Molecular Cloning of the Oncofetal Isoform of the Human Pancreatic Bile Salt-dependent Lipase*

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Specific transcripts for bile salt-dependent lipase (BSDL), a 100-kDa glycoprotein secreted by the human pancreas, were immunodetected in BxPC-3 and SOJ-6 pancreatic tumoral cell lines. Sequencing of fragments, obtained by mRNA reverse transcription and amplification, confirmed the presence of BSDL transcripts in these cancer cells. The protein was detected in lysates of pancreatic tumoral cells, where it was mainly associated with membranes. Only a minute amount of the enzyme was detected in the culture media. Immunofluorescence studies demonstrated that in SOJ-6 cells, BSDL colocalizes with the p58 Golgi protein and suggested that the protein may be sequestrated within the Golgi compartment. These results demonstrated that BSDL is expressed in human pancreatic tumoral cells and cannot be secreted (or for the least very poorly). Subsequently, a cDNA covering the entire sequence of BSDL was obtained by reverse transcription-polymerase chain reaction. The sequence of this cDNA indicated that the N-terminal domain encoded by exons 1-10 was identical to that of BSDL expressed by the human normal pancreas. However, the sequence corresponding to exon 11, which should code for the 16 tandem-repeated identical mucin-like sequences of BSDL, was deleted by 330 base pairs (bp) and encoded only 6 of these repeated sequences. We conclude that this truncated variant of BSDL would be its oncofetal form, referred to as feto-acinar pancreatic protein. We then investigated whether the deletion of 330 bp affected the secretion of the protein. For this purpose, the cDNA corresponding to the mature form of the BSDL variant expressed in SOJ-6 cells was cloned into an expression/secretion vector and transfected into CHO-K1 cells. Results indicated that the variant of BSDL isolated from SOJ-6 cells was expressed and secreted by these transfected CHO-K1 cells. However, the level of BSDL secreted by these transfected CHO-K1 cells was significantly higher than that observed for SOJ-6 cells. Consequently, the retention of the oncofetal variant of BSDL observed in human pancreatic tumoral cells might not result from inherent properties of the protein.

The bile salt-dependent lipase (BSDL, EC 3.1.1) is a 100-kDa glycoprotein secreted by the pancreas into the duodenum, where it is thought to play an important role in cholesterol and lipid-soluble vitamin ester hydrolysis and absorption (1, 2). The cDNA for pancreatic BSDL has been isolated and sequenced (3). The amino acid sequence is rich in proline (12%), the majority of these residues (68%) being located within 16 C-terminal tandemly repeated sequences (repeats numbered 1 to 16) (3, 4). Further studies have shown that exon 11 encoded these tandem repeats (5), the size of which varied by species (5, 6). This accounts, in part, for the previously observed species variation in BSDL size and amino acid composition (7). In contrast to the other secretory pancreatic enzymes, BSDL is associated with membranes during its intracellular processing (8, 9). This association involves a multimeric folding complex including p94, a protein immunologically related to the glucose-regulated protein of 94 kDa (Grp94) and two other proteins of 56 and 46 kDa (9). It has been suggested that the interaction of BSDL with the Grp94-related p94 protein is essential for the O-glycosylation of C-terminal tandem-repeated sequences (9). These repeated sequences contain PEST regions, which are signals for rapid degradation (10). It is therefore possible that glycosylation of these PEST regions may contribute to the removal of BSDL from a possible degradation route (11). Once fully glycosylated, the enzyme is phosphorylated and released from membranes either in or after the trans-Golgi compartment (12). It is then aggregated in the trans-Golgi network (8, 9) with other digestive enzymes and enters the regulated secretion pathway. In a previous study, we have isolated an oncofetal variant of BSDL (13), later identified as the feto-acinar pancreatic protein (FAPP) (14).

FAPP was first characterized by the monoclonal antibody J28 (mAb J28) (14). FAPP is expressed in human embryonic and fetal pancreas. The earliest expression of this protein was seen in undifferentiated mesenchymal cells and in nascent acini at the beginning of the morphological differentiation of the pancreas (15). Maximal synthesis of FAPP occurs at the time of intense proliferation of acinar cells and declines progressively thereafter (15) to reach a very low level of expression in normal adult pancreas (16). FAPP concentration is elevated in the blood of patients suffering from pancreatitis and pancreatic cancer, suggesting an enhanced synthesis in cases of pancreatic pathologies. This finding corroborates the increased level of FAPP in pathological pancreatic juices (17). FAPP was shown to be different from BSDL in many aspects: (a) FAPP is

