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Purification of a basic fibroblast growth factor-binding proteoglycan from bovine cardiac plasma membrane

Chris R. Ross [a], Susan Kubinak [b] and Calvin C. Hale [b]

[a] *Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS (USA)* and [b] *Department of Veterinary Biomedical Sciences, John M. Dalton Research Center, University of Missouri-Columbia, Columbia, MO (USA)*

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A heparan sulfate proteoglycan (HSPG) from bovine cardiac plasma membrane was purified to homogeneity using either isoelectric focusing or anion-exchange chromatography, followed by affinity chromatography on immobilized basic fibroblast growth factor (bFGF). Fractions were assayed for bFGF-binding activity using ^{125}I-bFGF as a probe. Purified proteoglycan ran as a broad band on SDS-PAGE, spanning an apparent molecular mass range of 100–200 kDa, and could be incorporated into liposomes. Digestion of radioiodinated proteoglycan with heparatinase yielded a product of 73 kDa, while digestion with chondroitinase ABC did not change the apparent molecular mass. Monoclonal antibody directed against the ectodomain of another plasma membrane HSPG, syndecan, failed to recognize the purified cardiac proteoglycan on immunoblots. We conclude that adult bovine myocardium contains a membrane-associated bFGF-binding heparan sulfate proteoglycan containing little or no chondroitin sulfate and that this HSPG may be distinct from those of the syndecan family of heparan sulfate proteoglycans.

Introduction

Both bFGF and its congener, acidic fibroblast growth factor, have received attention as potential mediators of coronary angiogenesis. Both FGF's are present in myocardial tissue [1–5]. In addition, Galloway and coworkers suggested that mitogens for 3T3 cells and endothelial cells could be released from hearts subjected to ex vivo ischemia [6]. In a similar study, Yang and colleagues found that the mitogenic activity released from hearts by ischemia is similar to bFGF [7].

Basic fibroblast growth factor is stored within the cell and as a complex with cell-surface and extracellular matrix heparan sulfate proteoglycans (HSPG). Although a definitive physiologic role of the bFGF–heparan sulfate interaction has not been demonstrated, several functions for heparan sulfates in bFGF physiology have been proposed. Endothelial cell-secreted heparan sulfate protects bFGF from proteolytic inactivation by plasmin [8] as well as from trypsin and chymotrypsin [9], suggesting that the protective action of heparan sulfate might serve to prolong bFGF action in the hostile microenvironment of a wound. Heparan sulfate may also serve to store bFGF for future interaction with cell surface receptors, [10,11] and this matrix-immobilized bFGF could be released in a soluble, active form by cell-produced heparanases [12,13]. Although the mechanism of bFGF export from the cell has not been defined, newly synthesized bFGF may cross the cell membrane in association with heparan sulfate [14] or be released by leakage following membrane damage [15]. Finally, recent evidence suggests that binding of bFGF to HSPG is an obligatory step in the presentation of bFGF to its cell-surface receptor [16,17]. Thus, regulation of bFGF action may be accomplished through regulation of HSPG.

Although bFGF and HSPG may be involved in the growth of coronary neovasculature, detailed information regarding the structure of bFGF-binding proteoglycans from the heart is lacking. Several groups have described the molecular structure of bFGF-binding proteoglycans, but no HSPG from differentiated myocardium has been examined in detail. Therefore, the elucidation of the structure and function of bFGF-binding proteoglycans in the myocardium may enhance our understanding of the pathophysiology of angiogenesis in this tissue.

In the present study, we describe the purification and further characterization of a previously reported

Correspondence to: C.R. Ross, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA.

heparan sulfate bFGF binding site on the cardiac plasma membrane [18]. The purification strategy reported here utilized ion exchange chromatography or isoelectric focusing, followed by affinity chromatography and was expedited by the use of a solid-phase detection assay for bFGF-binding activity. Our results indicate that the set of diffuse bands observed when total cardiac plasma membrane proteins are probed with ^{125}I-bFGF may be purified to a single proteoglycan species. Using these methods, bFGF-binding proteoglycan can be obtained with a degree of purity sufficient to allow further structural analysis and antibody generation, and is in a form suitable for amino acid sequencing and animal inoculation.

Materials and Methods

Preparation of cardiac plasma membranes

Plasma membranes from bovine ventricular tissue were prepared according to the method of Slaughter et al. [19]. No proteinase inhibitors were used for the preparation and purification of the proteins described here. Subsequent experiments in our laboratory using a combination of phenylmethylsulfonyl flouride (0.19 mM), aprotinin (0.025 μM), leupeptin (0.35 μM), and pepstatin A (0.24 μM) during membrane preparation and purification have shown no discernible increase in either modal molecular mass or molecular mass range on SDS-PAGE (data not shown).

