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Cloning, Chromosomal Mapping, and Expression of a Novel Human Secretory Phospholipase A_2*

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Lionel Cupillard‡§, Kamen Koumanov¶, Marie-Geneviève Mattéi‖, Michel Lazdunski‡, and Gérard Lambeau‡**

‡From the Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UPR 411, Sophia Antipolis, 660 route des Lucioles, 06560 Valbonne, France, ‖U406 INSERM Génétique Médicale, et Développement, Faculté de Médecine, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France, and ¶URA 1283 CNRS, Centre Hospitalier Universitaire Saint Antoine, 27 rue Chaligny, 75012 Paris, France

Secretory phospholipases A_2 ($sPLA_2$s) represent a rapidly expanding family of structurally related enzymes found in mammals as well as in insect and snake venoms. In this report, a cDNA coding for a novel $sPLA_2$ has been isolated from human fetal lung, and its gene has been mapped to chromosome 16p13.1-p12. The mature $sPLA_2$ protein has a molecular mass of 13.6 kDa, is acidic (pI 5.3), and made up of 123 amino acids. Key structural features of the $sPLA_2$ include: (i) a long prepropeptide ending with an arginine doublet, (ii) 16 cysteines located at positions that are characteristic of both group I and group II $sPLA_2$s, (iii) a C-terminal extension typical of group II $sPLA_2$s, (iv) and the absence of elapid and pancreatic loops that are characteristic of group I $sPLA_2$s. Based on these structural properties, this $sPLA_2$ appears as a first member of a new group of $sPLA_2$s, called group X. A 1.5-kilobase transcript coding for the human group X (hGX) $sPLA_2$ was found in spleen, thymus, and peripheral blood leukocytes, while a less abundant 0.8-kilobase transcript was detected in the pancreas, lung, and colon. When the hGX $sPLA_2$ cDNA was expressed in COS cells, $sPLA_2$ activity preferentially accumulated in the culture medium, indicating that hGX $sPLA_2$ is an actively secreted enzyme. It is maximally active at physiological pH and with 10 mM Ca^{2+}. hGX $sPLA_2$ prefers phosphatidylethanolamine and phosphatidylcholine liposomes to those of phosphatidylserine.

Phospholipases A_2 (PLA_2s; phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4)[1] represent a growing family of enzymes that catalyze the hydrolysis of glycerophospholipids at the *sn*-2 position, producing free fatty acids and lysophospholipids (1–3). PLA_2s generate rate-limiting precursors in the biosynthesis of various types of biologically active lipids, including prostaglandins, hydroxy fatty acids, leukotrienes, thromboxanes, and platelet-activating factor. Over the last two decades, two major classes of mammalian PLA_2s (intracellular and secretory) have been characterized and cloned. Secretory PLA_2s were first characterized and then classified into different groups according to their molecular structure and the localization of their disulfide bridges (4, 5). Recently, an updated classification of PLA_2s has been proposed that include both intracellular and secretory types of PLA_2s (2). A number of intracellular PLA_2s have been characterized and now comprise the well known Ca^{2+}-sensitive arachidonoyl-specific 85-kDa $cPLA_2$ (6, 7), multiple Ca^{2+}-independent PLA_2s (1, 2, 8) as well as several other types of cytosolic enzymes less extensively characterized (9–13).

Secretory low molecular mass PLA_2s ($sPLA_2$s, 13–18 kDa) form a class of structurally related enzymes whose members are also rapidly increasing (2). These enzymes are characterized by the presence of several disulfide bridges, an absolute catalytic requirement for millimolar concentration of Ca^{2+}, and a broad specificity for phospholipids with different polar head groups and fatty acyl chains (14). $sPLA_2$s have been purified from a variety of sources including not only mammalian pancreas, spleen, lung, platelets, and extracellular fluids, but also insect and snake venoms (1, 15–17). Pancreatic $sPLA_2$, nonpancreatic inflammatory $sPLA_2$, bee venom $sPLA_2$, and a novel $sPLA_2$ highly expressed in heart, are prototypes of group I, II, III, and V, respectively (2). The pancreatic group I $sPLA_2$, as its name indicates, was originally purified from pancreatic juice and then identified and cloned in other tissues, such as lung, spleen, kidney, and ovary (3, 18–20). Besides its primary function in digestion of dietary lipids, the pancreatic group I $sPLA_2$ has been proposed to play a role in cell proliferation (21), smooth muscle contraction (22, 23), as well as acute lung injury (24). The inflammatory group II $sPLA_2$ (initially called nonpancreatic $sPLA_2$) has been purified and cloned from various sources, including platelets and extracellular fluids (16, 25, 26). It is highly expressed in the plasma and synovial fluids of patients with various inflammatory diseases, such as rheumatoid arthritis, acute pancreatitis, Crohn's disease, and in endotoxic shock (27–31), as well as in various gastrointestinal cancers (32, 33). It is considered as a potent mediator of the inflammatory process (16, 27, 29, 30, 34) and has been recently proposed as a tumor suppressor gene of intestinal tumorigenesis (35). The human group V $sPLA_2$ has been cloned from brain and is found strongly expressed in heart (36, 37). More recently, this $sPLA_2$ has been detected in P388D1 murine macrophages where it may act as a novel $sPLA_2$ effector involved in lipid mediators production (38). A fourth $sPLA_2$ has been cloned in rat and mouse (39), but appears to be a nonfunctional pseudogene in humans (40). This $sPLA_2$ belongs to

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U95301.

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** To whom correspondence should be addressed: Tel.: 33 (0)4 93 95 77 02 or 03; Fax: 33 (0)4 93 95 77 04; E-mail: ipmc@unice.fr.

[1] The abbreviations used are: PLA_2, phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; hGX, human group X; EST, expressed sequence tag; PCR, polymerase chain reaction; bp, base pair; kb, kilobase pair; GC-MS, gas chromatography-mass spectrometry.

group IIC and is prevalently expressed in testis. Other low molecular mass PLA_2s have been characterized from various tissues including spermatozoa, brain, and lung, suggesting a larger diversity of PLA_2s (41–44). Moreover, a $sPLA_2$-related gene has been cloned from human teratocarcinoma cells and found to code for a protein of 689 amino acids containing two domains of high homology with $sPLA_2$s (45).

