Human fetoacinar pancreatic protein: an oncofetal glycoform of the normally secreted pancreatic bile-salt-dependent lipase

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A fetoacinar pancreatic protein (FAP) associated with the ontogenesis, differentiation and oncogenic transformation of the human exocrine pancreas has been purified from pancreatic juices of patients suffering from pancreatitis or duodenal cancers invading the pancreas [Escribano and Imperial (1989) *J. Biol. Chem.* **264**, 21865–21871]. This protein has striking similarities, i.e. M_r , amino acid composition and N-terminal sequence, to the bile-salt-dependent lipase (BSDL) of normal human pancreatic secretion. The aim of this study was to gain further insight into the nature of the two proteins. Reactivity with the mouse monoclonal antibody J28 (mAb J28), which characterizes FAP, and enzyme activity could not be dissociated during biochemical purification of BSDL. Furthermore, a polyclonal antiserum raised against purified human BSDL reacted completely with FAP in Western-blot analysis giving additional support to the idea of similar molecular structures for BSDL and FAP. However, by the same technique, mAb J28

reacted with a relatively restricted population of BSDL molecules. The classical BSDL preparation could be separated into molecules bearing the J28 epitope and those devoid of it by immunoaffinity on immobilized mAb J28. The two subpopulations had identical N-terminal sequences and some differences in their amino acid compositions. However, they had different carbohydrate compositions. J28-epitope-bearing molecules were active on BSDL substrates, although their specific activity was decreased. These results are consistent with the existence of two closely related polypeptide chains with different glycan counterparts. Therefore, if the name FAP is reserved for molecules bearing the J28 epitope, which is linked to a carbohydrate-dependent structure, FAP could represent an oncofetal-related variant of BSDL. Our result is the first demonstration of the existence of an oncofetal-type subpopulation of an otherwise normally secreted human pancreatic enzyme.

INTRODUCTION

The fetoacinar pancreatic protein (FAP) is a specific component of pancreatic acinar cells which is associated with the ontogenesis and development of the human pancreas. FAP was further defined as a member of the oncodevelopment-associated pancreatic antigens and was first identified using polyclonal antisera in hamster [1–3] and later in human [4]. FAP has been further characterized with a monoclonal antibody (mAb J28) [5]. The earliest expression of FAP is seen in undifferentiated mesenchymal cells and in nascent acini at the beginning of the morphological differentiation of the pancreas (9–10 weeks gestation). Maximal synthesis occurs at the time of intense acinar cell proliferation (15–25 weeks of gestation); it declines progressively thereafter [6] and reaches, in adults, levels far lower than those observed in fetuses [6,7]. Serum FAP determination provides a good test for pathologies of the pancreas and becomes an almost absolute requirement for the detection of cancer when combined with that of another marker, CA 19/9 [8]. FAP is also found in amniotic fluids and, in relatively high concentrations, in pathological pancreatic juices [9]. The latter source was used to purify FAP which was identified as a single-chain polypeptide glycoprotein, heterogeneous in Western blots, consisting of a main component at M_r 110 000 and a polydispersion in the range M_r 60 000–100 000. It is an acidic protein (pI 4.2–4.8) with no free thiol groups and a high proline content. The carbohydrate

moiety of FAP is probably responsible for the observed M_r dispersion [10].

Among the secreted proteins characterized in normal human pancreatic juice [11,12], five are glycosylated, and only glycoprotein G1 [11] has similar molecular properties (i.e. M_r and pH_i) to FAP. This G1 protein is probably the carboxylic ester hydroxylase found in the normal secretion of human pancreas [13]. This carboxylic ester hydroxylase (EC 3.1.1.1), also referred to as cholesterol ester acyl hydrolase (EC 3.1.1.13) or bile-salt-dependent lipase (BSDL; [14]), has striking similarities to FAP. From the literature, it appears that the two proteins have similar amino acid compositions and the same N-terminal sequences [10,15]. Pancreatic BSDL (which has a wide specificity) also has an M_r of 100 000, a high content of proline and is highly glycosylated [13]. It is found in the pancreatic secretion of all species so far examined [16] as well as in the milk of some primates [17] and carnivores [18], where it has been shown to be essential for newborn growth [19]. The enzyme is also present in sera of patients suffering from pancreatitis [20,21]. Nevertheless this protein has been demonstrated to be a normal enzyme of pancreatic secretion, in contrast with FAP which is an oncofetal-related protein.

