.png)

**Figure 4.** Glycosaminoglycan and disaccharide composition of recombinant biglycan expressed by HT-1080 cells. HT-1080 cells were co-infected with vBGN4 and vTF7–3 and incubated overnight in the presence of ^{35}S sulfate (○) and ^{3}H glucosamine (●). Recombinant biglycan from the culture medium was purified by nickel affinity chromatography as described for Fig. 2, and two peaks of radioactivity containing biglycan were resolved: pool II eluting at 60 mM imidazole; and pool III eluting in an imidazole gradient (60–400 mM). Aliquots of each pool were treated as described below, and then analyzed by gel filtration chromatography on Superose 6 eluted in a solvent of 4 M guanidine hydrochloride, 50 mM sodium acetate, pH 6.0, 0.5% CHAPS.

a, pool II untreated; b, pool III untreated; c, pool II treated with alkali borohydride; d, pool III treated with alkali borohydride; e, pool II treated with chondroitinase ABC; f, pool III treated with chondroitinase ABC. The bar in c and d indicates the elution of ^{3}H-labeled N-linked glycopeptides. The asterisk in e and f indicates the elution position of ^{3}H-labeled core protein.
chains with a median mass of 130 kDa. The glycoforms of biglycan isolated were a proteoglycan composed of two subpopulations, one substituted with two chondroitin sulfate chains with a median molecular size of 40 kDa and the other with 28 kDa. The disaccharide components of both the long and short chains were essentially identical: 75% 4-sulfated, 18% 6-sulfated, and 7% unsubstituted. All species were substituted with xylosyltransferase has acted, the molecule is then committed to becoming a proteoglycan; this potential regulatory step is dependent on secondary structure. However, the binding of biglycan and decorin in human intervertebral disc and articular cartilage (32, 33) suggests that enzymes important for polymerization of the glycosaminoglycan chain are present in excess in the cell (35) and, due to the overexpression of core protein exceeding the capacity of the enzymes required for initiation or polymerization of the glycosaminoglycan chain, differential glycosylation of recombinant biglycan may be due to the overexpression of core protein exceeding the capacity of the enzymes required for initiation or polymerization of the glycosaminoglycan chain. Studies using β-xylosides as alternative acceptors for assembly of glycosaminoglycan chains suggest that enzymes important for polymerization of the glycosaminoglycan chain are present in excess in the cell (35) and, under normal circumstances, it is availability of core protein that is rate-limiting. NH2-terminal sequencing of the recombinant biglycan core protein (i.e. the non-proteoglycan form) demonstrated that the serine acceptors for glycosaminoglycan initiation have not been modified, suggesting that the rate-limiting step may be at the level of xylose addition. Once the xylosyltransferase has acted, the molecule is then committed to becoming a proteoglycan; this potential regulatory step is under investigation.

Binding studies investigating the function of biglycan have traditionally used proteoglycan extracted from tissue or more recently recombinant core protein generated by bacterial expression systems. In both cases, chaotropic solvents have been utilized for efficient purification of biglycan. Using circular dichroism spectroscopy (36), we have demonstrated that the secondary structure of biglycan and decorin is disrupted when exposed to guanidine hydrochloride, whereas recombinant proteoglycan purified under non-denaturing conditions has a sec-ondary structure of biglycan and decorin in human intervertebral disc and articular cartilage (32, 33). Studies using β-xylosides as alternative acceptors for assembly of glycosaminoglycan chains suggest that enzymes important for polymerization of the glycosaminoglycan chain are present in excess in the cell (35) and, under normal circumstances, it is availability of core protein that is rate-limiting. NH2-terminal sequencing of the recombinant biglycan core protein (i.e. the non-proteoglycan form) demonstrated that the serine acceptors for glycosaminoglycan initiation have not been modified, suggesting that the rate-limiting step may be at the level of xylose addition. Once the xylosyltransferase has acted, the molecule is then committed to becoming a proteoglycan; this potential regulatory step is under investigation.

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biglycan to the complement protein C1q was independent of changes in secondary structure and is likely to involve specific amino acid sequences, since the binding of native and denatured biglycan to C1q was of a similar magnitude. Binding of native and denatured biglycan to C1q was of a similar magnitude. Binding of recombinant proteoglycan and core protein to C1q was also demonstrated. Decorin has been previously shown to bind to C1q, however, the interaction of biglycan with C1q has not previously been reported. The interaction of native biglycan with collagen type V and C1q are currently being further characterized.

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