Ectopic Epididymal Expression of Guinea Pig Intestinal Phospholipase B

POSSIBLE ROLE IN SPERM MATURATION AND ACTIVATION BY LIMITED PROTEOLYTIC DIGESTION

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Guinea pig intestinal phospholipase B is a calcium-independent phospholipase hydrolyzing sequentially the acyl ester bonds at sn-2 and sn-1 positions of glycerophospholipids, promoting the formation of sn-glycero-3-phosphocholine from phosphatidylcholine. This 140-kDa glycoprotein from the brush border membrane of differentiated enterocytes contributes to lipid digestion as an ectoenzyme. The cDNA coding for guinea pig phospholipase B was revealed to be the homologue of AdRab-B, an mRNA appearing in rabbit upon intestine development. The sequence predicts a polypeptide of 1463 amino acids displaying four homologous repeats, two of them containing the lipase consensus sequence GXSXG. A 5-kilobase transcript was particularly abundant in mature ileal and jejunal enterocytes but was also detected in epididymis, where phospholipase B displayed a higher molecular mass (170 kDa versus 140 kDa in intestine), with no obvious evidence for enzyme activity. Trypsin treatment of phospholipase B immunoprecipitated from epididymal membranes reduced its size to 140 kDa, coinciding with the appearance of a significant phospholipase A₂ activity. The same results were obtained in COS cells transfected with phospholipase B cDNA. Since sn-glycero-3-phosphocholine present at high concentrations in seminal plasma mainly stems from epididymis, this suggests a possible role of phospholipase B in male reproduction. This novel localization also unravels a mechanism of phospholipase B activation by limited proteolysis involving either trypsin in the intestinal lumen or a trypsin-like endopeptidase in the male reproductive tract.

PLB (EC 3.1.1.5) is defined as a hydrolyase displaying both a PLA₂ and a lyso phospholipase activity, thus achieving the full deacetylation of glycerophospholipids. Several PLB have been identified in various microorganisms (1–8) and in plants (9) as well as in the brush border membrane of mature enterocytes from three animal species including guinea pig (10–13), rat (14–17), and rabbit (17, 18). It was later found that intestinal PLB actually displays a broader substrate specificity, including diacylglycerol, monoacylglycerol (12), and retinyl esters (19). All of these data have led to the obvious conclusion that intestinal PLB participates in the digestion of various dietary lipids.

Despite its broad substrate specificity, PLB can be viewed as a PLA₂ due to its ability to release first the fatty acid esterifying the sn-2 position of glycerophospholipids. Indeed, guinea pig intestinal PLB was recently considered as a subgroup of calcium-independent PLA₂ (20). Moreover, a homologous sequence is shared by Penicillium notatum PLB and cytosolic PLA₂, the major enzyme responsible for intracellular liberation of arachidonic acid (21). Our knowledge of PLA₂ has considerably grown these last years with a number of enzymes being characterized at a molecular level (for a recent classification, see Ref. 22). This has allowed clarification of the role and the regulation of some of these enzymes, although there are still a number of uncertainties (23, 24).

The active site of guinea pig PLB is localized on the external face of the brush border membrane from which it can be solubilized either by detergents or by proteolytic cleavage (11), suggesting a structural arrangement similar to that of other brush border hydrolases such as sucrase-isomaltase, aminopeptidase N, or lactase-phlorizin hydrolase. All of these enzymes are stalked in the lipid bilayer by a hydrophobic segment connected to the larger, heavily glycosylated globular domain containing the catalytic site and protruding into the intestinal lumen (25–27). The mode of anchoring as well as other structural and functional features of these enzymes have been clearly determined by cloning and sequencing their cDNA (28–30). Thus, another interest of intestinal PLB is that, like other microvillar hydrolases, it might offer a very convenient tool to study general processes of cell biology such as protein glycosylation, differentiation of epithelial cells, or membrane traffic allowing proper addressing of proteins to apical membranes (25–27).

