Characterization of the Oligosaccharide Structures on Recombinant Human Prorenin Expressed in Chinese Hamster Ovary Cells

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ABSTRACT: Prorenin was isolated by immunoprecipitation from the culture medium of Chinese hamster ovary cells transfected with a human prorenin cDNA. The N-linked oligosaccharide structures on the in vivo [^{3}H]mannose-labeled, purified protein were characterized using a combination of serial lectin affinity chromatography, high-pressure liquid chromatography, ion-exchange chromatography, and size-exclusion chromatography and treatment with specific glycosidases and methylation analysis. Approximately 61% of the oligosaccharides on the molecule are complex type, in the form of tetraantennary (2%), 2,6-branched triantennary (13%), 2,4-branched triantennary (3%), and biantennary (43%) structures. The majority of all complex type structures are core-fucosylated. Sialic acids are linked at the C-3 position of terminal galactose, and the degree of sialylation of the bi- and triantennary structures varies between nonsialylated and fully sialylated; no tetraatennary structure contains more than three sialic acid residues. Recombinant prorenin contains 4% hybrid-type structures, all of which carry a terminal sialic acid residue. The remaining 35% of the structures on the molecule are high mannose type, composed of 5, 6, or 7 mannose residues. Approximately 6% of the high mannose type structures and 10% of the hybrid structures are phosphorylated, as judged by their susceptibility to treatment with alkaline phosphatase. Compositional analysis of an unlabeled preparation of the protein suggested the presence of approximately 1.4 oligosaccharide units per molecule.

Renin is an aspartyl protease with an important function in the regulation of blood pressure and electrolyte balance. The enzyme catalyzes the initial step in a series of reactions which ultimately leads to the generation of angiotensin II, a potent vasoconstrictor [reviewed in Ondetti and Cushman (1982)]. In addition to the kidney, human renin is synthesized in several extrarenal tissues such as uterine lining, ovarian theca, corpus luteum, pituitary, and adrenal. The initial translation product is a pre–pro form of the molecule, containing a 20 amino acid signal sequence and a 43 amino acid prosegment (Baxter et al., 1989; Hseuh & Baxter, 1991). In circulation, renin is found predominantly in the inactive proform, which is converted to active renin by cleavage of the 43 amino acid prosegment.

Human prorenin has a molecular mass of approximately 47 kDa and contains two sites for N-linked glycosylation (Sielecki et al., 1989). Several reports have demonstrated that secreted renin (both in circulating and in recombinant molecules) is glycosylated and that it appears to exist in more than one glycoform, the latter mainly defined by its capacity for interacting with Concanavalin A and/or its sensitivity to endoglycosidase H treatment (Printz et al., 1977; Hirose et al., 1985; Pinet et al., 1985; Fritz et al., 1986; Faust et al., 1987; Kim et al., 1988a, 1991). It has also been reported that recombinant human renin contains phosphorylated high mannose type structures, a characteristic probably related to the close homology (46% amino acid sequence identity) between renin and cathepsin D, a lysosomal enzyme (Faust et al., 1987).

The oligosaccharide structures on renin have been shown to influence the rate and amount of secretion (Paul et al., 1988; Hori et al., 1988; Nakayama et al., 1990) of the molecule as well as its clearance time in plasma (Kim et al., 1988b,c). In addition, the oligosaccharide structures on glycoproteins generally have been shown to be capable of influencing a very diverse spectrum of functions and properties. Thus, in order to be able to correctly evaluate further studies on the properties of the recombinant renin molecule, it was necessary to investigate its oligosaccharide structures. In this study, we have made a complete characterization of the N-linked oligosaccharide structures linked to recombinant human prorenin synthesized by Chinese hamster ovary cells.

EXPERIMENTAL PROCEDURES

Materials. [2-^{3}H]Mannose (18.5 Ci/mmol) was from Amersham. *Bacteroides fragilis* endo-β-galactosidase was from Boehringer Mannheim. Pronase and *Escherichia coli* alkaline phosphatase were from Calbiochem. *Aspergillus phoenicis* α-mannosidase, *Streptococcus pneumoniae* β-*N*-acetylglucosaminidase, and partially methylated alditol acetates of fucose, galactose, glucose, mannose, *N*-acetylgalactosamine, and *N*-acetylglucosamine were from Oxford Glycosystems. Concanavalin A–Sepharose and protein A–Sepharose were from Pharmacia. *Vibrio cholerae* neuraminidase, jack bean β-galactosidase, jack bean α-mannosidase, jack bean β-*N*-acetylglucosaminidase, bovine testis α-fucosidase, *Phaseolus vulgaris* erythroagglutinating phytohemagglutinin (E-PHA) and leukoagglutinating phytohemagglutinin (L-PHA), and *Ricinus communis* agglutinin II (RCA) were from Sigma. Pea lectin–agarose was from Vector. Peptide N-glycosidase F was from Genzyme. Endoglycosidase H was from ICN. Dulbecco's-modified Eagle's Medium (DMEM), heat-inactivated fetal bovine serum (FBS), nonessential MEM amino acids, HEPES, trypsin, penicillin, and streptomycin were from Gibco. Rabbit anti-renin antiserum was kindly provided by Dr. S. Sharma at The Upjohn Company. All other supplies were from standard sources.

Oligosaccharide Standards. Galβ1–4GlcNAcβ1–6(Galβ1–4GlcNAcβ1–2)Manα1–6[Galβ1–4GlcNAcβ1–4(Galβ1–

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4GlcNAcβ1–2)Manα1–3]Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc, Galβ1–4GlcNAcβ1–2Manα1–3[Galβ1–4GlcNAcβ1–6(Galβ1–4GlcNAcβ1–2)Manα1–6]Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc, Galβ1–4GlcNAcβ1–2Manα1–6[Galβ1–4GlcNAcβ1–4(Galβ1–4GlcNAcβ1–2)Manα1–3]Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc, and Galβ1–4GlcNAcβ1–2Manα1–6[Galβ1–4GlcNAcβ1–2Manα1–3]Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc were isolated from an in vivo [^{3}H]mannose-labeled tPA analog (Aeed & Elhammer, 1990). $Man_5GlcNAc$, $Man_6GlcNAc$, $Man_7GlcNAc$, $Man_8GlcNAc$, and $Man_9GlcNAc$ were a gift from Dr. R. Cummings, The University of Georgia, Athens, GA.

Preparation of Lectin–Agarose Gels. Lectin–agarose gels were prepared essentially as described in Cummings and Kornfeld (1982). Coupling efficiency (as determined by OD_{280}) was 85% for E-PHA and 95% for L-PHA and RCA.

Cells. Development of the prorenin-producing CHO clone has been described (Poorman et al., 1986). Cells were maintained in DMEM containing 10% FBS, 100 μM nonessential MEM amino acids, 20 milliunits/mL penicillin, 20 μg/mL streptomycin, and 100 nM methotrexate.

Metabolic Labeling. [^{3}H]Mannose-labeled prorenin was prepared as follows. Cells were grown to approximately 60% confluence in three 75-cm^2 tissue culture flasks. The cells were rinsed once with glucose-free DMEM and cultured in 5 mL of DMEM (per flask) containing 10% FBS, 2.5 mM glucose, 10 mM HEPES, and 1.67 mCi of [^{3}H]mannose (330 μCi/mL) for 24–36 h. Following labeling, the culture medium, containing secreted, labeled prorenin, was removed and cooled on ice. Aprotinin (0.1 TIU/mL) and 10 μg/mL each of antipain, chymostatin, leupeptin, and pepstatin were added, and the medium was centrifuged at low speed to remove remaining cells.

Immunoprecipitation and Preparation of Glycopeptides and Oligosaccharides. Prorenin was immunoprecipitated from 5-mL aliquots of the culture medium as follows. The medium was pre-cleared by incubated with 200 μL of protein A–Sepharose for 90 min. Following sedimentation of the gel, the supernatant was supplemented with 5 μL of anti-renin antiserum and incubated overnight. The antibody–antigen complex was precipitated by adding 40 μL of protein A–Sepharose and incubating for 2–4 h, after which the gel was sedimented and washed essentially as described by Dunphy et al. (1985). All incubations were carried out at 4 °C on a tube rotator. Prorenin was released from the immunomatrix by boiling for 10 min in 50 μL of 100 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 0.01% bromophenol blue, 0.3 M β-mercaptoethanol. Following sedimentation of the gel, the supernatant was subjected to SDS–PAGE and fluorography; the prorenin band was cut from the dried gel, and radiolabeled glycopeptides were released by Pronase treatment as described (Cummings et al., 1983, 1989). Oligosaccharides were liberated from the glycopeptides by hydrazinolysis (Takasaki et al., 1982) or endoglycosidase treatment (see below).

