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Novel Human Secreted Phospholipase A_2 with Homology to the Group III Bee Venom Enzyme*

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Venom and mammalian secreted phospholipases A_2 ($sPLA_2$s) have been associated with numerous physiological, pathological, and toxic processes. So far, structurally related group I and II $sPLA_2$s have been found in vertebrates such as mammals and snakes, whereas group III $sPLA_2$s have mainly been found in venom from invertebrates such as bees and scorpions. Here we report the cloning and expression of a cDNA coding for a human group III (hGIII) $sPLA_2$. The full-length cDNA codes for a signal peptide of 19 residues followed by a protein of 490 amino acids made up of a central $sPLA_2$ domain (141 residues) flanked by large N- and C-terminal regions (130 and 219 residues, respectively). The $sPLA_2$ domain is 31% identical to bee venom $sPLA_2$ and displays all of the features of group III $sPLA_2$s including 10 cysteines. The hGIII $sPLA_2$ gene consists of at least 7 exons and maps to chromosome 22q. By Northern blot analysis, a 4.4-kilobase hGIII transcript was found in kidney, heart, liver, and skeletal muscle. Transfection of hGIII $sPLA_2$ cDNA in COS cells led to accumulation of $sPLA_2$ activity in the culture medium, indicating that the cDNA codes for a secreted enzyme. Using small unilamellar vesicles as substrate, hGIII $sPLA_2$ was found to be a Ca^{2+}-dependent enzyme showing an 11-fold preference for phosphatidylglycerol over phosphatidylcholine and optimal activity at pH 8.

In recent years, it has been realized that phospholipases A_2 (PLA_2,[1] EC 3.1.1.4) form a superfamily of intracellular and secreted enzymes, which all catalyze the hydrolysis of glycerophospholipids at the *sn*-2 position to release fatty acids and lysophospholipids (1–4). To date, eight distinct mammalian secreted phospholipases A_2 ($sPLA_2$s) have been cloned and classified into groups I, II, V, and X (2, 4–9). Although the biological role of each of these enzymes has not yet been clearly defined, mammalian $sPLA_2$s have been implicated in various physiological and pathophysiological functions including lipid digestion, cell proliferation, neurosecretion, release of proinflammatory lipid mediators, antibacterial defense, cancer, and inflammatory diseases (3, 4). The level of identity between the eight mammalian $sPLA_2$s can be as low as 23% (8), but they have in common a low molecular mass (14–17 kDa), the presence of several disulfides, a similar Ca^{2+}-dependent catalytic mechanism, and a well conserved overall three-dimensional structure (10–13).

Numerous $sPLA_2$s have also been described in venoms from both vertebrate and invertebrate animals such as snakes and bees (14, 15). Similar to mammalian $sPLA_2$s, snake venom enzymes have been classified into groups I and II, and they all have a common catalytic mechanism and a very similar three-dimensional structure (1, 10–13). Snake venom $sPLA_2$s are often neurotoxins or myotoxins but can also promote physiological effects such as cell migration and cell proliferation (14, 16, 17). Using venom $sPLA_2$s as ligands, different types of $sPLA_2$ receptors have been identified (4). These receptors are likely to be involved in venom $sPLA_2$ toxicity, and recent studies have suggested that mammalian $sPLA_2$s can be the normal endogenous ligands (4, 18, 19). Invertebrate venom $sPLA_2$s are also disulfide-rich proteins, but they have a primary structure distinct from mammalian and snake venom $sPLA_2$s and have been classified into groups III and IX (2, 4). They have been found in bee, scorpion, jellyfish, and marine snail venoms (20–25), and the group III bee venom $sPLA_2$ has been the best studied enzyme. This $sPLA_2$ has been cloned (20), and determination of its three-dimensional structure (11) has revealed important differences with group I and II $sPLA_2$s, although the catalytic site is similar to that of vertebrate $sPLA_2$s (13). Interestingly, $sPLA_2$s similar to the bee venom enzyme were discovered in lizard venom (26, 27), indicating that group III $sPLA_2$s also exist in vertebrates and thus may occur in mammals as well.