Detection of bFGF binding activity by ligand blotting

Human recombinant bFGF (Chiron) was radioiodinated by the lactoperoxidase method of Sutter et al. [20] and repurified using heparin-Sepharose chromatography as previously described [18]. Radiolabelled bFGF (^{125}I-bFGF) was routinely obtained with a specific activity of about 75 μCi/μg ($1 \cdot 10^5$ cpm/ng).

Cardiac plasma membrane bFGF-binding proteoglycan was detected by probing blot-immobilized proteins with ^{125}I-bFGF. Two methods of protein transfer to blotting membranes were used. The first method utilized SDS-PAGE fractionation of membrane proteins, followed by electrophoretic transfer to poly(vinylidine difluoride) (PVDF) membrane, and has been described previously [18].

The second method utilized a slot-blot format and allowed rapid screening of chromatography column fractions for bFGF-binding activity. Samples were transferred by gentle suction to the transfer membrane, followed by one wash with 200 μl blotting buffer (192 mM glycine, 25 mM Tris base, 20% methanol (pH 8.3)) per slot. Following blocking in 5% nonfat dry milk solution for 45–60 min, blots were equilibrated for 20–30 min in 500 mM NaCl, 20 mM Tris-HCl, 0.5% Tween-20 (pH 7.5) (TTBS). Blot membranes were then probed with ^{125}I-bFGF ($1 \cdot 10^6$ cpm/50 ml TTBS) for 2–3 h at room temperature. Unbound ^{125}I-bFGF was removed by washing membranes four times for 5 min each with TTBS. Membranes were dried and subjected to autoradiography, and ^{125}I-bFGF binding was quantified by scanning densitometry where indicated. Using the two similar assay procedures, information regarding apparent molecular weight and purity could be obtained (electroblot) and column chromatography fractions could be quickly screened for activity (slot-blot).

Ion-exchange chromatography

Hydrated resin (DEAE-Sephadex A-50; Pharmacia) was washed with 20 bed volumes 2 M NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4), followed by equilibration in 20 bed volumes 150 mM NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4) (solubilization buffer). Membrane vesicles were pelleted by centrifugation at $2000 \times g$ for 15 min and resuspended in solubilization buffer (7.5–10 mg/ml) and rocked for 30 min at room temperature. Unsolubilized material was removed by centrifugation at $100\,000 \times g$ for 60 min at 4°C. The supernatant was applied to the column which was then washed with 7 ml solubilization buffer. The column was eluted with a 30 ml 150 mM to 2 M linear NaCl gradient also containing 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4). The flow rate was maintained at 7.5 ml/h throughout the run. 1-ml fractions were collected; 50-μl aliquots were screened for ^{125}I-bFGF-binding activity using the slot-blot assay described above.

Preparative isoelectric focusing

As an alternative to anion-exchange chromatography, cardiac membrane proteins enriched in heparan sulfate proteoglycans were obtained using preparative isoelectric focusing (Bio-Rad Rotofor apparatus). Cardiac plasma membranes containing 12–20 mg protein were centrifuged at $100\,000 \times g$ for 1 h, and the pellet solubilized by homogenizing with a teflon-glass homogenizer in 50 ml 4 M urea containing 2% ampholytes (Fisher Biotech), pH range 3–5 or 3–10. The protein solution was subjected to isoelectric focusing at 12 W, typically for 4–5 h. Fractions were then collected, dialyzed against 1 M NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.5), and assayed for bFGF-binding activity as described above. Active fractions were pooled and stored at −20°C until further use.

FGF affinity chromatography

Affinity column preparation: 10 mg of human recombinant bFGF was coupled to the chromatography support (Affi-Gel 10; Bio-Rad) according to the manufacturer's instructions. Immediately prior to use, the column was washed with 20 bed volumes 2 M NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4), followed by

20 bed volumes of solubilization buffer. Ion exchange column chromatography or isoelectric focusing fractions exhibiting bFGF-binding activity in the slot-blot assay were desalted by dilution with 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.5) to a final NaCl concentration of 0.5 M. The resulting material was applied to the bFGF affinity column twice, followed by washing with 5 ml 600 mM NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4) and elution with 3 ml of 2 M NaCl, 10 mM Tris-HCl, 0.5% Triton X-100 (pH 7.4). The flow rate was 7.5 ml/h and 0.5 ml fractions were collected throughout washing and elution. A 50-μl aliquot of each column fraction was used to screen for ^{125}I-bFGF-binding activity using the slot-blot assay as described above. Fractions exhibiting ^{125}I-bFGF binding activity were pooled and desalted by dilution as above, and subjected to a second round of bFGF-affinity chromatography. Binding activity was assayed as before and active fractions were pooled and precipitated using 10% trichloroacetic acid (TCA). All dialysis and affinity chromatography steps were carried out at 4°C. Detection of proteins in SDS-PAGE gels was performed using sequential Alcian blue staining [21], followed by silver staining [22]. Protein concentrations were determined using the bicinchonic acid assay (BCA; Pierce) using bovine serum albumin as a standard.

