of a water molecule in the sixth position prevents the cooperativity in a manner that is reminiscent of the displacement of O_2 by CO.

Registry No. Hb^+ A, 12646-21-8; NO, 10102-43-9; CO, 630-08-0; H_2O, 7732-18-5; L-histidine, 71-00-1; chelated protoheme, 108189-62-4; protohemin-2-methylimidazole, 88106-21-2.

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Prostatic Growth Factor: Purification and Structural Relationship to Basic Fibroblast Growth Factor†

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ABSTRACT: Prostatic growth factor (PrGF) was purified from alkaline homogenates of human benign prostatic hyperplastic tissue by a combination of ammonium sulfate precipitation, heparin affinity chromatography, and cation-exchange chromatography. The 17 600-dalton, basic (p*I* 10.2) PrGF is related to basic fibroblast growth factor (bFGF) since antisera raised against synthetic peptides with sequence homologies corresponding to an internal peptide and amino- and carboxyl-terminal peptides of bFGF react with the growth factor. The growth factor appears larger than bFGF, suggesting that additional amino-terminal sequences may be present as a result of alkaline extraction in the presence of protease inhibitors.

Jacobs and associates (Jacobs et al., 1979) first reported the presence of a growth-promoting factor in extracts of human tissue prepared from benign prostatic hyperplasia (BPH),[1] well-differentiated prostatic adenocarcinoma (pelvic lymph node), and normal postpubertal prostate. The factor was originally called prostatic osteoblastic factor because it enhanced radiolabeled nucleotide incorporation by cultured fetal rat osteoblasts and calvaria but has also been called prostatic growth factor (PrGF) (Story et al., 1984a; Nishi et al., 1985;

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[1] Abbreviations: aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; ECGF, endothelial cell growth factor; PrGF, prostatic growth factor; HDGF, hepatoma-derived growth factor; BPH, benign prostatic hyperplasia; NBS, newborn bovine serum; RMA, relative mitogenic activity; ELISA, enzyme-linked immunosorbant assay; CM, carboxymethyl; Tris, tris(hydroxymethyl)aminomethane; TBS, 50 mM Tris with 0.15 M NaCl, pH 7.6; PBS, 0.1 M sodium phosphate with 0.15 M NaCl, pH 7.4; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; KLH, keyhole limpet hemocyanin; MHS, *N*-hydroxysuccinimidyl maleimidohexanoate ester; DMF, dimethylformamide; EDTA, ethylenediaminetetraacetic acid disodium salt; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; dpm, disintegrations per minute; TURP, transurethral; kDa, kilodalton(s).

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Jinno et al., 1986). A convenient assay for PrGF using [^{3}H]thymidine uptake by confluent monolayers of quiescent human foreskin fibroblasts has been described (Story et al., 1983). The mitogenic activity of BPH extracts was not due to epidermal growth factor (Story et al., 1983) nor was it due to acid phosphatase or polyamines present in crude extracts (Jacobs & Lawson, 1980). Treatment of BPH extracts with lipid solvents did not remove growth factor nor was PrGF bound by concanavalin A. The factor was a heat- and acid-sensitive component that was destroyed by trypsin and inactivated by the dissociating agents urea and guanidine hydrochloride (Story et al., 1984b). The molecular weight of PrGF, estimated by gel filtration [>67 000 (Lawson et al., 1981), 300 000, 150 000, and 60 000 (Tackett et al., 1985), 80 000, 43 000, and 10 000 (Jinno et al., 1986), 11 000–13 000 (Nishi et al., 1985)], suggested multimolecular forms. However, Story et al. (1984a) showed that differences in molecular weight were largely a result of the ionic strength under which the estimates were made. Yet, multiforms of the growth factor may be present in BPH tissue since both acidic (Story et al., 1984a; Jinno et al., 1986) and basic (Nishi et al., 1985) isoelectric points have been reported.

A preliminary report (Story et al., 1986) confirmed by Nishi and associates (Nishi et al., 1985) showed that PrGF is a member of the class of heparin binding growth factors (Lobb et al., 1986). At this time, we report the purification of the major growth factor in BPH tissue by a combination of heparin–Sepharose chromatography and cation-exchange chromatography and demonstrate immunologically that the growth factor is related to basic fibroblast growth factor (bFGF).