In the last 3 years, a systematic search for $sPLA_2$ homologs in nucleic data bases has allowed us to clone four novel mammalian $sPLA_2$s that belong to groups II and X (6–8). Using the same strategy, we identified a human genomic sequence that displays significant homology with the bee venom group III $sPLA_2$. We report here the cloning, genomic organization, chromosomal mapping, tissue distribution, and heterologous expression of the first human group III $sPLA_2$.

EXPERIMENTAL PROCEDURES

Molecular Cloning of hGIII $sPLA_2$—Searching for $sPLA_2$ homologs in gene data bases stored at the National Center for Biotechnology using the tBLASTn sequence alignment program (28) resulted in the identification of a human genomic sequence (PAC clone DJ412A9, GenBank™ accession number AC005005) of 133,893 nucleotides containing several regions of homology to bee venom group III $sPLA_2$. This suggested that this large genomic clone contains a gene with several exons

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[1] The abbreviations used are: $sPLA_2$, secreted phospholipase A_2; hGIII $sPLA_2$, human group III $sPLA_2$; EST, expressed sequence tag; RACE-PCR, rapid amplification of cDNA ends by polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction.

This paper is available on line at http://www.jbc.org

and introns coding for a novel human group III sPLA$_2$. The exon-intron boundaries of the human sPLA$_2$ gene were deduced according to alignment with bee venom sPLA$_2$ and exon-intron consensus sequences (29) to provide a putative cDNA sequence. To demonstrate the presence of the putative cDNA sequence in human tissues, a first set of RT-PCR experiments (*RT-PCR 1* in Fig. 1) was performed on different human cDNAs with primers flanking the Ca^{2+}-binding loop and the active site domain of the novel sPLA$_2$ (sense and antisense primers correspond to nucleotides 445 to 468 and 655 to 679, respectively, Fig. 1). A DNA product was amplified from human fetal lung cDNA and found to have a nucleotide sequence corresponding to the putative cDNA. This sequence was then used to clone the entire cDNA sequence by 5′ and 3′ RACE-PCR experiments as described previously (7). Briefly, human fetal lung poly(A)$^+$ RNA (2 μg, CLONTECH) was reverse-transcribed, and double-stranded cDNA was ligated to adaptors containing sequences for the universal primers SP6 and KS. PCR reactions were performed using KS primer and a specific forward or reverse primer, for 3′ or 5′ RACE-PCR, respectively. PCR products were subcloned into pGEM-T easy vector (Promega), and colonies were screened using an internal ^{32}P-labeled oligonucleotide probe. 3′ RACE-PCR experiments led to the cloning of a 1458-nucleotide sequence that contained in its 3′ end an in-frame extension of 304 amino acids, a stop codon, and a 3′-noncoding region of 546 nucleotides containing a putative polyadenylation site. Searching in EST data bases resulted in the identification of an EST sequence (GenBank™ AI282787), and full sequencing of this EST clone revealed a 193-nucleotide sequence containing a 166-nucleotide sequence identical in its 5′ end to the genomic clone and a 27-nucleotide poly(A) sequence. 5′ RACE-PCR experiments were performed with an antisense primer (nucleotides 518–545 in Fig. 1) and led to the cloning of a 158-nucleotide sequence, extending the 5′ end sequence of the RT-PCR 1 DNA fragment by 20 amino acid residues. In-frame with this 158-nucleotide sequence, an initiator methionine followed by a 19-amino acid sequence presenting the features of a signal peptide sequence (30) was found in the upstream genomic sequence. A primer upstream of the putative initiator methionine (nucleotides −254 to −229 in Fig. 1) and an antisense primer (nucleotides 2205 to 2236 in Fig. 1) derived from the above EST sequence were designed and used to amplify the full-length hGIII cDNA sPLA$_2$ (*RT-PCR 2* in Fig. 1). This RT-PCR experiment was performed on the same human fetal lung cDNA using the proofreading *Pwo* DNA polymerase and led to the cloning of a cDNA fragment of 2564 nucleotides containing an open reading frame of 1530 nucleotides. To confirm that this long open reading frame resulted from a proper splicing of the hGIII sPLA$_2$ gene, exon-trapping experiments were performed. For this purpose, a genomic fragment encompassing the putative hGIII gene was amplified with the Expand long template PCR system (Roche Molecular Biochemicals), primers designed from the human PAC clone DJ412A9 (nucleotides 36143–36175 and 43062–43092 for sense and antisense primers, respectively), and human genomic DNA as template. An expected 6.95-kilobase pair genomic fragment was amplified and subcloned into the exon trapping pET01 vector (MoBiTech) and partially sequenced, and the resulting plasmid was transfected into COS cells. Three days after transfection, total RNA was prepared, reverse-transcribed with oligo(dT), and submitted to PCR with primers flanking the hGIII sPLA$_2$ open reading frame. A PCR fragment of 1530 nucleotides was amplified, cloned into pGEM-T easy vector (Promega), and found to encode for the full-length hGIII open reading frame. No amplification was observed with cDNA from COS cells transfected with the parent exon-trapping vector.