Enzyme Treatments. Oligosaccharides were released from glycopeptides by endoglycosidase treatment with peptide N-glycosidase F (20 units/mL) in 100 mM Tris-HCl, pH 8.6, for 24 h or endoglycosidase H (300 milliunits/mL) in 50 mM citrate–phosphate, pH 5.5, 20 mM EDTA, for 24 h. Treatment with *V. cholerae* neuraminidase (250 milliunits/mL) was in 50 mM acetate, pH 4.6, 150 mM NaCl, 10 mM $CaCl_2$ for 72 h; jack bean α-mannosidase (14 units/mL) was in 50 mM acetate, pH 5.0, for 48 h; *A. phoenicis* α-mannosidase (0.2 milliunit/mL) was in 100 mM acetate, pH 5.0, for 20 h; *B. fragilis* endo-β-galactosidase (250 milliunits/mL) was in 50 mM sodium acetate, pH 5.6, 0.2 mg/mL bovine serum albumin for 24 h; jack bean β-galactosidase (400 milliunits/mL) was in 50 mM citrate–phosphate, pH 4.6, for 48 h; jack bean β-*N*-acetylglucosaminidase (4.15 milliunits/mL) was in 50 mM sodium citrate, pH 5.6, for 18 h; *S. pneumoniae* β-*N*-acetylglucosaminidase (8 milliunits/mL) was in 100 mM citrate–phosphate, pH 6.0, for 24 h; bovine testis α-fucosidase (155 milliunits/mL) was in 50 mM citrate–phosphate, pH 4.6, for 48 h with an additional 8 milliunits of enzyme added after 24 h; *E. coli* alkaline phosphatase (6 units/mL) was in 50 mM Tris-HCl, pH 8.0, for 2 h. All enzyme treatments were in a 50-μL total volume, except peptide N-glycosidase F, which was in 100 μL. Incubations were at 37 °C under toluene atmosphere.

Column Chromatography. Serial lectin affinity chromatography was performed essentially as described by Merkle and Cummings (1987) and references therein.

Fractionation of [^{3}H]mannose-radiolabeled glycopeptides was done on Con A–Sepharose columns with 2.5-mL bed volumes; bound glycopeptides were sequentially eluted with 10 mM methyl α-glucoside and 100 mM methyl α-mannoside at 55 °C; fraction volume was 5 mL. Fractionation on E-PHA–agarose or L-PHA–agarose was performed on a 3-mL bed volume column (0.5 × 15 cm); fraction volume was 1 mL. Separation of glycopeptides on pea lectin–agarose was done on a 2.5-mL column (0.5 × 12.5 cm); bound material was eluted with 10 mM methyl α-glucoside followed by 500 mM methyl α-mannoside; fraction volume was 1 mL. Chromatography on RCA–agarose was performed on a 3-mL bed volume (0.5 × 15 cm) column; elution was with 0.1 M lactose; 1-mL fractions were collected. All lectin chromatography was done at room temperature.

Ion-exchange chromatography on QAE-Sephadex was carried out essentially as described (Varki & Kornfeld, 1983). Solutes were eluted from the columns (1-mL bed volume) stepwise with NaCl in 2 mM Tris base; 1.5-mL fractions were collected. Sialic acid residues were removed by either neuraminidase treatment or mild acid hydrolysis (2 M acetic acid at 100 °C for 1 h). Phosphomonoester groups were removed by alkaline phosphatase treatment; phosphate in diester form ("covered" phosphates) was converted to monoester by removal of the terminal GlcNAc residue by mild acid hydrolysis; the resulting monoester was cleaved as described above.

Ion-suppression high-pressure liquid chromatography (HPLC) was performed on neutral, endoglycosidase H released oligosaccharides using a Varian Micropack AX-5 column as described previously (Mellis & Baenziger, 1981).

Hydrazinolysis-released oligosaccharides were fractionated on a calibrated Bio-Gel P-4 column (1.5 × 200 cm) maintained at 55 °C. Sialic acid residues were removed by mild acid hydrolysis (see above) prior to loading the samples on the column. Flow rate was 0.6 mL/min; fraction volume was 900 μL. Standard glucose oligomers were derived from high molecular weight dextran by limited hydrolysis (Yamashita et al., 1982).

Partially methylated [^{3}H]mannose and [^{3}H]fucose residues (see below) were analyzed by high-pressure liquid chromatography essentially as described previously (Szilagyi et al., 1985).

Paper Chromatography. The extent of core fucosylation on the complex-type oligosaccharides was investigated by hydrolyzing aliquots of [^{3}H]mannose-labeled tetraantennary,

triantennary, and biantennary complex structures in 2 M TFA at 100 °C for 4 h followed by separation of the resulting radioactive monosaccharides on descending paper chromatography in ethyl acetate/pyridine/water (8:2:1) as described (Cummings et al., 1983).

Separation and identification of monosaccharides release by α-mannosidase treatment of phosphorylated hybrid-type oligosaccharides was done as above, on descending paper chromatography in ethyl acetate/pyridine/water (8:2:1).

Methylation Analysis. [^{3}H]Mannose-labeled oligosaccharides were released by hydrazinolysis and fractionated on Bio-Gel P-4 (see above). Aliquots of the material in the 20, 17, and 14.5 glucose units (GU) peaks, as well as an aliquot from the endoglycosidase H released neutral structures in Con A fraction III, were permethylated essentially as described by Ciucanu and Kerek (1984). The partially methylated alditols were dissolved in water (20–50 μL), filtered, and separated on HPLC (see above).

Samples of unlabeled renin (0.5 mg) were precipitated by the method of Wessel and Flugge (1984). The asparagine-linked oligosaccharides were liberated from the glycoprotein by hydrazinolysis and purified by descending paper chromatography in *n*-butanol/ethanol/water (4:1:1) as described previously (Takasaki et al., 1982). Released oligosaccharides were quantified by subjecting aliquots to hydrolysis in 6 M HCl followed by determination of the release aminosugars on a Dionex liquid chromatograph as described below. The purified oligosaccharides were reduced with sodium borohydride (250 μL of 25 mg/mL in 50 mM NaOH for 2 h at 30 °C). Following reduction, the samples were passed over columns of Dowex 50W-X8 (2-mL bed volume) to remove sodium ions. Methyl borates were removed by repeated evaporation with methanol/4 M acetic acid (9:1). Reduced oligosaccharides (15 nmol) were permethylated and hydrolyzed as described above, reduced with sodium borodeuteride, and O-acetylated as described in York et al. (1985). The partially methylated alditol acetates were analyzed by positive ion electron impact gas chromatography/mass spectrometry using a VG 70SE mass spectrometer equipped with a Hewlett Packard 5890 gas chromatograph and a VG 11-250J data system. A 15-m fused silica column (DB-1, J & W Scientific) was used with a temperature gradient from 50 °C to 170 °C at 30 °C/min, followed by 4 °C/min to 270 °C. Mass spectra were acquired with an electron energy of 70 eV, an accelerating voltage of 8 kV, and a scan rate of 1 s/dec with a 0.5-s interscan time over a mass range of 50–500 amu.

Compositional Analysis. Monosaccharide composition was determined on acid hydrolysates of unlabeled renin oligosaccharides using a Dionex liquid chromatograph equipped with a CarboPac PA1 column (4.6 × 250 mm) and pulsed amperometric detection (Hardy et al., 1988). Flow rate was 1 mL/min.

RESULTS

Serial Lectin Affinity Chromatography. Separation of glycopeptides prepared by pronase treatment of [^{3}H]mannose-labeled prorenin, on serial lectin affinity chromatography, resulted in the profiles shown in Figure 1. In the first step, the glycopeptides were applied to a column of Con A–Sepharose. Approximately 12% of the radioactivity in the [^{3}H]mannose-labeled glycopeptides did not interact with the lectin and was eluted in the run-through (Con A fraction I). This fraction should contain mainly tri- and tetraantennary complex-type oligosaccharides, structures containing bisecting GlcNAc residues, and O-linked oligosaccharides.