In an effort to understand both the enzymatic properties and the intracellular trafficking of guinea pig intestinal PLB in relation to enterocyte differentiation, we decided to clone the corresponding cDNA. The data reported below indicate that guinea pig PLB cDNA is actually the homologue of AdRab-B, an mRNA appearing in rabbit upon intestine development and enterocyte differentiation (18). However, an emerging observa-
FIG. 1. Nucleotide and deduced amino acid sequences of the cDNA coding for guinea pig PLB. A, The deduced amino acid sequence is shown below the nucleotide sequence. Both nucleotides and predicted amino acids are numbered on the right. Broken underlines indicate amino acid sequences of PLB tryptic fragments. The putative amino-terminal signal sequence and carboxyl-terminal transmembrane domain are underlined. Boldface asparagine residues represent potential N-glycosylation sites. Boldface serine residues indicate potential active serines surrounded by underlined lipase consensus sequence. The sugar transport signature is double underlined. The stop codon limiting the open reading frame is indicated by a hyphen. Potential substrates for cytosolic protein kinase C and casein kinase are indicated by asterisks. The arrows correspond to various oligonucleotides used for PCR. B, Schematic structure of guinea pig PLB. C, Comparison of deduced amino acid sequences of guinea pig PLB and rabbit AdRab-B (18). Those amino acids identical between the two sequences are indicated by vertical lines. Putative lipase consensus sequences are in boldface type.

The detection of the mRNA of intestinal PLB in epididymis. GPC is the final product resulting from phosphatidylcholine breakdown by PLB. It is present at 1–3 mM concentrations in the seminal plasma of various animal species, and epididymis represents its major site of synthesis in the male reproductive tract, epididymal fluid containing as much as 100 mM GPC (31–38).
indicated that rat epididymis contains an androgen-dependent phospholipase A as well as a lysophospholipase activity able to produce GPC from phosphatidylcholine; however, no further molecular characterization of these enzymes has been achieved so far (39, 40). Together with the evidence that epididymal PLB is synthesized as a proenzyme requiring limited proteolytic cleavage to reveal full enzymatic activity, our data open interesting issues concerning the biological function as well as the regulation of this subgroup of calcium-independent PLA2.

**EXPERIMENTAL PROCEDURES**

Isolation of Various Intestinal Cell Populations—Intestinal cells were isolated and separated as a villus to crypt gradient according to Weiser (41).

Immunoprecipitation—Intestinal brush border membrane vesicles were prepared according to Schmitz et al. (42). Proteins were solubilized by the addition of an equal volume of a twice concentrated immunoprecipitation buffer referred to as RIPA I buffer (43) and containing 40 mM Tris-HCl (pH 7.5), 300 mM NaCl, 4 mM EDTA, 1% (w/v) sodium deoxycholate, 2% (v/v) Triton X-100, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 5 mg/ml leupeptin). Samples were pre-cleared by adding 25 µl of a 10% (v/v) suspension of protein A-Sepharose (Sigma), mixing for 30 min at 4 °C, and centrifuging for 2 min at 13,000 × g. Supernatants were then incubated overnight at 4 °C with 10 µl of polyclonal anti-guinea pig PLB obtained as described previously (13). Immunocomplexes were precipitated by the addition of 50 µl of the 10% suspension of protein A-Sepharose. After 4 h of incubation at 4 °C under gentle shaking, samples were then centrifuged at 13,000 × g for 2 min, and the pellet was washed twice in RIPA I buffer and once in RIPA II buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA), resuspended in Laemmli buffer (44) for SDS-PAGE or in phosphate-buffered saline containing 1 mM phenylmethysulfonyl fluoride, 1 µg/ml leupeptin, and 1 mM benzamidine for determination of PLA2 activity.

Polymerase Chain Reaction Amplification—PCR was performed by standard techniques (46) with Taq DNA polymerase (Applied Biosystems) in a Crocodile III thermal cycler (Applied Biosystems, Les Ulis, France).
bromide staining. Oligonucleotides were synthesized in a PCR Mate DNA synthesizer (Applied Biosystems), except PLB5 and OI2, which were obtained from Isoprime (Toulouse, France). 