Therefore FAP, characterized by the mAb J28, could be an oncofetal form of BSDL. In this work we have compared immunochemical, molecular and enzymic properties of FAP and BSDL purified from pancreatic secretions.

Abbreviations used: BSDL, bile-salt-dependent lipase (EC 3.1.1.–); FAP, fetoacinar pancreatic protein; mAb, monoclonal antibody; pAs, polyclonal antiserum.

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MATERIAL AND METHODS

Chemicals

The sources of reagents used in the study were: alkaline phosphatase-labelled goat antibodies against mouse or rabbit IgG, 4-nitrophenyl acetate, 4-nitrophenyl hexanoate, Nitroblue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, Sigma (St. Louis, MO, U.S.A.); sodium taurocholate, Calbiochem (La Jolla, CA, U.S.A.); nitrocellulose membrane, Schleicher and Schuell (Dassel, Germany); CM-Sepharose and Ultrogel ACA54, Pharmacia (Uppsala, Sweden) and IBF (Villeneuve-la-Garenne, France) respectively. All other chemicals were of the best grade available.

Protein purification

Human pancreatic juices were collected by catheterization of the pancreatic duct from patients after either gastric or colonic surgery. BSDL was purified from normal pancreatic juices devoid of free proteolytic activity by classical physicochemical methods [13]. FAP was purified from pathological pancreatic juices as described by Escribano and Imperial [10]. Because of the strong inhibition effect of di-isopropyl phosphorofluoridate on BSDL activity [13,14], it was replaced by phenylmethanesulphonyl fluoride in FAP purification in this study. Proteins were assayed routinely using the micro-BCA kit from Pierce (Rockford, IL, U.S.A.).

Antibodies

Mouse mAb J28 directed against FAP was obtained as described previously [6,8]. A polyclonal antiserum against BSDL (pAs L64) was raised in rabbits [13].

SDS/PAGE and Western-blot analysis

SDS/PAGE was carried out in 10% (w/v) polyacrylamide slab gels as described by Laemmli [22]. After migration, proteins were visualized with either Coomassie Blue or silver stain or electrophoretically transferred to nitrocellulose membranes [23]. Immunodetection in nitrocellulose replicas was performed with either mAb J28 or pAs L64. Nitrocellulose membranes were then incubated in buffered BSA solution containing alkaline phosphatase-labelled anti-mouse or anti-rabbit goat IgG respectively. Development was achieved with a mixture of 0.5 mM Nitroblue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M Tris/HCl buffer, pH 9.5 (0.1 M NaCl, 1 mM MgCl₂) for 10 min.

Enzyme assays

The activity with 4-nitrophenyl acetate or 4-nitrophenyl hexanoate as substrate was measured spectrophotometrically at 400 nm and pH 7.4 in a thermostatically controlled cell at 25 °C using the method described by Erlanson [24]. The activity with cholesteryl esters was determined as described [25]. Units were defined as $\mu\text{mol}/\text{min}$ per ml and $\mu\text{mol}/\text{h}$ per ml respectively for hydrolysis of carboxyl esters and cholesteryl esters.

Semiquantitative dot-blot assay of mAb J28-reactive epitope

The semiquantitative dot-blot assay of FAP was performed by serial 2-fold dilution of FAP-containing solution applied to nitrocellulose membranes as previously described. Development using mAb J28 as primary antibody was performed as described above for Western blot. The amount of J28 epitope was calculated

by reference to the last dilution in a series using the following equation:

$$J28 \text{ (units/ml)} = 2^{(n-1)}$$

where n is the number of positive dilutions [10].

Amino acid analysis

Amino acid analyses were carried out on a Beckman System 6300 analyser. Suitable amounts of protein were hydrolysed in 6 M HCl in the presence of 1 mM phenol at 110 °C for 24 h in vacuum-sealed tubes.

Carbohydrate analysis

Monosaccharides were determined after acidic methanolysis and benzylation of samples. Derivatized carbohydrates were analysed by reversed-phase h.p.l.c. [25a]. Neutral sugars were determined by the phenol/H₂SO₄ method with mannose as standard [25b]. Amino acid analysis was used to obtain reliable quantification of proteins.

