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CHARACTERIZATION OF HUMAN FIBRONECTIN GLYCOPEPTIDES FROM CYSTIC FIBROSIS AND CONTROL SKIN FIBROBLASTS*,†

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ABSTRACT

Fibronectins isolated from different species and tissue sources are glycosylated differently. We report here a characterization of the glycopeptides of fibronectin isolated from the culture medium of skin fibroblasts from patients with cystic fibrosis together with age-, race-, and sex-matched control subjects. The characterization of this fibronectin is of special interest because it is derived from: (*1*) a non-fetal, cellular source; (*2*) eight different individuals; and (*3*) cystic fibrosis and control individuals. The fibronectin glycopeptides were purified by gel-permeation chromatography and Con A–Sepharose and were analyzed by anion-exchange chromatography and affinity columns of immobilized 5-hydroxytryptamine and lectins. One half of the glycopeptides of skin fibroblast fibronectin were shown to contain biantennary oligosaccharides which were core-fucosylated and partially sialylated. Although the remaining half was a complex mixture of glycopeptides, there was remarkably little inter-individual variation. No difference between cystic fibrosis and control subjects was discernible by the techniques employed here. Unlike the biantennary glycopeptides of human plasma fibronectin, those from skin fibroblast fibronectin were core-fucosylated and less highly sialylated. However, compared to human cellular fibronectin glycopeptides from fetal sources, those from skin fibroblast fibronectin were both more highly fucosylated and sialylated.

INTRODUCTION

The fibronectins are a class of high molecular weight glycoproteins found in the plasma and the pericellular matrix as well as on the cellular surface in many mammalian and avian species[1–3]. A number of differences in the oligosaccharide residues of human fibronectins from different sources have been described recently[4–14]. These differences seem to depend on the species, tissue, age, and physiologic state of the source of the fibronectin. Although the major residues

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appear to be biantennary, *N*-linked oligosaccharides, numerous variations of this structure have been described, including the number of oligosaccharide chains[5,8–10], the amount, linkage position, and type of sialic acids[5,7,8–12], and the degree of fucosylation[5–14]. In addition, oligosaccharides other than the predominant bi-antennary forms have been demonstrated including tri-[5,10,13,14] and tetra-antennary[5] moieties, *O*-linked oligosaccharides[10], and various polylactosaminated structures[5,10].

The carbohydrate structures of human fibronectins have been described; however, the only reports on non-fetal human fibronectin to date are for adult plasma fibronectin[6,7,9,10,13,14]. Given the differences between plasma and cellular fibronectins within one species[8,11,12], the unique structures found in human placenta[5,13] and amniotic fluid[10,14] fibronectins, and the fact that the only human fibroblast fibronectin that has been studied was from embryonic lung[7], we have characterized the carbohydrate residues of a cell-associated fibronectin from non-fetal sources. This characterization was performed using glycopeptides from fibronectin isolated from the culture medium of human skin fibroblasts. Only small amounts of material are available from a tissue-culture system; however, by extensive use of serial lectin chromatography, partial characterization of the oligosaccharides was obtained using microgram quantities of radiolabeled fibronectin.

A second aspect of this work was to extend the previous studies of glycoproteins in cystic fibrosis (CF) (refs. 15–20 and references therein) by isolating and characterizing a single glycoprotein. It was reported previously that fibronectin isolated from CF fibroblasts had altered fucosylation when compared to fibroblasts from age-, race-, and sex-matched control subjects[15]. In order to determine whether this difference represented a change in the structure of the oligosaccharides of CF fibronectin glycopeptides we have further compared glycopeptides derived from both CF and control fibroblast lines.

EXPERIMENTAL

Materials. — The following materials were obtained from the indicated sources: heparin (sodium salt), ICN Biomedicals; goat anti-human plasma fibronectin, Ig fraction, U.S. Biochemicals; gelatin, swine skin Type I 300 Bloom G-2500 and 5-hydroxytryptamine (5-HT), Sigma; Pronase, Calbiochem; Biogel P-10 and P-2, 200–400 mesh, Biorad; Con A–Sepharose, lentil lectin–Sepharose and Sepharose 4B, Pharmacia; *Arthrobacter ureafaciens* neuraminidase, E-PHA–agarose and L-PHA–agarose (special high density), E. Y. Laboratories; *Vibrio cholerae* neuraminidase, Boehringer; human plasma fibronectin, Pierce Chemical Co.; tissue-culture medium and fetal calf serum, Flow Laboratories; and L-[5,6-$^3H_2$]fucose ([^{3}H]Fuc; 60 Ci per mmol) and [6-^{3}H]- or [^{14}C]-2-amino-2-deoxy-D-glucose, ([^{3}H]GlcN; 19 Ci per mmol) or ([U-^{14}C]GlcN; 222 mCi per mmol), respectively, NEN Corp. All other materials were of reagent grade and used without further purification.