**Radioactive Probes**—The PLB1 probe (468 bp) was obtained by PCR using intestinal guinea pig cDNA, obtained by random priming reverse transcription of 60 mg of poly(A) + RNA, and hybridized to the cDNA. The oligonucleotides, cob3' and cob3, derived from the guinea pig PLB amino acid sequences B and C, respectively. The PCR protocol comprised 1 min at 94 °C, 1 min at 45 °C, and 1 min 20 s at 72 °C for 30 cycles. The PLB2 probe (460 bp) was obtained by PCR on 2a1 clone plasmid DNA with P205 and M10 oligonucleotides (1 min at 94 °C, 1 min at 52 °C, and 1 min at 72 °C for 30 cycles). The plaque lift gelatine dehydrogenase probe (350 bp) was prepared by PCR on human lung cDNA with specific oligonucleotides determined according to the sequence (47). The 30-cycle program consisted of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C. These probes were labeled by random primer extension using the Nonprimer Kit (AppiGene Oncor) and [α-32P]dCTP from Amersham Pharmacia Biotech. 

**Northern Blotting**—Total or poly(A) + RNA were run on 1% agarose gels in the presence of 0.37 μm formaldehyde, transferred by capillarity (46) to positively charged nylon membrane (Biohyb Z+, Bioprobe system) and covalently immobilized on the membrane by exposure to UV light. Filters were hybridized as described for library screening with a final wash at 65 °C in 0.1% SDS for PLB1 probe and in 0.2% SSC, 0.1% SDS for glyceraldehyde-3-phosphate dehydrogenase probe. Deblocting of the membranes was obtained by treatment in 0.1% SDS at 100 °C. 

**Epididymal Subcellular Fractionation**—Animals were killed by a blow to the head, and the epididymis was removed. It was rinsed with ice-cold isotonic NaCl and homogenized on ice in Tris-sucrose buffer (250 mM sucrose buffered with 10 mM Tris-HCl, pH 7) using a Potter-Elvejhem homogenizer. The subcellular fractionation was carried out according to Diagne et al. (10) by a sequential centrifugation procedure, and the different pellets were resuspended in the homogenization buffer. 

**Trypsin Digestion of PLB**—Transfected COS cell homogenate or epididymal 100 000 × g pellet were incubated for 30 min at 37 °C in the presence or in the absence of trypsin (50 μg/ml). The reaction was stopped by adding soybean trypsin inhibitor (30 μg/ml) in 5 mM potassium phosphate (pH 6.8) as described by Quaroni et al. (49). The sample was used for gel electrophoresis and determination of PLB activity. 

**Immunoblotting**—Proteins were subjected to SDS-PAGE on a 7% (w/v) polyacrylamide gel under reducing conditions according to Laemmli (44) and transferred to nitrocellulose membranes (Hybond C Extra; Amersham Pharmacia Biotech). Immunoreactive proteins were detected using a 1:500 dilution of the polyclonal anti-PLB antibody, second antibody (goat anti-rabbit, Promega, France) being conjugated to alkaline phosphatase. The substrate was 5-bromo-4-chloro-3-indolyl phosphate, and the chromogen was nitro blue tetrazolium (Promega, France). 

**Epididymal cDNA Synthesis**—About 2 μg of poly(A) + RNA from guinea pig epididymis were converted into single-strand cDNA using oligo(dT) primer and Moloney murine leukemia virus reverse transcriptase (Superscript Preamplification System; Life Technologies, Inc., Cergy, France) in a final volume of 10 μl, from which 10-μl aliquots were used for each PCR amplification. 

**Assays of PLB, Lyosphospholipase, and Lipase Activities**—These were achieved using 1-acyl-2-[14C]linoleoyl-sn-glycero-3-phosphocholine, 1-acyl-sn-glycero-3-phospho-[3H]choline, or [3H]oleoyl-labeled diolein as described previously (11, 12). 