A wealth of $sPLA_2$s have also been described in snake and insect venoms. Besides a role in prey digestion, several venom $sPLA_2$s are potent toxins that display neurotoxicity and myotoxicity among a variety of other toxic effects (17, 46, 47). Studies with several iodinated venom $sPLA_2$s, including OS_1 and OS_2 purified from Taipan snake venom, have shown the existence of various high affinity $sPLA_2$ receptors (48). N-type $sPLA_2$ receptors were first identified in rat brain membranes and are made up of several protein subunits of 36–51 kDa and 85 kDa (49). These receptors display a high affinity for neurotoxic $sPLA_2$s, but not for nontoxic $sPLA_2$s, suggesting that N-type receptors may contribute to $sPLA_2$ neurotoxic effects (49–51). M-type $sPLA_2$ receptors were first identified in skeletal muscle cells (52). They consist of a single 180-kDa subunit and bind a number of toxic and nontoxic venom $sPLA_2$s. The M-type $sPLA_2$ receptor has now been cloned in various species (53–56), and its molecular properties have been analyzed in detail (57–61). Although the physiological roles of M- and N-type receptors remain to be discovered, M-type receptors from various species have been shown to associate with high affinity pancreatic group I $sPLA_2$s as well as inflammatory group II $sPLA_2$s with K_d values of 1–10 nM, suggesting that mammalian endogenous $sPLA_2$s might be the natural ligands of these receptors (21, 48, 54, 61).

We now report the cloning, chromosomal mapping, and recombinant expression of a novel human $sPLA_2$. Based on its structural properties, this $sPLA_2$ appears as a first member of a new group of mammalian $sPLA_2$s, called group X, according to the PLA_2 nomenclature defined recently (2).

EXPERIMENTAL PROCEDURES

Identification of the 309343 3′ Expressed Sequence Tag (EST) Sequence—Protein sequences of different $sPLA_2$s were used to search for homologues in gene data bases stored at the National Center for Biotechnology (NCBI) by using the tBLASTn sequence alignment program (62). Translation of an EST sequence (I.M.A.G.E. Consortium clone identification 309343, 3′, GenBank™ accession no. N93958) (63) in one frame presented a significant sequence similarity ($p = 9.9\ e^{-21}$) with several $sPLA_2$s. This 445-bp sequence was originally obtained from a human fetal lung cDNA library. A 327-bp DNA fragment corresponding to the EST sequence was then amplified by reverse transcriptase-PCR using human fetal lung cDNA. For that purpose, poly(A)$^+$ mRNA (CLONTECH Laboratories, Inc.) was reverse-transcribed with the Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) according to the manufacturer's protocol and used for PCR with a sense primer (5′-GGAATTCGCCTATATGAAATATGGT-3′) and an antisense primer (5′-GGAATTCAAGGTAGTCAGTCACACTTG-3′) containing *Eco*RI restriction sites. PCR reactions were performed using a low error rate DNA polymerase (PWO polymerase, Eurogentec Corp., Belgium) in a total volume of 50 μl containing 10 mM Tris-HCl, pH 8.85, 25 mM KCl, 2.0 mM $MgCl_2$, 200 μM dNTPs, 200 μM of each primer, 20 ng of cDNA template, and 1 unit of PWO DNA polymerase. PCR conditions were: 94 °C, 30 s; 50 °C, 30 s, and 72 °C, 30 s, for 33 cycles. The 327-bp DNA fragment was then subcloned into pBlueScript (Stratagene Cloning Systems) and sequenced to verify its identity with the EST sequence by using an automatic sequencer (Applied Biosystems model 373A) with the dideoxy nucleotide method.

Chromosomal Mapping of the hGX $sPLA_2$ Gene—In situ hybridization was performed on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 5-Bromodeoxyuridine (60 μg/ml) was added for the final 7 h of culture to ensure a posthybridization chromosomal banding of good quality. The 5′ Alu repeat element was removed from the full-length hGX $sPLA_2$ cDNA clone by restriction with *Apo*I, and the resulting DNA fragment (nucleotides −370 to 580, see Fig. 1) inserted into pBlueScript was tritium-labeled by nick-translation to a specific activity of 10^8 dpm/μg. The radiolabeled probe was hybridized to metaphase spreads at a final concentration of 200 ng/ml of hybridization solution as described previously (64). After coating with nuclear track emulsion (Kodak NTB_2), slides were exposed for 20 days at 4 °C and then developed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphase-photographed. R-banding was then performed by the fluorochrome-photolysis-Giemsa method, and metaphases were rephotographed before analysis.

Northern Blot Analysis—Three human multiple tissue Northern blots (CLONTECH Laboratories, Inc., catalog nos. 7759-1, 7760-1, and 7770-1) were first probed with the randomly primed ^{32}P-labeled PCR fragment corresponding to a partial sequence of hGX $sPLA_2$ cDNA (nucleotide 179–507 in Fig. 1) in 50% formamide, 5 × SSPE (0.9 M NaCl, 50 mM sodium phosphate, pH 7.4, 5 mM EDTA), 5 × Denhardt's solution, 0.1% SDS, 20 mM sodium phosphate, pH 6.5, and 250 μg/ml denatured salmon sperm DNA at 42 °C for 18 h. Blots were washed to a final stringency of 0.1 × SSC (30 mM NaCl, 3 mM trisodium citrate, pH 7.0) with 0.1% SDS at 55 °C and then exposed to Kodak X-Omat AR films with two intensifying screens. Northern blots were then stripped and hybridized in the same conditions as above with the entire coding sequences of the human group V $sPLA_2$ (36), the human group II $sPLA_2$ (25), the human group I $sPLA_2$ (19), and finally with the manufacturer-supplied β-actin probe.