Cell growth and harvest of culture medium. — Skin fibroblasts were obtained

from posterior deltoid biopsies of individuals with CF. Biopsies were also obtained from age-, race-, and sex-matched individuals to provide control fibroblast cultures[17]. For studies of individual variations, the fibroblasts were derived from three individuals with CF (11 y, white male; 13 y, white female; 21 y, black male) and their age-, race- and sex-matched controls. All of the cells were within 8 and 15 passages of the original culture and were free of Mycoplasma[21]. The cells were grown in Eagle's Minimal Essential Medium containing 10% fetal calf serum on 75- or 150-cm^2 Falcon flasks for 7 days with fresh medium added on the third and fifth days. On the fifth day, medium containing 5 or 10 μCi of the stated isotope per 75- or 150-cm^2 flask, respectively, was added. On the seventh day, the radioactive medium was decanted from the cells and centrifuged at 800 x*g* for 15 min at 5°. The supernatant medium was stored at −40° until further processed.

The cells from each experiment were harvested and protein[22], radioactivity[15] and cell count determined. In all cases reported here, the cells had a protein and radioactivity content similar to that reported previously[15].

Heparin precipitation. — The frozen culture medium was thawed, dialyzed, and lyophilized. The powder was suspended in 1.5 mg of heparin per mL of 0.1M sodium acetate buffer, pH 5.5 (1 mL per 12.5 mL of medium) and precipitated at 4° as described[15]. The precipitate was washed twice in 0.1M sodium phosphate buffer, pH 7.0, and then resuspended in 0.05% sodium dodecyl sulfate[15]. All solutions contained 0.5mM phenylmethylsulfonyl fluoride. Typically, precipitation with heparin of 25 mL of medium provided 5000 c.p.m. of [^{3}H]Fuc-labeled precipitate, 60,000 c.p.m. of [^{3}H]GlcN-labeled precipitate, or 43,000 c.p.m. of [^{14}C]GlcN-labeled precipitate. The precipitates from medium of the different cell lines were never combined for any of the characterizations unless distinguished by isotopic label.

Polyacrylamide gel electrophoresis. — Gradient (7–14%) polyacrylamide gel electrophoresis was carried out under denaturing conditions[23] using standard proteins as previously noted[24] and human plasma fibronectin M_r = 220,000.

Two-dimensional immunodiffusion. — Ouchterlony immunodiffusion was carried out on microscope slides using 3 mL of 0.7% agarose in 0.02M sodium phosphate, pH 7.2 with 0.16M NaCl and cutting the gel with a template. The peripheral wells were loaded with up to 20 μL of goat anti-human plasma fibronectin (25 mg/mL of the same buffer). Diffusion was allowed to proceed for 4 h at 37°, and then at room temperature overnight.

Affinity chromatography on gelatin–Sepharose. — CNBr-activated Sepharose 4B was prepared[25] and allowed to react with 12 mg of porcine collagen per mL of settled beads overnight at 4° with shaking. The subsequent reaction with ethanolamine and washing have been described[25]. Greater than 70% of the protein was coupled to the gel as determined by absorbance at 280 nm in a Zeiss spectrophotometer.

Between 10^3 and 10^4 c.p.m. of the material, which was precipitated by heparin from the culture medium, was applied to a column (2.3 × 3.5 cm) of

gelatin–Sepharose in 2mM Tris, pH 8.8 with 0.02% sodium azide and 0.5mM phenylmethylsulfonyl fluoride. The column was washed stepwise with 0.1M NaCl and M NaCl, and then eluted with 4M urea, all in the Tris buffer[15].

Chromatography. — All of these experiments were carried out using glycopeptides generated by Pronase digestion[26] of the heparin-precipitated material from the fibroblast culture-medium. For analytical purposes, 100–600 c.p.m. of desalted glycopeptides were loaded onto columns and fractions were collected for the determination of radioactivity in a scintillation counter. Recoveries were always >90%. All affinity columns were standardized with glycopeptides of known binding characteristics[27,28].

To remove salts and sugars, a column (1 × 23 cm) of Biogel P-2, 200–400 mesh, in water, was eluted at a flow rate of 0.63 mL/min. A column (0.9 × 92 cm) of Biogel P-10, 200–400 mesh, in 0.05M ammonium acetate was used to size glycopeptides. Fractions of 1 mL were collected from both columns.

Affinity chromatography on a column (0.8 × 5.5 cm) of Con A–Sepharose and a column (1.3 × 1.7 cm) of lentil lectin–Sepharose was carried out as described[29–31]. Glycopeptides which were eluted from lentil lectin–Sepharose with 10mM and 0.2M methyl α-D-mannopyranoside (meMan) are referred to collectively as "lentil lectin-bound". Affinity chromatography using a column (0.3 × 60 cm) of E-PHA–agarose and a column (0.55 × 24 cm) of L-PHA–agarose has been described[29,32].

