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Note

Purification and partial characterization of fucose-rich glycopeptides from normal human pancreatic juice*

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Pancreatic juice is known to contain a large number of proteins[1,2], tissue-specific antigens[3], digestive enzymes, peptides, nucleic acids and glycoconjugates[4] (for reviews see refs. 5 and 6). Interest in the glycoconjugates of the pancreas and the secretion of this organ has recently increased considerably, especially because of the changes which occur in certain disease states including cancer[7,8] and pancreatic fibrosis[9]. While some knowledge of the chemical properties and metabolism of the glycoconjugates from animal pancreatic secretions is available[10–14], only a small number of studies have been reported on these constituents of the human secretion[1,2,15–19]. The present paper describes the purification and partial characterization of fucose-rich glycopeptides from human pancreatic juice.

EXPERIMENTAL

Starting material. — Human pancreatic juice (3.1 L) was obtained from three patients after partial excision of the pancreas‡ because of bile duct cancer. The secretion was collected through a Teflon tube that had been inserted into the main duct of the tail of the pancreas and connected to a bottle which was placed in an ice bath. The bottle contained 10 mL of buffer *A* (mM phosphate buffer, pH 7.3, 0.1M 6-aminohexanoic acid, 0.1mM phenylmethylsulfonyl fluoride, 10mM EDTA, 2mM iodoacetamide, 5mM benzamidine hydrochloride, and 10mM kanamycin) and 0.25 mL of toluene. The juice was removed every h over a period of 2 days, centrifuged

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‡The "tail" of the pancreas and the "head" of this organ were demonstrated by histological examination to be normal.

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at 4°, and dialyzed in the cold against buffer *A*. Dialysis tubing with a molecular weight cutoff of 1,000 (Fisher) was used throughout this study. After 3 days, the non-dialyzable fraction was lyophilized. The resulting pooled preparations (13 g) were suspended in buffer *A*, stirred gently for 3 h, and centrifuged at 30,000 r.p.m. for 1 h. The supernatant solution, the starting material for the present study, was used for the isolation of the pancreatic glycopeptides. The precipitate was stored at −20° for future investigation.

Reagents. — Phenylmethylsulfonyl fluoride and molecular-weight markers (dextran sulfates) were purchased from Sigma and the boronate affinity gel, Matrex Gel PBA-30, from Amicon. *Charonia lampas* α-L-fucosidase was obtained from Seikagaku Kogyo Co., Tokyo. All other reagents were commercial products of analytical grade.

Chromatographic procedures. — The essential details of these methods are included in the legend to Fig. 1. All chromatographic techniques were carried out at 4°.

Analytical high-voltage electrophoresis. — Electrophoresis of the glycopeptides (40 V/cm, 90 min) was carried out at pH 2.5 in 1% formic acid and pH 9.5 in 0.04M borate buffer[20] utilizing Whatman no. 1 chromatography paper. The electrophoretograms were stained with ninhydrin after neutralization of the borate buffer by spraying with 5% acetic acid in acetone.

Analytical procedures. — Total neutral sugar was quantified by the phenol–sulfuric acid method[21]. The content of each monosaccharide was measured by g.l.c. (JEOL, Model JCG-20KEP, 1% ECNSS-M on Gas-Chrom Q, temperature gradient of 2°/min from 150 to 200°) after hydrolysis of the glycopeptide with M HCl for 2 h at 100° and derivatization of the monosaccharides formed as alditol acetates[22]. The total hexosamine content (4M HCl, 4 h, 100°) was determined by the indole procedure[23], whereas the amount of each hexosamine was measured with the aid of a JEOL amino acid analyzer (Model JLC-6AH). Sialic acid was measured by the Warren method[24] and sulfate by the rhodizonic acid technique[25].