Further fractionation of Con A fraction I on E-PHA–agarose resulted in no radioactivity interacting with the column, suggesting a lack of biantennary structures with a bisecting *N*-acetylglucosamine residue. The glycopeptides in Con A fraction I were then chromatographed on a column of pea lectin–agarose. This resulted in two fractions, IA (approximately 30% of the applied radioactivity) which did not bind to the lectin and IB (approximately 70% of the applied radioactivity) which was eluted with 10 mM methyl α-glucoside. The bound material, fraction IB, should contain mainly core-fucosylated 2,6-branched triantennary structures. Both IA and IB were further fractionated on L-PHA–agarose. This lectin interacts with galactosylated tri- and tetraantennary structures containing one α-linked mannose substituted at carbon atoms 2 and 6. Essentially all the radioactivity eluted in fraction IB (from pea lectin–agarose) also bound to L-PHA, consistent with this fraction containing complex structures with the 2,6 branch. Fraction IA on the other hand, separated into two fractions, IA1 (approximately 45% of the applied radioactivity) and IA2 (approximately 55% of the applied radioactivity). By process of elimination, fraction IA1 should contain mainly 2,4-branched triantennary structures (with or without core fucose) while fraction IA2 could contain 2,6-branched triantennary structures without core fucose and/or triantennary structures.

The glycopeptides in Con A fraction II (approximately 32% of the labeled glycopeptides), predominantly biantennary structures, were further separated into core-fucosylated structures (IIB) and structures without core fucose (IIA), on pea lectin–agarose. Seventy-seven percent of the radioactivity in Con A fraction II bound to the column, suggesting that the majority of the biantennary structures on recombinant renin are core-fucosylated. (The amount of radioactivity bound to pea lectin–agarose showed considerable variation between different experiments suggesting batch-to-batch variations in the proportions of fucosylated vs unfucosylated molecules; see below.) The glycopeptides eluting in Con A fraction III, finally, should be enriched in high mannose type and hybrid-type oligosaccharides.

Separation of High Mannose Type Structures on HPLC. Fractionation of endoglycosidase H released neutral oligosaccharides from Con A fraction III on ion-suppression HPLC resulted in the three peaks shown in panel A of Figure 2. Four percent of the radioactivity comigrated with the $Man_5GlcNAc$ high mannose oligosaccharide standard, the remaining 46% and 50% comigrated with the $Man_6GlcNAc$ and $Man_7GlcNAc$ standards, respectively.

Ion-Exchange Chromatography of Charged Oligosaccharides. The presence of terminal sialic acid residues on the complex-type oligosaccharides on recombinant prorenin was investigated by ion-exchange chromatography on QAE-Sephadex. Oligosaccharides were liberated from labeled lectin fractionated glycopeptides by peptide N-glycosidase F treatment before separation on the ion-exchange column. Charged structures were eluted from the column by stepwise increases in NaCl concentration, with each step equaling one additional charge (sialic acid residue) (Varki & Kornfeld, 1983). The separations of the [^{3}H]mannose-labeled lectin fractions are summarized in Table I. All Con A I fractions (IA1, IA2, and IB) appear to contain structures ranging in sialylation from non- to trisialylated, with the majority of the structures containing at least one sialic acid residue. Con A fraction II ranges from non- to bisialylated, again with the majority of the structures (90%) containing at least one sialic acid.

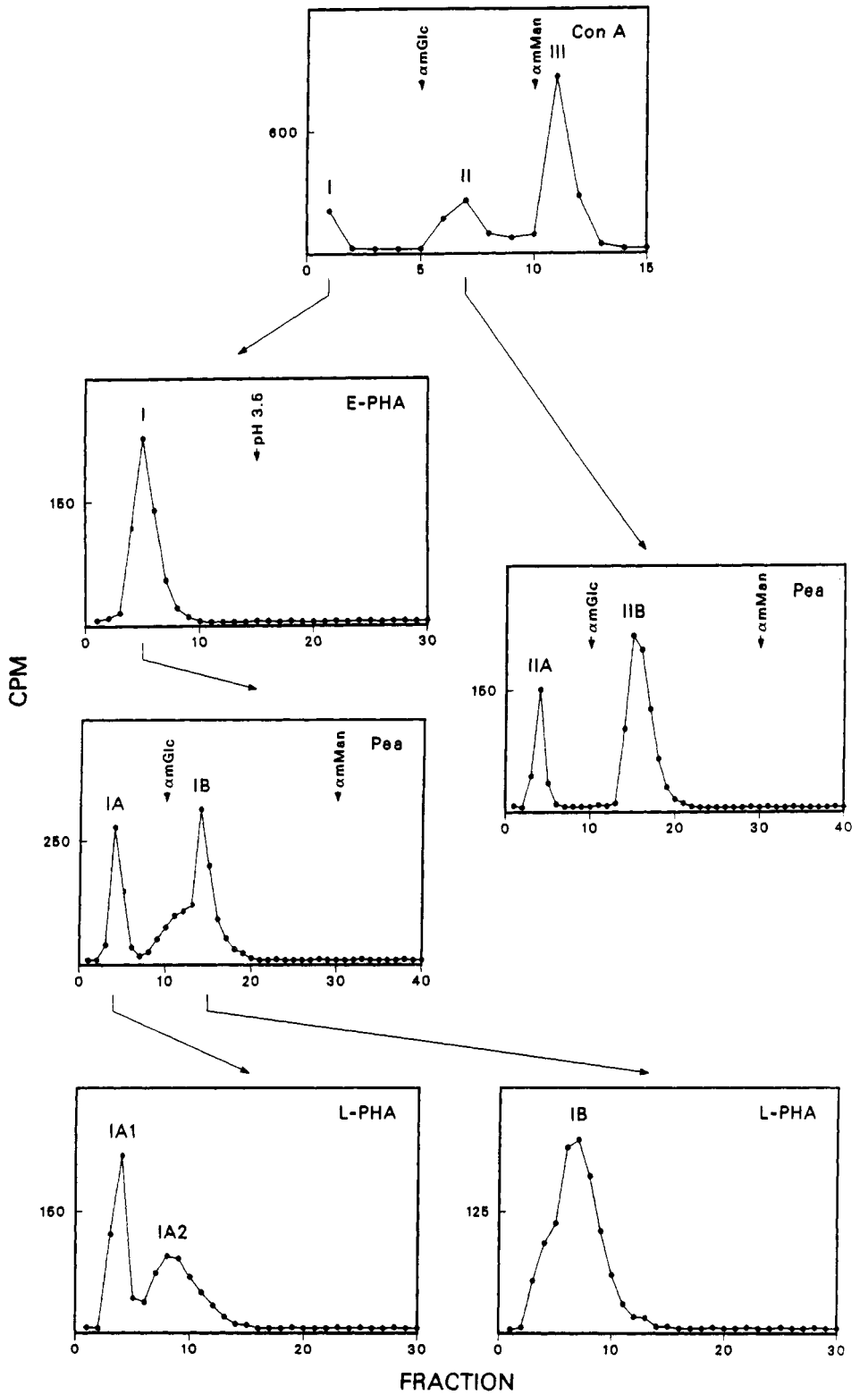


FIGURE 1: Serial lectin affinity chromatography of [^{3}H]mannose-labeled prorenin glycopeptides. [^{3}H]Mannose-labeled prorenin glycopeptides isolated by pronase treatment of the immunoprecipitated in vivo labeled molecule were subjected to serial lectin affinity chromatography. The separation profiles shown were obtained from chromatography on Con A–Sepharose, E-PHA–agarose, pea lectin–agarose, and L-PHA–agarose. Abbreviations: αmGlc, methyl α-glucoside; αmMan, methyl α-mannoside.

Chromatography of the oligosaccharide structures in Con A fraction III (after release by endoglycosidase H treatment) on QAE-Sephadex demonstrated that the majority (85%) of these structures are uncharged (data not shown). Further fractionation of this material on RCA–agarose resulted in no radioactivity interacting with the column (Figure 3, panel A) suggesting that the uncharged structures in Con A III are all high mannose type; hybrid structures are retarded on RCA–agarose (Merkle & Cummings, 1987). The presence of only high mannose type structures in this fraction was confirmed by separation of the material on HPLC (Figure 2, panel A).