Expression of hGX $sPLA_2$ in COS and HEK 293 Cells—The full-length 1020-bp cDNA clone coding for hGX $sPLA_2$ was subcloned directly into the expression vector pcDNA I (Invitrogen Corp.) by using convenient restriction enzyme sites. hGX $sPLA_2$ clones Met-42 and Met-32 were prepared by a PCR-assisted strategy using a low error rate PWO DNA polymerase (Eurogentec Corp., Belgium) and respective sense oligonucleotides primers (Met-42, 5′-GGAATTCGCGGCCGCCATGGGGCCGCTACCTGTGT-3′; Met-32, 5′-GGAATTCGCGGCCGCCATGCTGCTCCTGCTACTGC-3′) in combination with a common antisense oligonucleotide (5′-GTCTAGAGTCAGTCACACTTGGGCGAGT--3′). Primers contained restriction sites to facilitate subcloning of the PCR fragments and consensus Kozak sequences were added to the sense primers to enhance protein expression (65). The PCR products were subcloned into pcDNA I and sequenced to verify the integrity of DNA constructions. The various plasmids were then transfected into COS or HEK 293 cells by a modification of the DEAE-dextran/chloroquine method (66) or by the Ca/PO_4 procedure (67), respectively. Cells at 50% confluence were transfected with 5 μg of plasmid DNA/75-cm^2 Petri dish. Cell supernatants were harvested at different times post transfection and assayed for hGX $sPLA_2$ activity. On day 3, washed cells were harvested and disrupted by sonication in 300 μl of 20 mM Tris-HCl, pH 7.4, 2 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride to measure cell-associated $sPLA_2$ activity. To test whether hGX $sPLA_2$ is secreted as a proenzyme, serum-free cell supernatants containing hGX $sPLA_2$ activity were treated with 3 μg/ml trypsin (Sigma, T-1005 type XI from bovine brain) in 20 mM Tris-HCl, pH 8.0, 10 mM $CaCl_2$ for 3 h at 37 °C and then assayed for $sPLA_2$ activity with [^{3}H]oleate-labeled *Escherichia coli* membranes.

$sPLA_2$ Activity Assay on [^{3}H]Oleate-labeled E. coli Membranes—Preparation of autoclaved *E. coli* membranes and $sPLA_2$ assays were performed essentially as described previously (57). Unless otherwise specified, $sPLA_2$ assays were performed at 25 °C in a total volume of 100 μl consisting of 140 mM NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$, 0.1% bovine serum albumin, and 100,000 dpm of [^{3}H]oleate-labeled *E. coli* membranes. Incubation times and sample volumes were adjusted to ensure hydrolysis rates within the linear range of enzymatic assays. Typically, 3–10 μl of solution containing hGX $sPLA_2$ were incubated for 30–60 min at 20 °C to measure $sPLA_2$ activity. Reaction mixtures were stopped by adding 300 μl of 0.1 M EDTA, pH 8.0, and 1% fatty acid-free bovine serum albumin. After centrifugation at 10,000 × *g* for 3 min, 300 μl of supernatant containing hydrolyzed phospholipids were counted. Control incubations in the absence of added $sPLA_2$ were carried out in parallel and used to calculate specific hydrolysis.

$sPLA_2$ Activity Assay on Mixed Phospholipid Liposomes—Liposomes were prepared by sonication using different phospholipid mixtures (phosphatidylethanolamine, phosphatidylcholine, or phosphatidylserine, with D-α-dipalmitoylphosphatidylcholine, 75:25 mol%). D-α-Dipalmitoylphosphatidylcholine is a nonhydrolyzable phospholipid that is used for formation of liposomes with the various hydrolyzable phospholipids. L-α-Phosphatidylcholine (from egg yolk), L-α-phosphatidylethanolamine (from egg yolk), L-α-phosphatidylserine (from bovine brain), and D-α-dipalmitoylphosphatidylcholine were purchased from Sigma.

Phospholipids were dissolved in chloroform, dried under a nitrogen stream, and resuspended at 0.33 mM in 100 mM Tris-HCl buffer, pH 7.4. The lipid suspension was then sonicated twice for 2 min with a 20-kHz MSE tip probe at 100 W. Incubations were carried out for 15 min at 37 °C in a total volume of 500 μl containing 200 nmol of hydrolyzable phospholipids resuspended in 100 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$, and 0.1% fatty acid-free BSA. hGX $sPLA_2$ activity was measured by adding 20 μl of a 72-h COS cell supernatant, and incubation was carried out for 15 min at 37 °C. Released fatty acids were extracted by a modification of Dole's procedure (68), methylated with diazomethane, and quantified as described below by GC-MS measurements. Control incubations in the absence of added $sPLA_2$ were carried out in parallel and used to calculate specific hydrolysis.

$sPLA_2$ Activity Assay on Erythrocyte Membrane Phospholipids—Human erythrocytes were isolated from fresh citrated blood by the procedure of Steck *et al.* (69). Briefly, blood was centrifuged for 10 min at 4 °C at 100 × *g*. Red blood cells were then collected, washed several times with 5 volumes of an ice-cold phosphate saline buffer (150 mM NaCl, 5 mM sodium phosphate, pH 8.0), and hemolyzed in 40 volumes of 5 mM sodium phosphate, pH 8.0. Erythrocyte ghosts were collected by centrifugation for 20 min at 22,000 × *g* and washed several times with 5 mM phosphate buffer until "white ghosts" were obtained. $sPLA_2$ assays were performed in a total volume of 500 μl containing erythrocyte membranes (representing 200 nmol of hydrolyzable phospholipids) resuspended in 100 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$, and 0.1% fatty acid-free bovine serum albumin. Incubations were carried out for 15 min at 37 °C with translational shaking. 20 μl of a 72-h COS cell supernatant were used to measure hGX $sPLA_2$ activity. The released fatty acids were extracted by a modification of the Dole's procedure (68), methylated with diazomethane, and quantified by GC-MS measurement as described below. Control incubations in the absence of added $sPLA_2$ were carried out in parallel and used to calculate specific hydrolysis.

GC-MS Measurements—The released fatty acids extracted by Dole's procedure were methylated with diazomethane and separated by gas chromatography on a capillary column of Supelcowax-10 bonded phase (0.32 mmx30 m, Supelco) with a Hewlett Packard 5890 Series II gas chromatograph. Fatty acids were detected by mass spectrometry (Nermag 10-10C, France) in the chemical ionization mode with ammonia (0.1 bar) as the reagent gas. The positive quasi-molecular ions were monitored and time-integrated. Quantification referred to an internal standard of heptadecanoic methyl ester with response factors was calculated with the various fatty acid methyl ester calibrators.