N-Terminal sequence determination

N-Terminal sequence analyses were performed by stepwise Edman degradation using a gas-phase sequencer (Applied Biosystems, model 470 A). The resulting phenylthiohydantoins were analysed by h.p.l.c. using a C₁₈ column (Brownlee, 5 μm , 2.1 mm $\times$ 220 mm). They were eluted with a methanol gradient (10–46%) in a 7 mM sodium acetate buffer (pH 4.84).

RESULTS

BSDL has, so far, been described as an enzyme of normal adult pancreatic secretion, and the oncofetal behaviour of FAP has been assessed by its reactivity with the mouse mAb J28. We have therefore looked at the immunological and enzymic properties of classical preparations of the two proteins [10,13,15].

Co-elution of esterase activity and mAb J28 reactivity during classical purification of BSDL

This purification is achieved by two chromatographic steps [13]. The first consists of gel filtration of pure pancreatic juice on Ultrogel ACA54. As shown in Figure 1(a), the activity of BSDL with 4-nitrophenyl acetate parallels the reactivity with mAb J28. The second step on CM-Sepharose (Figure 1b) shows that the reactivity with mAb J28 was eluted with the linear gradient of NaCl and that once again this reactivity is strictly superimposed on the BSDL activity.

Immunological relationships between FAP and BSDL

The electrophoretic patterns of preparations of FAP and BSDL are in agreement with previously published results (Figure 2a). Both consist of a predominant compound at an average M_r of 100 000–110 000. Some minor components in FAP are absent from the BSDL preparation.

Immunodetection of nitrocellulose-transferred proteins (Western blots) shows (Figure 2b) that pAs L64 reacts with the same intensity with the predominant compound in both preparations, indicating that FAP and BSDL are immunologically related. The minor compound (doublet at M_r 54 000–58 000) in FAP is also recognized by this polyclonal antiserum, suggesting it could result from enzymic degradation or deglycosylation, since such a doublet has previously been seen after complete deglycosylation of FAP [10].

A quite different pattern is obtained after immunodetection

Figure 1 Co-purification of BSDL and mAb J28 reactivity

(a) Gel filtration of human pancreatic juice on Ultrogel ACA 54. The column (2 cm $\times$ 400 cm) was equilibrated and developed (20 ml/h) with buffer containing 500 mM NaCl, 1 mM benzamidine and 10 mM Tris/HCl (pH 8.0). The sample was 5 g of freeze-dried pancreatic juice (i.e. 900 mg of protein) dissolved in 50 ml of elution buffer containing 0.5 mM phenylmethanesulphonyl fluoride and 2 mM 3-phenylpropionate as proteinase inhibitors. BSDL activity (■) was determined with 4-nitrophenyl acetate as substrate, and the mAb J28 reactivity (Δ) was semiquantitatively determined by dot-blot dilution. —, A_{280} . (b) Chromatography on CM-Sepharose. Fractions from (a) that were active with 4-nitrophenyl acetate and reactive with the mAb J28 were concentrated and dialysed against 0.1 M NaCl, 10 mM Mes buffer and loaded (22 ml, 70 mg of protein) on to a CM-Sepharose column (18 cm $\times$ 8 cm) developed at 50 ml/h with the same buffer. After washing, a linear gradient from 0.1 M to 0.3 M NaCl was applied. BSDL activity (■) with 4-nitrophenyl acetate as substrate and mAb J28 reactivity (Δ) were eluted at about 0.20 M NaCl. —, A_{280} .

Figure 2 Electrophoretic and immunological comparison of BSDL and FAP

(a) SDS/PAGE (with Coomassie Blue staining) of BSDL (10 μ g) and FAP (10 μ g). (b) Western-blot analysis of BSDL (10 μ g) and FAP (10 μ g) after electrophoretic migration and transfer to nitrocellulose film. Immunodetection was with either pAs L64 or mAb J28.

with mAb J28. This antibody stains a large band (100000–110000) in FAP in agreement with previous results, but it recognizes only a narrow band in the BSDL preparation (Figure 2b). This result suggests that the structure recognized by mAb J28 is unequally represented in FAP and BSDL. In other words,

Figure 3 Immunoabsorption on immobilized mAb J28

Freeze-dried BSDL was dissolved in phosphate-buffered saline solution (pH 7.4) with 1 mM benzamidine, and incubated for 1 h at 4 $^{\circ}$ C with immobilized mAb J28. Unbound fractions were eluted and the column was washed with the same buffer without benzamidine until zero absorbance. Bound fractions were eluted with 0.5 M glycine, pH 2.3. Enzyme activity (■) was determined with 4-nitrophenyl hexanoate as substrate and mAb J28 reactivity (Δ) was determined as in Figure 1. —, A_{280} .

if the name FAP is reserved for molecules bearing the epitope recognized by mAb J28, FAP would account for only a small percentage of the classical BSDL preparation.