Liposome incorporation of HSPG

Liposome incorporation of cardiac HSPG was performed after the method of Lories et al. [23]. DEAE-Sephadex column fractions exhibiting bFGF-binding activity using the slot-blot assay were pooled, dialyzed overnight against 4 M urea, 0.5% Triton X-100, 50 mM Tris-HCl (pH 8.0), and adsorbed on 2 ml DEAE-Trisacryl M beads (Pharmacia) for 2 h. The beads were rinsed twice with 100 mM Tris-HCl, 10 mM octyl glucoside (pH 7.5), followed by elution with 2.5 ml guanidine-HCl buffer (4 M guanidine-HCl, 50 mM Na acetate (pH 5.8), containing 75 mM octyl glucoside). Phosphatidylcholine dissolved in guanidine-HCl buffer was added to a final concentration of 5 mg/ml. Liposomes were formed by dialysis against guanidine buffer containing no detergent, followed by dialysis against 150 mM NaCl, 10 mM Tris-HCl (pH 7.5), and were pelleted by centrifugation at 100 000 $\times g$ for 1 h.

Enzymatic digestion of ^{125}I-proteoglycan

Purified bFGF-binding HSPG was radiolabelled with ^{125}I using the chloramine-T method and repurified on a bFGF affinity column as described above. Fractions that eluted following the 2 M NaCl step were pooled and TCA precipitated. The pellet was washed twice with ether and resuspended in 100 μl 100 mM Tris-HCl, 30 mM Na acetate, 10 μM EDTA (pH 8.0). Aliquots were incubated with 0.02 U chondroitinase ABC (proteinase-free; Seikagaku America, Rockville, MD), 0.1 U heparatinase I (EC 4.2.2.8; Sigma), both chondroitinase ABC and heparatinase, or with no enzyme, for 30 min at 37°C. The reaction was stopped by TCA precipitation. The pellets were washed with ether, and solubilized in SDS-PAGE sample buffer.

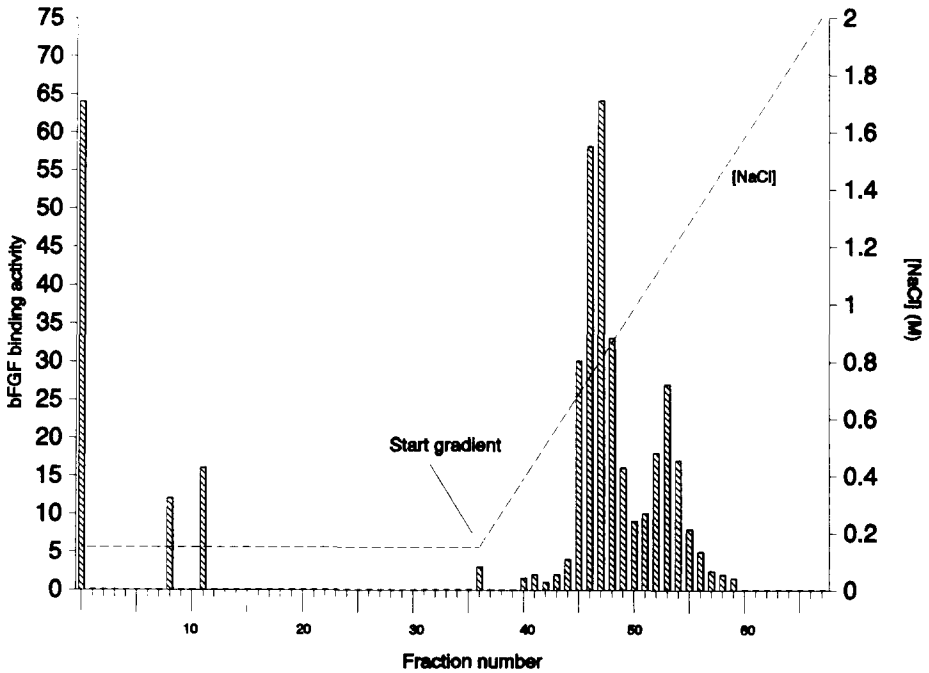

Fig. 1. DEAE-Sephadex column chromatography fractionation of bFGF-binding membrane proteins. Detergent-solubilized cardiac plasma membrane proteins were subjected to DEAE-Sephadex as described in Materials and Methods. ^{125}I-bFGF binding was quantified using the slot-blot screening format followed by densitometric analysis of the autoradiogram. Fractions indicated by the horizontal bar were pooled for subsequent purification by bFGF affinity chromatography.