RESULTS AND DISCUSSION

Molecular Cloning of hGX $sPLA_2$ and Chromosomal Localization of Its Gene—Protein sequences of various $sPLA_2$s were used to search for related sequences in gene data bases by using the tBLASTn sequence alignment program (62). As a result, we identified a human EST of 445 bp that was originally obtained from a fetal lung cDNA library (Fig. 1). The deduced amino acid sequence of the EST displayed a high homology to other $sPLA_2$s, including several amino acids invariably conserved in catalytically active $sPLA_2$s. We thus postulated that this EST was a partial copy of a mRNA coding for a novel low molecular mass $sPLA_2$. This $sPLA_2$ was assigned as hGX $sPLA_2$ in accordance with the recent numbering system proposed by Dennis (2). Two primers were then designed from the EST sequence and used in PCR amplification to screen for the presence of hGX $sPLA_2$ in human testis, human placenta, and human fetal lung cDNAs. Only human fetal lung cDNAs produced the expected 327-bp PCR fragment predicted from the EST sequence. This fragment was cloned and sequenced, confirming its identity with the EST sequence. This PCR fragment was then used as a probe to hybridize to several human multiple Northern blots to analyze the transcription pattern of hGX $sPLA_2$. A 1.5-kb transcript coding for this $sPLA_2$ was found in spleen, thymus, and peripheral blood leukocytes, while no such expression was observed in several other human tissues, including human fetal lung (see Fig. 5). These data indicate that this novel $sPLA_2$ is expressed at a fairly low level in a limited number of human tissues. This prompted us to request the clone from which the EST sequence was derived at

FIG. 1. **Nucleotide and deduced amino acid sequences of hGX $sPLA_2$.** The predicted prepropeptide segment is *boxed* and possible initiator methionines are shown in *bold*. The arginine doublet preceding the mature protein is indicated by *squares*. The putative Asn-glycosylation site is marked with a *triangle*. The putative polyadenylation signal is *underlined* and shown in *bold*. The original EST sequence (I.M.A.G.E. Consortium Clone identification 309343, 3′) comprised nucleotides 117–580 in a reverse complement orientation.

the "I.M.A.G.E. consortium" (LLNL) cDNA clones data base (63). This clone was found to contain a cDNA insert of 1020 bp bearing an open reading frame of 166 amino acid residues, thus coding for the entire protein sequence of the novel $sPLA_2$ (Fig. 1). The 5′-noncoding region was found to contain an Alu repetitive element, while the short 3′-noncoding region contained a canonical polyadenylation site located 18 bases upstream from a poly(A) tail (Fig. 1).

After removal of the 5′ Alu repeat, the cDNA clone was used as a probe to map the hGX $sPLA_2$ gene by *in situ* hybridization on human metaphase chromosomes. In the 100 metaphase cells examined after *in situ* hybridization, there were 191 silver grains associated with chromosomes and 59 of these (30.9%)

were located on chromosome 16. The distribution of grains on this chromosome was not random, since 74.6% (44/59) of them mapped to the p13.1-p12 region of chromosome 16 short arm (Fig. 2). These results indicate that the hGX $sPLA_2$ gene maps to the 16p13.1-p12 region of the human genome. The chromosomal localization of this novel $sPLA_2$ is thus distinct from those of other known $sPLA_2$s which have been mapped on chromosomes 1 and 12 (Table I) (40, 70, 71).

Structural Features of hGX $sPLA_2$ and Comparison with Other $sPLA_2$s—The hGX $sPLA_2$ cDNA clone predicts a mature $sPLA_2$ protein of 123 amino acids (calculated molecular mass, 13.6 kDa) and the presence of a signal peptide with a maximal length of 42 amino acids (Fig. 1). The calculated isoelectric point of the mature protein is 5.29, thus placing hGX $sPLA_2$ as the most acidic human $sPLA_2$ (Table I). Sequence pattern analysis indicates that hGX $sPLA_2$ contains a potential glycosylation site at position 71, which is also found in the sequence of the mouse group IIC $sPLA_2$ (Table I and Fig. 3). Two potential initiator methionines located at positions −42 and −32 relative to the mature protein are found in the hGX $sPLA_2$ sequence (Fig. 1). Interestingly, two potential initiator methionines were also observed in the mouse group IIC $sPLA_2$ (39). Although the second methionine of the hGX $sPLA_2$ sequence does not appear in a more favorable context for initiation of translation as compared with the first methionine (65), the use of this second methionine as a translation initiation site will reduce the length of the prepropeptide to 32 amino acids, *i.e.* to a size more consistent with those of other $sPLA_2$ signal peptide sequences (19, 25, 26, 36, 37, 39). The presence of an arginine doublet and of other polar residues preceding the mature $sPLA_2$ protein strongly suggests that the signal sequence of hGX $sPLA_2$ is a prepropeptide. It was, however, difficult to assign the position of the signal peptidase cleavage site that separates prepeptide and propeptide sequences (72). In addition, no evident homology was observed between the putative hGX $sPLA_2$ propeptide sequence and that of other $sPLA_2$s containing propeptides (19, 39). Although the maturation processing of hGX $sPLA_2$ remains to be elucidated, cleavage at dibasic amino acid motifs, including an arginine doublet, is known to be efficiently catalyzed by subtilisin-like protein convertases in the Golgi apparatus (73), thus suggesting that hGX $sPLA_2$ might be secreted as an active enzyme after removal of its propeptide sequence into the cell interior.

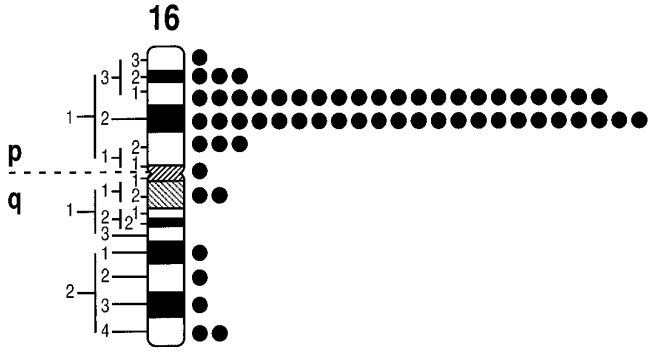

FIG. 2. **Chromosomal localization of the hGX $sPLA_2$ gene.** Idiogram of the human G-banded chromosome 16 illustrating the distribution of labeled sites for hGX $sPLA_2$ probe.