Experimental Procedures

Materials. (Carboxymethyl)Sepharose, heparin–Sepharose, and chromatographic columns were obtained from Pharmacia (Piscataway, NJ). Tris(hydroxymethyl)aminomethane (Tris), *N*-hydroxysuccinimidyl maleimidohexanoate ester, dimethylformamide, phenylmethanesulfonyl fluoride, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone, *N*-ethylmaleimide, and soybean trypsin inhibitor were from Sigma (St. Louis, MO). Disodium ethylenediaminetetraacetate (Na_2EDTA) was from J. T. Baker Chemical Co. (Phillipsburg, NJ). Ammonium sulfate and sodium chloride were from Mallinckrodt (Paris, KY). All tissue culture medium, antibiotics, and serum were from Grand Island Biological Co. (Grand Island, NY), and all culture flasks and multiwell plates were from Falcon (Becton Dickinson Labware, Oxnard, CA). Econofluor scintillation cocktail, Protosol tissue solubilizer, and [*methyl*-^{3}H]thymidine were from New England Nuclear (Boston, MA). All reagents for SDS–PAGE and electrofocusing were from LKB (Gaithersburg, MD). Protein was assayed with a kit with bovine serum albumin as standard (Bio-Rad, Richmond, CA).

Cell Culture and Assay for Growth Factor Activity. The bioassay was based on the ability of a sample to stimulate [^{3}H]thymidine uptake by quiescent confluent monolayers of human foreskin fibroblasts as described elsewhere (Story et al., 1983). The amount of radioactivity was determined with a Model 1219 liquid scintillation counter (LKB) interfaced with an Apple IIE computer for conversion of cpm to dpm. The mean of triplicate samples was determined, and relative mitogenic activity (RMA) was calculated:

$$\text{RMA} = \frac{\text{dpm(test sample)} - \text{dpm(buffer)}}{\text{dpm(10\% NBS)} - \text{dpm(buffer)}}$$

The buffer was the same as in the test sample. One unit of activity was defined as an RMA equal to 1, i.e., a response equal to that produced by 10% NBS.

Table I: Growth Factor Activity of BPH Homogenates Prepared at pH 7.6 in Low and High Ionic Strength Buffer and at pH 4.5[a]

source of BPH tissue	extraction conditions: ionic strength	pH	activity: units/mg of protein	activity: units/g wet wt
open prostatectomy	low[b]	7.6	20 (10–47)	420 (300–890)
open prostatectomy	high[c]	7.6	143 (112–165)	2949 (2758–3086)
TURP	high	7.6	151 (101–234)	1761 (1198–2568)
TURP	*d*	4.5	161 (87–344)	217 (48–375)

[a] Mean and range ($N \geq 4$). [b] 50 mM Tris/50 mM NaCl. [c] 50 mM Tris/1.55 M NaCl with EDTA (10 mM), phenylmethanesulfonyl fluoride (1 mM), L-1-(tosylamido)-2-phenylethyl chloromethyl ketone (0.03 mM), ethylmaleimide (0.05 mM), and soybean trypsin inhibitor (10 mg/L). [d] As described by Gospodarowicz et al. (1985a).

Tissue Homogenization and Ammonium Sulfate Precipitation. Human prostate tissue was from surgical specimens obtained at transurethral (TURP) and open prostatectomy for BPH. All tissues used in this study were from TURP specimens except in Table I. Tissue was transported in saline in an ice bath and stored frozen (−80 °C). All steps in preparing tissue homogenates and in isolating growth factor were at 0–4 °C. The tissue was thawed, rinsed in saline, and minced with scissors. The tissue was weighed and homogenized with a Polytron (Brinkmann Instruments, Inc., Westbury, NY) at a setting of 8 for 30 s at 10-s intervals with 30-s cooling between homogenizations. The homogenization buffer (1:2 tissue wet weight:volume), except as indicated in Table I, was 50 mM Tris with 1.55 M NaCl, pH 7.6, containing EDTA (10 mM), phenylmethanesulfonyl fluoride (1 mM), L-1-(tosylamido)-2-phenylethyl chloromethyl ketone (0.3 mM), *N*-ethylmaleimide (0.05 mM), and soybean trypsin inhibitor (10 mg/L). The homogenate was centrifuged at 23000*g* for 40 min in a Sorvall Model RC-2B centrifuge (Du Pont Instruments, Newtown, CT), and the supernatant was transferred to a clean beaker. An equal volume of buffer was added to the pellet, and the homogenization and centrifugation were repeated. The supernatants from the two centrifugations were pooled and filtered through a Metrigard glass fiber membrane (Gelman Sciences, Inc., Ann Arbor, MI) with positive nitrogen pressure. A small portion of the filtrate was dialyzed against 50 mM Tris with 0.15 M NaCl, pH 7.6 (TBS), in bags made of Spectra/Por3 tubing, 3500 molecular weight cutoff (Spectrum Medical Industries, Los Angeles, CA). A precipitate was removed by centrifugation, and the supernatant was sterilized by filtration (0.2-μm Acrodisc membranes; Gelman) and assayed for activity and protein. The remaining filtrate was brought to 25% ammonium sulfate by the slow addition of saturated ammonium sulfate, pH 7.6. After being mixed 2 h, the preparation was centrifuged at 23000*g* for 30 min. The pellet was discarded, and the supernatant was brought to 75% ammonium sulfate. After the solution was mixed and centrifuged, the pellet was dissolved in TBS and dialyzed against the same buffer. The preparation was centrifuged and Metrigard filtered, and a small portion was sterilized by filtration and assayed for activity and protein.