Analysis of the Tissue Distribution of hGIII sPLA$_2$—A human Northern blot (CLONTECH Catalog No. 7780-1) was probed with a ^{32}P-labeled riboprobe corresponding to the nucleotide sequence 445 to 679 of hGIII sPLA$_2$ (Fig. 1) in ULTRAHyb hybridization buffer (Ambion, Catalog No. 8670) for 18 h at 70 °C. High sensitivity strippable antisense riboprobe was synthesized using the Strip-EZ RNA Ambion kit (Catalog No. 1360). The blot was washed to a final stringency of 0.1× SSC (30 mM NaCl, 3 mM trisodium citrate, pH 7.0) in 0.1% SDS at 70 °C and exposed to Kodak Biomax MS films with a transcreen-HE intensifying screen.

Recombinant Expression of hGIII sPLA$_2$ in COS Cells—The full-length cDNA sequence coding for hGIII sPLA$_2$ was subcloned into the expression vector pRc/CMVneo (Invitrogen), and a consensus Kozak sequence was added to enhance protein expression as described previously (6). The DNA construct was sequenced after subcloning and transiently transfected into COS cells using DEAE-dextran (7). Five days after transfection, cell medium was collected and partially purified on an anion exchange column. Briefly, COS cell culture medium (9 ml) was loaded at 1 ml/min onto a 10-ml column of Q-Sepharose Fast Flow (Amersham Pharmacia Biotech) previously equilibrated in 25 mM Tris, pH 8.0, at 4 °C. After washing with equilibration buffer to remove unbound protein, the solvent program was started (10 min in equilibration buffer followed by a linear gradient of NaCl from 0 to 1 M NaCl over 40 min). hGIII sPLA$_2$ enzymatic activity was detected using the fluorimetric assay with 1-palmitoyl-2-(10-pyrenedecanoyl)-*sn*-glycero-3-phosphomethanol as described (8). The pool of hGIII-containing fractions was concentrated approximately 10-fold by centrifugal ultrafiltration (YM-10 membrane, Amicon) at 4 °C, and the concentrate was stored at −20 °C. Using this assay, no phospholipase A$_2$ activity was detected in culture medium from COS cells transfected with the parent expression vector.

PLA$_2$ Activity Studies—Studies to measure the initial rate of hydrolysis of small unilamellar vesicles of phosphatidylglycerol (1-palmitoyl-2-[9,10-^{3}H]palmitoyl-*sn*-glycero-3-phosphoglycerol in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol at 50 Ci/mol) and phosphatidylcholine (1-palmitoyl-2-[9,10-^{3}H]palmitoyl-*sn*-glycero-3-phosphocholine, 50 Ci/mol) were carried as described (8) using Q-Sepharose-purified hGIII sPLA$_2$. Initial rates were calculated from 3 time points in the linear portion of the product *versus* time curve. pH-rate profiles for the hydrolysis of phosphatidylcholine were obtained as described (8). The Ca^{2+} dependence of phospholipid hydrolysis was carried out with the fluorimetric assay (described above) with 10 μM EGTA (no Ca^{2+}) or with $CaCl_2$ in excess of EGTA to give 10–650 μM Ca^{2+}.