An alignment of the amino acid sequence of various mammalian $sPLA_2$s with the sequence of hGX $sPLA_2$ is presented in Fig. 3, and the biological properties of these $sPLA_2$s are compared in Table I. Fig. 3 shows that hGX $sPLA_2$ displays only 27–35% identity with other mammalian $sPLA_2$s, indicating no preferential homology with most of the other $sPLA_2$s. However, comparison of the complete hGX protein sequence with data bases indicates a slightly higher level of identity with group II $sPLA_2$s including those of snake venom origin. For example, hGX $sPLA_2$ displays 35% identity with human group II $sPLA_2$, while only 29% identity is observed with human group I $sPLA_2$. As a hallmark of all other $sPLA_2$s, the hGX $sPLA_2$ protein contains a large number of cysteines that are most probably assembled into disulfide bridges. Of the 16 cysteines found in the structure of hGX $sPLA_2$, two are located at positions 11 and 77, which are characteristic of group I $sPLA_2$s, while two others are found at positions 50 and 137, which are typical of group II $sPLA_2$s (Table I). The other cysteines are located at positions observed in both group I and II $sPLA_2$s, but not at positions that are characteristic of group IIC $sPLA_2$s (Fig. 3 and Table I). This cysteine pattern places the newly cloned $sPLA_2$ in a novel group, assigned as group X, according to the PLA_2 nomenclature recently refined by Dennis (2). In fact, the cysteine pattern of hGX $sPLA_2$ resembles that of PLA_2L3′ (Table I), except that this latter $sPLA_2$ contains an extra cysteine at position 18 (Fig. 3). It is also interesting to note that hGX $sPLA_2$ has an amino acid C-terminal extension, which is characteristic of group II $sPLA_2$s, while it contains neither the elapid loop characteristic of group IA nor the pancreatic loop, a particular feature of group IB $sPLA_2$s, or the 4 amino acid insertion found in group IIC $sPLA_2$s (Table I and Fig. 3) (5). Fig. 3 also shows that hGX $sPLA_2$ contains all of the amino acids that are absolutely conserved in active $sPLA_2$ enzymes, indicating that the newly cloned $sPLA_2$ could be catalytically active.

A phylogenetic tree was derived from an alignment of all

TABLE I
The different groups of mammalian $sPLA_2$s

$sPLA_2$	Group	Major sources	Molecular mass[a]	pI[a]	*N*-glycosylation site[a]	No. of cysteines	Specific disulfide bridges[b]			Other specific features	Chromosomal localization
							11–77	50–137	86–92		
			kDa								
Pancreatic	IB	Pancreas, lung, spleen	13.2	7.6		14	+	−	−	Pancreatic loop propeptide	12
Inflammatory	IIA	Synovial fluid, platelets	13.9	9.3	101	14	−	+	−	C-terminal extension	1
PLA_2-8	IIC	Testis	14.6	8.3	72	16	−	+	+	C-terminal extension	1
PLA_2-10	V	Heart, lung	13.6	8.5		12	−	−	−		1
hGX	X	Spleen, thymus, peripheral blood leukocytes, lung	13.6	5.3	71	16	+	+	−	C-terminal extension putative propeptide	16
PLA_2L5′	?	Teratocarcinoma cell lines	13.0	4.7	105	16	+	−	−		?
PLA_2L3′	?	Teratocarcinoma cell lines	13.4	5.6	104	17	+	+	−	C-terminal extension	?

[a] As determined from the sequence of mature proteins. For PLA_2L5′ and PLA_2L3′, the protein mature sequences correspond to those shown in Fig. 3.
[b] Refer to Fig. 3 for the position of disulfide bridges.