Immunoblotting of cardiac HSPG and syndecan

Cardiac HSPG and murine syndecan (ectodomain) were subjected to SDS-PAGE as described by Koda et al. [24], except that a 3.8% acrylamide gel was used. Following electrophoresis, proteins were transferred to cationic nylon membrane (Zeta-Probe; Bio-Rad) overnight at 100 mA. The membrane was blocked in 100 mM Tris-HCl, 150 mM NaCl, 3% nonfat dry milk, 0.5% BSA, 0.3% Tween-20 (pH 7.5) (blocking buffer) for 45 min at room temperature. The membrane was then incubated in monoclonal antibody 281–2 [25], diluted to 2 μg/ml in blocking buffer, for 4 h at room temperature, followed by washing four times, 5 min each, with 100 mM Tris-HCl, 150 mM NaCl, 0.3% Tween-20 (pH 7.5) (wash buffer). Bound antibody was detected by incubation for 2 h at room temperature with goat anti-rat antisera conjugated to alkaline phosphatase, diluted 1:200 in blocking buffer. Following washing, the blot was developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium in 100 mM Tris-HCl (pH 9.5).

Reagents

Human recombinant bFGF was kindly supplied by Dr. Lawrence Cousens, Chiron, Emeryville, CA [26]. Monoclonal antibody 281–2 and murine syndecan ectodomain were graciously provided by Dr. Merton Bernfield, Harvard Medical School, Boston, MA. PVDF membrane was obtained from Millipore, Bedford, MA. Lactoperoxidase was obtained from Calbiochem, San Diego, CA. DEAE-Sephadex and DEAE-Trisacryl M were supplied by Pharmacia, Uppsala, Sweden. Prestained SDS-PAGE molecular weight standards were obtained from Amersham, Arlington Heights, IL. All other reagents, except where indicated, were supplied by Sigma, St. Louis, MO.

