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CHARACTERIZATION OF TWO GLYCOPROTEINS OF HUMAN PANCREATIC JUICE: P35, A TRUNCATED PROTEASE E AND P19, PRECURSOR OF PROTEIN X

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Four glycoproteins were separated by SDS-polyacrylamide gel electrophoresis of proteins of human pancreatic juice devoid of free proteolytic activity. The two low molecular weight glycoproteins were isolated and characterized. Protein P19, the precursor family of protein X, was analyzed by its carbohydrate content which seemed to play an important role in protein solubility at pH 8.0. Protein P35was found to be a Con A-binding protein rich in mannose. Its N-terminal amino acid sequence covering 33 residues revealed a strong homology with human protease E without the dipeptide Val-Val. Is P35 a protein homologous to the subunit III of bovine procarboxypeptidase A ?

If the protein composition of human pancreatic juice has been extensively studied and is now well elucidated (1), little is known about glycoproteins. During the purification of pancreatic proteins, we have characterized two lipolytic enzymes as glycoproteins : carboxylester hydrolase of 100 kDa which presents a high content of sugars (20 % in weight) (2) and lipase of 48 kDa which separates in two glycosylated isolipases containing only glucosamine and neutral sugars (3). Moreover, human pancreatic kallikrein, a minor component of pancreatic juice of 35 kDa was found to give a positive staining with the Schiff reagent (4). By two dimensional gel electrophoresis, Scheele et al. have separated five glycoproteins giving a positive staining with the Schiff reagent (5)but except lipase, the four other glycoproteins were not identified.

In this paper we report the characterization of two glycoproteins, a protein of 19 kDa (P19) whose carbohydrates content seems to play an important role in protein solubility and a protein of 35 kDa (P35) not yet identified in human pancreatic juice.

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MATERIAL AND METHODS

Material. Human pancreatic juice was collected by catheterization of the main pancreatic duct after surgery for biliary or pancreatic diseases and lyophilise . Only samples devoid of free proteolytic activty measured by chymotrypsin assay on N-acetyl-L- tyrosine ethylester as substrate, were used.

Polyacrylamide gel electrophoresis. Gel electrophoresis was performed in slab gel in 15 % polyacrylamide in the presence of 0.1 % sodium dodecyl sulfate according to the method of Laemmli (6) without treatment with 2-mercaptoethanol. Proteins were stained by Coomassie Blue R 250. Glycoproteins were identified with periodic acid Schiff reagent according to the method of Zacharius et al. (7). Glycoproteins with affinity for Con A were detected by affinoblotting after electrophoretic separation followed by transfer to nitrocellulose paper and specific reaction with Con A as described by Faye et al. (8).

Carbohydrate analysis The identification and estimation of carbohydrates were performed after methanolysis (methanol/0.5M HCl,80°C, 24 h) by gas-liquid chromatography of trifluoroacetylated methyl glycosides (9).

Sequence determination. Amino acid sequencing was performed with an Applied Biosystem (model 470 A) gas phase sequencer.Phenylthiohydantoin derivatives of amino acids were analyzed by HPLC using a C-18 column (Brownlee, 5 µm, 2.1 x 220 mm) (10).

Purification of proteins P19 and P35 .The first step of purification of proteins P19 and P35 was a chromatography of human pancreatic juice on DEAE-Trisacryl at pH 8.0 in the presence of high amounts of trypsin inhibitors (1 mM benzamidine and lima bean inhibitor, 5 % of protein weight) as described before (11). Then, fractions containing P19 and P35 determined by SDS polyacrylamide gel electrophoresis were purified separately. Fractions containing P19 were submitted to a chromatography on CM-Sephadex at pH 6.5 as described for protein X purification (11). Purified P19 migrated like a family of proteins of close molecular weight, heterogeneity due to the presence of carbohydrates . P35 was purified by an affinity chromatography on Con A-Ultrogel (IBF) equilibrated in a 20 mM Tris, 0.5 M NaCl buffer pH 7.6 containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$. Protein P35 was eluted by the addition of 0.2 M α-D-mannopyranoside to the chromatography buffer, but with a low yield. In some cases, fractions containing P35 eluted from DEAE-Trisacryl were purified using the Pharmacia FPLC system in a Mono-S column equilibrated in a 50 mM Mes buffer pH 6.5 and eluted by a NaCl concentration gradient.

RESULTS

Characterization of glycoproteins of human pancreatic juice

Fig. 1 shows the electrophoresis pattern of proteins of human pancreatic juice separated on polyacrylamide slab gel in the presence of SDS. As shown by Coomassie staining, 11 protein bands were separated according to their molecular weight. After staining with the Schiff reagent, a pink reaction was observed with four protein bands with the respective molecular weights of 100 kDa, 48 kDa, 35 kDa and 19 kDa. When glycoproteins were characterised by affino-blotting with Con A, two out of the four glycoproteins were positive, one of them corresponded to lipase with a molecular weight of 48 kDa and the other glycoprotein was a protein of 35 kDa.

Carbohydrate content of P19 and P35

The carbohydrate analysis of proteins P19 and P35 is given in table I, calculated on the basis of 3 mannoses. While P19 contains all neutral and amino sugars, P35 contains only mannose, galactose and glucosamine.