Six percent of the radioactivity in Con A III was eluted from QAE-Sephadex with 20 mM NaCl (QAE 20; structures containing one charge), 5% with 70 mM NaCl (QAE 70; structures containing two charges), and the remaining 4% with 140 mM NaCl (QAE 140; structures containing three

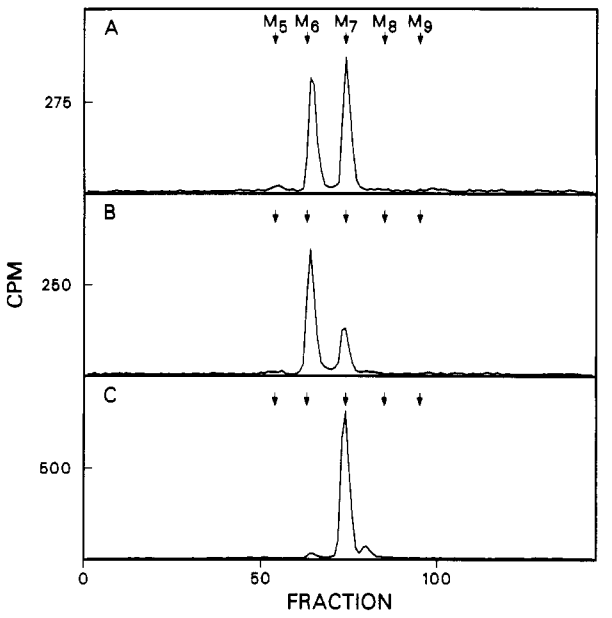


FIGURE 2: Separation of [^{3}H]mannose-labeled high mannose type oligosaccharides on ion suppression HPLC. [^{3}H]Mannose-labeled oligosaccharides were released by endoglycosidase H treatment of glycopeptides in Con A fraction III and fractionated on QAE-Sephadex. Following removal of charged substituents (panels B and C), the neutral oligosaccharides were fractionated on a Micropak AX-5 column in a linear gradient (65–35%) of acetonitrile in water. Panels: A, neutral oligosaccharides; B, oligosaccharides eluted with 70 mM NaCl (following treatment with alkaline phosphatase); C, oligosaccharides eluted with 140 mM NaCl (following treatment with alkaline phosphatase). M_5–M_9 indicate the elution positions of oligosaccharide standards $Man_5GlcNAc$ through $Man_9GlcNAc$.

Table I: Distribution of Sialic Acid Residues on [^{3}H]Mannose-Labeled Prorenin Complex-Type Oligosaccharides[a]

	percent of fraction containing				
fraction	no charge	1 charge	2 charges	3 charges	4 charges
IA1	6	12	25	44	13[b]
IA2	10	16	28	46	
IB	2	15	33	50	
II	17	13	70		

[a] [^{3}H]Mannose-labeled prorenin glycopeptides were isolated and fractionated by lectin chromatography. The lectin fractions were treated with peptide: N-glycosidase F and the released oligosaccharides were fractionated by ion-exchange chromatography on QAE-Sephadex. Charges were identified by neuraminidase treatment or mild acid hydrolysis. [b] The radioactive material in this fraction did not shift to neutral upon mild acid hydrolysis.

charges). The nature of the charges associated with these salt-eluted fractions was investigated in the experiments summarized in Table II. The fractions were individually characterized as follows. Neuraminidase treatment of the material with one charge (QAE 20) removed the charge from 75% of the radioactivity, and essentially all of these (desialylated) structures were retarded upon subsequent chromatography on RCA–agarose (Figure 3, panel B), suggesting that this fraction contains mainly sialylated hybrid-type oligosaccharides. The remaining 25% of the radioactivity eluted with 20 mM NaCl was resistant to direct treatment with alkaline phosphatase. However, an additional 9% shifted to neutral after a mild acid hydrolysis followed by alkaline phosphatase treatment, indicating that some of the radioactivity in this fraction is incorporated into structures containing a phosphate in diester linkage. Alternatively, the additional charge removed represents sialic acid residues resistant to neuraminidase treatment. The small amounts of radioactivity did not allow us to further characterize this fraction.

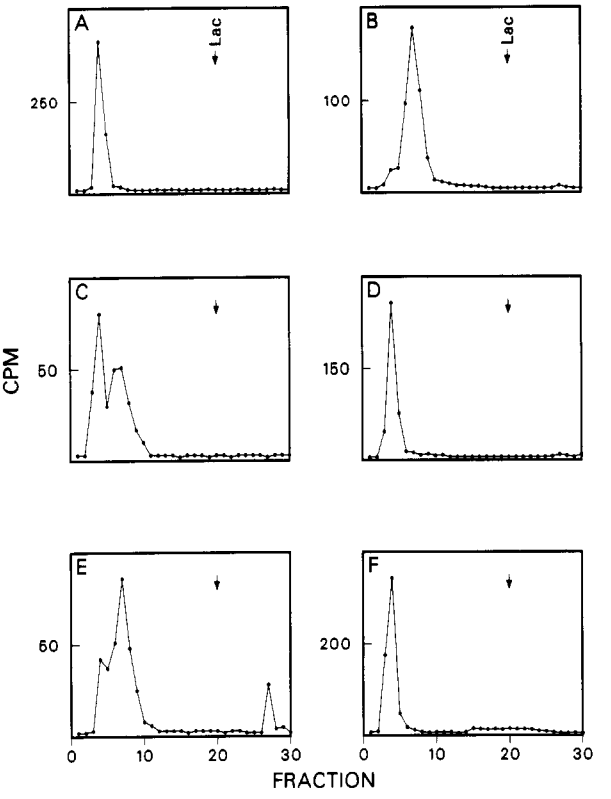


FIGURE 3: *Ricinus communis* lectin affinity chromatography of oligosaccharides in Con A fraction III. [^{3}H]Mannose-labeled prorenin Con A fraction III oligosaccharides were fractionated by ion-exchange chromatography on QAE-Sephadex. Following removal of the charged substituents (sialic acid and/or phosphate), the neutral oligosaccharides were fractionated on columns of RCA–agarose as described in Experimental Procedures. Panels: A, neutral structures; B, charged structures eluting with 20 mM NaCl which shifted to neutral following treatment with neuraminidase; C, charged structures eluting with 70 mM NaCl which eluted with 20 mM NaCl after treatment with neuraminidase and shifted to neutral following treatment with alkaline phosphatase; D, charged (neuraminidase-resistant) structures eluting with 70 mM NaCl which shifted to neutral after treatment with alkaline phosphatase; E, charged structures eluting with 140 mM NaCl which were eluted with 70 mM NaCl after treatment with neuraminidase and shifted to neutral after treatment with alkaline phosphatase; F, charged (neuraminidase-resistant) structures eluting with 140 mM NaCl which shifted to neutral after treatment with alkaline phosphatase. Lac represents lactose.

Neuraminidase treatment of the radioactive structures eluting with 70 mM NaCl (QAE 70) resulted in a one charge shift of 8% of the radioactivity. Further treatment of this material with alkaline phosphatase caused approximately 40% of the radioactivity (3% of QAE 70) to shift to neutral; treatment of the remaining 60% with mild acid hydrolysis followed by alkaline phosphatase treatment cause an additional 16% (1% of QAE 70) to shift to neutral. Thus, approximately 4% of the radioactivity in QAE 70 appears to represent phosphorylated (monoester and diester), sialylated hybrid structures. This conclusion is supported by the lectin chromatography experiment shown in panel C of Figure 3. A large portion of the radioactivity was retarded on RCA–agarose consistent with mainly hybrid-type structures in this fraction. The majority (92%) of the radioactivity in QAE 70 was resistant to neuraminidase. A large portion (52%), however, shifted to neutral following treatment with alkaline phosphatase. This material did not interact with RCA (Figure 3, panel D), suggesting that it represents phosphorylated high mannose type structures (Varki & Kornfeld, 1980). When examined by HPLC, 70% of the radioactivity in this fraction comigrated with the $Man_6GlcNAc$ standard and 30% comi-

Table II: Distribution of Sialic Acid and Phosphate Residues on [^{3}H]Mannose-Labeled Prorenin Con A Fraction III Oligosaccharides[a]

initial fraction[b]	subfraction following neuraminidase treatment[c]	percent of initial fraction	retarded by RCA	percent of initial fraction that shifts to neutral following treatment with: alkaline phosphatase	percent of initial fraction that shifts to neutral following treatment with: mild acid hydrolysis/ alkaline phosphatase
QAE 20	QAE 20 → 0	75%	+	ND[d]	ND
	QAE 20 → 20	25%	-	0%	9%
QAE 70	QAE 70 → 20	8%	+	3%	1%
	QAE 70 → 70	92%	-	52%	8%
QAE 140	QAE 140 → 70	25%	+	10%	3%
	QAE 140 → 140	75%	-	55%	0%

[a] [^{3}H]Mannose-labeled prorenin glycopeptides were isolated and fractionated on Con A–Sepharose. The glycopeptides in Con A fraction III were treated with endoglycosidase H and the released oligosaccharides were fractionated by ion-exchange chromatography on QAE-Sephadex. Charges were identified by neuraminidase treatment, mild acid hydrolysis, and alkaline phosphatase treatment. The identity of hybrid-type structures was confirmed by chromatography on RCA–agarose (Figure 3). [b] Fraction designations refer to the NaCl concentration (millimolar) needed to elute the materials in the initial separation on QAE-Sephadex. [c] Fraction designations refer to the change (or absence of change) in elution conditions required for recovery from QAE-Sephadex chromatography following neuraminidase treatment. [d] Not done; structures in this subfraction were neutral following neuraminidase treatment.

grated with the $Man_7GlcNAc$ standard (Figure 2, panel B). The remaining neuraminidase-resistant material in fraction QAE 70 was also resistant to alkaline phosphatase. Approximately one-fifth of this (8% of QAE 70) did, however, shift to neutral following sequential mild acid hydrolysis and alkaline phosphatase treatment, suggesting that some of the structures in this fraction contain phosphodiesters.