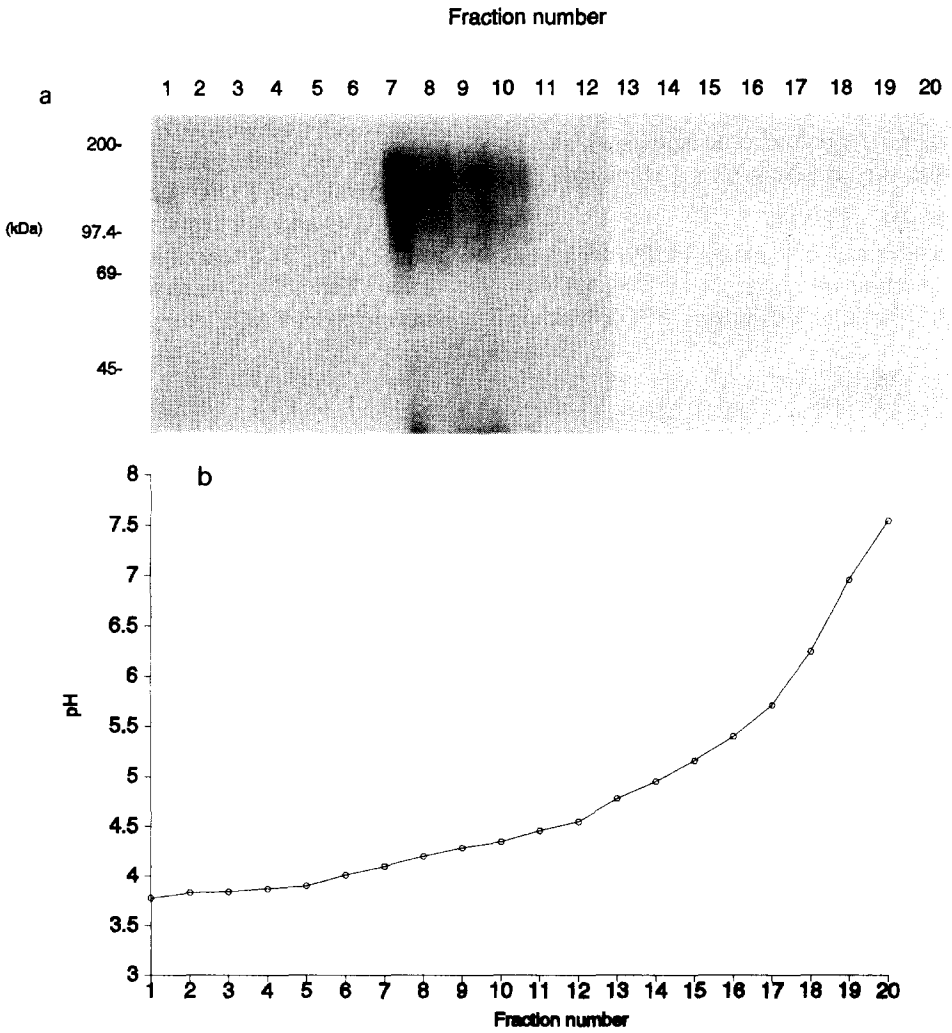

Fig. 2. Partial purification of bFGF-binding proteoglycan by preparative isoelectric focusing. Membrane proteins were solubilized in 4 M urea and subjected to isoelectric focusing using pH 3–5 ampholytes. (a) Aliquots of each fraction were dialyzed against 1 M NaCl, precipitated with 20% trichloroacetic acid, and subjected to SDS-PAGE. Gels were electroblotted and probed with ^{125}I-bFGF as described in Materials and Methods. (b) pH of each isoelectric focusing fraction.

Results

Proteoglycans responsible for bFGF-binding activity from cardiac plasma membranes were purified by column chromatography using a two-step strategy based upon the high negative charge content of heparan sulfate and affinity for its ligand, bFGF.

Representative results showing fractionation of bFGF binding activity on a DEAE-Sephadex column are shown in Fig. 1. The column load, wash, and elution fractions were assayed using a ligand binding assay in a slot-blot format. ^{125}I-bFGF binding activity bound to the column under the low-salt loading conditions and eluted from the DEAE column over a broad range of salt concentrations, behavior consistent with a high degree of heterogeneity in the charged side chain content of the proteoglycan. In addition, bFGF-binding activity sometimes exhibited a biphasic elution profile, suggesting that more than one discrete species may exist in this membrane preparation. Since the majority of the bFGF-binding material eluted from the DEAE-Sephadex column at approx. 0.8 M NaCl, these fractions were pooled and utilized for further purification. DEAE column chromatography routinely yielded a 5-fold purification with a 40% recovery based upon protein values and densitometric quantification of the load and eluted fractions.

Due to the highly charged nature of the protein under study, preparative isoelectric focusing was also used as an initial purification step. bFGF-binding material was found in the fractions with pH values ranging from 4 to 4.5 (Fig. 2). Some loss of material occurred during this step due to the formation of an insoluble precipitate in the fractions containing bFGF-binding activity. This precipitate only partially redissolved during dialysis.

Basic FGF-binding DEAE column fractions were pooled and desalted, and subsequently applied to a bFGF affinity column. Basic FGF-binding isoelectric focusing fractions were pooled following dialysis and diluted 1:5 with 10 mM Tris, 0.5% Triton X-100 and applied to the bFGF affinity column. In both cases, following column washing with buffer containing 0.6 M NaCl, bFGF-binding activity eluted from immobilized bFGF following the application of 2 M NaCl. Results of two cycles of bFGF affinity column chromatography are shown in Fig. 3. The recovery of bFGF binding activity following the second affinity column chromatography purification was 50% with a purification of approx. 30-fold compared with the initial bFGF column load.

The purity of fractions containing bFGF-binding activity from the final bFGF affinity column was assessed by silver staining of SDS-PAGE gels, and by probing electroblots with ^{125}I-bFGF followed by autoradiography. Results of this analysis are shown in Fig.