Fig. 1. Purification and partial characterization of glycopeptides from human pancreatic juice. *1a.* Chromatography of pooled pancreatic juice on a DEAE-cellulose column (3 × 40 cm, equilibrated with buffer *A*, flow rate 24 mL/h, 10 mL/test tube). After elution of Fraction A, a linear ionic-strength gradient of NaCl from 0.0 to 0.3M in the same buffer was utilized to displace fractions B and C. The effluents of this and the experiments indicated in frame 1b were monitored at 280 and 490 nm (phenol–sulfuric acid method for total neutral hexoses), while those of the experiments indicated in frames 1c and 1d were analyzed only at 490 nm. *1b.* Fraction A was next chromatographed on a CM-cellulose column (3 × 40 cm, equilibrated with buffer *A*, flow rate 24 mL/h, 10 mL/test tube). After elution of fraction $A^{(1)}$, the retained components were displaced by using a linear ionic-strength gradient of NaCl from 0.0 to 0.5M. *1c.* Fraction $A^{(1)}$ after further purification by gel filtration through Sephadex G-100 and G-50, was chromatographed on an AG 50W-X2 column (1.8 × 36 cm, 200–400 mesh, mM pyridine–formate buffer, pH 2.8, flow rate 24 mL/h, 5 mL/test tube). This column was first washed with the same buffer and then eluted with a linear ionic-strength gradient (0.001 to 0.2M pyridine–formate buffer) at the same pH and subsequently with a pH gradient (pH 2.8 to 5.4) at the ionic strength of 0.2 (pyridine–formate and pyridine–acetate). *1d.* Chromatography of glycopeptide fraction GP-1 (frame 1c) was carried out on a Matrex Gel PBA-30 column (1.6 × 28 cm, equilibrated with 0.05M ammonium acetate adjusted to pH 9.2, flow rate 30 mL/h, 5 mL/test tube). The glycopeptides were eluted with the same buffer followed by a pH gradient from pH 9.2 to 4.5 using 0.05M ammonium acetate–acetic acid buffer.

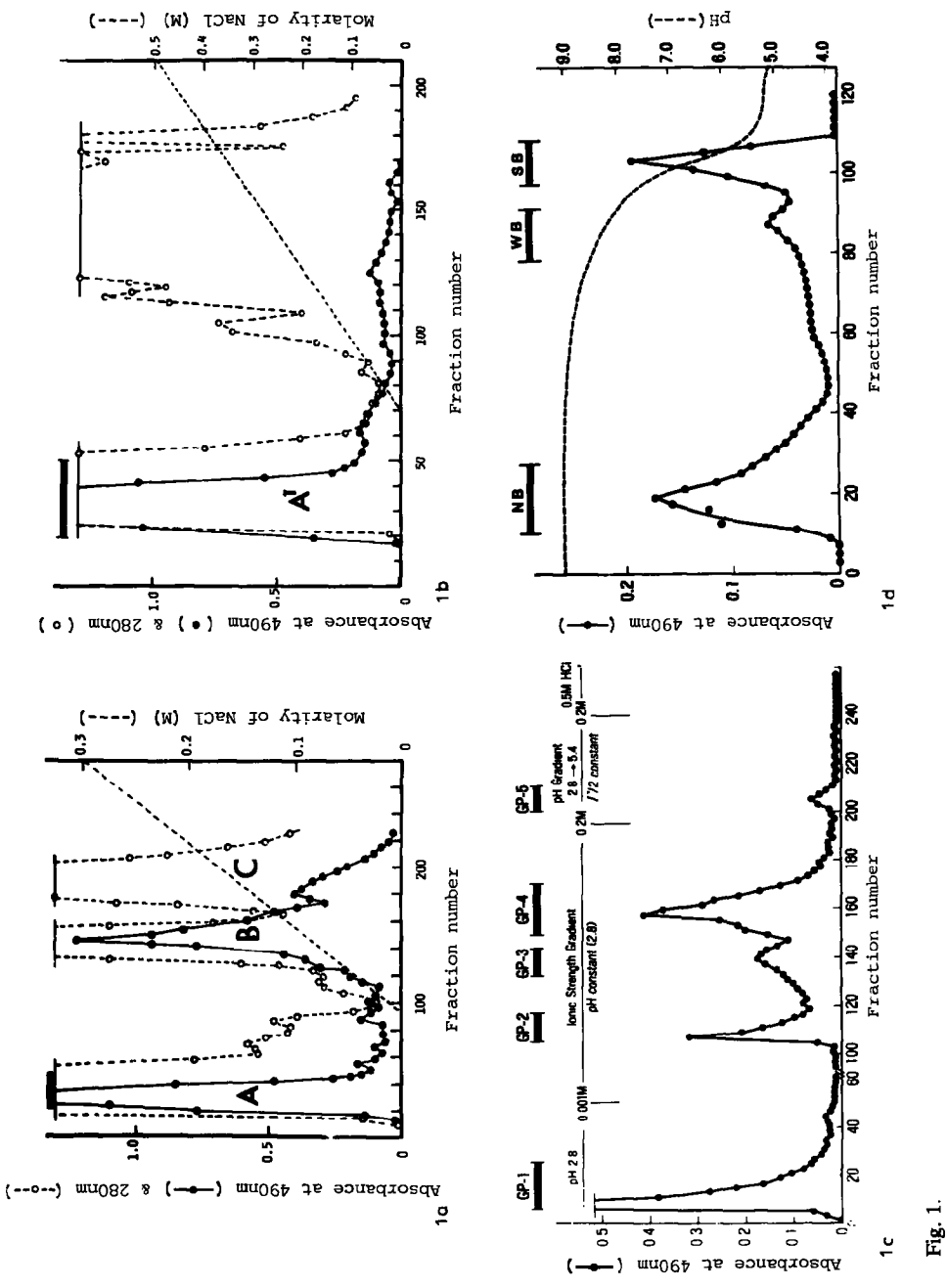
Absorbance at 490nm (—●—) & 280nm (---○---)
Molarity of NaCl (M) (----)
Fraction number
A
B
C
1a
Absorbance at 490nm (●) & 280nm (○)
Molarity of NaCl (M) (----)
Fraction number
A'
1b
Absorbance at 490nm (—●—)
pH (----)
Fraction number
NB
WB
SB
1d
Absorbance at 490nm (—●—)
Fraction number
GP-1
GP-2
GP-3
GP-4
GP-5
pH 2.8
0.001M
Ionic Strength Gradient
pH constant (2.8)
0.2M
pH Gradient
2.8 → 5.4
I/2 constant
0.5M HCl
0.2M
1c