Sepharose 4B was activated[25] and coupled to 5-HT as described[33]. Affinity chromatography was performed using a column (0.8 × 5.5 cm) of 5-HT–Sepharose[28,33]. Glycopeptides eluted with 0.1M Tris buffer, pH 7.2 or Tris buffer containing 0.1M NaCl are referred to as "5-HT-Sepharose-bound". Some fractions were pretreated with neuraminidase by incubating 100–200 c.p.m. of glycopeptides with 5 mU of *V. cholerae* neuraminidase and 5 mU of *A. ureafaciens* neuraminidase for 24 h at 37°, followed by filtration on Biogel P-2 prior to chromatography on 5-HT–Sepharose.

Fractionation of glycopeptides by charge was carried out by anion-exchange l.c. as described[34] using a Varian model 5000 chromatograph with a Micropak AX-5 column (30 cm). The column was calibrated with milk oligosaccharides containing no, one, or two sialic acid residues, LNF 1, LsTc, and DSL, respectively (obtained from Drs. V. Ginsburg, NIH and D. Smith, Virginia Polytechnic Institute).

RESULTS

Characterization of the glycoprotein precipitated by heparin. — The culture medium obtained after growth of human skin fibroblasts, metabolically labeled with [^{3}H]Fuc, [^{14}C], or [^{3}H]GlcN was precipitated with heparin. The precipitated material, referred to as "heparin precipitate", was solubilized in 0.05% sodium dodecyl sulfate and partially characterized.

The [^{3}H]GlcN-labeled heparin precipitate contained only one major radio-

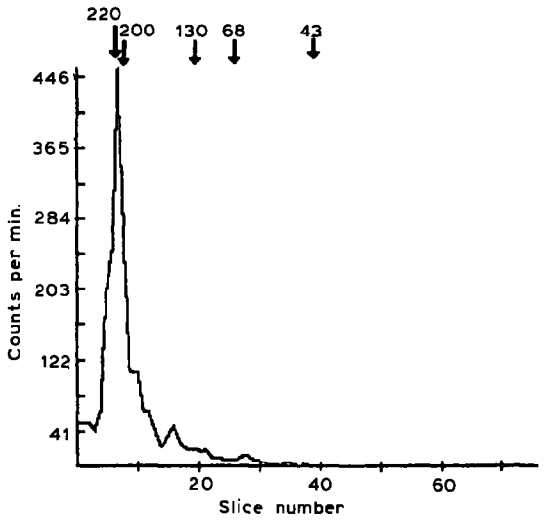


Fig. 1. Polyacrylamide gradient (7–14%) gel electrophoresis under denaturing conditions of [^{3}H]GlcN-labeled heparin precipitate. The gel was cut into 2-mm slices and the radioactivity determined by liquid-scintillation counting. The molecular weights and positions of standard proteins are indicated by numbers ($\times 10^3$) and arrows: 220, fibronectin; 200, myosin; 130, β-galactosidase; 68, bovine serum albumin; and 43, ovalbumin. See Experimental for details.

active glycoprotein, as shown by polyacrylamide gradient gel electrophoresis under denaturing conditions (Fig. 1). The mobility was identical to that of authentic human plasma fibronectin (M_r = 220,000). This finding was similar when [^{3}H]Fuc served as the metabolic label[15].

The heparin precipitates were applied to a column of gelatin–Sepharose and chromatographed as described[15]. At least 95% of the applied [^{3}H]GlcN-labeled heparin precipitate was retained by the column. Of the amount retained, ~15% was eluted with M NaCl and 80% with 4M urea.

When the heparin precipitate was subjected to two-dimensional immunodiffusion using goat anti-human plasma fibronectin, a single line of identity was formed with a sample of human plasma fibronectin. Moreover, the fractions which were eluted from the gelatin–Sepharose column with 4M urea and M NaCl also formed a single precipitation line with plasma fibronectin and the heparin precipitate. Therefore, on the basis of molecular weight, immunoreactivity, precipitability by heparin at 5°, and ability to bind gelatin, the heparin precipitate was identified as fibronectin.

Characterization of fibronectin glycopeptides. — Human fibroblast fibronectin glycopeptides were generated by Pronase digestion and characterized. The results are summarized in Table I for material derived from one matched pair of control and CF cell lines metabolically labeled with [^{3}H]Fuc and three different matched pairs of control and CF cell lines metabolically labeled with [^{14}C]- or [^{3}H]GlcN.