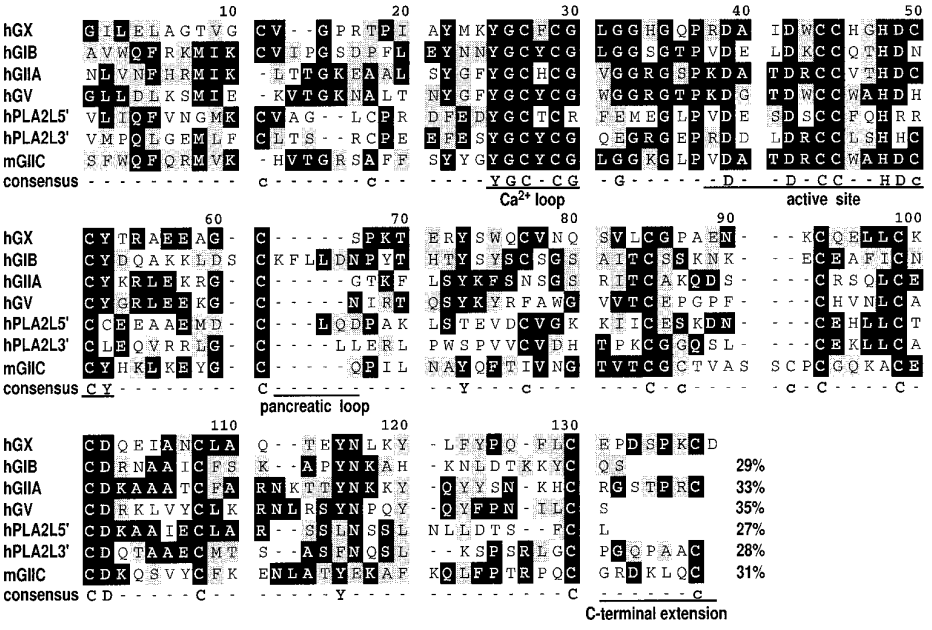

FIG. 3. **Alignment of the amino acid sequence of hGX $sPLA_2$ with other human $sPLA_2$s and mouse group IIC $sPLA_2$.** Only mature protein sequences of $sPLA_2$s are shown. *hGX*, human group X $sPLA_2$; *hGIB*, human pancreatic group IB $sPLA_2$ (19); *hGIIA*, human inflammatory group IIA $sPLA_2$ (25, 26); *hGV*, human group V $sPLA_2$ (36); *hPLA$_2$L5′*, human $sPLA_2$-like gene 5′ (45); *hPLA$_2$L3′*, human $sPLA_2$-like gene 3′ (45); *mGIIC*, mouse group IIC $sPLA_2$ (39). The level of identity between hGX $sPLA_2$ and each other $sPLA_2$ is indicated. The consensus sequence of active $sPLA_2$s was refined from that published previously (5). Cysteines that are conserved throughout all $sPLA_2$s are indicated in *capital letters*, while those conserved in some $sPLA_2$ subgroups are indicated in *lower case letters*. Note that hPLA$_2$L5′ and hPLA$_2$L3′ do not share some of the active site residues, indicated in the consensus sequence of catalytically active $sPLA_2$s, and thus may belong to catalytically inactive enzymes.

known protein sequences of mammalian $sPLA_2$s (Fig. 4). A first division was observed in this tree between PLA$_2$L3′/PLA$_2$L5′ and other mammalian $sPLA_2$s, that places these two $sPLA_2$ domains on a branch distinct from that of other mammalian $sPLA_2$s. This is particularly interesting if one considers that these two branches correspond to a separation between catalytically active and inactive $sPLA_2$s. Indeed, it is likely that the PLA$_2$L3′ and PLA$_2$L5′ domains (45), if expressed separately as $sPLA_2$ proteins, would correspond to catalytically inactive enzymes, since these structures do not have several of the residues found in catalytically active $sPLA_2$s (Fig. 3). According to this tree, a second duplication event would have occurred to generate the present group I $sPLA_2$s and another branch from which the other mammalian $sPLA_2$s originate. Interestingly, hGX $sPLA_2$ appears to result from an ancestral duplication event occurring after the separation of group I $sPLA_2$s and other $sPLA_2$s, but before the divergence between group II and group V $sPLA_2$s (Fig. 4). Therefore, it is likely that $sPLA_2$s from groups II, V, and X have emerged from a common ancestor, while $sPLA_2$s from group I are more distantly related and arose before the development of these more recent groups. This dendrogram is also in accordance with the chromosomal localization of $sPLA_2$s (Table I). Indeed, the three closely related human $sPLA_2$s from group IIA, IIC, and V, which would have emerged from recent gene duplication events, map to chromosome 1, while the more distant human group I and human group X $sPLA_2$s map to chromosome 12 and 16, respectively. Finally, it appears from this tree that group IIC $sPLA_2$s are more distant from group IIA and group V $sPLA_2$s that would have emerged from a common ancestor (Fig. 4).

Transcription Pattern of hGX $sPLA_2$ and Comparison with That of Other Human $sPLA_2$s—The tissue expression pattern of hGX $sPLA_2$ was analyzed by probing several human multiple tissue Northern blots at high stringency (Fig. 5). Transcripts of 1.5 kb coding for hGX $sPLA_2$ were detected in adult spleen and at a lower level in adult thymus and peripheral blood leukocytes. This result could indicate a specific expression of hGX $sPLA_2$ in cells related to the immune system and inflammation. It is then possible that hGX $sPLA_2$, as with group II and group V $sPLA_2$s (3, 16, 27, 38, 74), may participate in the release of lipid mediators of inflammation. Whether the expression of hGX $sPLA_2$, as with human group II $sPLA_2$ (3, 16, 27, 74), is up-regulated in certain pathological conditions remains to be

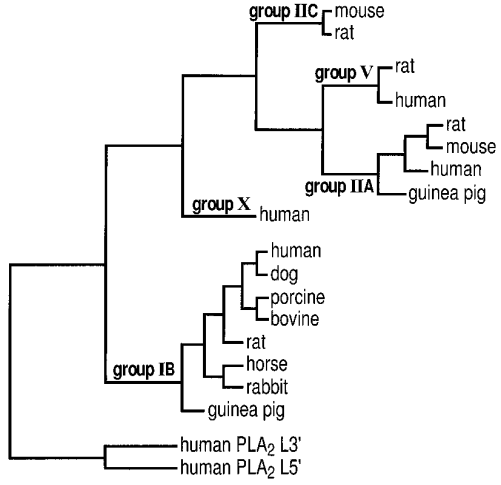

FIG. 4. **Phylogenetic tree of mammalian $sPLA_2$s.** $sPLA_2$s sequences were analyzed using the Genetics Computer Group (GCG) package. The phylogenetic tree was generated using successively pileup, distances, and growtree programs. $sPLA_2$ sequences used have been retrieved from Refs. 19 and 86–92 for group I $sPLA_2$s; Refs. 25, 26, and 93–95 for group IIA $sPLA_2$s; Ref. 39 for group IIC $sPLA_2$s; Refs. 36 and 37 for group V $sPLA_2$s; and Ref. 45 for hPLA$_2$L5′ and hPLA$_2$L3′.

determined. Although the cDNA coding for hGX $sPLA_2$ was originally cloned from a human fetal lung library, no transcript was detected in human fetal lung, indicating that hGX $sPLA_2$ is not highly expressed in this fetal tissue (although the absence of expression may also be due to differences in sample preparations from different individuals). No expression was detected in other fetal tissues such as heart, liver, and brain (Fig. 5). Another low abundance 0.8-kb transcript was detected in the colon, lung, and pancreas, which could result from distinct sites of initiation or termination of transcription or from alternative splicing.