Separation of mAb J28 reactivity from enzyme activity by immunoaffinity on immobilized mAb J28

BSDL obtained by CM-Sepharose chromatography was first dialysed against phosphate-buffered saline (10 mM sodium phosphate, pH 7.4, 0.15 M NaCl) in the presence of proteinase inhibitor (1 mM benzamidine), then applied to an immunoaffinity column made by covalent fixation of mAb J28 to CNBr-activated Sepharose 4B. The column was first washed with the same buffer until the absorbance of unabsorbed proteins fell to zero. Absorbed proteins were then eluted with 0.5 M glycine, pH 2.3. Unabsorbed and mAb J28-reactive (absorbed) fractions were then analysed for enzyme activity using 4-nitrophenyl hexanoate as sensitive substrate for BSDL, for immunoreactivity using mAb J28 in semiquantitative dot-blot assay, and for protein as measured by absorbance at 280 nm. In terms of protein, the unabsorbed fraction accounted for more than 90 % of the BSDL loaded. As shown in Figure 3, this fraction is devoid of mAb J28 reactivity and is fully active with 4-nitrophenyl hexanoate. As expected, the eluted fraction contains all of the mAb J28 reactivity and also hydrolyses the ester substrate. The relatively low amount of J28-epitope-bearing molecules obtained after immunoaffinity is in agreement with the Western blot presented in Figure 2.

Enzyme activity of mAb J28-reactive and -unreactive fractions

BSDL has been shown to be implicated in the intraduodenal hydrolysis of cholesteryl ester [16]; this substrate was tested and compared with the non-specific esterase activity on 4-nitrophenyl acetate using the classical preparations of FAP and BSDL. The enzyme activity of BSDL, when determined on cholesteryl ester and 4-nitrophenyl acetate, is in accordance with previously determined values [13,14,25]. Although less active, FAP is able to hydrolyse both substrates as well. Moreover, the activity ratios on 4-nitrophenyl acetate versus cholesteryl ester are almost identical for FAP and BSDL (Table 1). The activity of the mAb J28 reactive and -unreactive fractions obtained by immunoaffinity chromatography were also determined on cholesteryl ester and

Table 1 Cholesteryl and non-specific esterase activities of mAb J28-reactive and -unreactive fractionsResults are means $\pm$ S.E.M. for 3–4 determinations. N.D., not determined.

Substrate...	Specific activities (units/mg)			Ratio
	4-Nitrophenyl acetate	4-Nitrophenyl hexanoate	Cholesteryl ester	
BSDL*	45.0 $\pm$ 3.7	N.D.	48.4 $\pm$ 15.7	0.9 $\pm$ 0.3
mAb J28-unreactive fraction	N.D.	175 $\pm$ 55	11.6 $\pm$ 1.2	15.1 $\pm$ 1.6
FAP†	6.8 $\pm$ 0.9	N.D.	8.5 $\pm$ 1.2	0.8 $\pm$ 0.1
mAb J28-reactive fraction	N.D.	71 $\pm$ 36	4.1 $\pm$ 0.6	17.3 $\pm$ 3.3

* Purified as in ref. [13].

† Purified as in ref. [10].

Table 2 Amino acid composition of mAb J28-reactive and -unreactive fractions eluted from the immunoabsorption column

The composition for BSDL was deduced from the cDNA sequence [26,27]. Results for mAb J28-reactive and -unreactive fractions are the means of two determinations. Residues that clearly differ in amount are underlined. n.d., not determined.