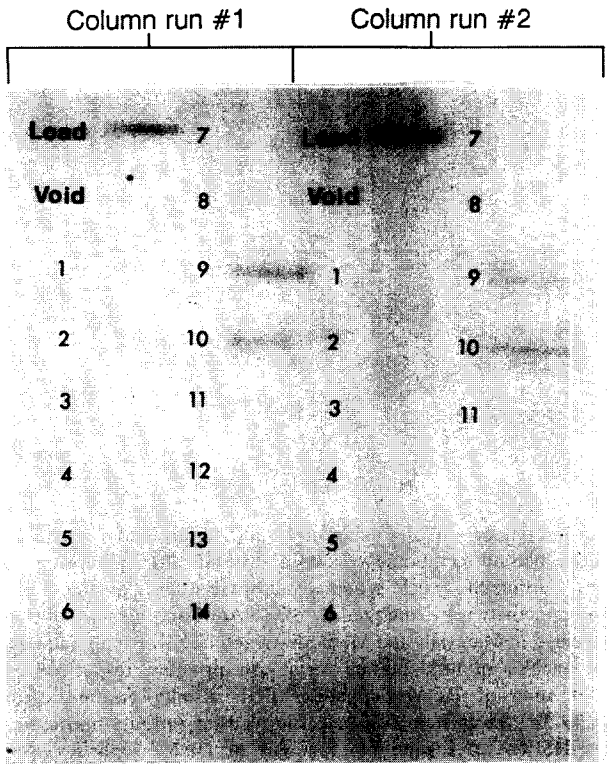

Fig. 3. bFGF affinity column chromatography of bFGF-binding plasma membrane proteins. DEAE-Sephadex column fractions exhibiting bFGF binding activity on slot blots were pooled, desalted, subjected to bFGF affinity column chromatography, and eluted with a stepwise NaCl gradient as described in Materials and Methods. Fractions 1–8 were collected during a 600 mM NaCl wash, and fractions 9–11 were collected during a 2 M NaCl wash. Fractions eluted with 2 M NaCl were desalted, reapplied to the column, and eluted again with a step gradient. Shown is a representative elution pattern of bFGF-binding activity from the first and second bFGF affinity column runs.

4. Lanes a and b contain total cardiac membrane proteins; the faint banding pattern within the overall proteoglycan smear seen in Fig. 4b (see also Fig. 2a) is presumably due to interfering proteins within the total membrane protein population. Lanes c and d contain affinity-purified HSPG derived from DEAE-Sephadex and preparative isoelectric focusing, respectively. A lack of contaminating proteins within the HSPG smear in both cases indicates that the proteoglycan has been purified to homogeneity. The similarity in electrophoretic mobility of the product in either lane suggests that both ion exchange chromatography and isoelectric focusing give comparable qualitative results as a first purification step. We have only been able to stain the HSPG described here using a combination of Alcian blue staining and silver staining; attempts to use Coomassie blue, Alcian blue, or silver stain alone have been unsuccessful.

Ligand blot analysis of affinity-purified cardiac HSPG is shown in Fig. 4e. The discrete negative band-

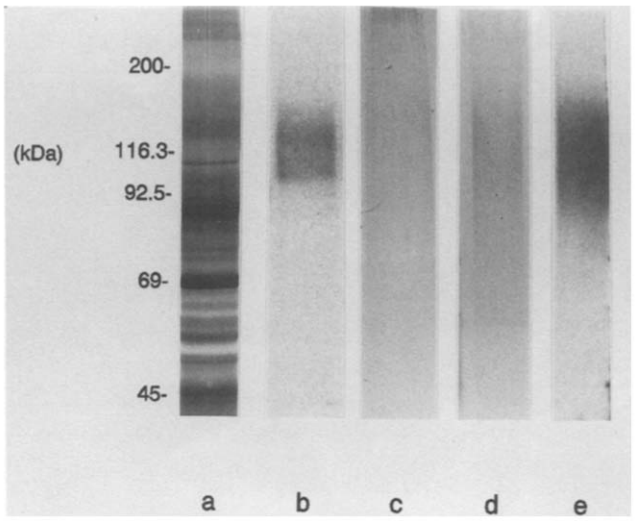

Fig. 4. Comparison of total protein and bFGF-binding activity in cardiac plasma membrane vesicles and bFGF affinity purified fractions. Cardiac SL vesicle proteins and bFGF affinity purified bFGF-binding activity were analyzed by SDS-PAGE and ligand blotting as described in Materials and Methods. (a and b) Silver stain and ligand blot analysis of total plasma membrane proteins, respectively. (c) Silver stain of DEAE-Sephadex/bFGF affinity purified cardiac HSPG. (d) Silver stain of isoelectric focusing/affinity purified cardiac HSPG. (e) Ligand blot analysis of DEAE-Sephadex/bFGF affinity purified HSPG.

ing pattern which appeared within the ^{125}I-bFGF-probed total membrane proteins (Figs. 2a and 4b) were not observed in the purified proteoglycan.

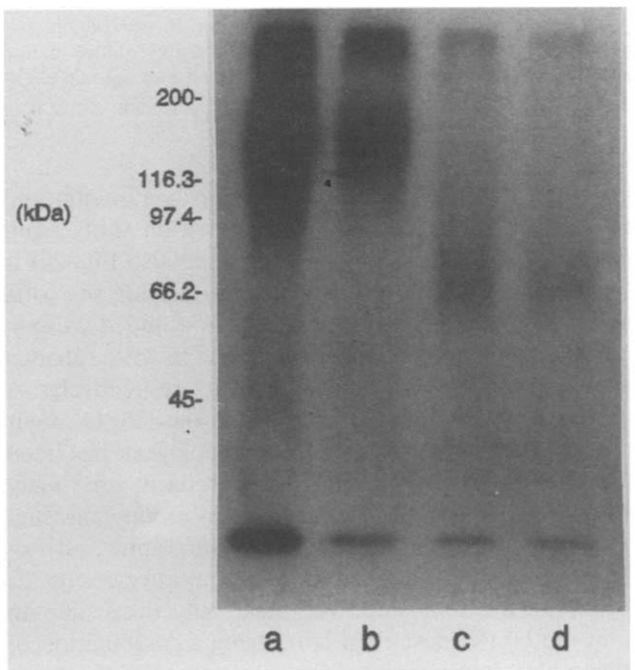

Fig. 5. Enzymatic deglycosylation of ^{125}I-labelled HSPG. Purified HSPG was radiolabelled, treated as indicated, and subjected to SDS-PAGE and autoradiography. (a) No treatment. (b) Chondroitinase ABC. (c) Heparatinase. (d) Chondroitinase ABC and heparatinase.