### RESULTS

The cDNA Coding for Guinea Pig Intestinal PLB Is the Homologue of AdRab-B—Guinea pig PLB was immunoprecipitated from brush border membranes with a specific polyclonal antibody and migrated on SDS-PAGE under reducing conditions. The 140-kDa Coomassie Blue-stained band was excised, treated with trypsin, and partially sequenced as described under “Experimental Procedures.” Three amino acid sequences (A, VAGFFNQA; B, LYNLVDIFMN; C, LGDLSLA) were obtained, and the B and C sequences were used to design two oligonucleotides (cob3' and cob3, respectively). A 486-bp fragment (PLB1) was PCR-amplified from intestinal guinea pig cDNA with these two primers and used for screening a cDNA library prepared in λ-ZAP II vector. Eight positive clones were isolated and analyzed. They carried 3-3.5-kbp cDNA insertions, and they were all sequenced on a few bases at their 3' and 5'-terminal ends. Six of them displayed an identical 3'-terminal exon (initiated at the poly(A) tail), indicating that these were subclones of the same cDNA clone. Oligo(dT) primer fusing 2a1 cDNA (346 kbp, 2a1 clone) was totally sequenced, but it did not contain the ATG initiation codon. In order to isolate the full-length cDNA, a 460-bp probe (PLB2) was PCR-amplified on the 2a1 5'-terminal end between the two specific primers: P205 and M10 (Fig. 1A). This probe was used for a second screening of the cDNA library and allowed the isolation of the 4a clone.
containing a 3.679-kbp cDNA.

Since we could not identify any ATG initiation codon on this clone, we chose to isolate the 5′-terminal end of PLB cDNA by the 5′-RACE method. The primers used are shown in Fig. 1A. The mRNA isolated from jejunal mucosa was reverse transcribed using an oligo(dT) primer. The single-stranded cDNA was converted to double strand, and a 44-bp adaptor was ligated at both ends. PCR amplification with M10 primer, located 660 bp downstream of the 5′-terminal end of the 4a clone (Fig. 1A), and with the adaptor primer 1 allowed us to obtain a 1.5-kbp band. This was reamplified using internal ol2 primer and adaptor primer 2 as a single band of about 1.1 kb. The nucleotide sequence of this 1.1-kbp RACE product displayed an initiator ATG codon at its 5′-terminal end.

The full-length PLB nucleotide sequence (Fig. 1A) consists of 4608 bp. We have assigned the first ATG codon (nucleotides 39–41) as the initiator one, since the surrounding sequence meets the criteria for a translation initiation site, i.e. GCCATGG compared with the consensus ACCATGG (50). Moreover, this ATG codon is preceded four codons upstream by an in frame termination codon (TGA). The 184 bases of 3′-untranslated sequence contains a consensus polyadenylation signal sequence (AATAAAA), 14 bp upstream of the poly(A) tail.

The single long open reading frame of 4.392 kbp predicts a primary translation product of 1463 amino acids (Fig. 1, A and B). The amino-terminal sequence shows a typical cleavable signal sequence for translocation through the membrane of the endoplasmic reticulum. Maximal cleavage propensities were found following residues 20 and 21 (51). The sequence of the primary translation product, coded by nucleotides 99–4431, comprises 1463 amino acids, with a calculated molecular mass of 158,692 Da. Hydropathy analysis by the method of Kyte and Doolittle (48) identified the N-terminal signal sequence and a C terminus-spanning domain of 29 amino acids (residues 1415–1443). It defined a short cytoplasmic carboxyl-terminal domain and a very large extracellular domain. In the cytoplasmic domain, serines 1145 and 1153 are potential phosphorylation sites for protein kinase C and casein kinase, respectively. Amino acids 38–1403 can be divided into four repeated sequences (I, 38–346; II, 364–705; III, 707–1053; IV, 1063–1403). Two lipase consensus sequences (GXSXG), which are found in several serine esterases (52, 53), are present at amino acid residues 452–456 (domain II) and 1153–1157 (domain IV). The entire primary structure contains 12 asparagine residues that are part of the consensus sequence NX(S/T) for N-glycosylation sites (54). A sugar transport signal is also indicated on the sequence.

As shown in Fig. 1C, the amino acid sequence shares 72% identity with AdRab-B, an intestine-specific cDNA cloned in the adult rabbit (18), indicating that the PLB cDNA described herein represents the guinea pig homologue of AdRab-B. Indeed, at the exception of Cys-1444 present exclusively in AdRab-B, there is a perfect conservation of the 32 cysteine residues and of the two lipase consensus sequences, AdRab-B containing, however, an additional lipase consensus sequence (GSSEG, residues 1222–1226) in domain IV.