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The abbreviations used are: BSDL, bile salt-dependent lipase; FAPP, feto-acinar pancreatic protein; CHO, Chinese hamster ovary; 4-NPH, 4-nitrophenyl hexanoate; bp, base pairs; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; PBS, phosphate-buffered saline; LDH, lactate dehydrogenase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; RT, reverse transcription; kb, kilobase(s).
more less active than BSDL; (b) the N-linked glycosylation of FAPP seems to be a high-mannose type, whereas that of BSDL is complex (18); (c) the amount of O-linked structures is largely decreased in FAPP (18); (d) carbohydrate accounts for 47% of the FAPP mass (14) instead of 20% for BSDL (19); and (e) the amino acid composition of FAPP differs from that of BSDL (13), although their N-terminal sequences are identical (20). Data suggest that the two proteins may have different C-terminal tails (18, 20). Finally, FAPP migration on SDS-PAGE is diffuse and lower than that of BSDL (13). Other studies have shown that FAPP is expressed in pancreatic tumor cells; however, the secretion of the protein was not detected (21). Miralles et al. (21) have postulated that the absence of secretion was due to the retention of the protein within the endoplasmic reticulum (ER) and suggested that FAPP would remain associated to ER resident protein(s) as a consequence of its improper folding. Recent findings suggested that glycosylation of the C-terminal region of BSDL regulates the secretion of the protein (11). The aim of this study was 2-fold: first, to investigate the molecular properties of BSDL expressed by human pancreatic tumoral cells; second, to determine whether the retention of the variant expressed by tumoral cells is due to inherent properties of the protein. We showed that part of C-terminal tandem repeated sequences were deleted in the BSDL variant expressed by pancreatic tumoral cells, suggesting that we are dealing with FAPP. Moreover, CHO-K1 cells transfected with the truncated cDNA secreted the protein. This result indicates that the retention of FAPP within tumor cells cannot be due to the truncation of the C-terminal region of the protein.

**EXPERIMENTAL PROCEDURES**

**Materials**—Glutamine, penicillin, trypsin-EDTA, and streptomycin were from Life Technologies, Inc. Fetal calf serum was from Dutscher (Brumath, France). Phenylmethylsulfonyl fluoride, benzamidine, and 2 mM benzamidine and sonicated for 10 s. The cell membrane fraction was separated from the soluble fraction by centrifugation at 30,000 g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. Cell culture media wereGFP CATCTG) and using the GC-rich PCR kit from CLONTECH. These primers were used to amplify the FAPP above-mentioned PCR products in pSecTag (see below) was confirmed by digestion with EcoRI restriction sequences (lower-case letters), which were used for the subsequent cloning of transcripts. Bases were randomly added in 5’ of these primers to allow restriction sites for mammalian expression. cDNA transcripts were amplified using the following reaction cycles program as follows: denaturation (94 °C, 1 min), annealing (64 °C, 1 min), and extension (68 °C, 4 min). The reaction was terminated by an incubation at 68 °C for 10 min. PCR products were then analyzed on a 1% agarose gel. Prehybridization, probes were 32P-labeled by random priming (Life Technologies, Inc.) using [α-32P]dCTP at a specific radioactivity of 4,000 cpm/μg DNA probe. In vitro translations of RNA were performed using the rabbit reticulocyte lysate system from Promega in accordance with the manufacturer’s instructions. 10 μg of RNA were incubated with the reticulocyte lysate and [35S]methionine (0.4 mCi/ml) in a final volume of 50 μl. After 2 h of incubation at 30 °C, the translated products were immunoprecipitated with pAbL64 and analyzed by SDS-PAGE followed by autoradiography.

**Transfection**—Transcripts obtained by RT-PCR were digested by HindIII and EcoRI and ligated into pSecTag expression vector (Invitrogen). Stable transfection of CHO-K1 cells was performed with the pSecTag vector comprising RT-PCR transcripts and using the LipofectAMINE-mediated transfection procedure according to the manufacturer’s instructions. The selection of stable clones was performed for 6 weeks in medium with zeocin (500 μg/ml). Control cells, transfected with the empty pSecTag vector, were cloned under the same conditions.

**Immunofluorescence**—Cells grown to confluence were rinsed twice with incomplete PBS buffer (10 mM sodium phosphate buffer, pH 7.4, with 0.15 M NaCl and no Ca2+ and no Mg2+ ions) and harvested with 0.25% trypsin-EDTA. Cells were suspended in culture medium and centrifuged at 400 × g for 3 min. Pellets were washed twice with PBS and suspended in PBS (500 μl) containing 100 μg/ml soybean trypsin inhibitor, 2 mM phenylmethylsulfonyl fluoride, 2 mM β-mercaptoethanol, and sonicated for 10 s. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22). The pellet containing membrane proteins was solubilized in 0.125 mM Tris/HCl, pH 7.0, buffer (3% SDS) containing 0.15 M NaCl and no Ca2+ ions. The cell membrane fraction was separated from the soluble fraction by centrifugation at 12,000 × g for 20 min at 4 °C (22).
secretion of BSDL was first investigated by Western blotting using polyclonal antibodies specific for BSDL and \( 125^\text{I}-\text{protein A} \) overlay. This very sensitive assay allowed us to detect BSDL in cell culture medium of BxPC-3 and SOJ-6 cell lines (Fig. 2). The migration of BSDL secreted by these tumoral cells, although diffuse, appeared lower (arrow, 110–125 kDa, p125) than that of the protein (arrow, 100 kDa, p100) present in normal human pancreatic juice (Fig. 2, HPJ). This could be related to high \( M_r \) glycoforms of BSDL also referred to as concanavalin A reactive forms (13, 18), which preferentially display the \( J28 \) epitope. Two immunoreactive forms of BSDL were detected in cell culture of SOJ-6 cells, the lower \( M_r \) form having the same electrophoretic migration than that of the protein detected in normal pancreatic juice (100 kDa).