RESULTS AND DISCUSSION

Molecular Cloning of hGIII sPLA$_2$—Screening of mammalian nucleic sequence data bases with various venom sPLA$_2$s led us to identify a large human genomic fragment of 133,893 nucleotides presenting several regions of homology with bee venom group III sPLA$_2$. This suggested that the genomic clone contains a complete gene with several exons and introns coding for a putative human group III (hGIII) sPLA$_2$. A first set of sense and antisense primers was designed from the genomic sequences homologous to bee venom sPLA$_2$ and used for RT-PCR experiments (*RT-PCR 1* in Fig. 1*A*) on human cDNAs from brain, pancreas, spleen, skeletal muscle, and fetal lung. A DNA fragment was amplified from fetal lung cDNA, and its sequence was found to correspond to the expected spliced exons from the genomic sequence. 5′ and 3′ RACE-PCR experiments followed by a second round of RT-PCR (*RT-PCR 2* in Fig. 1*A*) on human fetal lung cDNA led to the cloning of a cDNA fragment of 2564 nucleotides containing a large open reading frame of 1530 nucleotides (see Fig. 1 and "Experimental Procedures" for details). Screening of EST data bases resulted in the identification of a single human EST sequence (GenBank™ AI282787) of 193 nucleotides containing a poly(A) tail, suggesting that this EST sequence corresponds to the 3′ end of the hGIII sPLA$_2$ mRNA (Fig. 1*A*). Comparison of the 2564-nucleotide cDNA sequence with the PAC genomic sequence indicated that the hGIII sPLA$_2$ gene is composed of at least 7 exons and 6 introns spanning about 7 kilobase pairs (Fig. 1*A*). Exon-trapping experiments were performed and found to confirm the exon-intron structure and the sequence of the complete hGIII sPLA$_2$ open reading frame of 1530 nucleotides (see "Experimental Procedures"). The PAC clone DJ412A9 (GenBank™ AC005005) containing the hGIII sPLA$_2$ gene was generated by the sequencing program of human chromosome 22 (31), indicating that the hGIII sPLA$_2$ gene maps to this chromosome between the Genethon markers D22S1150 and D22S273. The location of the hGIII gene is thus distinct from those of genes for human group IB, IIA, IID, V, and X sPLA$_2$s (8, 9).

Similar to other mammalian sPLA$_2$s, the open reading frame of hGIII sPLA$_2$ begins with a signal peptide of 19 amino acids (30), indicating that the novel enzyme could be secreted. In contrast to other mammalian sPLA$_2$s (117 to 148 amino acids), the hGIII open reading frame codes for a much larger protein of 490 amino acids (calculated molecular mass 55.3 kDa, calculated pI 9.1) containing five putative *N*-glycosylation sites (Fig. 1*B*). This protein is made up of a central sPLA$_2$ domain (141