Approximately 25% of the radioactivity in the Con A fraction III material eluting with 140 mM NaCl (QAE 140) shifted one charge upon treatment with neuraminidase. Forty percent of this (neuraminidase-sensitive) material (10% of QAE 140) shifted further to neutral following alkaline phosphatase treatment and another 12% (3% of QAE 140) shifted to neutral following sequential mild acid hydrolysis and alkaline phosphatase treatment, again suggesting the presence of sialylated phosphorylated hybrid-type structures in Con A III; both monoesters and diesters and more than one phosphate group per molecule. This conclusion is supported by the RCA–agarose binding experiment shown in panel E of Figure 3 (more than 90% of the loaded radioactivity was retarded on the column) and by the experiment shown in Figure 4. Aliquots from the sialylated, phosphorylated hybrid structures in QAE 140 were first treated with neuraminidase and alkaline phosphatase to remove sialic acid and phosphates. The resulting uncharged structures were treated with either *A. phoenicis* α-mannosidase or jack bean α-mannosidase. The released mannoses were separated by descending paper chromatography. All the structures in this fraction were resistant to *A. phoenicis* α-mannosidase, indicating a lack of outer α1–2-linked mannose residues (Figure 4, panel A). Jack bean α-mannosidase, however, released 60% of the radioactivity as free mannose, suggesting that three out of five mannose residues in the molecule were susceptible to the enzyme (Figure 4, panel B). Taken together, these experiments suggest that all outer α1–2-linked mannose residues are trimmed on these molecules and that the phosphates probably are linked to the α1–3-linked and/or the α1–6-linked mannoses; data suggesting structures phosphorylated on the outer α1–6-linked mannose have been published (Varki & Kornfeld, 1983). The remining 75% of fraction QAE 140 was resistant to neuraminidase treatment. Approximately 75% of this material (55% of QAE 140) shifted to neutral upon treatment with alkaline phosphatase. Furthermore, the structures in this fraction were not retarded on RCA–agarose (Figure 3, panel F); instead they comigrated with the $Man_6GlcNAc$ (4%) and $Man_7GlcNAc$ (96%) standards on HPLC (Figure 2, panel C), suggesting that the majority of this material represents high mannose type structures containing more than one phosphate in monoester linkage. Further treatment of the alkaline phosphatase-resistant material did not result in any significant changes in the charge distribution.

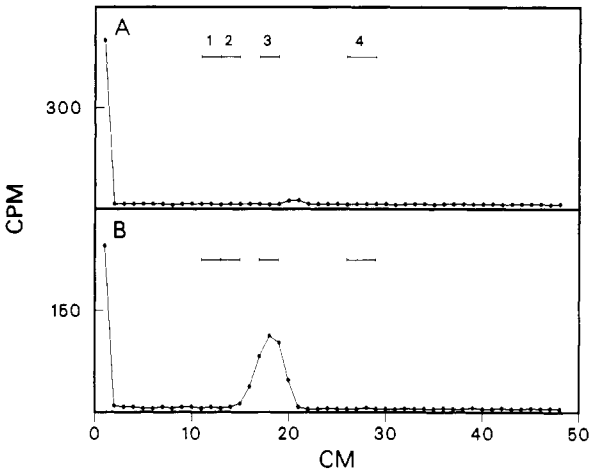


FIGURE 4: Descending paper chromatography of [^{3}H]mannose-labeled phosphorylated hybrid-type prorenin oligosaccharides. Phosphorylated hybrid-type oligosaccharides from [^{3}H]mannose-labeled prorenin were isolated from Con A fraction III by ion-exchange chromatography on QAE-Sephadex. Charged substituents were removed by treatment with neuraminidase and alkaline phosphatase, and the resulting neutral oligosaccharides were subjected to descending paper chromatography in ethyl acetate/pyridine/water (8:2:1) following treatment with (A) *Aspergillus phoenicis* α-mannosidase and (B) jack bean α-mannosidase. The migration distances of standard monosaccharides are indicated: 1, galactose; 2, glucose; 3, mannose; 4, fucose.

Taken together, the data strongly suggest that approximately 10% percent of the structures in Con A fraction III (4% of total) are hybrid type. Ten percent of these structures are phosphorylated (mono- and diesters). All nonphosphorylated hybrid structures are sialylated. Ninety percent of the oligosaccharides in Con A fraction III (35% of total) are high mannose, in the form of $Man_5GlcNAc_2$ (5%), $Man_6GlcNAc_2$ (41%), and $Man_7GlcNAc_2$ (44%). Approximately 6% of these structures ($Man_6GlcNAc_2$ and $Man_7GlcNAc_2$) are phosphorylated.

Bio-Gel P-4 Chromatography. *N*-Glycans from [^{3}H]mannose-labeled prorenin were released by hydrazinolysis and characterized on a calibrated Bio-Gel P-4 column. The complex-type structures were eluted in three peaks having the elution volumes of dextran oligomers composed of 20, 17, and 14.5 GU, respectively. The sequential degradation of the oligosaccharides in these peaks by exoglycosidase treatment is summarized in Figure 5. Treatment of the material in the 20 GU peak with jack bean β-galactosidase resulted in a shift of essentially all the radioactivity to a broad peak eluting at approximately 16 GU. Further treatment of this material with jack bean β-*N*-acetylglucosaminidase produced two peaks with elution volumes of 12 (60% of the radioactivity) and 8 GU (40% of the radioactivity), respectively (panel A). Thus, 40% of the radioactivity in the 20 GU peak is incorporated in structures having the characteristics of a tetraantennary complex oligosaccharide. The 8 GU degradation product from

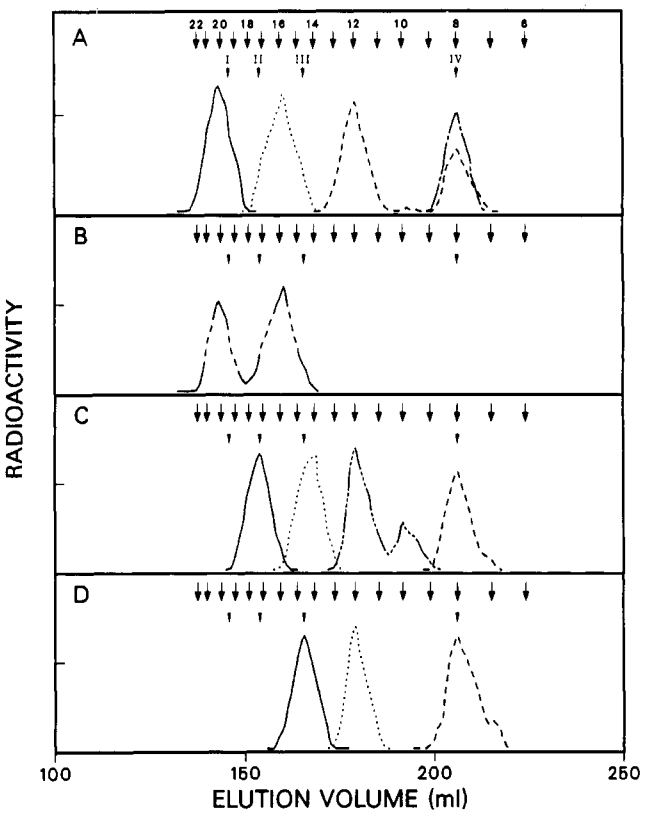


FIGURE 5: Sequential exoglycosidase treatment of [^{3}H]mannose-labeled prorenin oligosaccharides. Hydrazinolysis-released, [^{3}H]-mannose-labeled prorenin oligosaccharides having the elution volumes (on Bio-Gel P-4; 1.6 × 200 cm) of glucose oligomers composed of 20 (panels A and B), 17 (panel C) and 14.5 (panel D) glucose units were subjected to sequential exoglycosidase treatment and re-chromatography on Bio-Gel P-4. Lines: (—) the untreated oligosaccharide; (···) product from treatment with jack bean β-galactosidase; (– –) product from treatment with jack bean β-galactosidase followed by jack bean β-*N*-acetylglucosaminidase; (— -) product from treatment with jack bean β-galactosidase and jack bean β-*N*-acetylglucosaminidase simultaneously; (— · -) product from treatment with *Bacteroides fragilis* endo-β-galactosidase; (— · · ·), product from treatment with jack bean β-galactosidase followed by *Streptococcus pneumoniae* β-*N*-acetylglucosaminidase. Numbers 6–22 indicate the elution volumes of dextran oligomers composed of 6–22 glucose units. I, II, and III indicate the elution volumes of standard core-fucosylated tetra-, tri-, and biantannary complex type oligosaccharides, respectively; IV indicates Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc.