Second, BxPC-3 and SOJ-6 cells were incubated for 6 h in fresh medium, and BSDL activity was then recorded on 4-NPH. This activity was found in cell culture medium of SOJ-6 cells, whereas no activity could be detected in that of BxPC-3. BSDL activity represented approximately 12% of the total esterolytic activity detected in SOJ-6 cell line (Table 1). The presence of BSDL in the extracellular medium did not correlate with that of the cytoplasmic marker LDH, the activity of which never exceeded 5% of its intracellular activity. This indicated that cell lysis may not be responsible for the presence of extracellular BSDL activity. Because BSDL activity represented at least 85% of the total esterolytic activity associated with tumoral cells (22), it seems that only a minute amount of BSDL activity was secreted by SOJ-6 cells. Attempts to detect \( \alpha \)-amylase in the cell culture medium by Western blot or by recording activity were unsuccessful.

**BSDL Is Associated with Membranes in Pancreatic Tumoral Cells**—We next attempted to determine whether the BSDL expressed by tumoral cells was associated with membranes, as found in normal pancreatic tissue (8). For this purpose, cells were grown to 80% confluence and lysed, and the lysate was clarified as described previously (22). The pellet was then solubilized, in the same volume as the clarified lysate, with a 10 mM Tris/HCl, pH 7.4 (0.1% SDS), buffer. The presence of BSDL was then analyzed in clarified lysate and solubilized pellet. As shown in Fig. 3, BSDL could be detected in both fractions obtained from BxPC-3 and SOJ-6 cell lines. The detected protein migrated at the same position as BSDL present in microsomes isolated from a normal human pancreas (Fig. 3, HPM). From this figure, it is obvious that BSDL (arrow, p100) was mainly associated with the membrane pellet. Intriguingly, the p46 immunoreactive form of BSDL (22) was detected in clarified lysates of BxPC-3 and SOJ-6 cells (arrowhead, p46). This pattern is similar to that obtained with the clarified lysate of normal pancreatic tissue (22). The release of BSDL from membranes can be obtained by treatment of the pellet with a 0.1 M sodium carbonate buffer (pH 10.0) or 0.25 M KBr (not shown). This suggests that the association of BSDL with
TABLE I

| Cells       | Activity (10⁻³ units) | Extracellular activity (% Total activity) |
|-------------|-----------------------|----------------------------------------|
|             | Intracellular         | Extracellular                          |
| SOJ-6       | 29.5 ± 1.3            | 4.1 ± 0.7                              | 12.2 ± 2.4 |
| BxPC-3      | 45.1 ± 4.1            | 0                                      | 0          |
| CHO-pSecFAPP| 2.8 ± 0.7             | 3.6 ± 0.4                              | 56.3 ± 4.7 |
| CHO-pSecBSDL| 11.2 ± 2.0            | 130.7 ± 4.6                            | 92.1 ± 1.4 |

**Fig. 3. Presence of bile salt-dependent lipase in tumoral cell lines.** Proteins (50 μg) from membrane fraction (lane 1) and soluble fraction (lane 2) were separated on SDS-PAGE and electrotransferred to a nitrocellulose membrane. The analysis and detection were then performed as described in Fig. 2. HPM, human pancreatic microsomes.

membranes involves ionic interactions, as already described in normal human pancreatic tissue (8). Attempts to detect α-amylase using specific antibodies and specific enzymatic assays were unsuccessful.

The distribution of BSDL inside cells has been examined by immunofluorescence studies using pAbL64 antibodies on permeabilized SOJ-6 cells. Although BSDL appeared dispersed throughout the ER, which in pancreatic cells largely occupied the cytoplasm (Fig. 4C), a typical Golgi region with a punctuated staining around the nucleus was also revealed (Fig. 4C). Antibodies directed against the p58 Golgi protein detected identical structures around the nucleus (Fig. 4D). This pattern indicated that in SOJ-6 cells, BSDL colocalizes with Golgi probe and, as a consequence, would be sequestered within this compartment.