The pattern of expression of hGX $sPLA_2$ was then compared with those of other human $sPLA_2$s. In accordance with previous data (19, 55), a huge amount of human group I $sPLA_2$ transcript was detected in adult pancreas and at lower levels in the spleen, lung, and prostate. No detectable expression was observed in the kidney and small intestine, contrasting with previous data (18, 75). Interestingly, a 0.5-kb transcript was

also observed in the ovary as well as in fetal lung and fetal liver, while a transcript of higher size was detected in the testis (Fig. 5). Fig. 5 shows that human group II $sPLA_2$ is widely distributed in several human adult tissues, including heart, liver, skeletal muscle, small intestine, and prostate. As for the human pancreatic $sPLA_2$, human group II $sPLA_2$ is expressed also in fetal liver (Fig. 5). These results are in accordance with the previous detection of human group II $sPLA_2$ in a variety of mammalian tissues and cells, although the expression levels might be rather variable, depending on cell activation, which is known to highly modulate the expression of human group II $sPLA_2$ (3, 16, 27, 29, 75). For example, no human group II $sPLA_2$ transcripts are detected in spleen and peripheral blood leukocytes, from which human group II $sPLA_2$ was characterized (3, 16, 27, 76). As previously observed (36), human group V $sPLA_2$ is strongly expressed in adult heart and at lower levels in the lung and liver (Fig. 5). A low expression is also observed in the prostate, testis, ovary, small intestine, and colon, while no expression is detected in fetal tissues (Fig. 5). Taken together, it appears that the tissue distribution of these four human $sPLA_2$s is different, suggesting diverse biological functions for each of them. Besides the expression of hGX $sPLA_2$ in spleen, where both human group I (Fig. 5) and human group II $sPLA_2$s (76) are also expressed, hGX $sPLA_2$ is expressed in the thymus, a tissue in which other human $sPLA_2$s are not detected (Fig. 5). The presence of a secretory PLA_2 activity in the thymus either at adult stages or during the fetal thymic development is not well documented. Group II $sPLA_2$ is known to be strongly expressed in rat thymus after endotoxic shock induced by lipopolysaccharides (77), and PLA_2 activity has been detected in T lymphocytes and epithelial cells from rat thymus,

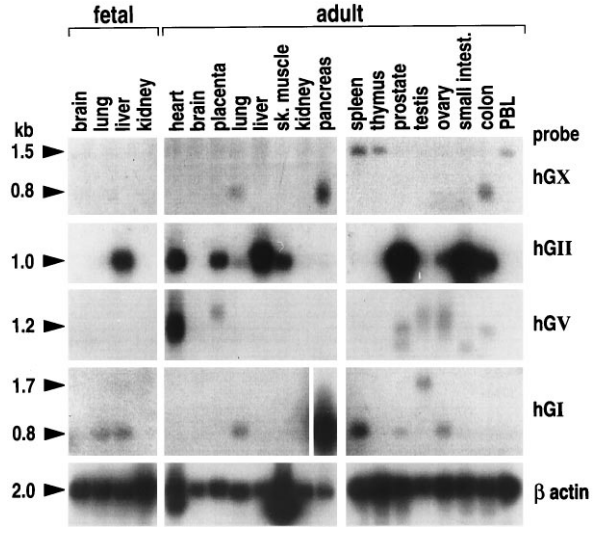

FIG. 5. **Northern blot analysis of the tissue distribution of hGX $sPLA_2$ and of other human $sPLA_2$s.** Fetal and adult human multiple Northern blots (2 μg of poly(A^+) mRNA/lane) were first hybridized at high stringency with a ^{32}P-labeled insert of hGX $sPLA_2$ (*hGX probe*) as described under "Experimental Procedures." *Sk. muscle*, skeletal muscle; *PBL*, peripheral blood leukocytes. The same blots were then hybridized at high stringency successively with the entire coding sequences of human group II $sPLA_2$ (*hGII probe*), human group V $sPLA_2$ (*hGV probe*), and human group I $sPLA_2$ (*hGI probe*). Filters were exposed for 7 days for each panel except for hybridization of human group I $sPLA_2$ to pancreas (3 h of exposure). The same blots were finally hybridized with the commercial β-actin probe (7 h of exposure).