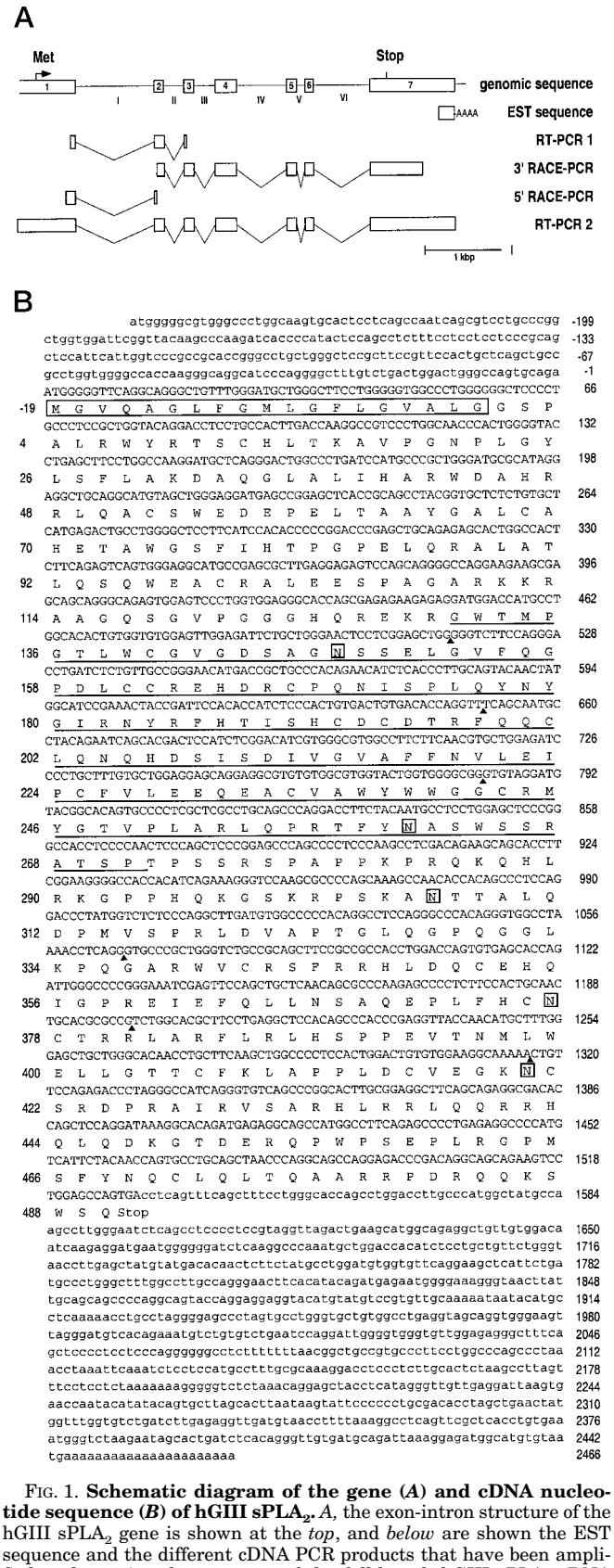

FIG. 1. **Schematic diagram of the gene (*A*) and cDNA nucleotide sequence (*B*) of hGIII sPLA$_2$s.** *A,* the exon-intron structure of the hGIII sPLA$_2$ gene is shown at the *top*, and *below* are shown the EST sequence and the different cDNA PCR products that have been amplified to determine the sequence of the full-length hGIII sPLA$_2$ cDNA (*Panel B*). Exons and introns are represented as *open boxes* and *straight lines*, respectively. The methionine initiation codon and stop codon of the hGIII sPLA$_2$ gene are located in exons 1 and 7. The sPLA$_2$ domain is encoded by exons 1 to 4. *B,* the consensus cDNA sequence is shown. The predicted signal peptide segment is *boxed*. The five putative *N*-glycosylation sites are *squared*. The sPLA$_2$ domain is *underlined*. The exon-intron boundaries are indicated by *arrowheads*.

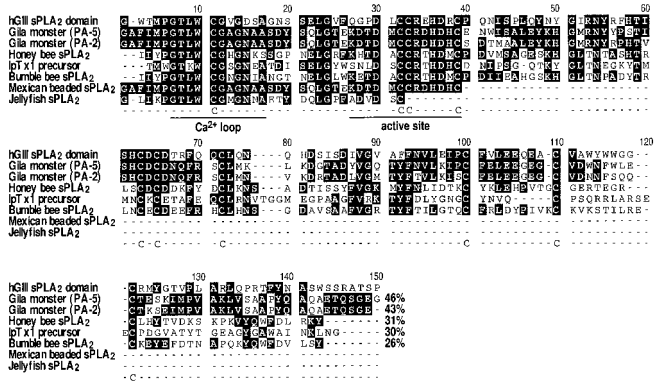

FIG. 2. **Alignment of the amino acid sequences of group III sPLA$_2$s.** Sequences of mature sPLA$_2$ proteins are shown. sPLA$_2$ sequences are from Refs. 20, 22, 23, and 25–27. Only partial sequences have been reported for jellyfish and Mexican beaded lizard sPLA$_2$s (25, 26).

residues) flanked by N- and C-terminal regions (130 and 219 residues, respectively). Based on the alignment with venom group III sPLA$_2$s (Fig. 2), the sPLA$_2$ domain comprises 141 amino acids (calculated molecular mass 16 kDa, calculated pI 5.4) and displays the typical features of group III sPLA$_2$s including the 10 cysteines specific for group III sPLA$_2$s and the key residues of the Ca^{2+} loop and catalytic site. The sPLA$_2$ domain contains 2 putative *N*-glycosylation sites that are not conserved with that of bee venom sPLA$_2$ located at position 15 in Fig. 2. However, one of them is located only 4 residues downstream of the glycosylation site in bee venom sPLA$_2$. Interestingly, the hGIII domain is more similar to venom group III sPLA$_2$s identified from vertebrates. Indeed, higher levels of identity are found with the isoforms PA-2 and PA-5 (43 and 46%, respectively) purified from the lizard Gila monster (27), whereas lower levels are observed with venom group III sPLA$_2$s from honey bee, bumble bee, and the scorpion *Pandinus imperator* (Fig. 2).