**Sequence of the PCR Transcript**—The next objective was to obtain the entire BSDL transcript sequence expressed by tumoral cells. Because the mAb2J82, which characterizes FAPP (14), recognized a carbohydrate-dependent epitope (20), the screening of a cDNA library was precluded. Consequently, a pair of primers (BSDL-5 and BSDL-3) covering the entire sequence of the mature BSDL was used. As shown in Fig. 5, transcripts of approximately 1.8 kb were obtained using RNA extracted from BxPC-3 and SOJ-6 cells. Transcripts obtained from SOJ-6 cells were cloned into the pCR2.1 TOPO vector, amplified, and sequenced. Both strands of two different clones obtained from independent RT-PCR experiments were sequenced to confirm dissimilarities. The sequence matched 99.8% that of human BSDL including the sequence of exons 1–10, from nucleotide 1 to nucleotide 1424 (3). Two mutations, T→C and C→T at nucleotides 489 and 612, respectively, which did not change the amino acid sequence (residues Gly-163 and Thr-204, see below and Fig. 8) were detected. However, the sequence of the SOJ-6 transcript corresponding to that of exon 11, which encodes C-terminal tandem-repeated sequences of BSDL, showed a major difference (Fig. 6). Although the SOJ-6 transcript sequence from nucleotide 1425 to 1736 matched 99.7% that of exon 11 of BSDL up to the end of repeat number 4 (nucleotide 1736), it seemed that 330 bp were deleted. Two other repeated sequences, from nucleotide 1737 to 1803, were detected in the cancer cell transcript. The sequence of these two repeats was compared with that of repeats 1 to 16 of BSDL, successively. As shown in Table II, the sequence matched that of repeats 15 and 16 of BSDL. The remaining sequence of the SOJ-6 transcript, from nucleotide 1804 to the stop codon (nucleotide 1836), was identical to that of BSDL. Consequently, the sequence of BSDL between nucleotide 1736 and nucleotide 2066 (or between nucleotides 2476 and 2806 according to the numbering used in Ref. 3) was deleted in the SOJ-6 transcript. This nucleotide deletion leads to the excision of 10 repeated sequences, indicating that repeats 5 to 14 were missing in the SOJ-6 transcript. Therefore, this transcript possessed tandem repeats 1 to 4 and 15 to 16 of BSDL (Fig. 6).

**Fig. 4. Detection of BSDL in SOJ-6 cells.** Detection of BSDL in SOJ-6 cells using pAbL64. A, SOJ-6 cells labeled with pAbL64 and FITC-conjugated antibodies directed against rabbit immunoglobulins. B, SOJ-6 cells treated as in A but omitting pAbL64 prior to immunofluorescence (control). C, magnification of SOJ-6 cells treated as in A prior to immunofluorescence. D, SOJ-6 cells labeled with mouse antibodies specific for the p58 Golgi protein and TRITC-conjugated antibodies directed against mouse immunoglobulins.

**Fig. 5. Amplification of RNA specific for bile salt-dependent lipase.** RNA was extracted from SOJ-6 (lane 2) and BxPC-3 (lane 3) cells. 1 μg of each RNA was reverse transcribed and amplified using sense and antisense primers designed to cover the entire sequence of the mature BSDL. Amplification products were separated on 1% agarose gel. Lane 1 represents the migration of the normal transcript of BSDL (2.2 kb).

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showed that a reaction product can be immunoprecipitated as one band at approximately 80 kDa when the translation program used RNA extracted from normal human pancreatic tissue (p80, lane 1). The size of this product correlates with the translation of a 2.2-kb mRNA corresponding to that encoding BSDL (3). However, when RNA extracted from pancreatic tumoral cells were translated under identical conditions, a protein migrating at approximately 70 kDa was detected (Fig. 7, lane 2, arrowhead). The size of this protein suggests that it could be the translation product of the 1.8-kb transcript. Further translational modifications such as N- and O-glycosylation lead to the 100–125-kDa protein (Fig. 2). Another band, weakly stained and associated with an Mr of 90–100 kDa, can be immunoprecipitated from the translated material using RNA of tumoral cells. It is possible that RNA extracted from tumor cells encoded two different forms of BSDL. However, this material could also represent part of the membrane-folding complex of BSDL, which includes the chaperone Grp94-related 94-kDa protein (8, 9).