Heparin–Sepharose Chromatography. The growth factor from 150–200 g of tissue, precipitated with ammonium sulfate and dialyzed against TBS, was applied to a 5 cm × 2 cm, 40-mL bed volume, column of heparin–Sepharose equilibrated with TBS. The column was washed with TBS until the ab-

sorbency of the eluate at 206 nm became negligible. The absorbed proteins were then eluted with a gradient (480 mL) formed from TBS and 50 mM Tris with 3.0 M NaCl. The conductivity of fractions was determined with a conductivity bridge (Yellow Springs Instrument Co., Yellow Springs, OH). A portion of each fraction was diluted in minimum essential medium (Eagle's) with 0.5% NBS, sterile filtered, and assayed for activity. The growth factor eluting between 1.2 and 2.1 M NaCl was concentrated by ultrafiltration [YM5-type membrane in a stirred cell apparatus; Amicon (Danvers, MA)] and dialyzed against 50 mM Tris with 0.5 M NaCl, pH 7.6. A portion of the sample was assayed for activity and protein.

The growth factor recovered from two 40-mL heparin affinity columns was applied to a 1.6 cm × 7.5 cm, 15-mL bed volume, column of heparin–Sepharose equilibrated with 50 mM Tris with 0.5 M NaCl. The column was washed with the equilibration buffer until the absorbency of the eluate reached base line. The absorbed proteins were then eluted with a gradient (280 mL) formed from 50 mM Tris with 0.5 M NaCl and 50 mM Tris with 3.0 M NaCl. The conductivity and activity of fractions were measured. The growth factor eluting between 1.4 and 2.0 M NaCl was concentrated by ultrafiltration, the salt content was lowered by the addition of 30 volumes of 50 mM Tris with 50 mM NaCl, and the sample was reconcentrated. A portion of the sample was assayed by analytical SDS–PAGE.

Cation-Exchange Chromatography. A portion of the sample from the second heparin affinity column was further purified by cation-exchange chromatography. Chromatography was performed with a fast protein liquid chromatography system and a Mono S column (Pharmacia). The sample (100 μL) was diluted in 400 μL of 50 mM 3-(cyclohexylamino)-1-propanesulfonic acid, pH 10 (CAPS), and applied at 1 mL/min to the column equilibrated with the same buffer. After the absorbency of the eluate at 280 nm returned to base line, the absorbed proteins were eluted with a 0–0.15 M NaCl gradient, formed over 24 min, and a 0.15–0.5 M NaCl gradient, formed over 10 min. The salt gradients were formed from CAPS and CAPS with 0.5 M NaCl. One-minute fractions were collected, and 0.25% of each fraction was assayed for activity. Fractions eluting at 0.5 M NaCl were pooled, dialyzed, and concentrated. Portions of the sample were assayed for activity, and purity was assessed by SDS–PAGE.

SDS–PAGE and Electroblotting. Analytical SDS–PAGE was performed on a vertical slab gel apparatus (LKB) using 10–22.5% linear gradient gels prepared by standard techniques (Laemmli, 1970) in the presence of thiol reducing agent. Molecular weight markers (Bethesda Research Laboratories, Gaitherburg, MD) were insulin (A and B chains), 2300 and 3400; bovine trypsin inhibitor, 6200; lysozyme, 14 300; β-lactoglobulin, 18 400; α-chymotrypsinogen, 25 700; and ovalbumin, 43 000. Some gels were silver stained (Wray et al., 1981). Other gels were electroblotted with a Transphor Unit (LKB) to nitrocellulose paper, 0.2-μm pore size (Schleicher & Schuell, Keene, NH). The transfer was performed in 25 mM Tris-HCl, 0.15 M glycine, and 20% methanol, pH 8.3, at 10% voltage for 15 h.

Electrofocusing and Dot Blotting. Analytical electrofocusing was performed in a tube gel electrophoresis unit (LKB). Gels, 7.5% acrylamide/4% cross-linking with 2% Ampholyte carrier (3.5–10 or 9–11 pH range) and 10% glycerol, were cased in 3 mm × 120 mm tubes. Samples were applied in 10% glycerol/1% Ampholyte and overlayed with 5% glycerol/1% Ampholyte. The cathode electrode solution was 0.5 M NaOH, and the anode electrode solution was 0.5 M H_3PO_4. Gels were electrophoresed at 15 °C, 1 W/tube constant power, for 3 h. Gels were removed from the tubes, cut in 5-mm pieces, and eluted 2 days at 4 °C in degassed water in sealed tubes that were gassed with N_2. The pH of eluates of slices from gels receiving sample buffer only was determined. Eluates of slices from gels receiving growth factor were dot blotted in duplicate on nitrocellulose paper.