Fig. 1.

Amino acid analysis of the hydrolyzed glycopeptides (constant boiling HCl, 20 h, N_2, sealed tube, 110°) was performed with the amino acid analyzer mentioned. The polypeptide content was calculated from the amino acid composition.

The carbohydrate–peptide linkage. — The type of linkage between carbohydrate and polypeptide chain was assessed by β-elimination as described by Carlson[26]. The resulting hydrolyzate was acidified, desalted by gel filtration through Sephadex G-25, and the non-retained fraction analyzed for amino acids.

RESULTS

Isolation of glycopeptides. — For this purpose, a five-step chromatographic procedure was employed. *1.* Chromatography of the starting material on DEAE-cellulose resulted in three fractions (Fig. 1a). Fraction A, which was not retained and contained the glycopeptides, was dialyzed against buffer *A* for the subsequent fractionation. The retained material (fractions B and C) was eluted with an ionic-strength gradient and consisted of proteins and glycoproteins which were stored at −20° for future studies. *2.* Fraction A was chromatographed on a column of CM-cellulose (Fig. 1b). Fraction $A^{(1)}$ was not retained and was dialyzed and lyophilized. Subsequent elution with a linear ionic-strength gradient displaced carbohydrate-free material. *3.* Fraction $A^{(1)}$ was then passed through a column of Sephadex G-100. The resulting hexose-containing fraction was well separated from the carbohydrate-free material. After concentration, this fraction was passed through a column of Sephadex G-10 in order to remove additional carbohydrate-free material