this material has a shoulder at 7 GU suggesting that some of these oligosaccharides may not contain a core fucose. This was confirmed by the experiment shown in panel A of Figure 8. The extent of core-fucosylation was investigated by determining the mannose-to-fucose ratio (radioactivity) in the peak. On the basis of this experiment, the distribution of radioactivity in the Bio-Gel P-4 peaks and the methylation analysis data (Table IV), approximately 60% of the tetraantennary structures contain a core-fucose residue. Further evidence for the identity of the 8 GU peak with a core-fucosylated Man_3 oligosaccharide is provided by the sequential exoglycosidase treatment experiment shown in Figure 6 and the results from the methylation analysis (Table IV). The 12 GU product from treatment with jack bean β-*N*-acetylglucosaminidase could be quantitatively converted to an 8 GU Man_3 core oligosaccharide by simultaneous treatment with jack bean β-galactosidase and jack bean β-*N*-acetylglucosaminidase (Figure 5, panel A). Further, treatment of the radioactive material in the 20 GU peak with endo-β-galactosidase resulted in a shift of approximately 55% of the radioactivity to 16 GU (Figure 5, panel B), consistent with

Table III: Permethylated Alditol Acetates Identified in Oligosaccharides Isolated from Recombinant Renin

partially methylated monosaccharides identified
1,5-diacetyl-2,3,4-trimethylfucitol
1,3,5,6-tetraacetyl-2,4-dimethylmannitol
1,2,5,6-tetraacetyl-3,4-dimethylmannitol
1,2,4,5-tetraacetyl-3,6-dimethylmannitol
1,2,5-triacetyl-3,4,6-trimethylmannitol
1,5-diacetyl-2,3,4,6-tetramethylmannitol
1,3,5-triacetyl-2,4,6-trimethylgalactitol
1,5-diacetyl-2,3,4,6-tetramethylgalactitol
1,4,5,6-tetraacetyl-3-methyl-2-*N*-acetylglucosaminitol
1,4,5-triacetyl-3,6-dimethyl-2-*N*-acetylglucosaminitol

the removal of one terminal *N*-acetyllactosamine repeat unit (Galβ1–4GlcNAcβ1–3Gal). Together with the methylation data (Table IV), these experiments strongly suggest that 55–60% of the oligosaccharides in the 20 GU peak are 2,6-branched triantennary with one poly(*N*-acetyllactosamine) repeat unit. Panel C of Figure 5 shows the sequential degradation of the oligosaccharides eluted in the 17 GU peak. The untreated material coelutes with standard II, a core-fucosylated triantennary structure. Treatment with jack bean β-galactosidase results in a 3 GU shift in elution volume, consistent with the removal of three terminal galactose residues. Further treatment with *S. pneumoniae* β-*N*-acetylglucosaminidase resulted in two products. Eighty percent of the radioactivity eluted at 12 GU, and the remaining 20% eluted at 10 GU. *S. pneumoniae* β-*N*-acetylglucosaminidase cleaves β1–2-linked *N*-acetylglucosamine residues from 2-substituted and 2,4-substituted mannose, but not from 2,6-substituted mannose, under the conditions used. It can therefore be used to distinguish 2,4- from 2,6-branched triantennary structures (Yamashita et al., 1981). Thus, both types of triantennary structures are present on recombinant prorenin. Sequential treatment of the 17 GU material with jack bean β-galactosidase and jack bean β-*N*-acetylglucosaminidase resulted in only one peak at 8 GU with a shoulder at 7 GU, suggesting that not all of the triantennary structures are core-fucosylated. The extent of fucosylation was investigated in the experiment shown in panel B of Figure 8. The ratio of mannose to fucose suggests that 65% of the triantennary structures are core-fucosylated; this estimate is further supported by the distribution of radioactivity in the methylation analysis experiment (Table IV). The untreated 14.5 GU peak elutes in the position of standard III, a core-fucosylated biantennary structure (Figure 5, panel D). Treatment of this material with jack bean β-galactosidase results in a shift to 12 GU. Further treatment with jack bean β-*N*-acetylglucosaminidase produced two peaks eluting at 8 and 7 GU, respectively, again consistent with the presence of fucosylated and nonfucosylated core structures on the biantennary oligosaccharides on renin. Further support for this conclusion comes from the results of the pea lectin chromatography of Con A II (Figure 1), from the ratio of radioactive mannose to fucose (Figure 8, panels C and D), and from the methylation data (Table IV). Taken together, these results suggest that 74% of the biantennary structures are core-fucosylated.

The sequential degradation of the 8 GU core from the biantennary structure (all 8 GU structures yielded similar results) is shown in Figure 6. Treatment with bovine testis α-fucosidase resulted in two peaks of 7 GU (75% of the radioactivity) and 1 GU (25% of the radioactivity) (Panel B), consistent with the release of one fucose residue. Further treatment of the 7 GU material with jack bean α-mannosidase yielded two peaks eluting at 1 GU and 5 GU in a 2:1 ratio

Table IV: Relative Proportions of Partially Methylated Mannose and Fucose Residues Isolated from [^{3}H]Mannose-Labeled Prorenin Oligosaccharides

permethylated sugar	linkage	molar radio[a] of permethylated sugar found in fraction			
		20 GU	17 GU	14.5 GU	Con A III
3,4-dimethylmannitol	1,2,6	0.9 (1.0)[b]	1.1 (0.8)		
3,6-dimethylmannitol	1,2,4	0.4 (0.4)	0.2 (0.2)		
2,4-dimethylmannitol	1,3,6	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)
3,4,6-trimethylmannitol	1,2	0.5 (0.6)	0.9 (1.0)	1.6 (2.0)	0.9 (0.7)
2,3,4,6-tetramethylmannitol	terminal				1.3 (1.5)
2,3,4-trimethylfucitol	terminal	0.7 (0.8)	0.3 (0.6)	0.4 (0.7)	

[a] Ratios calculated by setting 2,4-dimethylmannitol to 1.0. [b] Numbers in parentheses represent values predicted by oligosaccharide analysis.

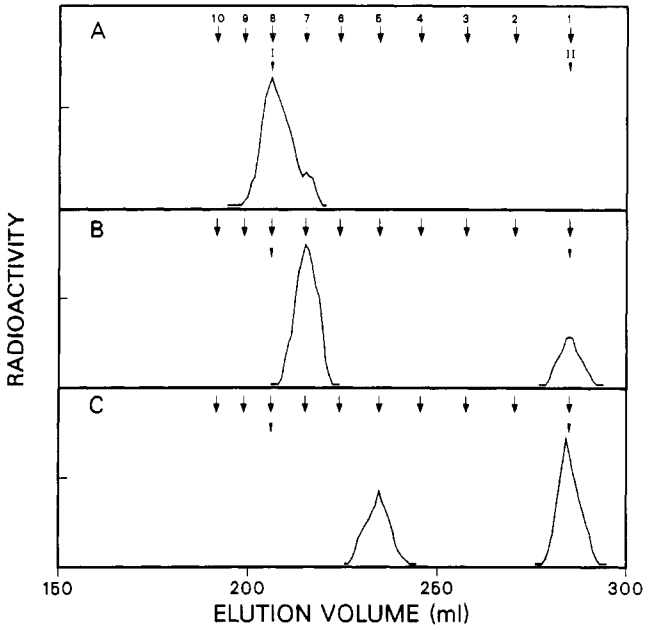


FIGURE 6: Sequential exoglycosidase treatment of an 8 glucose unit core structure isolated from [^{3}H]mannose-labeled prorenin oligosaccharides. The 8 glucose unit product from exoglycosidase treatment of the 14.5 (or 20 or 17) glucose unit [^{3}H]mannose-labeled prorenin oligosaccharide was subjected to further sequential exoglycosidase treatment and re-chromatography on Bio-Gel P-4. Panels: A, the intact 8 glucose unit oligosaccharide; B, product from treatment with bovine epididymis α-fucosidase; C, product from treatment with bovine epididymis α-fucosidase followed by jack bean α-mannosidase. Numbers 1–10 indicate the elution volumes of dextran oligomers composed of 1–10 glucose units. I indicates Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc; II indicates mannose.