Residue	Fractions		
	BSDL	Unreactive	Reactive
Ala	10.9	10.9	10.1
Asx	12	12.4	12.2
Arg	3.2	3.0	2.7
Gly	11.5*	11.5*	30.3*
Glx	6.5	<u>7.6</u>	<u>15.6</u>
His	1.4	1.5	1.6
Ile	4.4	4.2	3.5
Leu	6.6	7.3	6.3
Lys	5.8	5.7	4.7
Met	2.0	1.9	1.4
Phe	4.1	3.9	3.1
Pro	14.6	<u>14.7</u>	<u>10.3</u>
Ser	6.5	<u>5.8</u>	<u>11.3</u>
Thr	9.3	8.9	7.6
Tyr	4.1	3.9	3.0
Trp	—	n.d.	n.d.
Val	8.4	8.3	6.7
$\frac{1}{2}$ -Cys	—	n.d.	n.d.
Total	100	100.0	100.1

* These values were not taken into account for total amino acid and Δn determination (see the text).

compared with the activity on 4-nitrophenyl hexanoate. Whatever the fraction used as catalyst, the hydrolysis of each substrate was effective and the ratios of specific activity determined on 4-nitrophenyl hexanoate to that determined on cholesteryl ester were 17.3 ± 3.3 and 15.1 ± 1.6 for mAb J28-reactive and -unreactive fractions respectively (Table 1). Again the mAb J28-reactive fraction possesses a lower catalytic efficiency. The mAb J28-reactive fraction was completely inhibited by di-isopropyl phosphorofluoridate and incorporated stoichiometric amounts of tritiated serine as also observed for the BSDL and the mAb J28-unreactive fractions. These data confirm those obtained with classical preparations of BSDL and FAP and suggest that we are dealing with a similar enzymic protein.

Molecular characterization of mAb J28-reactive and -unreactive fractions isolated by immunoabsorption

The amino acid composition of the BSDL fraction devoid of J28 epitope is, within experimental error, identical with that of crude BSDL [13] and the composition deduced from the cDNA sequence [26,27] (Table 2). When compared with the composition of J28 epitope-bearing molecules, significant differences were noted in the content of glutamate/glutamine, proline and serine. Methionine, lysine, phenylalanine, tyrosine and valine also appear a little different in the absorbed and unabsorbed fractions. The glycine content, which is much higher in the absorbed fraction, is probably residual glycine from the elution buffer despite exhaustive dialysis before the analysis and the fact that only a small amount of glycine was found as a contaminant during N-terminal sequence analysis. From these compositions, we can deduce that the mAb J28-reactive and -unreactive molecules differ by about 40 sites over 100 (not taking the results for glycine into account), since Cornish-Bowden index Δn is equal to 61.4 [28], suggesting that we are dealing with similar but not completely identical proteins. We determined the N-terminal sequences of the mAb J28-reactive and unreactive fractions (ten and four residues respectively) and found the alignments to be identical with those of BSDL and FAP (Table 3).

The carbohydrate compositions of the reactive and unreactive fractions show important differences (Table 4). The presence of mannose and *N*-acetylgalactosamine indicates that the mAb J28-unreactive fraction contains *N*- and *O*-linked oligosaccharides. The ratio Man/GalNAc (approx. 3/7) suggests a preponderance of *O*-glycans over *N*-glycans. Assuming a minimal structure of diantennary three mannoses for the minimal structure of *N*-glycosylation (hybrid or complex-type) and Gal-GalNAc for minimal *O*-glycosylation, it can be deduced that 12–14 *O*-linked glycans for one *N*-linked glycan are present in the mAb J28-unreactive fraction (E. Mas, N. Abouakil, S. Roudani and D. Lombardo, unpublished work). This fraction also contains fucose and sialic acid in equal amounts. Neutral sugar, as determined by the phenol/H₂SO₄ method, accounted for $6.1 \pm 0.6\%$ of the total mass of protein. This value, which agrees with previous determinations for BSDL [15,29], also agrees with the sugar composition given in Table 4. From this composition, it can be calculated that sugars represent approximately 12.6% of the mass of the mAb J28-unreactive protein, which corresponds quite well with the experimentally determined value of 12.1%.

The ratio Man/GalNAc in the mAb J28-reactive fraction (approx. 1) shows that *N*-glycosylation is predominant over

Table 3 N-Terminal sequences of mAb J28-reactive and -unreactive fractions eluted from the immunoabsorption column

Sample	N-Terminal sequence
BSDL (from ref. [14])	Ala-Lys-Leu-Gly-Ala-Val-Tyr-Thr-Glu-Gly-Gly-Phe-Val-Glu-Gly-Val-Asn-Lys-Lys-Leu Gly-Leu-Leu-Gly-Asp-Ser-Val-Ile-Phe
FAP (from ref. [10])	Ala-Lys-Leu-Gly-Ala-Val-Tyr-Xaa-Glu-Gly-Gly-Phe-Val-Glu-Gly-Val-Asn-Lys-Lys-Leu Gly-Leu-Leu
mAb J28-unreactive fraction	Ala-Lys-Leu-Gly-Ala
mAb J28-reactive fraction	Xaa-Lys-Leu-Gly-Ala-Val-Tyr-Thr-Glu-Gly