**Intestinal Expression of Guinea Pig PLB Gene Is Maximal in Jejunum/Ileon and Follows Enterocyte Differentiation**—Using the Weiser procedure, expression of guinea pig PLB mRNA was analyzed along the crypt/villus axis with the PLB1 probe. As shown by Northern blot (Fig. 2A), a single 5-kilobase transcript was detected in all fractions, and we observed an increase of this specific mRNA level from undifferentiated cells (fraction 9) to differentiated ones (fractions 1–3). The same distribution was previously observed for protein expression (13), suggesting a transcriptional regulation of intestinal phospholipase B gene expression. The filter was debotted and hybridized with a glyceraldehyde-3-phosphate dehydrogenase probe demonstrated that the amount of RNA loaded onto each lane was very similar.

To complete analysis of phospholipase B expression in guinea pig intestine, a regional dissection was accomplished. Total RNA from duodenum, jejunum/ileon, and colon mucosa was extracted and hybridized to PLB1 probe. The highest PLB mRNA level was observed in jejunum/ileon and little was found in duodenum, whereas no expression was detected in colon (Fig. 2B, right).
Intestinal and Epididymal Phospholipase B

Epididymis Appears as a Second Site of PLB Gene Expression—PLB mRNA expression was also investigated in other guinea pig tissues, confirming the selective localization of PLB in the digestive tract. However, an additional site of PLB expression was observed in epididymis, as clearly illustrated in Fig. 2B. Northern blot data were confirmed by PCR analysis of single-stranded epididymal cDNA. Four couples of intestinal PLB-specific primers (Fig. 2C) located on the four repeated domains were used in amplification reactions with epididymal cDNA or 4a cDNA as a matrix. With each couple of primers, epididymal cDNA gave a specific PCR amplification product of the expected size (Fig. 2C), indicating that it contains the four repeated sequences. Moreover, the cob5/cob3’ 486-bp product was sequenced and revealed a total identity with intestinal PLB cDNA.

This led us to investigate PLB protein expression in epididymis by subcellular fractionation of epididymal homogenates. Using a polyclonal anti-PLB antibody, a single 170-kDa polypeptide was detected by Western blotting in the different subcellular fractions of epididymis (500 μg of protein each), and the anti-PLB immunoprecipitate obtained from 1 mg of protein of the 100,000 × g pellet were subjected to SDS-PAGE on 16 × 17.5-cm gels and analyzed by Western blotting using the polyclonal anti-PLB antibody. B, effect of N-glycosidase F on epididymal PLB. The 100,000 × g fraction from epididymis was heated for 5 min at 100 °C in denaturing buffer containing SDS and mercaptoethanol, incubated overnight at 37 °C in the absence (−) or in the presence (+) of N-glycosidase F. After treatment, the proteins were analyzed by Western blotting.

FIG. 3. Expression of PLB protein in guinea pig epididymis. A, subcellular localization of epididymal PLB. Intestinal homogenate, the different subcellular fractions of epididymis (500 μg of protein each), and the anti-PLB immunoprecipitate obtained from 1 mg of protein of the 100,000 × g pellet were subjected to SDS-PAGE on 16 × 17.5-cm gels and analyzed by Western blotting using the polyclonal anti-PLB antibody. B, effect of N-glycosidase F on epididymal PLB. The 100,000 × g fraction from epididymis was heated for 5 min at 100 °C in denaturing buffer containing SDS and mercaptoethanol, incubated overnight at 37 °C in the absence (−) or in the presence (+) of N-glycosidase F. After treatment, the proteins were analyzed by Western blotting.