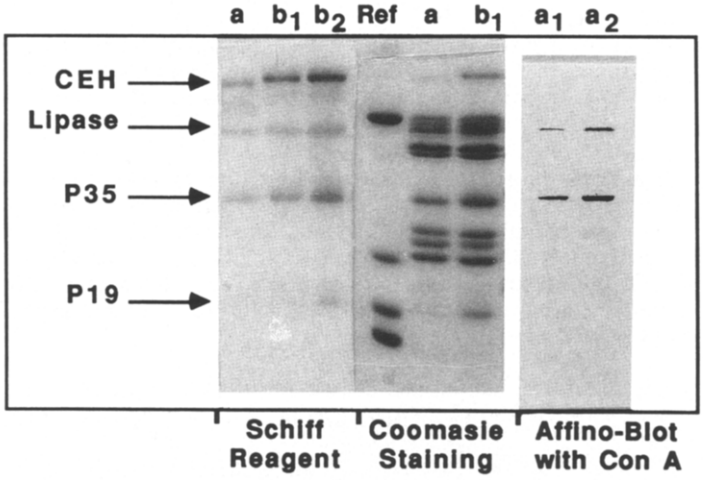

Figure 1 : Polyacrylamide slab gel of proteins of human pancreatic juice after electrophoresis in the presence of SDS and different stainings.
a and *b* are two samples of pancreatic juice loaded with different amounts: *a* , 14 µg ; a_1 , 1 µg ; a_2 , 2 µg ; b_1 , 18 µg ; b_2 , 36 µg. *Ref.* represents the reference proteins: albumin (66 kDa), bovine trypsinogen (24 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.3 kDa).

N-terminal sequence of P35

The N-terminal sequence of P35 determined on 33 residues is given in fig. 2 with five unidentified residues in position 1, 23, 26, 27, 28. The N-terminal residue could not be identified because of the presence of some free amino acids in the sample of protein.

DISCUSSION

Four glycoprotein bands have been characterized after separation by SDS polyacrylamide gel electrophoresis of proteins of human pancreatic juice devoid of free proteolytic activity. The same pattern was obtained by Scheele et al. after one dimensional analysis of human pancreatic proteins.

Table I

Carbohydrates Analysis of Proteins P 19 and P 35

Carbohydrates	P 19	P 35
Mannose	3.00	3.0
Galactose	2.00	2.1
Fucose	2.00	-
Glucose	2.80	-
Galactosamine	1.20	-
Glucosamine	0.45	2.7
Sialic acid	0.00	0.0

(calculated on the basis of 3 mannose residues)

X - Gly - Glu - Asp - Ala - Val - Pro - Tyr - Ser - Trp - Pro- Trp - Gln -
1 10

Val - Ser - Leu- Gln - Tyr - Glu - Lys - Ser - Gly - X - Phe - Tyr - X -
20

X - X - Gly - Gly - Ser - Leu - Ile -
30

Figure 2 : N-terminal sequence of protein P35.

After a two-dimensional urea isoelectric focusing/SDS gel electrophoresis (5) the authors have separated one additional minor component with a molecular weight slightly lower than that of P35 . It may correspond to kallikrein (4). The two high molecular weight glycoproteins of 100 kDa and 48 kDa that we have separated correspond to carboxyl ester hydrolase and lipase characterized before (2, 3) .

The band of 19 kDa contains the family of glycoproteins P19 that are the precursors of the protein X of 14 kDa which was isolated from slightly autoactivated pancreatic juice (11) and which was found homologous to pancreatic thread protein (12) and to a form of pancreatic stone protein (13). Glycoproteins P19 present in native pancreatic juice (devoid of free proteolytic activity) generate protein X by proteolysis after normal or pathological spontaneous trypsinogen activation (11, 14). The results of carbohydrate analysis indicate that the transformation of P19 into protein X corresponds to the liberation of a small glycopeptide of approximately 5 kDa since protein X was found devoid of sugars by the Schiff reagent as well as by carbohydrate analysis. The carbohydrate moiety seems inefficient to protect proteins P19 from proteolytic degradation as reported for many glycoproteins (15). However it could play an important role in protein solubility at pH 8.0 since P19, the native proteins are soluble in pancreatic juice and chromatography fractions while proteinX precipitates easily after its liberation in activated pancreatic juice or chromatography fractions (11).

The Con A-binding glycoprotein P35 has been characterized by its N-terminal sequence determined on 33 residues. It corresponds to the N-terminal sequence of human protease E recently determined by Shen et al. (16) without the dipeptide Val-Val. This is the first characterization of a truncated protease E in human pancreatic juice. In this respect P35 is highly homologous to the subunit III of procarboxypeptidase AS6 complex present in bovine (17) and some other ruminants (18) but with a higher molecular weight probably due to the presence of carbohydrates . The characterization of this novel human pancreatic protein suggests the coexistence, in human like in bovine, of two proteins closely related, protease E and the protein P35 homologous to the subunit III of procarboxypeptidase A which would be free in human juice.

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