TABLE I

CARBOHYDRATE AND AMINO ACID COMPOSITIONS OF SIX HIGHLY PURIFIED GLYCOPEPTIDES DERIVED FROM HUMAN PANCREATIC JUICE

Residues	*Glycopeptides*					
	GP-1-NB	*GP-1-WB*	*GP-1-SB*	*GP-2-SB*	*GP-3-SB*	*GP-4-SB*
Monosaccharides						
Man[a]	3.0[b]	3.0	3.0	3.0	3.0	3.0
GlcN	4.0	4.9	6.4	6.8	5.4	4.0
Gal	3.0	5.1	7.3	7.2	5.0	3.1
Fuc	2.6	4.0	4.7	4.5	3.6	3.1
Amino acids						
Asx[a]	1.0[b]	1.0	1.0	1.0	1.0	1.0
Thr	0.8	0.5	0.2	0.5	0.8	1.4
Gly	0.9	0.4	0.1	0.6	0.8	1.2

[a]The following abbreviations are used: Man, mannose; GlcN glucosamine; Gal, galactose; Fuc, fucose; Asx, aspartic acid plus asparagine; Thr, threonine, and Gly, glycine. [b]Contents of the sugars are expressed in mol per 3 mol of Man and those of the Thr and Gly in terms of mol per mol of Asx (assumed). These values are not corrected for losses due to hydrolysis. For further information pertaining to the chemical composition of these glycopeptides see text.

(elution patterns not shown). *4.* The carbohydrate-containing fraction was next chromatographed on an AG 50W-X2 column (Fig. 1c), and the resulting five hexose-containing fractions, designated GP-1 to GP-5, were lyophilized. Fraction GP-5 was not further investigated because of its low yield. *5.* Final purification was achieved by boronate affinity-gel chromatography, which resolved fraction GP-1 into 3 subfractions (Fig. 1d) designated GP-1-NB (not bound to the gel), GP-1-WB (weakly bound), and GP-1-SB (strongly bound). Fractions GP-2, GP-3 and GP-4, which proved to be strongly bound to the borate gel, were eluted with the same pH gradient and afforded in each case a major peak designated GP-2-SB, GP-3-SB, and GP-4-SB, respectively. After lyophilization, each fraction was desalted with the aid of a Sephadex G-10 column. The recovery of these fractions was found to be 19.5, 16.2, 11.9, 4.1, 6.6, and 9.6 mg for glycopeptides GP-1-NB, GP-1-WB, GP-1-SB, GP-2-SB, GP-3-SB, and GP-4-SB, respectively.

Assessment of homogeneity. — The six glycopeptide fractions appeared homogeneous on high-voltage electrophoresis in the aforementioned system.

Chemical composition of the glycopeptides. — The carbohydrate composition of the six highly purified glycopeptides (Table I) revealed the following interesting findings: Each glycopeptide possessed a high content of fucose, but was devoid of sialic acid, galactosamine, and sulfate. The peptide moiety of each glycopeptide accounted for 3.4–19%. As to their amino acid compositions, aspartic acid, threonine, and glycine were found to be predominant (Table I). Small amounts of 2 to 6 other amino acids were also present, indicating the action of unspecific proteases. The molecular mass of these peptides were found to range from 3,500–5,000, as judged by chromatography on columns of Sephadex G-50 or G-75 using appropriate molecular-weight markers.

Carbohydrate–peptide linkage. — Before and after β-elimination the amino acid compositions of these glycopeptides were essentially unchanged. Therefore, the carbohydrate moiety is probably *N*-glycosylically[27–29] bound to the peptide chain. The absence of galactosamine in these glycopeptides (Table I) is in agreement with this conclusion.

Fucose linkage. — The glycopeptides after treatment with α-L-fucosidase[30] were chromatographed on a column of Sephadex G-50. The fucose residues of each glycopeptide were found to be essentially completely released. It was concluded that these residues are in terminal positions.

DISCUSSION

This is the first report on the isolation and partial characterization of glycopeptides from human pancreatic juice. These compounds are characterized by their unusually high fucose content and lack of sialic acid, galactosamine, and sulfate. The fucose content may account for 3–5 residues per oligosaccharide unit, assuming the presence of N-glycans. It should also be noted that these peptides were probably formed by proteolytic digestion of a glycoprotein(s) during secretion

rather than during collection, because the pancreatic juice was collected at a temperature close to 0° and in presence of several protease inhibitors. Therefore, it appears that the pancreatic glycoprotein(s), from which these fucose-rich glycopeptides was derived, perhaps belongs to a new class of conjugated proteins with an as yet unknown biological role(s).

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