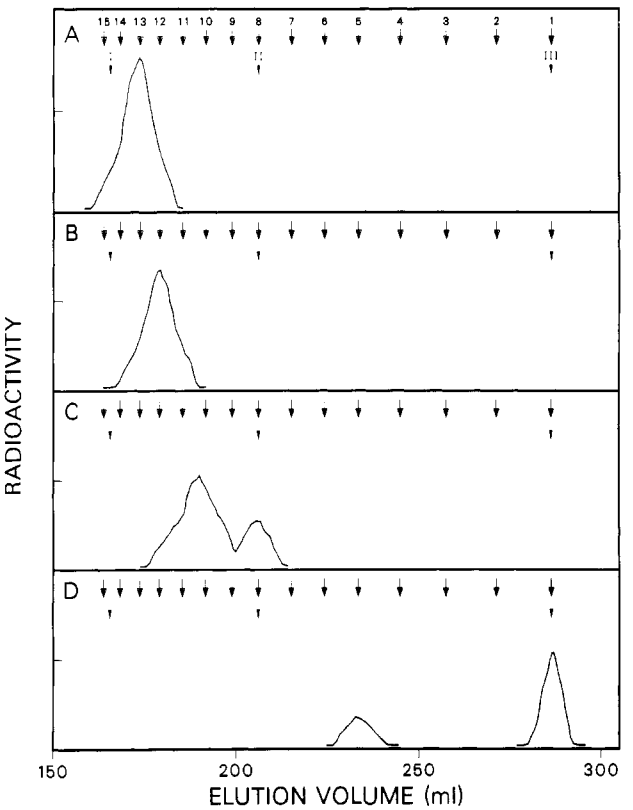


FIGURE 7: Sequential exoglycosidase treatment of a 13 glucose unit [^{3}H]mannose-labeled prorenin oligosaccharide. A hydrazinolysis-released, [^{3}H]mannose-labeled oligosaccharide having the elution volume of an dextran oligomer composed of 13 glucose units was subjected to sequential exoglycosidase treatment and re-chromatography on Bio-Gel P-4. Panels: A, the untreated 13 glucose unit oligosaccharide; B, product from treatment with jack bean β-galactosidase; C, product from treatment with jack bean β-galactosidase followed by jack bean β-*N*-acetylglucosaminidase; D, product from sequential treatment with jack bean β-galactosidase, jack bean β-*N*-acetylglucosaminidase, and jack bean α-mannosidase. Numbers 1–15 indicate the elution volumes of dextran oligomers composed of 1–15 glucose units. I indicates a core-fucosylated biantennary standard oligosaccharide; II indicates Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4(Fucα1–6)GlcNAc; III indicates mannose.

(panel C). These results demonstrate that the 8 GU peak contains a fucosylated trimannosyl structure.

Finally, the sequential exoglycosidase treatment of an oligosaccharide eluting at 13 GU is shown in Figure 7. This material shifted to 12 GU following treatment with jack bean β-galactosidase (panel B) and to 10 GU upon further treatment with jack bean β-*N*-acetylglucosaminidase (panel C). Further treatment of the 10 GU peak with jack bean α-mannosidase results in two peaks of 1 GU and 5 GU with a 4:1 distribution of recovered radioactivity (panel D). These results are consistent with a hybrid-type structure containing one terminal galactose, one outer *N*-acetylglucosamine, and four α-linked mannose residues. The additional 8 GU peak which appeared after β-*N*-acetylglucosaminidase treatment (panel C) represents contaminating biantennary structures in this fraction (data not shown).

Methylation Analysis. Samples of hydrazinolysis-released oligosaccharides from unlabeled renin were subjected to methylation analysis and gas chromatography/mass spectrometry (Table III). All partially methylated alditol acetates predicted by the oligosaccharide analysis, and no additional ones, were detected in the analysis. Galactose was found as both terminal and 1,3-linked, suggesting that the terminal sialic acid residues are linked to carbon 3 of galactose. The presence of 3-methyl-(1,4,6-substituted)-*N*-acetylglucosamine indicates that core-fucosylation occurs on carbon 6 of the reducing *N*-acetylglucosamine.

Separation (by HPLC) of partially permethylated monosaccharides isolated from the neutral structures in Con A fraction III and the oligosaccharides eluting at 20, 17, and 14.5 GU from Bio-Gel P-4 is summarized in Table IV. The presence of 3,4,6-trimethylmannose and the molar ratios of the permethylated sugars found in the 20 GU Bio-Gel P-4 peak are

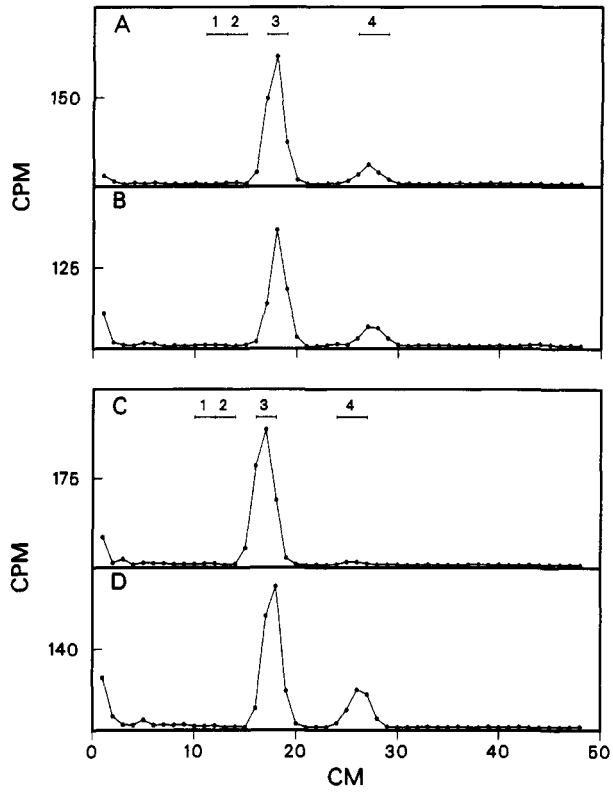


FIGURE 8: Descending paper chromatography of acid hydrolysates of [^{3}H]mannose-labeled prorenin oligosaccharides. Labeled prorenin glycopeptides and oligosaccharides were hydrolyzed in 2 M trifluoroacetic acid, and the released radioactive monosaccharides were separated on descending paper chromatography in ethyl acetate/pyridine/water (8:2:1). Panels: A and B, products from hydrolysis of teraaantennary (20 glucose units) and triantennary (17 glucose units) prorenin oligosaccharides, respectively; C and D, hydrolysis products from lectin fractions IIA and IIB, respectively. The migration distances of standard monosaccharides are indicated: 1, galactose; 2, glucose; 3, mannose; 4, fucose.

consistent with this peak containing two structures, a fucosylated tetraantennary complex type oligosaccharide and a fucosylated 2,6-branched triantennary complex structure with one poly(*N*-acetyllactosamine) repeat in a ratio of approximately 2:3. The presence and proportions of 3,4- and 3,6-dimethylmannose in the 17 GU peak are consistent with this peak containing both 2,4-branched and 2,6-branched triantennary structures. The data are also consistent with the 14.5 GU peak containing biantennary complex structures and with the neutral structures in Con A fraction III being high mannose type oligosaccharides in the form of Man_5-, Man_6-, and $Man_7GlcNAc_2$. The recovered fucose-to-mannose ratios of permethylated monosaccharides are, in all fractions containing complex-type oligosaccharides, lower than what would be predicted from the size-exclusion chromatography, lectin chromatography, and paper chromatography data. This may be due to the poor stability of fucose (vs mannose) during acid hydrolysis or, more likely, reflect batch-to-batch variations in the degree of core-fucosylation of the complex oligosaccharides on the molecule (compare above). Control experiments investigating the degree of core-fucosylation on the biantennary oligosaccharides from different batches of in vivo [^{3}H]-mannose-labeled recombinant prorenin demonstrated variations between 50% and 80% in the percentage of fucosylated molecules (data not shown).