Examination of the BSDL sequence, deduced from the transcript obtained from SOJ-6 cells, indicated that the four Cys residues involved in disulfide bridges (Cys-64 linked to Cys-80 and Cys-246 linked to Cys-257), the amino acids involved in the catalytic site (Ser-194, Asp-320, and His-435) and the site for N-linked glycosylation (Asn-186) are well conserved. These data strongly suggested that the N-terminal domain of this protein should be correctly folded and, consequently, enzymatically active. The deletion of the repeats 5 to 14 leads to the presence of only six putative sites for O-linked glycosylation (4, 33) on Thr-538, -549, -559, -576, -587, and -577 (Fig. 8). When the amino acid sequence of the SOJ-6 transcript was compared

### Table II

Comparison of repeated sequences 5 and 6 of the SOJ-6 transcript with the 16 repeated sequences of BSDL

| BSDL repeat | Nucleotides | Match |
|-------------|-------------|-------|
| Start | End | % |
| 1–2 | 1608 | 1675 | 85.9 |
| 2–3 | 1641 | 1704 | 85.9 |
| 3–4 | 1676 | 1737 | 83.9 |
| 4–5 | 1705 | 1765 | 86.4 |
| 5–6 | 1718 | 1803 | 86.4 |
| 6–7 | 1771 | 1836 | 86.4 |
| 7–8 | 1804 | 1869 | 84.6 |
| 8–9 | 1837 | 1902 | 84.6 |
| 9–10 | 1870 | 1935 | 83.3 |
| 10–11 | 1903 | 1968 | 81.5 |
| 11–12 | 1936 | 2001 | 83.3 |
| 12–13 | 1969 | 2034 | 84.8 |
| 13–14 | 2002 | 2067 | 87.9 |
| 14–15 | 2035 | 2097 | 86.9 |
| 15–16 | 2068 | 2133 | 98.5 |
above, tumoral cells secreted, although poorly, a high reticulocyte lysate for pancreatic tissue and from tumor cells was used to program a rabbit transcript matched repeats 15 and 16 of BSDL (Fig. 9). The alignment (Clustal W program, Infobiogen, Paris) also suggests that repeats 5 and 6 of the SOJ-6 gen, Paris) also suggests that repeats 5 and 6 of the SOJ-6 sequence were obvious. The alignment (Clustal W program, Infobiogen, Paris) also suggests that repeats 5 and 6 of the SOJ-6 sequence were obvious. The SOJ-6 Transcript Likely Encodes FAPP—As mentioned above, tumoral cells secreted, although poorly, a high M\(_v\) variant of BSDL. Two high M\(_v\) glycoisofoms of BSDL were previously isolated. The first one was a concanavalin A-reactive or ConA-reactive form (18), the second one was reactive with the mAbJ28 and was referred to as FAPP (13). We have further shown that the ConA-reactive form of BSDL was related to FAPP (18). Both the ConA-reactive form of BSDL and FAPP were poorly active (13, 18). They also differed from the normal form of BSDL (ConA-unreactive and mAbJ28-unreactive) at the level of amino acids involved in tandem-repeated sequences, which are located on the C-terminal peptide (13, 18). The amount of sugar involved in the minimal O-linked structure, such as galactose and N-acetylgalactosamine residues, was largely decreased in FAPP (13) and in the ConA-reactive fraction as well (18), where it seems that no more than six N-acetylgalactosamine residues may initiate O-linked oligosaccharide structures (20). On BSDL, 14–16 sites for O-linked glycosylation, which are located on the C-terminal tail of BSDL, were described (18, 33). Only six of those sites were still present on the sequence of the SOJ-6 transcript (see Fig. 8). Therefore, the amino acid composition of the C-terminal peptide isolated from the ConA-reactive form of BSDL was compared with that deduced from the sequence of the SOJ-6 transcript from Met-510 to Met-606 (Fig. 8). As shown in Table III, the amino acid composition (in % of total amino acid) of the C-terminal peptide of the ConA-reactive form of BSDL was, within experimental error, very close to that deduced from the sequence of the SOJ-6 transcript. Of course, the experimental results obtained with the C-terminal tail of the ConA-reactive fraction could be partially erroneous due to contamination by an amino acid such as Gly or to some degradation of an amino acid such as Thr. However, the amino acid composition of the C-terminal peptide of BSDL expressed in SOJ-6 cells seemed far different than that of the C-terminal tail of the ConA-unreactive BSDL (18) or that predicted from the cDNA of BSDL (3). Consequently, one may suggest that the cDNA transcript isolated from SOJ-6 cells would encode FAPP.

Transfection of CHO Cells—To determine whether the impaired secretion of FAPP by tumoral cells was due to the shortened C-terminal tail compared with BSDL, the transcript obtained from SOJ-6 cells was subcloned, in frame, into the pSecTag vector; the plasmid thus obtained, referred to as pSec-FAPP, carries the V-J2-C region of the mouse IgK chains, which might drive expressed proteins toward secretion. The pSecFAPP plasmid was transfected into CHO-K1 cells. A clone expressing FAPP was selected in the presence of zeocin. As shown in Fig. 10A, immunofluorescence studies using pAbL64 revealed that the enzyme was expressed by pSecFAPP-transfected CHO-K1 cells (CHO-pSecFAPP), whereas CHO-pSecTag transfected control cells displayed no reactivity (Fig. 10B). The labeling of CHO-pSecFAPP transfected cells with pAbL64 (Fig. 10C) located in structures where the staining of the p58 Golgi protein was also detected (Fig. 10D). Therefore, these structures likely represent Golgi stacks. The full-length cDNA of the human BSDL, including the sequence coding for the 16 C-terminal repeats, in pS429 (34) was amplified by PCR using primers BSDL-5’ and BSDL-3’. The transcript of 2.2 kb was subcloned directly into pSecTag under the same conditions as the 1.8-kb transcript amplified from SOJ-6 cells. The plasmid (pSecBSDL) was also transfected into CHO-K1 cells, and a homogeneous clone (CHO-pSecBSDL) was selected.