Preparation of Synthetic Peptides. Peptide fragments corresponding to sequences located within bovine bFGF were synthesized by solid phase methods (Merrifield, 1963; Sakakibara, 1971) using an automated Applied Biosystems (Foster City, CA) 430A A peptide synthesizer. Peptide 1 has 12 amino acid residues (Pro-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala-Phe) and corresponds to the amino-terminal residues 1–12 of FGF, peptide 2 has 11 residues (Arg-Ile-His-Pro-Asp-Gly-Arg-Val-Asp-Gly-Val) and is located at positions 33–43, and peptide 3 has 10 amino acid residues (Ala-Ile-Leu-Phe-Leu-Pro-Met-Ser-Ala-Lys) at positions 136–145 of the carboxyl-terminal region of FGF. All peptides were synthesized with an additional cysteine residue at the carboxyl terminus to facilitate conjugation to the carrier protein.

Production of Polyclonal Antisera. (A) Conjugation of Hapten to Carrier Protein. The synthetic peptide fragments were conjugated to keyhole limpet hemocyanin, KLH (Sigma), as a carrier using *N*-hydroxysuccinimidyl maleimidohexanoate ester, MHS (Boehringer Mannheim Biochemicals, Indianapolis, IN), as a cross-linking agent. For this purpose, 30 mg of KLH was dissolved in 3 mL of phosphate-buffered saline, pH 7.4 (PBS), and 10 mg of MHS was dissolved in 0.3 mL of dimethylformamide (DMF). With constant stirring, the dissolved MHS was then added dropwise to KLH over a 5-min period. The mixture was allowed to react at room temperature with continued stirring for 45 min. To separate the activated carrier protein from the excess of MHS, the reaction mixture was immediately applied to a 60 cm × 0.7 cm gel filtration column packed with Trisacryl 05 (LKB) and equilibrated in PBS/10% DMF, pH 6.0. The flow rate was 50–60 mL/hr, and the eluant was monitored at 280 nm. The activated KLH eluting at the void volume was collected (ca. 10-mL total volume). Thirty milligrams of peptide was dissolved in 3 mL of PBS and the pH adjusted to 7.4 with 1 M NaOH. The dissolved peptide was combined with the activated KLH and was allowed to react at room temperature under continuous shaking. The peptide–carrier conjugate was dialyzed against PBS and stored frozen at −20 °C.

(B) Immunization. Eight-pound male New Zealand white rabbits were injected at multiple dorsal intradermal sites with 0.5 mg of KLH–peptide conjugate emulsified in complete Freund's adjuvant. Animals were boostered regularly at 4–6-week intervals with 0.2 mg of KLH–peptide conjugate emulsified in incomplete Freund's adjuvant. Rabbits were bled from a central ear vein at various time intervals following the initial immunization and the subsequent booster injections. The sera were aliquoted and stored at −20 °C. The titer was determined by an enzyme-linked immunosorbant assay (ELISA) using unconjugated peptide as antigen and peroxidase-conjugated goat anti-rabbit IgG as secondary antiserum.

Immunodetection by Anti-bFGF Antisera. Samples transferred to nitrocellulose paper were stained with antiserum as follows: incubated with 5 mg/mL gelatin in PBS for 30 min to block nonspecific binding sites, washed 3 × 5 min, incubated with anti-peptide antiserum at 1:1000 dilution for

Table II: Isolation of Growth Factor from BPH Tissue

steps	protein (mg)	activity		x-fold purification	% recovery
		units/mg	total		
homogenate[a]	4439	156.7	695741	1	100
ammonium sulfate precipitate (25–75%)	3430	165.6	567895	1.1	82
heparin–Sepharose (40 mL)	3.04	105396	320403	673	46
heparin–Sepharose (15 mL)	0.18	1330000	239400	8488	34

[a] From 361 g of tissue.

60 min, washed 3 × 5 min, incubated with biotinylated goat anti-rabbit IgG (Zymed, San Francisco, CA) at 1:1000 dilution for 60 min, washed 3 × 5 min, incubated with streptavidin–biotinylated peroxidase complex (Amersham, Arlington Heights, IL) at 1:330 dilution for 30 min, washed 3 × 5 min, incubated with enzyme substrate (see below) until color develops, washed with distilled water, and stored in the dark. All antibody dilutions were done in 2 mg/mL gelatin/0.1% Tween 20/4% goat serum in PBS. All washing steps were performed with PBS, pH 7.4, containing 0.1% Tween 20. The enzyme substrate was prepared as follows: solution A, 100 μL of 30% hydrogen peroxide added to 100 mL of PBS; solution B, 40 mg of 4-chloro-1-naphthol dissolved in 5–10 drops of dimethylformamide. Solution A was transferred to a 0.8-μm nitrocellulose filter (Nalge Rochester, NY); solution B was then added rapidly under stirring, mixed, and filtered.