No protein data base entries with significant homology to the N- and C-terminal regions flanking the sPLA$_2$ domain of the hGIII sPLA$_2$ gene could be found. They are both basic (calculated pI 9.1 and 11.3 for N- and C-terminal regions, respectively) and contain 4 and 8 cysteines, suggesting that they may fold separately from the sPLA$_2$ domain. The function of these two domains is completely unknown at present. One possibility is that these domains could be involved in the maturation of hGIII sPLA$_2$ during or after its secretion from cells. Although the maturation processing of hGIII sPLA$_2$ clearly remains to be elucidated, the presence of a basic doublet KR at the end of the N-terminal domain (Fig. 1*B*) suggests that this domain could serve as a long propeptide that can be cleaved by subtilisin-like protein convertase in the Golgi apparatus (32). Interestingly, the mature protein sequence of bee venom sPLA$_2$ is preceded by an arginine residue (20), and a short propeptide sequence ending with an arginine doublet has been found in human group X sPLA$_2$ (6). The C-terminal region also contains several basic residues including basic doublets, which may be involved in protein maturation as well. In addition, the C-terminal domain contains numerous prolines and a pentapeptide RRLAR similar to that found in Imperatoxin I from *P. imperator* venom (22). In this regard, it is not yet clear whether some venom group III sPLA$_2$s also have such large N- and C-terminal regions, because only mature protein sequences and partial cDNA sequences have been determined so far (20, 23, 25–27), except for the *P. imperator* venom sPLA$_2$s (22, 24). A second possibility may be that the N- and C-terminal domains are involved in sPLA$_2$ dimerization, cell targeting, or interaction

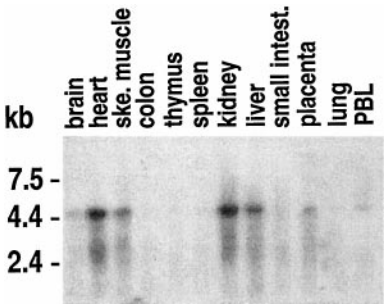

FIG. 3. **Northern blot analysis of the tissue distribution of hGIII sPLA$_2$.** A commercial Northern blot containing 2 μg of poly(A)$^+$ RNA from different human adult tissues was hybridized at high stringency with ^{32}P-labeled sPLA$_2$ RNA probe as described under "Experimental Procedures." *ske. muscle,* skeletal muscle; *small intest*., small intestine; *PBL*, peripheral blood leukocytes; *kb*, kilobase. The blot was exposed for 7 days.

with cellular proteins, possibly including sPLA$_2$ receptors (4). The last possibility may be that these domains play a role in regulating hGIII sPLA$_2$ activity. Unlike group I and II sPLA$_2$s, which contain a hydrogen bond network linking the N terminus to catalytic residues, the x-ray structure of bee venom sPLA$_2$ shows that the N terminus does not form part of the active site structure (11). Indeed, recombinant bee venom sPLA$_2$ expressed as an N-terminal fusion protein exhibits the same catalytic activity as the cleaved fusion or the native enzyme (33). This suggests that the presence of the N-terminal extension (and presumably the C-terminal region, which is also not part of the catalytic site (11)) would not interfere with the catalytic activity of hGIII sPLA$_2$. Full-length or partially cleaved hGIII sPLA$_2$ may thus be catalytically active, and N-and C-terminal domains may participate to the hGIII enzymatic properties. Further studies are clearly needed to elucidate the maturation process of the hGIII sPLA$_2$ protein and the role of these additional N- and C-terminal regions.