When [^{3}H]Fuc was used as the metabolic label, 94–95% of the radioactive

TABLE I

CHARACTERIZATION OF FIBRONECTIN GLYCOPEPTIDES[a]

Method of analysis	*Metabolic label*			
	[^{3}H]Fuc		*[^{3}H]-[^{14}C]GlcN*	
	Fibroblast lines			
	C	*CF*	*C*	*CF*
	Percent retained[b]			
Con A–Sepharose[c]	95	94	56 ±5	58 ±5
Lentil lectin–Sepharose[d]	84	81	38 ±2	34 ±2
Biogel P-10[e]	100	100	92 ±2	92 ±2
5-HT–Sepharose[f]	71	71	69 ±4	64 ±4
Anion-exchange l.c.[g]	72	77	n.d.	n.d.

[a]Obtained by Pronase digestion of fibronectin from culture medium after metabolic labeling of control (C) and CF skin fibroblasts with [^{3}H]Fuc or [^{3}H]- or [^{14}C]-GlcN. [b]The results with the [^{3}H]Fuc-labeled glycopeptides were obtained with one pair of control and CF cell lines. The results with the [^{3}H]- or [^{14}C]-GlcN-labeled glycopeptides were the mean (±) standard deviations of determinations performed on fibronectin glycopeptides derived from three sets of matched control and CF cell lines. See Experimental for details. [c]Eluted by 10mM meMan. [d]Eluted by 0.2M meMan. [e]Included in a column (0.9 × 92 cm) of Biogel P-10 in 0.05M ammonium acetate. [f]Eluted by 0.1M Tris, pH 7.2 containing 0.1M NaCl. [g]Eluted in mono-anion position.

glycopeptides was retained by Con A–Sepharose and was eluted with 10mM meMan (Fig. 2A). Thus the fucosylated glycopeptides of fibronectin were biantennary. Less than 5–6% of the total was either unbound or was eluted with 0.2M meMan. Not all of the fucosylated biantennary glycopeptides, however, fulfilled the requirements for binding to lentil lectin–Sepharose since only 81–84% of the [^{3}H]Fuc-labeled glycopeptides bound and were eluted with 0.2M meMan. Therefore some Fuc residues may not be located on the core 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc), or some other structural feature of the biantennary glycopeptides may preclude interaction with lentil lectin–Sepharose[31].

The Fuc-labeled glycopeptides were included in a column of Biogel P-10 (Table I) and were eluted in a symmetrical peak. The net-charge-distribution was ascertained by anion-exchange l.c., which showed that 72–77% of the glycopeptide radioactivity was eluted in the mono-anion position and 23–28% was eluted in the neutral position. That the negative charge was contributed by a sialic acid group was supported by the finding that 71% of the [^{3}H]Fuc-labeled glycopeptides bound to 5-HT–Sepharose and that the binding was eliminated by prior incubation of the glycopeptides with neuraminidase.

Using either [^{3}H]- or [^{14}C]-GlcN as the metabolic label produced a more-complex picture for all of the cell lines examined (Table I). Only 56–58% of the radioactivity of the GlcN-labeled glycopeptides bound to immobilized Con A and

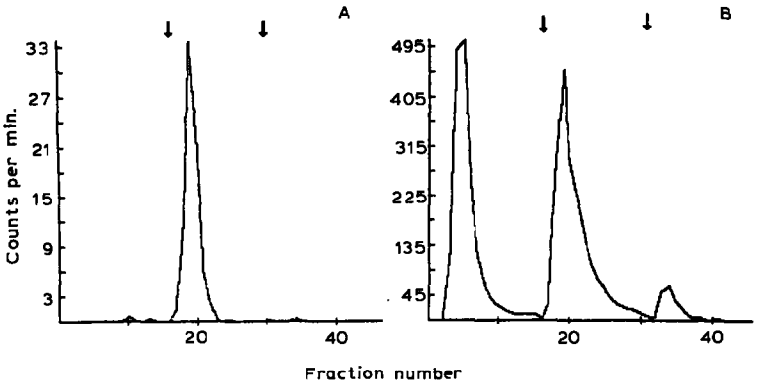


Fig. 2. Con A–Sepharose affinity chromatography of fibronectin glycopeptides from control skin fibroblast culture-medium metabolically labeled with (*A*) [^{3}H]Fuc or (*B*) [^{3}H]GlcN. The arrows, from left to right in each figure, indicate the fraction where elution with 10mM and 0.2M meMan, respectively, was begun. See Experimental for details.

was eluted with 10mM meMan (Fig. 2B). Less than 5% of the radioactivity was eluted with 0.2M meMan. The remaining radioactivity was unbound, suggesting the presence of more-complex structures. Indeed, some of these Pronase-digested glycopeptides were large since 8% of the radioactivity of the total GlcN-labeled glycopeptides was excluded from Biogel P-10. A portion (34–38%) of the radioactivity of the GlcN-labeled glycopeptides was retained by lentil lectin–Sepharose and 64–69% bound to 5-HT–Sepharose.