Results

Tissue Homogenization. Human BPH tissue obtained by open prostatectomy or by transurethral resection was homogenized in low or high ionic strength buffer, pH 7.6, or at pH 4.5 (Gospodarowicz et al., 1985a) and assayed for growth factor activity (Table I). The activity and yield (units per gram of tissue) of homogenates prepared in high ionic strength buffer were about 7-fold higher than in homogenates prepared in low ionic strength buffer. Nearly twice as much activity was recovered from tissue obtained by open prostatectomy than by transurethral resection. In addition, the yield of growth factor was 8-fold higher from tissue homogenized at high ionic strength, pH 7.6, than when homogenized under acidic conditions, pH 4.5.

Heparin–Sepharose Chromatography. Figure 1A illustrates a typical elution profile from a column of heparin–Sepharose of growth factor activity from BPH tissue homogenized in high ionic strength buffer, pH 7.6, and precipitated with ammonium sulfate. Two peaks of activity were seen. About 60% of the activity applied to the column was recovered in fractions that eluted between 1.2 and 2.1 M NaCl. A smaller peak of activity, 7.5%, was recovered in fractions that eluted between 0.3 and 1 M NaCl. Typically, 1–2% of the activity does not bind to heparin–Sepharose. Only the growth factor in the major peak of activity was subjected to further fractionation procedures.

The major peak of activity from two 40-mL columns of heparin–Sepharose was applied to a second heparin affinity column (Figure 1B). The predominate activity was recovered in fractions 18–24 eluting between 1.4 and 2 M NaCl.

The isolation scheme, consisting of tissue homogenization at pH 7.6 in high ionic strength buffer, ammonium sulfate precipitation, and two cycles of heparin affinity chromatography, was used to recover the major growth factor from four separate preparations totaling 1467 g of BPH tissue. The recovery of the growth factor from one preparation of 361 g of tissue is summarized in Table II. Precipitation by ammonium sulfate did not substantially increase activity. However, precipitation was necessary to remove lipids from the

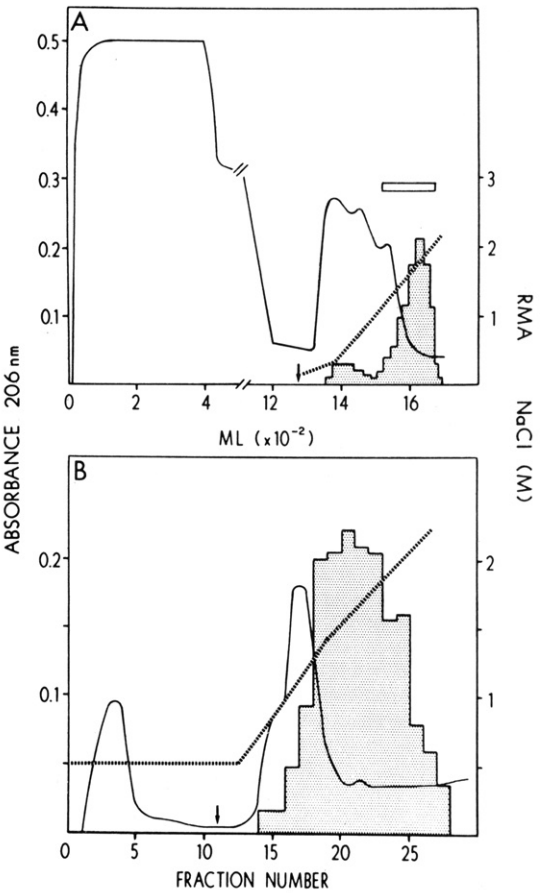

FIGURE 1: Heparin–Sepharose chromatography. (A) BPH tissue (361 g) was homogenized in 50 mM Tris/1.55 M NaCl containing protease inhibitors, pH 7.6, precipitated with ammonium sulfate (25–75% saturation, pH 7.6), and dialyzed against 50 mM Tris/0.15 M NaCl, pH 7.6 (TBS). Half of the sample was applied to a column of heparin–Sepharose (5 cm × 2 cm, 40-mL bed volume) equilibrated with TBS. The column was washed with 870 mL of TBS, and a gradient (480 mL) of TBS to TBS with 3 M NaCl was started (arrow). Fractions were collected, and the absorbance (—), conductivity (···), and growth factor activity (shaded area) were measured. The activity eluting between 1.2 and 2.1 M NaCl (bar) from two 40-mL columns was concentrated, dialyzed against TBS with 0.5 M NaCl, and applied to a 1.6 cm × 7.5 cm, 15-mL bed volume, column of heparin–Sepharose (B) equilibrated with TBS/0.5 M NaCl. The column was washed with equilibration buffer until the absorbance reached base line. A gradient (280 mL) of TBS/0.5 M NaCl to TBS with 3 M NaCl was started (arrow). Fractions (12 mL) were collected, and the absorbance, conductivity, and growth factor activity were measured. The legends are as in (A). Fractions 18–25 were dialyzed against 50 mM Tris/50 mM NaCl, pH 7.6, concentrated, and analyzed by SDS–PAGE (Figure 3, lane 2).