Table 4 Carbohydrate composition of mAb J28-reactive and -unreactive fractions eluted from the immunoabsorption column

For each fraction, the molar ratio is expressed for three mannose residues. The composition (i.e. mol of sugar/mol of protein) was calculated using the percentage of neutral sugar determined for each fraction (see the text). Results are the means $\pm$ S.E.M. for three determinations.

	mAb J28-unreactive fraction		mAb J28-reactive fraction	
	Molar ratio	(mol of sugar/mol of protein)	Molar ratio	(mol of sugar/mol of protein)
Fuc	6.2 $\pm$ 1.1	10.4 $\pm$ 1.8	4.6 $\pm$ 1.4	11.8 $\pm$ 3.6
Gal	7.6 $\pm$ 1.4	12.7 $\pm$ 2.4	2.6 $\pm$ 0.6	6.7 $\pm$ 1.5
Man	3	5	3	8
GalNAc	7.0 $\pm$ 1.2	11.7 $\pm$ 2.0	3.1 $\pm$ 0.6	7.9 $\pm$ 1.5
GlcNAc	2.1 $\pm$ 0.3	3.5 $\pm$ 0.5	1.0 $\pm$ 0.2	2.6 $\pm$ 0.5
NeuAc	6.3 $\pm$ 0.2	10.0 $\pm$ 0.6	1.2 $\pm$ 0.6	3.1 $\pm$ 1.5

O-glycosylation. Interestingly, in this fraction, mannose and GlcNAc residues appear in amounts compatible with high-mannose structure for N-glycosylation. This fraction is also fucosylated, but a few residues are sialylated (Fuc/NeuAc = 5/1). When compared with the carbohydrate composition of the unreactive fraction, it can be seen that the sugars mainly involved in O-glycosylation, such as Gal and GalNAc, are significantly decreased, as is NeuAc (Table 4). From this composition it can be calculated that neutral sugars represent some 4.6% in weight (which agrees with the experimentally determined value) and total sugars represent 8.3%. Therefore the mAb J28-reactive fraction appears to be less, and differently, glycosylated than the unreactive fraction.

DISCUSSION

FAP is a specific component of acinar cells of the human pancreas and is associated with ontogenesis and development of the gland. It has been characterized using the monoclonal antibody mAb J28 [4]. Maximum synthesis of FAP, determined as emergence of the J28 epitope, occurs when acinar cell proliferation is maximal; it then declines to parturition [6]. Thereafter, FAP, defined by the presence of the J28 epitope, behaves as an oncodevelopment-associated antigen [10].

BSDL is an enzyme found in the normal secretion of the pancreas, and has been found in all species examined so far [16]. The enzyme is non-specific with regard to substrate, being able to hydrolyse cholesteryl esters, tri-, di- and mono-acylglycerols and esters of fat-soluble vitamins, but the maximum activity is obtained with esters of 4-nitrophenyl. Phylogenetically, BSDL appears to be an ancient pancreatic protein [30,31].

Classical (i.e. biochemical) preparations of BSDL and FAP

from pancreatic secretions either normal (BSDL) or pathological (FAP), have been used for molecular characterization. Previous separate studies have indicated that the two proteins have similar molecular properties including size, N-terminal sequence, isoelectric pH and amino acid composition [10,13,15]. In addition, both are heavily glycosylated, although the carbohydrate composition differs. The molecular microheterogeneity of FAP was attributed to variation in the sugar content [10].

The data presented here reveal that the enzymic activity of BSDL cannot be separated from the mAb J28 reactivity by enzyme-purification steps. Also a polyclonal antibody against BSDL recognized all M_r variants of the FAP preparation. The latter protein was, in addition, able to hydrolyse carboxyl esters with the same specificity as BSDL. From all these results, it can be concluded that FAP and BSDL are in fact the same enzyme, differing in their carbohydrate compositions.

In these studies, FAP was purified by biochemical procedures whereas its oncodevelopmental profile was assessed using mAb J28. Application of this mAb to analysis of BSDL preparations, by two different approaches, i.e. Western blot and immunoaffinity, revealed the presence of a minor population of BSDL molecules bearing the J28 epitope.