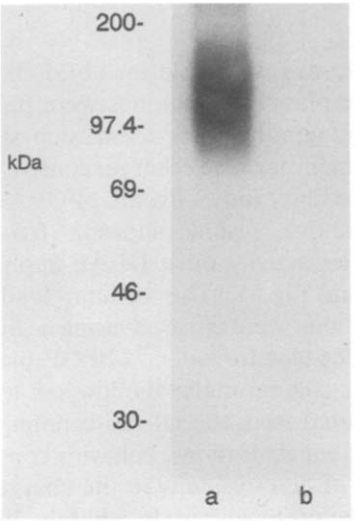

Fig. 6. Incorporation of bFGF-binding proteoglycan into liposomes. DEAE-purified proteoglycans were incorporated into phosphatidylcholine liposomes, and centrifuged at 100000 × g for 1 h. Supernatant and pellet were analyzed by SDS-PAGE and ligand blotting as described in Materials and Methods. (a and b) Ligand blot analysis of pellet and supernatant, respectively.

bFGF-binding HSPG proteoglycans have been reported to exist in both heparan sulfate and hybrid heparan sulfate/chondroitin sulfate forms, and en-

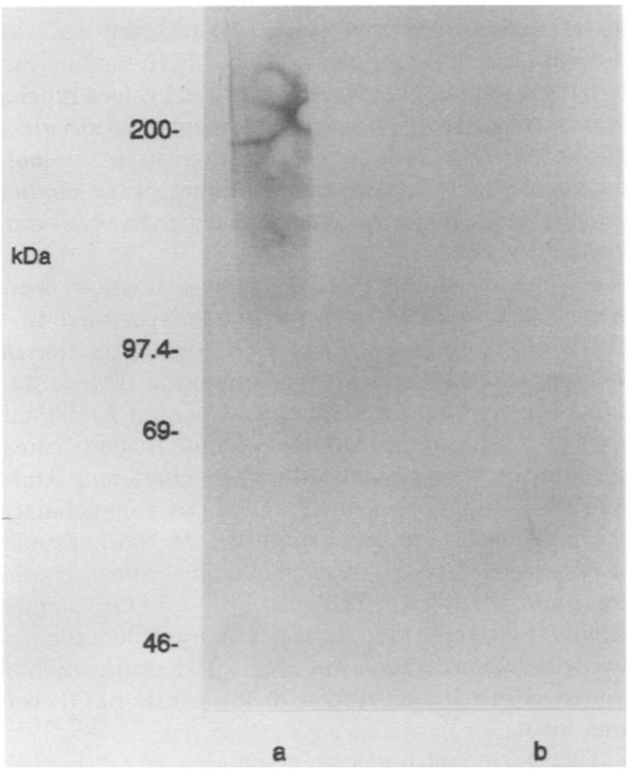

Fig. 7. Western blot assay using anti-syndecan antibody. Murine syndecan and purified cardiac HSPG were subjected to TBE-urea SDS-PAGE and electroblotting. The blot was probed with monoclonal antibody which recognizes murine and bovine syndecan. (a) Syndecan, ectodomain; 10 ng. (b) Cardiac HSPG; 2 μg.

zymes specific for these glycosaminoglycans were used to characterize purified cardiac HSPG. Radiolabelled proteoglycan, when treated with heparatinase, yielded a product of 73 kDa (Figs. 5c and d). Treatment with chondroitinase ABC did not change the apparent molecular mass of the labelled proteoglycan (Figs. 5b and d). The product of heparatinase digestion migrates as a heterodisperse species, making it likely that the core protein has not been completely deglycosylated.

Since HSPG may be present in both membrane-bound and free forms, we tested the ability of partially purified cardiac bFGF-binding HSPG to intercalate into liposomes. As shown in Fig. 6, bFGF-binding activity differentially associates with the liposome fraction in this preparation, confirming our earlier observation that the cardiac HSPG is an integral membrane protein [18]. This technique also has potential as an alternative purification step.

Monoclonal antibody (281–2) directed against the ectodomain of murine syndecan was used to probe the purified HSPG described above. Although this antibody crossreacts with bovine syndecan (Bernfield, M., personal communication) it did not react with purified cardiac HSPG in a western blot assay (Fig. 7).