tissue homogenate. The first heparin–Sepharose step resulted in greater than a 600-fold increase in activity. The second affinity step increased activity an additional 12-fold. The overall purification was nearly 8500-fold. One unit of activity resulted from the addition of 0.75 ng/mL to cultured fibroblasts. The isolation scheme recovered 34% (27–41%, range of four preparations) of activity in tissue homogenates. Analyses of the preparation by SDS–PAGE (Figure 3, lane

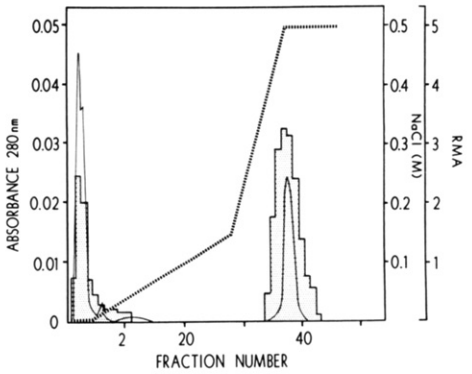

FIGURE 2: Cation-exchange chromatography. The growth factor obtained by two cycles of heparin affinity chromatography (0.2 mg, 1×10^6 units/mg) was applied to a Mono S (Pharmacia) column at 4 °C equilibrated with 50 mM 3-(cyclohexylamino)-1-propanesulfonic acid, pH 10 (CAPS), at 1 mL/min. After the absorbance (—) returned to base line, the column was eluted with a 0–0.15 M NaCl, 24-min, and a 0.15–0.5 M NaCl, 10-min, gradient (···) formed from CAPS and from CAPS with 0.5 M NaCl. Fractions were diluted and assayed for growth factor (shaded area). Fractions 35–41 were pooled, dialyzed, and concentrated. A portion of the sample was tested for activity and analyzed by SDS–PAGE (Figure 3, lane 4).

2) showed a predominate protein with an apparent molecular weight of 17 600. In addition, other prominent proteins were seen at 33.4, 29.3, and 12.2 kDa, indicating that the sample was not pure.

Cation-Exchange Chromatography. Cation-exchange chromatography at pH 10 (Mono S column) was used to further purify the growth factor obtained from the second heparin affinity step. Two peaks of activity were resolved by the column (Figure 2). Ninety-five percent of the applied activity was recovered in fractions 35–41 that eluted from the column at 0.5 M NaCl. The remaining activity was in the column flow-through. Analysis of a concentrate of fractions 35–41 by SDS–PAGE (Figure 3, lane 4) showed a single protein with an apparent molecular weight of 17 600. The staining in the upper region of the gel (M_r >43 000) appears to be an artifact of the sample buffer. Similar staining was also seen in this region of lane 3 on the original gel which received only one-fourth the amount of sample buffer that was applied to lane 4.

Immunodetection of PrGF by Anti-bFGF Antisera. The predominate growth factor in BPH tissue was partially purified by two cycles of heparin affinity chromatography (Table II) and subjected to SDS–PAGE followed by transfer to nitrocellulose paper. Nitrocellulose strips were incubated with rabbit antisera prepared against synthetic peptides with sequence homologies to amino acid residues 33–43 (internal peptide), 1–12 (amino-terminal peptide), and 136–145 (carboxyl-terminal peptide) of bovine bFGF. The nitrocellulose strips were incubated with biotinylated goat anti-rabbit IgG, and the bound antibody was detected with streptavidin–biotinylated peroxidase complex as described under Experimental Procedures. As seen in Figure 3, lanes 5–7, antiserum to each of the three synthetic peptides of bFGF reacted with a single band of protein resolved by SDS–PAGE at M_r 17 600.

In addition, the heparin affinity-purified growth factor was electrofocused in 3.5–9.5 and 9–11 pH Ampholyte gradients. Gels were cut in 5-mm pieces and eluted in water. The pH of eluates of slices from gels receiving sample buffer only was determined. Eluates of slices from gels receiving growth factor were blotted in duplicate on nitrocellulose paper and reacted with antisera to the amino-terminal and carboxyl-terminal synthetic peptides and then visualized by peroxidase staining. Antisera to both synthetic peptides reacted with material that