PLB Is Synthesized as a Proenzyme Activated by Proteolytic Cleavage—To verify the presence of a proenzyme, PLB was immunoprecipitated from the 100,000 × g epididymal pellet, submitted to trypsin digestion, characterized by Western blotting, and tested for PLA2 activity. As shown in Fig. 4A, trypsin treatment decreased the molecular mass of epididymal PLB from 170 to 140 kDa, which corresponds to the size observed in intestinal brush border membrane. Identical results were obtained upon trypsin treatment of whole membranes from the 100,000 × g pellet under conditions allowing a total conversion of the 170-kDa band into a 140-kDa polypeptide. Occasionally, a smaller fragment also appeared at 116 kDa (data not shown). Interestingly, this shift in mass was associated with the appearance of PLA2 activity (Fig. 4B), clearly demonstrating a proteolytic regulation of the enzyme.

Properties of Recombinant PLB Expressed in COS Cells—To follow PLB expression with the pcDNA3 construction and to confirm the data obtained with epididymal PLB, COS cells were transfected as described under “Experimental Procedures.” Analysis of cell homogenates and immunoprecipitates by Western blotting (Fig. 4C) indicated that PLB was not expressed in pcDNA3-transfected COS cells (lanes 1–3), whereas a 170-kDa polypeptide was recognized by the anti-PLB antibody in COS cells transfected with pcDNA3-PLB (lanes 4–6). In immunoprecipitated fractions (lane 5), the 140-kDa proteolytic form was slightly detectable but increased significantly upon trypsin treatment (lane 6). Here again, trypsin digestion was concomitant with a dramatic increase in PLA2 activity (Fig. 4D), demonstrating that proteolytic cleavage was required for the protein to express a full enzymatic activity.
DISCUSSION

This study was based on the molecular cloning of the cDNA coding for guinea pig intestinal PLB, which revealed its identity with AdRab-B previously isolated in rabbit (18). Although both proteins are expected to display very similar structures and properties, one small difference concerns the lipase consensus sequence GXSXG, which was identified in domains II and IV of guinea pig PLB, whereas an additional motif (GS-SEG) is present in repeat IV of AdRab-B. In a recent study, Wacker et al. (55) identified the active serine of the product of AdRab-B, which they now call “adult only” esterase/phospholipase A. Unexpectedly, serine 400 was the only residue labeled with $^{14}$Cdiisopropyl fluorophosphate under conditions where enzyme activity toward both long chain fatty acyl esters and phosphatidylcholine was abolished. This active serine is located in a sequence (GDSSLT) similar to lipase consensus sequence and is absolutely conserved in repeats II, III, and IV of both rabbit and guinea pig phospholipases B (see Fig. 1C).

Further studies based on site-directed mutagenesis will be necessary to identify other residues of the catalytic triad and to explain why only one of the four repeated domains apparently bears the enzymatic activity. In addition, there might be some significant differences between guinea pig and rabbit PLB. For instance, the latter one was found to be insensitive to sulfhydryl blocking reagents (18), whereas the guinea pig enzyme is fully inhibited by N-ethyl maleimide (12). Interestingly, cytosolic PLA$_2$ is inhibited by SH-blocking reagents; however, a serine but not a cysteine residue was identified in the catalytic triad, where histidine is replaced by an arginine residue (21, 56). This apparently small difference would deserve some attention in future studies dealing with the identification of PLB active site(s).

A first use of PLB cDNA was to follow the pattern of mRNA expression in guinea pig intestine, which was identified so far as the only site of PLB synthesis. These and previous data all support the view that the PLB gene is expressed as a function of enteroctye differentiation, probably at a transcriptional level, resulting in a maximal synthesis of PLB in cells that are the most implicated in lipid digestion, i.e. mature enteroctyes of jejunum and ileon (10–18).