To determine whether the translated products can be secreted, CHO-pSecFAPP and CHO-pSecBSDL cells were allowed to stand in fresh RPMI medium for 6 h. At the conclusion of the incubation, the cell-free medium was withdrawn and stored. Cells were washed, harvested, pelleted, and lysed. The lysate was cleared, and BSDL and LDH activities were recorded in cell-free medium and lysate. The LDH activity recorded in the extracellular medium of the two clones was very low (<5% of total activity) and indicated that no lysis of transfected cells occurred during the incubation time. As shown in Table I, secreted BSDL activity (corrected for the endogenous esterolytic activity of control cells transfected with the empty pSecTag vector), which represents 56% of the total esterolytic activity expressed in CHO-pSecFAPP cells, was detected in the cell-free medium. This value was higher than those determined with tumoral cells but lower than those recorded in extracellular medium of CHO-pSecBSDL. These data strongly suggest that the FAPP, which contains six repeated sequences, can be secreted once transfected into CHO-K1 cells.

**DISCUSSION**

Most of the human pancreatic cell lines were established from tumors displaying a ductal morphology. This is not surprising, as 90% of human pancreatic tumors are adenocarcinoma, with most of these presenting a ductal phenotype (35). A significant variability in the expression of different biochemical markers has been observed. Ductal phenotype marker such as carbonic anhydrase II was detected in some cell lines such as BxPC-3 but not in PANC-1 (36). Trypsinogens 1 and 2, which are markers for the acinar phenotype, were detected in CF-PAC-1 and CAPAN-1 cells but not in PANC-1 cells (37). In a previous study, we showed that human pancreatic tumoral cell lines BxPC-3 and SOJ-6 and human pancreatic adenocarcinoma tissue expressed a 46-kDa (p46) immunofrom of BSDL, whereas the 100-kDa (p100) immunofrom could not be detected in clarified cell extracts (22). In this study, we demonstrated that the p100 immunofrom of BSDL is expressed by BxPC-3 and SOJ-6 cell lines. This protein is also expressed by human pancreatic adenocarcinoma, and BSDL was mainly found associated with intracellular membranes of tumoral cells as also described in normal human pancreatic tissue (8). Providing an explanation to previous results (22), the p100 immunofrom of BSDL cannot be detected in the soluble fraction of tumor cells, whereas the p46 immunoreactive form partitioned between soluble and membrane fractions. The expression of BSDL by SOJ-6 cells was ascertained by RT-PCR and DNA sequencing. PCR amplification, using RNA extracted from BxPC-3 cells, also confirmed the presence of a BSDL mRNA in these cells. Attempts to detect a-amylase were all negative. Consequently, it is suggested that BSDL is expressed in tumoral pancreatic cell lines and in tumoral pancreatic tissue, whereas a-amylase cannot be detected. The still unanswered question concerns the nature of the p46 immunoreactive form of BSDL, which was
Fig. 8. Amino acid sequence of the BSDL expressed by SOJ-6 cells. The amino acid sequence of the BSDL expressed in SOJ-6 cells was deduced from the cDNA sequence of the RT-PCR transcript. **Bold double underlined** amino acids are involved in the catalytic site; **arrowheads** indicate disulfide bridges. The **asterisk** and **dots** indicate putative sites for N- and O-linked glycosylation, respectively. **Numbers** locate tandem-repeated sequences. Residue Gly-599 in **bold** represents the mutated residue Asp → Gly.
detected in tumoral pancreatic cells (Ref. 22 and this study). In light of recent data showing that BSDL secretion involves an association with a membrane-folding complex, including proteins of 94 kDa (Grp94-related p94 protein) and 46 kDa (9), one may wonder if p46 is complexed with BSDL within cancer cells and therefore coprecipitates with the enzyme. Accordingly, p46 may not be related to BSDL, and studies are in progress to clear this specific point. We went further in this investigation and examined the secretion of the p100 immunoreactive form of BSDL. For this purpose, BSDL activity was recorded in the culture medium of SOJ-6 cells. Extracellular LDH activity was extremely weak, and consequently cell lysis may not be responsible for the presence of BSDL in SOJ-6 cell culture medium. Thereby, immunoreactive forms of BSDL associated with Mr; 100,000 and 125,000 were detected in culture medium of SOJ-6 cells. Although no BSDL activity was recorded in BxPC-3 cell culture medium, Western blots allowed us to detect the protein. An explanation for that could be the different sensitivity of methods used (colorimetric enzyme assay and 125I-protein A overlay) or a poor activity of BSDL expressed by tumoral cells. The high Mr form of BSDL (125 kDa) was the unique form detected in BxPC-3 cell culture medium.