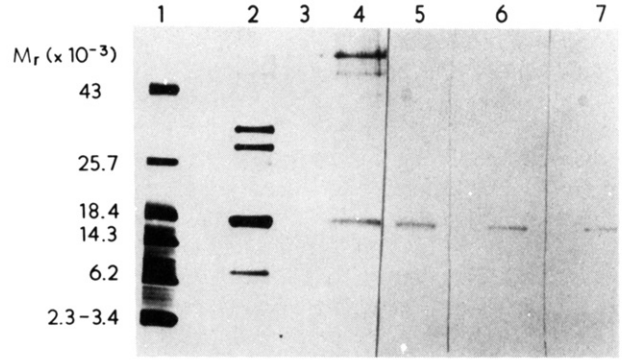

FIGURE 3: SDS–PAGE of PrGF and immunodetection by anti-bFGF antisera. A preparation of PrGF partially purified by two cycles of heparin–Sepharose chromatography as described in Figure 1 and PrGF purified by cation-exchange chromatography as described in Figure 2 were electrophoresed by SDS–PAGE, Laemmli system with a 10–22.5% acrylamide gradient. The proteins were transferred electrophoretically in replicate to nitrocellulose paper. The SDS–PAGE gel was silver stained for protein (lanes 1–4), and the nitrocellulose-transferred protein was incubated with antisera against synthetic peptides with sequence homologies to bovine bFGF. Bound antibody was visualized by incubation with biotinylated goat anti-rabbit IgG which was detected with streptavidin–biotinylated peroxidase complex (lanes 5–7). Lane 1, molecular weight markers; lane 2, heparin–Sepharose partially purified PrGF; lane 3, sample buffer; lane 4, cation-exchange purified PrGF. Lanes 5–7, heparin–Sepharose partially purified PrGF incubated wth antisera to bFGF peptides: lane 5, internal peptide; lane 6, amino-terminal peptide; lane 7, carboxyl-terminal peptide.

eluted from gel slices at pH 10.2 (not shown). No immunoreactivity was seen in the acidic region of electrofocusing gels.

Discussion

A growth factor (PrGF) has been isolated from human BPH tissue that is structurally related to bovine bFGF. The evidence for this relationship is (1) that PrGF, like bFGF (Lobb et al., 1986), binds tightly to heparin–Sepharose, requiring 1.4–2 M NaCl for dissociation (Figure 1), and (2) that antisera prepared against synthetic peptides with sequence homology corresponding to amino acid residues 33–43, as well as amino-terminal sequences 1–12 and carboxyl-terminal sequences 136–145 of bovine bFGF, recognize PrGF (Figure 3). The complete amino acid sequence for bovine bFGF is known (Esch et al., 1985), and 40 of the identified amino-terminal 41 residues of human bFGF are identical with bovine bFGF (Gimenez-Gallego et al., 1986). It is likely, given the high degree of homology existing between the growth factors in the two species, that antisera prepared against sequences in bovine bFGF reacted with highly homologous sequences in the growth factor from human BPH tissue. It is possible that antisera against bovine bFGF synthetic peptide sequences 33–43 and 136–145 also react with highly homologous sequences in human aFGF (Jaye et al., 1986). However, bFGF residues 1–12 have no homology with aFGF sequences, and thus, antiserum to these sequences does not recognize aFGF. In addition, antiserum to synthetic bFGF residues 1–12 reacted with growth factor with a basic p*I*, 10.2 (not shown). Therefore, it is likely that the growth factor that we have isolated from human BPH tissue is related to bFGF.

Both PrGF (M_r 17 600, Figure 3) and M_r 18 500–19 000 growth factor (HDGF) synthesized by cultured human hepatoma cells and shown to be structurally related to bFGF (Klagsbrun et al., 1986) appear larger than bFGF (M_r 16 415; Esch et al., 1985). The existing sequence data of a HDGF–tryptic peptide indicate that HDGF has an additional peptide domain on its amino terminus, but not on its carboxyl terminus, that is absent from bFGF (Klagsbrun et al., 1986). The

nucleotide sequence of a bovine brain clone encoding bFGF has recently been reported (Abraham et al., 1986). The nucleotide sequence of the cloned cDNA had an open reading frame extending upstream from the codon for the amino-terminus bFGF, suggesting that the growth factor may be synthesized initially as a precursor with a long amino-terminal extension. It is possible that the differences in molecular weight of HDGF, PrGF, and bFGF are due to the conditions by which the growth factors were isolated. HDGF and PrGF were isolated at alkaline pH, pH 7.5 and 7.6, respectively. In contrast, bFGF was isolated under acidic conditions, pH 4.5, from both bovine (Gospodarowicz et al., 1978; Lobb & Fett, 1984) and human tissues (Bohlen et al., 1985; Gospodarowicz et al., 1985b). It is tempting to speculate that variations in the apparent molecular weight of the growth factors are due to cleavage of the amino-terminal extension during isolation under acid conditions in the absence of protease inhibitors. Alternatively, as suggested by Klagsbrun et al. (1986), amino-terminal extensions might represent an important difference between growth factors in tumors and normal tissue. The extent of homology between tumor-derived growth factors, such as PrGF and HDGF, and bFGF will not be known until the full sequences are determined.