The BSDL fraction devoid of this epitope, which accounted for 90% of the total BSDL in normal pancreatic juice, was, as expected, structurally indistinguishable from crude BSDL. Thus both had the same amino acid composition which in addition was identical with that deduced from the cDNA sequence of BSDL [15,26,27]. The sequence of 29 N-terminal residues of BSDL [15] coincided exactly with the cDNA alignment. The first five residues of the mAb J28-unreactive fraction found in this study are also identical. Enzyme activity was also fully conserved and carbohydrate composition is compatible with a protein having O- and N-linked oligosaccharides [29]. From all these

data we conclude that there is total identity between 'classical' BSDL and the mAb J28-unreactive BSDL fraction.

In contrast, we noted substantial variation in the amino acid composition of BSDL bearing the J28 epitope. For example, the number of proline residues was considerably reduced, 10.3 compared with 14.7, i.e. five residues less per 100 residues. BSDL, as deduced from its cDNA, is 723 amino acids in length, and therefore has an M_r 78000; this means that there is a deletion of 35–40 proline residues. Serine and glutamine residues were, in contrast, increased, i.e. 57–60 more Glx residues and 35–40 more Ser residues per mol of protein. Minor differences, although beyond experimental error, were found for other residues, in particular methionine which is three to four residues light. However, the composition as a whole was typical of BSDL, which is characterized by a high content of proline, glycine, asparagine/aspartate and alanine. This result is consistent with two probably closely related but not identical polypeptides. Biochemically purified FAP showed microheterogeneity at the amino acid composition level (proline, serine and glutamine/glutamate to be precise) of two samples of FAP. One composition was similar to that of BSDL but the second sample had an intermediate amino acid composition. Since the FAP had been obtained from pancreatic juices, the preparations were probably composed of J28-negative (that is BSDL) and J28-positive (FAP) subpopulations. Possible variations on the peptide sequence between these two populations should not be located in the N-terminal portion, as no variation in the first ten residues was seen by Edman's alignment of BSDL molecules bearing the J28 epitope (this paper). This is in agreement with evolutionary conservation of the N-terminal portions of BSDL which includes the active-site consensus sequence and *N*-glycosylation site (residues Ser194 and Asn186). Modifications at the C-terminal portions are more probable for two reasons: (1) this is a non-conserved highly evolution-associated modified region [26,27,32,33]; (2) most variant residues (Pro, Glx and Ser) are mainly located at the C-terminus [26,27].

This hypothesis is supported by comparison of the carbohydrate composition of J28-reactive and -unreactive BSDL subpopulations. The FAP carbohydrate composition indicates a decrease in *O*-glycosylation; in BSDL, *O*-linked oligosaccharides are clustered in the proline-rich C-terminal tandem repeated sequence [27] (E. Mas, N. Abouakil, S. Roudani and D. Lombardo, unpublished work). Therefore sequence modification of these C-terminal repeats in FAP could be associated with a default in *O*-glycosylation.

J28-reactive BSDL, although still active on ester substrates, showed a decrease in specific activity, as was the case for the biochemical FAP preparation. This may be due to the handling conditions, for example the very acid pH used for elution from the mAb J28-immobilized Sepharose column. However, in a similar situation immunologically purified BSDL showed no alteration in its esterolytic activity [15]. Alternatively, *N*-glycosylation, which appears to be of the high-mannose unprocessed type in FAP and which greatly differs from that in BSDL, may have a structural effect, causing the lower catalytic efficiency of FAP. The *N*-glycosylation site, which is near the active-site serine of BSDL [26], may hinder the catalytic site of FAP. Whether the glycosylation can modulate or modify the enzyme activity has not been demonstrated, but this could be one example of such an effect.

The mAb J28 recognized a particular population of BSDL molecules which are oncofetal-related. Therefore the name FAP should be reserved for molecules bearing the J28 epitope. The question arises whether FAP and BSDL are the products of a single gene with post-translational carbohydrate modifications

(unlikely) or products of closely related but development-balanced genes, as is the case for α -fetoprotein and albumin, for example [34]. The third possibility is an alternative splicing as described for normal and oncofetal fibronectin [35]. Whatever the mechanism involved, our results show for the first time that a pancreatic enzyme normally secreted in adult is described as having an oncofetal glycovariant associated with ontogenic and oncogenic processes of the exocrine pancreas.

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