Therefore, tumoral cells expressed a high Mr variant of BSDL, the nature of which was further investigated. The cDNA covering the entire sequence of BSDL expressed in SOJ-6 cells was obtained by RT-PCR, and data indicated that the N-terminal domain encoded by exons 1–10 was strictly identical to that of BSDL expressed by normal human pancreas (3). However, the size of the C-terminal region encoded by the SOJ-6 transcript was shorter than that of BSDL (3), and a deletion of 330 bp was observed. The 1.8-kb cDNA fragment, amplified from the SOJ-6 cell RNA, could be produced by eliminating a large loop occurring in the 2.2-kb mRNA structure in RT or in PCR reactions. Prediction of the putative secondary structures of mRNA sequence coding for the C-terminal repeats of BSDL was performed according to the stems-and-loops program (Stemlo program, Infobiogen) and the free energy minimization model for RNA folding (38). Both programs predict that stems

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**FIG. 8**—continued
and loops can be formed within this GC-rich sequence. However, neither of them lead to the excision of a 330-bp loop giving rise to the exact deletion of ten repeats. Moreover, a reading frameshift with the occurrence of new stop codons would occur. However, the amplification of a 2.2-kb transcript of BSDL has been obtained from the complete cDNA (i.e. including the 16 repeated sequences) using the same protocol. This ruled out any PCR artifacts. Northern blot experiments, using a probe that hybridizes with the 5' region of the BSDL mRNA, suggested that an mRNA strand of approximately 1.8–2.0 kb was often associated with the 2.2-kb mRNA encoding for human BSDL (3, 32, 39). In this study using pancreatic tumoral cells, the shorter mRNA could have been amplified as the 1.8-kb cDNA. The translated product corresponding to this mRNA should have a protein core of approximately 70 kDa as indeed was found after in vitro translation of RNA from tumoral cells. However, taking into account possible post-translational modifications of the protein (9, 40), it is conceivable that variants of BSDL expressed by tumoral cells may be the product of this short mRNA, which possibly lacks the nucleotide fragment encoding repeats 5 to 14. Obviously, glycan structures present on this protein should be larger than those present on BSDL and may correlate with previous data showing that the carbohydrate moiety of FAPP accounted for some 47% of its apparent mass (14). All observations and results (13, 18, 20, 33) suggested that the cDNA transcript isolated from SOJ-6 cells would encode for the fet-to-acinar glycoisoform of BSDL, referred to as the fet-to-acinar pancreatic protein or FAPP (13, 14).

![Fig. 9. Comparison of the amino acid sequence of the SOJ-6 transcript and BSDL.](image)

| SOJ-6 | HUMSDL |
|-------|-------|
| AKLGVATQGFWEGVNYKLLGGGDLGSDVDFKGLPFPAAPTPAKLEPNQHPGQWYQSTLAKNYPK | AKLGVATQGFWEGVNYKLLGGGDLGSDVDFKGLPFPAAPTPAKLEPNQHPGQWYQSTLAKNYPK |
| KRCIQATTTQDSYGDCLHLNYWIFQGRQRQVSRDLHVMWYIYGAGLMSSGSGANFLNN | KRCIQATTTQDSYGDCLHLNYWIFQGRQRQVSRDLHVMWYIYGAGLMSSGSGANFLNN |
| YLYDGQATKNYVTVFPQGFLGSRDHLPNCGGUIQMAIIWVPERNNIAAFQG | YLYDGQATKNYVTVFPQGFLGSRDHLPNCGGUIQMAIIWVPERNNIAAFQG |
| DPNITLQGESAVQSLQTVLYKHLRRIASIQSGVQLPFWIQPNPLWAKWKAVK | DPNITLQGESAVQSLQTVLYKHLRRIASIQSGVQLPFWIQPNPLWAKWKAVK |
| GCCVQDFADAMQCKLTQVPTAALQYVLQLAGLEYPMLHYVGFVPVPFDGIFAPNPILY | GCCVQDFADAMQCKLTQVPTAALQYVLQLAGLEYPMLHYVGFVPVPFDGIFAPNPILY |
| NAADIXYAGTNNMDQHFAISDMPA15KGNKKTVEDEYKLVSEIFITLKGLWARKTTTDDV | NAADIXYAGTNNMDQHFAISDMPA15KGNKKTVEDEYKLVSEIFITLKGLWARKTTTDDV |
| YTESWQDFQSENKKTVDQFEDWTLQTVPEIAAQHRANAKSAKTQAYLFHPSRMVY | YTESWQDFQSENKKTVDQFEDWTLQTVPEIAAQHRANAKSAKTQAYLFHPSRMVY |
| PWWYGDADAOQVVFYKPRATPGYRFQDVQTVKAMAYWNTFARTGDPNMGDSAVPTWH | PWWYGDADAOQVVFYKPRATPGYRFQDVQTVKAMAYWNTFARTGDPNMGDSAVPTWH |
| EPTYTSSGELYITKKGMSGSSMRSLRNLFPNLTMLYALIPTDQQPVTVPDGEAEAT | EPTYTSSGELYITKKGMSGSSMRSLRNLFPNLTMLYALIPTDQQPVTVPDGEAEAT |
| FVPPPTGDSITAPVPTGDSAGPPVPFTGDS | FVPPPTGDSITAPVPTGDSAGPPVPFTGDS |
| SGAPPVPTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDS | SGAPPVPTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDS |
| TPGQSEDAPFPVPPGSSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPP | TPGQSEDAPFPVPPGSSAGPPVPFTGDSAGPPVPFTGDSAGPPVPFTGDSAGPP |