Compositional Analysis. The result from the compositional analysis of the oligosaccharides on renin is summarized in Table V. The molar ratios of the recovered monosaccharides are in good agreement with that predicted by the oligosaccharide analysis. The amounts of sugar recovered per mole of protein are, however, less (1.4 mol/mol of protein) than predicted if all renin molecules were fully (both sites) glycosylated.

Table V: Monosaccharide Composition of Recombinant Human Renin

sugar	amount (mol/mol of protein)	molar ratio[a]
mannose	5.5 (8.2)[b]	4.0 (4.0)
galactose	1.9 (2.9)	1.4 (1.5)
fucose	1.0 (1.2)	0.8 (0.6)
N-acetylglucosamine	4.8 (7.0)	3.5 (3.4)

[a] Ratios were calculated by setting the number of mannose residues to 4.0. [b] The values in parentheses represent the amounts theoretically obtainable from a population of oligosaccharide structures equal to that predicted by our analysis and assuming that both glycosylation sites are occupied on 100% of the protein molecules.

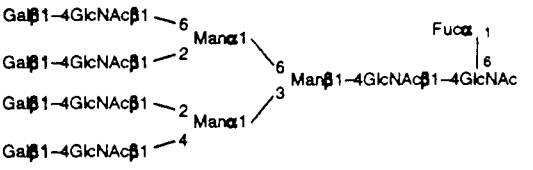
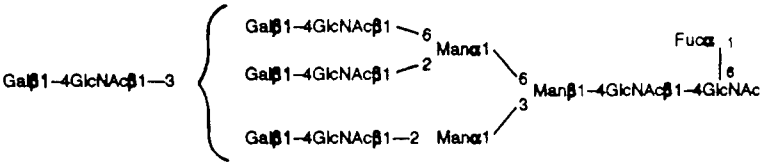
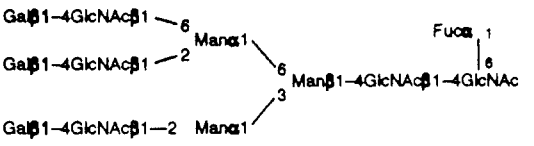
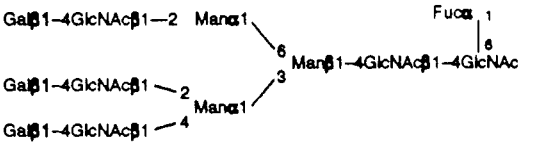
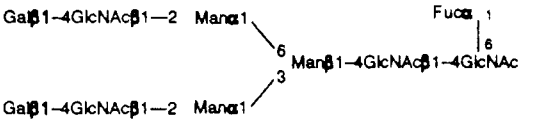
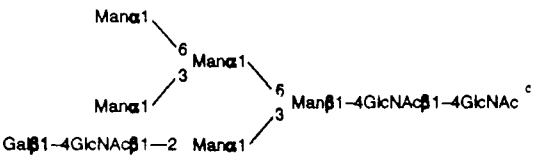
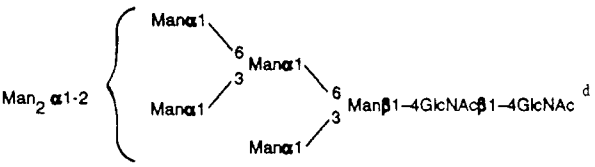
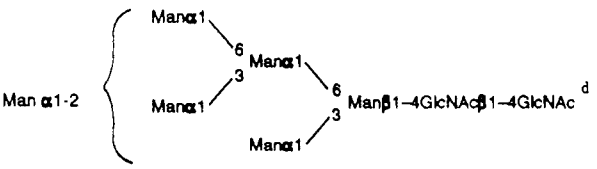
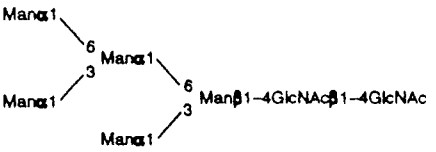
DISCUSSION

A summary of the N-linked oligosaccharide structures on recombinant prorenin suggested by the data in this report is shown in Table VI. Clearly, the two glycosylation sites on the molecule contain a number of different structures. This has frequently been shown for glycoproteins synthesized by mammalian cells [e.g., Takeuchi et al. (1988), Geyer et al. (1988), Spellman et al. (1989), and Endo et al. (1989)]. Furthermore, evidence for heterogeneity in the glycosylation of human renin has been presented previously [e.g., Pinet et al. (1985), Fritz et al. (1986), Faust et al. (1987), and Kim et al. (1991)]. More than half of the structures on the molecule are complex-type; however among these structures the majority (70%) are biantennary. Less than one-third of the complex structures on the molecule are of more extensively processed, multiantennary type, and of these structures no more than 11% (2% of total) are tetraantennary. This general distribution of oligosaccharide types is consistent with previous lectin chromatography separations of human prorenin and prorenin glycopeptides (Faust et al., 1987), synthesized by mouse L cells. Coupled with the fact that almost half (40%) of the total structures are of high mannose type or hybrid-type, this suggests that the oligosaccharides on recombinant prorenin are generally not subjected to extensive processing. Faust et al. (1987) showed that some molecules synthesized by mouse L cells contain almost exclusively high mannose type structures; this may also be the case for renin synthesized by CHO cells.

Similar to other recombinant molecules expressed in CHO cells (Sasaki et al., 1987; Takeuchi et al., 1988; Mizuochi et al., 1988), CHO-produced human prorenin contains structures with poly(*N*-acetyllactosamine) repeats, although the percentage is quite low. The limited extent of poly(*N*-acetyllactosamine) substitution may be related to the relatively low percentage of extensively processed oligosaccharides on the molecule; the β1–3-*N*-acetylglucosaminyltransferase reportedly has a preference for high branched structures (van den Eijnden et al., 1991). This transferase also prefers the 1,6-branch of complex-type structures (Sasaki et al., 1987; van den Eijnden et al., 1988). Interestingly, none of the teraantennary structures on recombinant prorenin contain poly(*N*-acetyllactosamine) repeats.

Recombinant human prorenin synthesized by CHO cells contains phosphorylated oligosaccharides. Previous work has shown that human renin synthesized by *Xenopus* oocytes and mouse L cells contains these types of structures, in varying amounts (Faust et al., 1987). The reason for this appears to be similarities in sequence (46% identity) and three-

Table VI: Proposed Structures of N-Linked Oligosaccharides Isolated from Recombinant Prorenin

Structure	Elution Volume (GU)[a]	Percent of Major Structures
	20	2 (0.9)[b]
	20	3
	17	10 (3.5)
	17	3 (1.1)
	14.5	43 (11.2)
	13	4
		16
		17
		2

[a] Glucose units after sialic acid residues and/or phosphates are removed. [b] Numbers in parentheses give the percentage of structures without core fucose. [c] Approximately 10% of the structures are phosphorylated. [d] Approximately 6% of the structures are phosphorylated.

dimensional structure between human renin and cathepsin D, a lysosomal enzyme (Tang, 1979; Faust et al., 1985). Cathepsin D, like most lysosomal enzymes, contains high mannose structures with the Man-6-P recognition signal essential for targeting of these molecules to the lysosomes (Hasilik & Neufeld, 1980; Gieselman et al., 1983). The similarities in protein structure between the two molecules apparently make renin a substrate for UDP-GlcNAc:lysosomal-enzyme *N*-acetyl-

glucosamine phosphotransferase, the enzyme responsible for the transfer of a GlcNAc–phosphate group to form a diester with certain mannose residues in high mannose and hybrid structures on lysosomal enzymes (Hasilik et al., 1981; Reitman & Kornfeld, 1981). Processing of this structure by removal of the terminal GlcNAc residue to generate a phosphomonoester, the Man-6-P recognition signal, appears also to take place. Approximately 6% of the high mannose structures and 10% of the hybrid structures on human recombinant prorenin (3% of the total structures) are phosphorylated. Thus, the total percentage of phosphorylated structures detected on these molecules is quite comparable to that found for secreted renin synthesized by mouse L cells (Faust et al., 1987). The phosphorylated structures on renin synthesized by CHO cells include both high mannose type and hybrid-type structures and phosphates in both mono- and diester linkage. Phosphates were detected on Man_6 and Man_7 structures (not on Man_5), and two phosphates were detected only on Man_7. Again, these results are in good agreement with reported data on the phosphorylated oligosaccharides synthesized by mammalian cells [e.g., Varki and Kornfeld (1980, 1983) and Gabel et al. (1982)].

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