The bFGF-related protein, PrGF, was isolated from human prostate under alkaline rather than acidic conditions because of an 8-fold greater yield of the growth factor (Table I). It is not clear if the increased yield was a result of the extremely acid labile nature of PrGF (Story et al., 1984b) or due to a difference in ionic strength during homogenization. Acidic extractions were performed in 0.15 M ammonium sulfate, whereas alkaline extractions utilized 1.55 M sodium chloride. Clearly, homogenization of prostate at pH 7.6 in low ionic strength buffer yielded less growth factor than when performed in high ionic strength buffer (Table I).

The behavior of alkaline-extracted PrGF after ammonium sulfate precipitation and acid-extracted bFGF differs on cation-exchange chromatography. Carboxymethyl cation-exchange chromatography is a valuable tool in isolating bFGF extracted under acidic conditions (Lobb & Fett, 1984). However, when the prostate preparation was fractionated on a column of CM-Sepharose, 50% of the activity was recovered in the column flow-through. The remaining activity eluted between 0.1 and 0.3 M NaCl (data not shown). Since the major contaminating proteins were also present in fractions with growth factor activity, it was concluded that cation-exchange chromatography was not of value as an initial step in the isolation of PrGF. The difference in behavior of the growth factors on cation-exchange fractionation may be due to (1) possible structural differences in the growth factors (previously discussed) or (2) the presence of unique binding proteins in prostate, or higher levels of binding proteins in alkaline tissue extracts, than are present when tissues are extracted under acid conditions. Following two cycles of heparin affinity chromatography, cation-exchange chromatography was found to be valuable in the purification of PrGF. A Mono S cation-exchanger was utilized at pH 10 to take advantage of the reported p*I* (pH 10.5) of the growth factor (Nishi et al., 1985) that agrees closely with the value (pH 10.2) reported here. Two peaks of activity were separated by the column (Figure 2). The bFGF-like activity was eluted from the column at 0.5 M NaCl. However, about 7% of the activity was recovered in the column flow-through. Because of insufficient material, it has not been determined if the latter is aFGF, bFGF bound to other protein, or a truncated form of the major bFGF-like molecule. The evidence to suggest that BPH prostate contains an aFGF or a related molecule is that about 7.5% of the activity eluted between 0.3 and 1 M NaCl from heparin–Sepharose (Figure 1A). Brain aFGF elutes between 0.9 and 1.1 M NaCl (Lobb et al., 1986). Second, activity has been detected in extracts of BPH tissue after electrofocusing in the pH range of 4.3–5.4 (Story et al., 1984a). The reported p*I* of authentic aFGF is about 5 (Klagsbrun & Shing, 1985). Conformation of aFGF in prostate will require further study. However, if aFGF is present in BPH tissue, this would be the first reported nonneural source of the growth factor. It appears likely that we failed to find the predominate cationic growth factor in extracts of BPH tissue in an earlier study (Story et al., 1984a) because electrofocusing was performed on a flatbed apparatus that did not establish a pH gradient above pH 9 and allowed the basic growth factor to exit the gel.

The identification of PrGF as a bFGF-related molecule suggests that the growth factor is not unique to BPH. Acidic and basic FGFs have been demonstrated in a wide variety of tissue including bovine brain (Thomas et al., 1984; Gospodarowicz et al., 1984), hypothalamus (Lobb & Fett, 1984; Klagsbrun & Shing, 1985; Maciag et al., 1982), pituitary (Bohlen et al., 1984), kidney (Baird et al., 1985a), cartilage (Sullivan & Klagsbrun, 1985), retina (Baird et al., 1985b), macrophages (Baird et al., 1985c), corpus luteum (Gospodarowicz et al., 1985a), testes (M. T. Story et al., submitted for publication), rat chondrosarcoma (Shing et al., 1984), and human brain (Bohlen et al., 1985; Conn & Hatcher, 1984), placenta (Gospodarowicz et al., 1985b), and hepatoma-derived cells (Klagsbrun et al., 1986). In addition, we have found growth factor activity in normal postpubertal prostate and adenocarcinoma of the prostate (lymph node metastases) (Jacobs et al., 1979). FGF is a mitogen for a wide variety of mesoderm- and neuroectoderm-derived cells (Gospodarowicz, 1984), and PrGF is mitogenic for fibroblasts derived from normal and BPH prostate (M. T. Story et al., unpublished results) and in rat calvaria-derived cells (Jacobs et al., 1979). It is possible that the growth factor may function in the development of the fibrostromal nodule characteristic of BPH (Bartsch et al., 1979, 1984) and the osteoblastic response to boney metastasis seen in prostate cancer. In addition, both bFGF and aFGF are potent mitogens for capillary endothelial cells (Gospodarowicz, 1984) and are known to be angiogenic factors (Esch et al., 1985; Lobb et al., 1985). It is conceivable that this property of PrGF may play a role in the development of BPH and contribute to the invasiveness of prostate cancer.

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