FIG. 9. Comparison of the amino acid sequence of the SOJ-6 transcript and BSDL. The amino acid sequence deduced from the SOJ-6 transcript (SOJ-6) was compared with that of the human pancreatic BSDL (BSDL) (3). Matches are indicated by an asterisk, mismatching amino acids are indicated with a dot, and the deleted sequence is denoted by the dashed line. Numbers locate tandem-repeated sequences.

Previous studies have shown that FAPP (characterized by its reactivity with the mAb28) cannot be detected in conditioned medium of pancreatic tumoral cells (14, 21). It has been postulated that the absence of secretion was due to the retention of the protein within the ER and suggested that FAPP would remain associated with ER resident protein(s) as a consequence of its improper folding (21). We have further shown that a multiprotein membrane complex including the chaperone Grp94-related p94 protein may play an essential role in the folding and transport of BSDL (9). Moreover, the glycosylation of the C-terminal tandem-repeated sequences regulates the secretion of BSDL (11). The distribution of FAPP in tumoral cells indicated that the protein, which is mainly membrane associated, distributes within the ER and the Golgi where FAPP colocalizes with the p58 Golgi protein. These data suggested that FAPP would be sequestered within either of these...
Amino acid composition of C-terminal fragment of BSDL

| Residue | Predicted | Reported | Deduced |
|---------|-----------|----------|---------|
| Ala     | 9.2       | 9.1      | 8.3     |
| Asx     | 9.2       | 9.3      | 8.8     |
| Arg     | 1.5       | 1.0      | 2.3     |
| Gly     | 12.6      | 12.4     | 16.5    |
| Gln     | 3.9       | 3.6      | 7.7     |
| His     | 0.0       | 0.0      | 0.7     |
| Ile     | 0.0       | 0.0      | 1.5     |
| Leu     | 2.4       | 2.8      | 4.2     |
| Lys     | 1.0       | 0.7      | 2.8     |
| Met     | 0.5       | 0.0      | 4.1     |
| Phe     | 0.5       | 0.9      | 1.9     |
| Pro     | 29.1      | 30.1     | 18.6    |
| Ser     | 7.5       | 7.0      | 9.0     |
| Thr     | 12.6      | 12.5     | 6.8     |
| Tyr     | 1.0       | 1.1      | 1.6     |
| Trp     | 0.5       | ND       | ND      |
| Val     | 8.3       | 8.7      | 6.7     |
| Cys     | 0.0       | ND       | ND      |
| Total   | 100.1     | 99.8     | 100     |

*The predicted amino acid composition indicates the amino acid composition corresponding to that of the C-terminal peptide of BSDL from Met-510 to Met-716 (3).

* Reported amino acid composition of the C-terminal peptide of BSDL either unreactive (ConA-unreactive) or reactive (ConA-reactive) to concanavalin A (18).

"The amino acid composition of the C-terminal fragment of SOJ-6 cells was deduced from the sequence of the cDNA transcript from Met-510 to Met-606 (see Fig. 8). ND, not determined.

FIG. 10. Transfection of CHO-K1 cells. The RT-PCR transcript obtained with SOJ-6 cells was cloned into pSecTag vector and used to transfect CHO-K1 cells. A, immunofluorescence using pAbL64 and FITC-conjugated antibodies directed against rabbit immunoglobulins was performed on an homogeneous transfected clone. B, control CHO cells transfected with the empty pSecTag vector. C, magnification of transfected CHO-K1 cells treated as in A prior to immunofluorescence. D, transfected CHO-K1 cells labeled with mouse antibodies specific for the p58 Golgi protein and TRITC-conjugated antibodies directed against mouse immunoglobulins.

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Bile Salt-dependent Lipase in Pancreatic Tumoral Cells