Tissue Distribution of hGIII sPLA$_2$—The tissue distribution of hGIII sPLA$_2$ was analyzed by hybridization at high stringency to a human Northern blot (Fig. 3). The hGIII sPLA$_2$ is expressed as a single transcript of 4.4 kilobases, which is abundant in kidney, heart, liver, and skeletal muscle, and is also present at low levels in placenta and peripheral blood leukocytes. Little, if any, expression was detected in brain, colon, thymus, spleen, small intestine, and lung. The pattern of expression of hGIII sPLA$_2$ is distinct from that of other human sPLA$_2$s, suggesting that this novel enzyme has specific function(s). Notably, hGIII sPLA$_2$ is expressed in kidney although no expression was previously detected in this tissue for human group IB, IIA, IID, V, and X sPLA$_2$s (6, 9). On the other hand, hGIII sPLA$_2$ is co-expressed in heart with human group IIA and V sPLA$_2$s and in liver and skeletal muscle with human group IIA sPLA$_2$ (6).

Recombinant Expression of hGIII sPLA$_2$ and Enzymatic Properties—When the hGIII sPLA$_2$ cDNA was transiently transfected in COS cells, sPLA$_2$ activity accumulated in the culture medium, indicating that the hGIII sPLA$_2$ cDNA codes for a secreted active enzyme. The level of PLA$_2$ activity measured after washing the cells with high salt buffer containing 1 M NaCl and in cell lysate was low, suggesting that hGIII sPLA$_2$ is not tightly bound to the cell surface and is efficiently secreted. The hGIII sPLA$_2$ was partially purified by chromatography on a Q-Sepharose fast flow column, and the eluted sPLA$_2$ fraction was used to analyze the enzymatic properties.

Like all mammalian sPLA$_2$s that have been kinetically characterized (7, 8, 34, 35), hGIII sPLA$_2$ is considerably more active (11-fold based on initial velocities) on anionic phosphatidylglycerol vesicles *versus* zwitterionic phosphatidylcholine vesicles

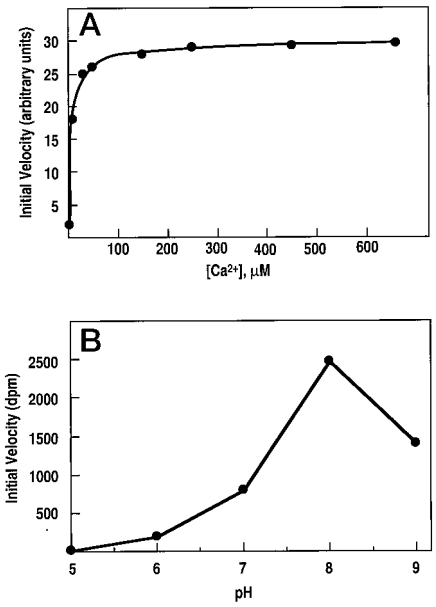

FIG. 4. **Enzymatic properties of hGIII sPLA$_2$.** *A,* Ca^{2+} dependence of the hydrolysis of 1-palmitoyl-2-(10-pyrenedecanoyl)-*sn*-glycerol-3-phosphomethanol vesicles by Q-Sepharose-purified hGIII sPLA$_2$. *B,* pH dependence of the hydrolysis of phosphatidylcholine vesicles by Q-Sepharose-purified hGIII sPLA$_2$.