Fractionation of fibronectin glycopeptides. — Since the Pronase-generated fibronectin glycopeptides proved to be heterogeneous, affinity chromatography on Con A–Sepharose was used to provide Con A-bound and Con A-unbound glycopeptides for subsequent characterization. Fibronectin glycopeptides from control and CF fibroblasts, metabolically labeled with either [^{3}H]- or [^{14}C]-GlcN, were combined and fractionated on Con A–Sepharose. The Con A-unbound fraction and a fraction which was eluted with 10mM meMan (Con A-bound) were lyophilized and freed of salts or meMan by several passages over a column of Biogel P-2. The Con A-bound and -unbound fractions were then further individually characterized and these results are shown in Table II. The percentages shown are the means of the results obtained from three different sets of matched cell lines.

Less than 10% of the Con A-unbound glycopeptide radioactivity was bound to lentil lectin–Sepharose. This result was consistent with the previous observation (Table I) that the Con A-unbound fraction was not metabolically labeled with [^{3}H]Fuc. The radioactivity in this fraction was also 24–26% excluded from Biogel P-10 (Table II and Fig. 3B). Although the P-10 excluded material was enriched in the Con A-unbound fraction, it still represented only 8% of the radioactivity from the total fibronectin glycopeptides. The Con A-unbound radioactivity was further shown to be composed of 70–72% mono-anionic species and 28–30% neutral species by anion-exchange l.c. Since 61–63% of the Con A-unbound radioactivity

TABLE II

CHARACTERIZATION OF CON A–SEPHAROSE FRACTIONS OF FIBRONECTIN GLYCOPEPTIDES[a]

Method of analysis	*Con A–Sepharose*			
	Unbound		*Bound*	
	Fibroblast lines			
	C	*CF*	*C*	*CF*
	Percent retained[b]			
Lentil lectin–Sepharose	8 ±3	4 ±3	66 ±3	70 ±3
Biogel P-10	76 ±5	74 ±4	100 ±2	100 ±2
5-HT–Sepharose	61 ±3	63 ±4	68 ±2	67 ±4
Anion-exchange l.c.	70 ±2	72 ±2	68 ±3	67 ±2

[a]Obtained by Pronase digestion of fibronectin from culture medium after metabolic labeling of control and CF skin fibroblasts with [^{3}H]- or [^{14}C]-GlcN. [b]The results are the mean (±) standard deviations of determinations performed as dual-isotope experiments on three sets of matched control and CF cell lines. See Table I and Experimental for details.

bound to 5-HT–Sepharose, most of the charge may be contributed by sialic acid.

As shown in Table II, 66–70% of the radioactivity of the GlcN-labeled, Con A-bound glycopeptides was bound to lentil lectin–Sepharose. This finding was consistent with the preceding observation that 81% of the Fuc-labeled, Con A-bound fibronectin glycopeptides also bound to lentil lectin–Sepharose (Table I). In both cases, the Con A-bound glycopeptides which failed to bind to lentil lectin–Sepharose may be biantennary structures lacking core Fuc[31].

All of the GlcN-labeled, Con A-bound glycopeptides were included in a column of Biogel P-10 (Table II and Fig. 3A). Based on anion-exchange l.c. and affinity chromatography on 5-HT–Sepharose, the Con A-bound glycopeptide radioactivity was composed of biantennary residues of which 32–33% contained no sialic acid and 67–68% contained one sialic acid residue.

Sub-fractionation and subsequent characterization of Con A-bound glycopeptides. — The [^{3}H]- or [^{14}C]-GlcN-labeled, Con A-bound glycopeptides were further fractionated by chromatography on lentil lectin–Sepharose (Table II). The lentil lectin-unbound and -bound fractions were lyophilized, desalted by chromatography on Biogel P-2, and analyzed by chromatography on 5-HT–Sepharose. More of the lentil lectin-bound radioactivity was bound to 5-HT–Sepharose (65–68%) than that of the lentil lectin-unbound (17–18%).