We would like to focus on the most crucial point of this study, i.e. our finding that guinea pig intestinal PLB is also strongly expressed in epididymis. This is actually not the first example of a pancreatic or intestinal enzyme with an ectopic expression suggesting other roles than a simple digestive function. For instance, pancreatic (type I) PLA$_2$ has been found in lung (57, 58), spleen (59), gastric mucosa (58, 60–61), pancreatic $\beta$-cells (62), and kidney (63). It is noteworthy that a membrane receptor specific for secretory PLA$_2$ was also found in some of these tissues (64–66). A pancreatic lipase was identified in mouse cytotoxic T lymphocytes (67) and is now classified in a subgroup of pancreatic lipase-related proteins (68–73), whose first member was actually discovered in guinea pig pancreas (74). Finally, intestinal dipeptidyl peptidase IV (also called CD26) is expressed in T-lymphocytes and thymocytes, where it appears as an activation signal-transducing molecule as well as having its intrinsic enzymatic activity (75, 76). A first issue of these various observations would be to investigate which factors regulate a so tight but so different tissue expression of these enzymes. This could be particularly interesting for guinea pig PLB, insofar as its appearance in the male genital tract might be under the influence of androgen secretion, as described for the PLB activity previously observed (39, 40).

In the latter case, it is tempting to suggest that epididymal PLB might fulfill a very critical role in sperm maturation. Spermatozoa are known to reside for about 12–14 days in epididymis, where they acquire a number of proteins essential for their motility and for oocyte fertilization (77–81). Moreover, during epididymal transit, spermatozoa loose up to 50% of their membrane phospholipids (82–85). This balances the production of up to 80% of GPC (a main component of seminal plasma probably essential for sperm protection and/or metabolism) by epididymis (31–38). However, spermatozoa might not represent the unique source of epididymal GPC, which could also be derived from phosphatidylcholine of blood lipoproteins (86).

The detection in epididymis of both mRNA and protein corresponding to intestinal PLB stimulated us to search for mechanisms(s) able to trigger the corresponding enzyme activities. The fact that trypsin digestion activates this latent enzyme strongly argues in favor of the view that a similar mechanism might operate in vivo. Proteolytic maturation of intestinal PLB has been suggested to occur during intracellular traffic in a way similar to that of other brush border hydrolases such as lactase-phlorizin hydrolase (18). More recently, Wacker et al. (55) found that a trypsin cleavage site after Arg-363, which is strictly conserved between rabbit and guinea pig proteins (Arg-362 in guinea pig), is present at the junction between repeats I and II and might be responsible for enzyme processing in the intestinal lumen. Roughly, this would result in a 25% reduction of PLB molecular mass, which is identical to the 26% difference determined between 146 and 108 kDa (deglycosylated forms). However, proteolytic digestion of the glycosylated form resulted in a reduction of apparent molecular mass limited to 18% (170 versus 140 kDa). This could be explained by the fact that the N-terminal part of the molecule until Arg-362 contains only one of the 12 N-glycosylation sites present in the entire molecule.

Our observation that native PLB needs limited proteolysis to acquire its full enzymatic activity is reminiscent of pancreatic PLA$_2$, which is produced as a zymogen activated upon removal of its N-terminal heptapeptide (87, 88). This has been the object of a number of functional and structural studies showing that activation of pro-PLA$_2$ results in a conformational change allowing the enzyme to interact with the lipid-water interface by an “interface recognition site” (87, 88). In the case of PLB, trypsin treatment induces a more drastic reduction in the protein size, probably upon the removal of repeat I, as discussed above. Since a priori this domain does not display any enzymatic activity, it will be interesting in future studies to define whether it can act as an intrinsic inhibitor of PLB, impairing for instance its interaction with the lipid-water interface. This can be suggested by the fact that recombinant 170-kDa PLB displayed a significant esterase activity toward water-soluble substrates (18), whereas both recombinant and epididymal 170-kDa PLB were virtually inactive against phospholipids (this study).

The point of PLB adsorption at lipid-water interfaces would be particularly crucial to understand a possible interaction between epididymal PLB and sperm membrane. In our hands, guinea pig intestinal PLB is a poorly penetrating enzyme requiring biliary salts to achieve significant degradation of phospholipids. In a recent study, we provided evidence that secretory nonpancreatic PLA$_2$ (type II PLA$_2$), which is largely involved in inflammatory processes, is another poorly penetrating phospholipase unable to attack phospholipids in the membrane of intact cells (89). However, we also showed that type II PLA$_2$ becomes active on membranes having lost their asymmetric transverse distribution of phospholipids. Our present finding will certainly stimulate further studies dealing with the characterization of sperm membrane organization in relation to its susceptibility to intestinal/epididymal PLB.