Discussion

The existence of integral membrane proteoglycans containing heparan sulfate has been reported by several workers (see review by Yanagishita and Hascall [27] and references therein). These proteoglycans may function as a modulator of cell adhesion [28], as receptors [29,30], or as a storage depot for other biomolecules, including growth factors such as bFGF [10,13,31–33]. The latter function is of particular interest in the case of bFGF which may thus be held in close proximity to cell surface receptors in a biologically stable form [8,9,12]. In addition, the interaction of bFGF and heparan sulfate may serve as a point at which growth factor activity may be regulated [34,35]. In the present study, we report the purification of a bFGF-binding proteoglycan from the bovine cardiac plasma membrane. Based upon previously reported kinetic and enzymatic characterization, it is likely that this proteoglycan is a heparan sulfate that may function as an extracellular storage site for bFGF in the heart.

Our purification strategy was predicated on the high negative charge content of heparan sulfate and its high affinity for bFGF. Elution of bFGF-binding proteins from DEAE-Sephadex using a linear NaCl gradient resulted in a broad, biphasic elution profile (Fig. 1). While this profile suggests the presence of more than one population of bFGF-binding proteins, the difference may also be due to variations in degree of glycosylation and/or sulfation of the proteoglycan. We have not investigated the bFGF-binding materials that eluted from DEAE columns in minor quantities.

It is unlikely that a complex tissue such as the myocardium contains only one species of HSPG. Several factors may explain the recovery of a predominant form as reported here. Our purification and detection strategy was biased toward HSPG capable of binding ^{125}I-bFGF. Turnbull et al. have recently reported a bFGF binding sequence contained in the glycosaminoglycan portion of HSPG [36], which raises the possibility of differential binding properties between proteoglycan forms. In addition, the initial stages of our purification procedure excludes extracellular matrix material, which likely contains substantial amounts of HSPG. Studies describing the total content and compartmentation of HSPG in the bovine heart have not been reported.

The overall degree of purification achieved in these studies was approx. 150-fold. This value only represents the purification from the starting point of the plasma membrane preparation and does not take into account that derived from the preparation of membrane vesicles from the original tissue. We were unable to obtain a specific activity value for bFGF-binding activity from whole myocardium due to limitations in the sensitivity of the ligand blotting assay used here.

The purification of three distinct bFGF-binding heparan sulfate proteoglycans has been reported by other workers. Saksela and colleagues reported bFGF affinity purification of secreted or trypsin-released heparan sulfate-containing proteoglycans from cultured bovine capillary endothelial (BCE) cells [8]. The proteoglycan they identified had an apparent molecular mass of greater than 200 kDa after treatment with trypsin and contained heparan sulfate side chains, but was not further characterized. As in the case of the BCE proteoglycan, treatment with chondroitinase ABC had no effect on the molecular mass of the proteoglycan described in this report.

A unique hybrid membrane proteoglycan that binds bFGF has been cloned and sequenced by Saunders and colleagues [37]. This proteoglycan, termed syndecan, was originally isolated from the surfaces of mouse mammary epithelia, and is largely limited to epithelial cells, with cultured mesenchymal cells containing about 100-fold less [28]. In addition, syndecan produced by mesenchymal cells is larger than that of epithelial cells due to an increase in the degree of glycosylation of the mesenchymal form. The syndecan core protein bears both heparan sulfate and chondroitin sulfate side chains in varying proportions depending on the cell type of origin and developmental circumstances. Neither syndecan nor mRNA for syndecan are detectable in mature myocardium [37,38]. Another syndecan-like cell surface heparan sulfate proteoglycan, denoted FGF-HSPG, was described by Kiefer and co-workers [39],

who probed a cDNA library derived from BHK-21 cells.

Another widely studied bFGF-binding heparan sulfate is produced by an extracellular matrix-secreting tumor, the Engelbreth-Holm-Swarm (EHS) sarcoma. This proteoglycan has a core protein of 400 kDa, with two to three side chains attached to a terminal globular structure [40,41]. Specific binding of EHS heparan sulfate to ^{125}I-bFGF in vitro was demonstrated by Vigny and co-workers [42] who found that ^{125}I-bFGF also bound strongly to basement membrane in frozen sections of EHS. The tissue distribution of bFGF-binding in this tumor was also examined and endogenous bFGF was found to co-distribute with ^{125}I-bFGF.

Although these heparan sulfate-containing proteoglycans share bFGF-binding capability with that described in the present report, they differ either in their tissue distribution, developmental expression, molecular size, or antigenic characteristics. The relationship between the bFGF-binding HSPG described here and other bFGF-binding proteoglycans will not be clarified until the primary structures of each core protein is known.

Acknowledgments

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