Sub-fractionation and subsequent characterization of Con A-unbound glycopeptides. — The scheme for the serial sub-fractionation of the Con A-unbound glycopeptides by sequential chromatographic techniques and the results obtained are shown in Fig. 4. The dual-isotope glycopeptides from control and CF fibronectins which did not bind to Con A–Sepharose were desalted by chromatography

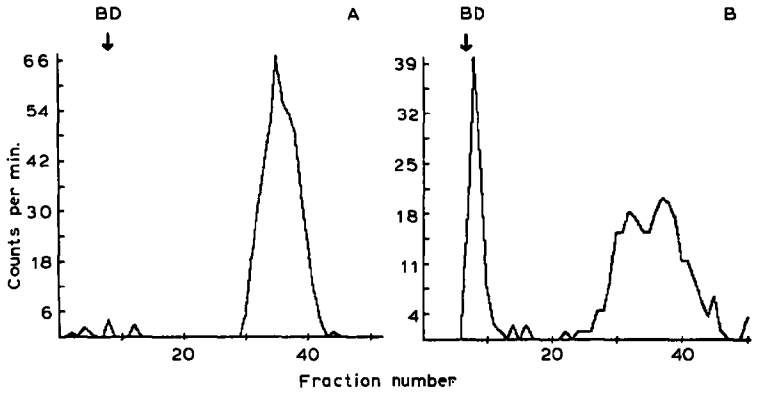


Fig. 3. Gel-permeation chromatography on Biogel P-10 of (*A*) Con A-bound and (*B*) Con A-unbound fibronectin glycopeptides from control skin fibroblast culture medium metabolically labeled with [^{14}C]GlcN. Cyanocobalamine was eluted in fractions 49–51. BD, blue dextran 2000. See Experimental for details.

on Biogel P-2 then applied to a column of Biogel P-10. The Biogel P-10-excluded fraction comprised 27–29% of the Con A-unbound radioactivity. Repeat Pronase digestion did not reduce the proportion of the Con A-unbound fraction which was excluded from Biogel P-10. This excluded fraction was desalted by chromatography on Biogel P-2 and an aliquot was applied to a column of 5-HT–Sepharose. All of the Biogel P-10-excluded material bound to the 5-HT–Sepharose column (Fig. 5A) and binding was completely eliminated by prior incubation of this fraction with neuraminidase, suggesting that it was sialylated. Because of the limited amounts of the Biogel P-10-excluded fraction, which comprised only 8% of the total fibronectin glycopeptide radioactivity, it was not further characterized.

The fraction which was included in the Biogel P-10 column was further characterized after desalting on Biogel P-2. Only 41–47% of the radioactivity in this fraction bound to 5-HT–Sepharose (Fig. 5B). This Biogel P-10-included fraction was then applied to a column of E-PHA–agarose (Fig. 4). Only 7–9% of the radioactivity (representing 2–3% of the total fibronectin glycopeptide radioactivity) was retarded, suggesting that bi- or tri-antennary glycopeptides containing the sequence GlcNAcβ1→4Manβ1→4GlcNAc were only minor components of the Con A-unbound glycopeptides[32]. Failure to bind to E-PHA–agarose was not due to the presence of Fuc on the *N*-acetyllactosaminyl branch[27] since these fractions were not fucosylated. The glycopeptides which were unbound to E-PHA–agarose were concentrated two-fold, then applied directly to a column of L-PHA–agarose (Fig. 4). Only 5–11% of the glycopeptide radioactivity (representing 1–3% of the total fibronectin glycopeptide radioactivity) was retarded. This small percentage may be tetra- or tri-antennary oligosaccharides with a 2,6-disubstituted α-mannosyl residue. The L-PHA-unbound glycopeptides may have triantennary oligosaccharides with a 2,4-disubstituted α-mannosyl residue and therefore did not bind to this lectin[32]. Thus, the L-PHA-unbound glycopeptides which contained 23–26%