(not shown). Further studies with pure hGIII sPLA$_2$ in larger quantities are required to determine if this rate difference is due to an increased fraction of enzyme bound to the anionic *versus* zwitterionic interface, a lower value of the interfacial K_m for phosphatidylglycerol *versus* phosphatidylcholine, or both. As shown in Fig. 4*A*, the rate of phosphatidylmethanol vesicle hydrolysis by hGIII is completely Ca^{2+}-dependent with a K_d of 6 $\pm$ 0.8 μM. The K_d for Ca^{2+} of 6 μM for the action of hGIII sPLA$_2$ on phosphatidylmethanol vesicles is considerably lower than the submillimolar to millimolar values reported for other sPLA$_2$s. However, the K_d value measured in this study is an apparent value. For sPLA$_2$s, phospholipid binding to the active site is Ca^{2+}-dependent, and thus the observed apparent K_d for Ca^{2+} depends on the affinity of the enzyme's active site for phospholipid substrate (36). K_d for Ca^{2+} is also modulated by the affinity of the enzyme for the vesicle interface because interfacial binding is a prerequisite for the binding of long chain phospholipids to the enzyme's active site. In this context, it may be noted that human group IIA sPLA$_2$ binds Ca^{2+} with millimolar affinity in the absence of substrate (37, 38), but the K_d for Ca^{2+} in the presence of phosphatidylglycerol (which supports tight interfacial and active site binding) is in the low micromolar range (39). Once large amounts of recombinant hGIII sPLA$_2$ are available, it will be possible to use spectroscopic methods to measure the affinity of the enzyme for Ca^{2+} in the absence of substrate. As shown in Fig. 4*B*, hGIII sPLA$_2$ is optimally active on phosphatidylcholine vesicles at pH 8. The pH-rate profile of hGIII is similar to most sPLA$_2$s (12). The increase in rate up to pH 8 probably reflects deprotonation of the active site histidine so that it can function as a general base for the attack of a water molecule on the substrate ester carbonyl group (13).

Concluding Remarks—Over the past few years, the molecular biology approach has revealed the presence of a diversity of sPLA$_2$s in mammals (5–9). The mammalian sPLA$_2$ family comprises eight members of 14–17 kDa including a group I, 5 group II, a group V, and a group X sPLA$_2$s. It also includes otoconin-95, a major protein of the extracellular otoconial complex of inner ear, which consists of a large secreted protein of 469 residues containing two sPLA$_2$-like domains (40, 41). All these

sPLA$_2$s have a conserved primary structure, have in common various disulfides, and several have a similar genomic organization. These sPLA$_2$s are thus structurally related enzymes that fall within the same set of proteins, namely the I/II/V/X sPLA$_2$ collection. It should be noted however that they all have distinct tissue distribution and function. The mammalian sPLA$_2$ family now also comprises the human group III sPLA$_2$, which does not belong to the I/II/V/X sPLA$_2$ collection. hGIII sPLA$_2$ has a distinct sPLA$_2$ primary sequence from the above sPLA$_2$s, contains extra N- and C-terminal regions, and has a different genomic organization. Together, this indicates that mammals can express sPLA$_2$s of the group I/II/V/X collection and of the distinct group III collection. Interestingly, the same can be observed in reptiles, because sPLA$_2$s found in snake venoms are group I or II enzymes whereas those found in lizard venoms belong to group III (15). In addition, as previously pointed out (15), it is likely that a single snake species can express several sPLA$_2$s from different groups that are present in various tissues other than the venom gland. Finally, while most sPLA$_2$s reported so far in the venom of invertebrates appear to be group III enzymes (20, 22–25), scanning of nucleic data bases indicates that invertebrates also express sPLA$_2$s from the group I/II/V/X collection in other tissues. In short, this makes likely that both vertebrates and invertebrates express a variety of sPLA$_2$s of the group I/II/V/X collection and of group III and that these sPLA$_2$s are present in various tissues to deserve specific functions. Lastly, based on the current sPLA$_2$s found in mammals, it is tempting to speculate that vertebrates have "chosen" to generate a sPLA$_2$ diversity from the group I/II/V/X collection and not from the group III collection. It remains however to determine if more than one group III sPLA$_2$ is expressed in mammals, and if reptiles and invertebrates have made the same "choice" to make their own sPLA$_2$ diversity.

In conclusion, we cloned a novel human sPLA$_2$ that clearly belongs to group III. This sPLA$_2$ seems to have a number of distinct structural features compared with the known venom group III sPLA$_2$s, suggesting that hGIII sPLA$_2$ may not be the structural "equivalent" of these venom sPLA$_2$s (4). Besides the evolutionary point of view, the biological role of hGIII sPLA$_2$ is unknown. Its tissue distribution appears nonredundant with other human sPLA$_2$s, suggesting particular function(s). Whether hGIII sPLA$_2$ is involved in lipid metabolism, arachidonic acid release, binding to specific receptors, and/or defense mechanisms against bacteria and viruses like human immunodeficiency virus type-1 (42) remains to be analyzed.

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