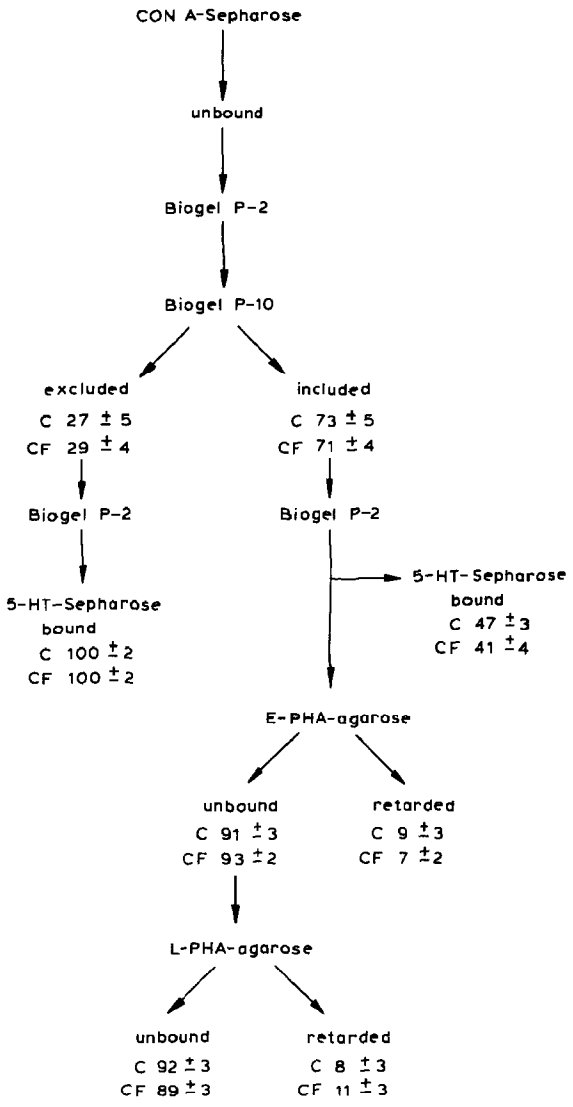


Fig. 4. Fractionation and characterization of Con A–Sepharose-unbound glycopeptides of fibronectin. The glycopeptides were obtained by Pronase digestion of fibronectin derived from the culture medium of control (C) and CF skin fibroblasts, metabolically labeled with [^{3}H]- or [^{14}C]-GlcN. The results are expressed as percentages of radioactivity applied to each column and are the mean (±) standard deviations of determinations performed as dual-isotope experiments on three sets of matched control and CF cell lines. See Experimental for details.

of the total radioactivity of the fibronectin glycopeptides remained uncharacterized with the exception of size and 5-HT–Sepharose binding.

Comparison of glycopeptides from control and CF fibroblast fibronectin. — No clear differences between the glycopeptides from control and CF fibronectin

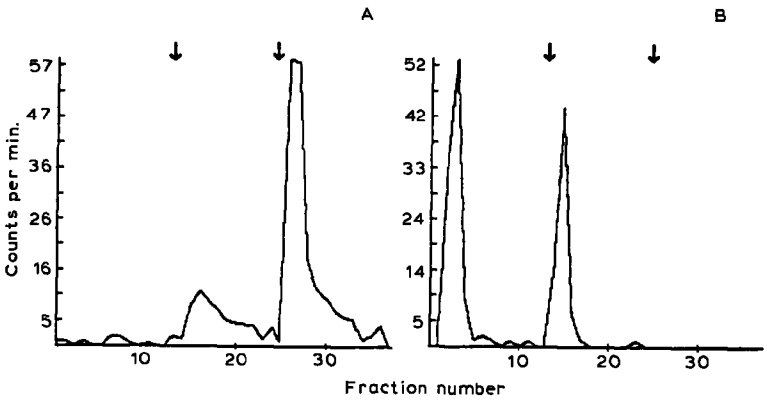


Fig. 5. Affinity chromatography on 5-HT–Sepharose of Con A-unbound fibronectin glycopeptides (see Fig. 3B) which were (*A*) excluded or (*B*) included in Biogel P-10. Fibronectin was from control skin fibroblast culture medium metabolically labeled with [^{3}H]GlcN. The arrows indicate the start of the eluting buffers. See Experimental for details.

derived from the fibroblast culture-medium could be discerned by the techniques employed in this study. At each step during fractionation and characterization, comparisons were made between control and CF fibronectin glycopeptides using four matched sets of fibroblast lines (Tables I and II, Fig. 4).

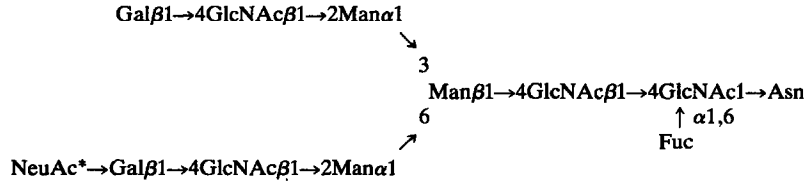
The use of these different fibroblast lines also allowed a comparison of fibronectin glycopeptides from individuals disparate for age, race, and sex. There was a remarkable similarity in the fibronectin glycopeptides, from all six (three controls and three CF) individuals, which were examined extensively. In addition, no difference was observed in two cell-lines labeled with [^{3}H]Fuc (Table I), although they were not examined as extensively as the radioactive GlcN-labeled glycopeptides.

DISCUSSION

The fibronectins are closely related glycoproteins which, nonetheless, are structurally and biologically diverse[3]. This diversity is reflected in the variations in glycosylation which depend on the species and tissue source as well as a number of other parameters[5–14]. We report here the characterization of the carbohydrate groups from human, cellular fibronectin. This fibronectin differed from others described in the literature in that it was derived from: (*1*) non-fetal cell lines; (*2*) eight different individuals, thereby permitting inter-individual comparison; and (*3*) CF fibroblast lines and age-, race-, and sex-matched fibroblast lines.

One-half of the radioactive glycopeptides from human skin fibroblast fibronectin was found to consist of biantennary oligosaccharides which were fucosylated, mostly on the core GlcNAc. About one-third of these glycopeptides contained no sialic acid whereas two-thirds contained one residue in an undefined

position. A structure can be proposed based on the results reported in Tables I and II with NeuAc arbitrarily placed on the α1→6 antenna:



The remaining half of the radiolabeled fibronectin glycopeptides which were Con A-unbound consisted of a mixture, one quarter of which were excluded from Biogel P-10. The majority of the Biogel P-10-included glycopeptides may be triantennary oligosaccharides with 2,4-disubstituted α-mannosyl residues. This conclusion was based on the results of serial lectin affinity chromatography (Fig. 4), and the absence of core Fuc (Tables I and II).

The characterization reported here was based on the fractionation of radiolabeled glycopeptides by several chromatographic techniques. As the precise carbohydrate structures of the different glycopeptides comprising the mixture are not known, it is difficult to interpret the results quantitatively. Nonetheless, these results provide valuable structural information using a limited amount of material.

Similar results were consistently found using fibronectin from six individuals; three CF and three control fibroblast lines radiolabeled with GlcN. In addition, supporting information was obtained with one CF and one control fibroblast line radiolabeled with Fuc. Although many differences between the fibronectin carbohydrates from different species and tissues have been described, they were usually based on analysis of material from a single individual or from pooled material. Given the consistency of our results, we have not described an aberrant individual nor have the data been obtained from fibronectin combined from several individuals.

The glycopeptides of human skin fibroblast fibronectin reported here are unlike those of the human plasma fibronectins in that: (*1*) they contain a larger proportion of Con A-unbound material; and (*2*) the biantennary glycopeptides are core fucosylated and partially sialylated. Moreover, though more closely resembling the other human cellular fibronectins, which are of fetal origin, skin fibroblast fibronectin has a larger complement of biantennary glycopeptides that are more-highly fucosylated and sialylated.

The human plasma fibronectins contain from 65% (refs. 7, 13) to 85% (refs. 5, 6, 10) of biantennary oligosaccharides whereas the cellular fibronectins have a smaller complement ranging from ~30% for amniotic fluid fibronectin[10], embryonic lung fibroblasts[7] and the gelatin-binding chymotryptic fragment of placental fibronectin[5], to 72% for the gelatin-unbound fragment of placental fibronectin[5]. The human plasma fibronectins contain little[5] or no[6,7,9,10,13,14] Fuc, but the various cellular fibronectins generally are fucosylated[5,7,10,14] on the core

*This group may be present or absent.

GlcNAc[7,10,14]. The human plasma fibronectins, however, are more highly sialylated than the cellular forms containing at least one mol of sialic acid per mol of bi-antennary oligosaccharide[5,7,10,14] and as much as 2 mol per mol[9]. The sialic acid is usually linked to the 6 position of the terminal D-galactose in plasma fibronectin[9–11] and to the 3 position in the cellular fibronectins[9,11,14] although other linkages have been demonstrated[9,11,14].

Variability has also been described in the Con A-unbound oligosaccharides from the human fibronectins. Although human plasma fibronectin contains mainly biantennary oligosaccharides[9,10,14], Con A-unbound moieties have been identified as triantennary oligosaccharides[10,13]. Triantennary oligosaccharides have also been described in amniotic fluid fibronectin[10,14]. Polylactosaminated bi- and tetra-antennary oligosaccharides have been detected in fibronectin from human placenta[5], and amniotic fluid[10]. The source and significance of the variation in glycosylation is unknown. Although differences in the functional and biochemical properties of fibronectins are described[3], there are few instances where the changes seem to be directly related to glycosylation[4]. The variety of glycosylation patterns of the fibronectins remains an intriguing natural experiment.

By the methods of analysis used here, no differences in glycosylation could be demonstrated between the fibronectins of CF and control fibroblasts. We have not ruled out, however, several parameters which could be responsible for the differences in fucosylation observed when the CF and control fibroblast fibronectins metabolically labeled with [^{3}H]Fuc[15] were compared. An even more pronounced difference in fucosylation, as indicated by different specific activities (c.p.m. per nmol Fuc) and affinity for immobilized lentil lectin, was demonstrated between CF and control fibroblast membrane glycopeptides[16]. Interestingly, the difference in binding to lentil lectin–Sepharose observed in the membrane glycopeptides was not observed among the fucose-labeled glycopeptides from fibronectin (Table I). This finding suggested that the oligosaccharides from the many membrane glycoproteins do not reflect those of a single glycoprotein. Alternatively, new glycoproteins may be present in the CF fibroblast membranes. However, only one glycoprotein, M_r = 190,000 was noted as different in previous experiments examining proteins of isolated surface membranes[18]. To aid in resolving this issue, fibronectin and membrane glycopeptides will be prepared in larger quantities and analyzed by high-resolution proton magnetic resonance spectroscopy[35].

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