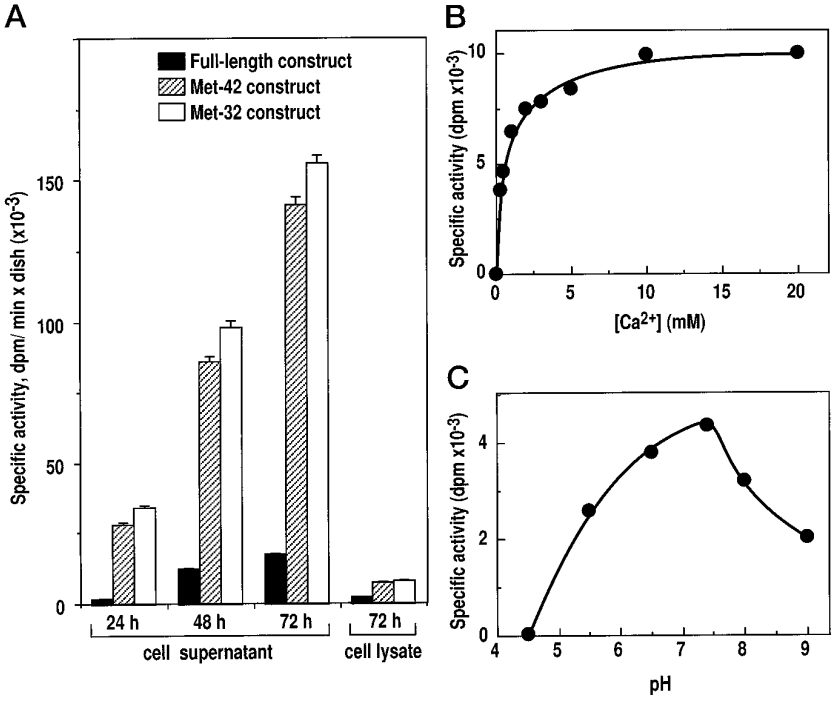

FIG. 6. **Recombinant expression of the hGX $sPLA_2$ cDNA in eukaryotic cells.** *Panel A*, hGX $sPLA_2$ activity measured in cell supernatants and cell lysate of COS cells transiently transfected with the full-length hGX $sPLA_2$ cDNA and Met-42 or Met-32 cDNA constructs (see "Experimental Procedures" for details). Cell supernatants were collected at 24, 48, and 72 h after transfection and assayed for enzymatic activity. $sPLA_2$ activity was also measured in cell lysates prepared at 72 h post transfection. Results are expressed as the mean value ± S.E. of three independent transfection experiments. $sPLA_2$ activity was measured by hydrolysis of [^{3}H]oleate-labeled *E. coli* membranes as described under "Experimental Procedures." Cells transfected with the parent expression vector did not show hGX $sPLA_2$ activity in the cell supernatant. *Panel B*, Ca^{2+} dependence of hGX $sPLA_2$ activity, enzymatic activity was determined by hydrolysis of [^{3}H]oleate-labeled *E. coli* membranes as described under "Experimental Procedures" in the presence of 2 mM EDTA (0 Ca^{2+} free) or of increasing concentrations of $CaCl_2$. *Panel C*, pH dependence of hGX $sPLA_2$ activity, enzymatic activity was determined by hydrolysis of [^{3}H]oleate-labeled *E. coli* membranes as described under "Experimental Procedures" in the presence of 25 mM sodium acetate buffer at pH range 4.5–6.5 or 25 mM Tris-HCl buffer at pH range 7.0- 9.0. Characterization of hGX $sPLA_2$ activity (*panels B* and *C*) was performed using supernatants of cells transfected with the Met-32 construct.

TABLE II
Substrate specificity of hGX $sPLA_2$

hGX $sPLA_2$ activity was measured on phospholipid liposomes and erythrocyte ghost membranes. 200 nmol of phospholipids with various fatty acid chains were used in $sPLA_2$ assays as described under "Experimental Procedures." A single set of data from one of three experiments is presented.

Substrate	*sn*-2 fatty acid	Substrate available	Substrate hydrolyzed	
		nmol	*nmol*	*% of release*
Phosphatidylcholine	C18:1	134.96	2.80	2.07
	C18:2	51.44	1.71	3.32
	C20:4	10.84	0.44	4.05
	C22:6	2.76	0.10	3.62
Total		200.00	5.05	2.53
Phosphatidylethanolamine	C18:1	117.72	1.84	1.56
	C18:2	63.16	2.06	3.26
	C20:4	15.70	2.45	15.61
	C22:6	3.42	0.13	3.80
Total		200.00	6.48	3.24
Phosphatidylserine	C18:1	143.08	0.174	0.12
	C18:2	2.27	0.000	0.00
	C20:4	1.77	0.003	0.17
	C22:6	52.88	0.000	0.00
Total		200.00	0.177	0.09
Ghost	C18:1	81.98	0.25	0.30
	C18:2	34.93	0.22	0.63
	C20:4	73.34	0.33	0.45
	C22:6	9.75	0.05	0.51
Total		200.00	0.85	0.43

but the molecular nature of these PLA_2s has not been identified (78, 79). The biological role of PLA_2 activity in thymus is poorly understood, although certain reports suggest a role in rat thymocyte apoptosis (80, 81). Interestingly, *p*-bromophenacyl bromide, a known inhibitor of $sPLA_2$s (14, 30, 82, 83), prevents apoptosis of rat thymocytes and lymphoma cells (84).

Recombinant Expression of hGX $sPLA_2$ and Characterization of $sPLA_2$ Activity—To test whether the hGX $sPLA_2$ cDNA really encodes a catalytically active $sPLA_2$, this cDNA was inserted into the expression vector pcDNA I and then transfected into eukaryotic cells. Three different constructs of hGX $sPLA_2$ were prepared. The first construct was generated by insertion of the full-length cDNA clone (1020 bp) into the expression vector. Two other constructs were prepared by a PCR-assisted strategy to insert into the expression vector only the cDNA sequence coding for the mature protein preceded by the prepropeptide sequence, starting either at methionine-42 (Met-42 construct) or at methionine-32 (Met-32 construct). In addition, a consensus site for initiation of translation was inserted in both of these latter constructs to increase the efficiency of expression of hGX $sPLA_2$ (65). The three different constructs were transfected into COS cells, and the cell supernatants were assayed for $sPLA_2$ activity at different times after transfection (Fig. 6). Cell supernatants from COS cells transfected with the three different constructs accumulated hGX $sPLA_2$ activity, whereas cells transfected with the parent vector did not show $sPLA_2$ activity (Fig. 6*A*). A $sPLA_2$ activity representing 6% of the total cellular activity was recovered from the cell lysate obtained 72 h after cell transfection, indicating that hGX $sPLA_2$ was actively secreted by COS cells (Fig. 6*A*). The expression level of hGX $sPLA_2$ activity obtained with Met-42 and Met-32 clones was about 10-fold higher relative to that observed with the full-length cDNA construct, probably due to the presence of the consensus leader sequence added in the PCR-made constructs (Fig. 6). Furthermore, the expression level of $sPLA_2$ activity was not significantly modified when the translation of hGX $sPLA_2$ started at methionine-32, suggesting that this methionine may be functional *in vivo* as an initiator site of translation. Finally, based on the presence of a propeptide sequence ending with an arginine doublet in the structure of hGX $sPLA_2$, it was of interest to verify whether hGX $sPLA_2$ could be secreted as a zymogen. For that purpose, COS cells were transfected and transferred into serum-free medium, and the resulting supernatants were treated with trypsin and then assayed for $sPLA_2$ activity. Treatment with trypsin (which is assumed to cleave efficiently at the propeptide arginine doublet if present) did not significantly increase the supernatant $sPLA_2$ activity, suggesting that most of the $sPLA_2$ released into the cell incubation medium was already active and thus that the cleavage of the propeptide sequence might have already occurred during the cell maturation of hGX $sPLA_2$. When human HEK 293 cells were used instead of COS cells for the same experiments, similar results were obtained, suggesting that hGX $sPLA_2$ is an active enzyme efficiently secreted by diverse eukaryotic cells (data not shown). As shown in Fig. 6, *B* and *C*, recombinant $sPLA_2$ required 10 mM Ca^{2+} for maximal enzymatic activity and was optimally active at physiological pH, with a rapid drop in $sPLA_2$ activity observed below pH 6.0 and above pH 8.0.

The substrate preference of hGX $sPLA_2$ was determined using as substrate artificial liposomes of natural lipids as well as erythrocyte ghost membranes. Table II indicates that hGX $sPLA_2$ hydrolyzes phosphatidylethanolamine slightly more efficiently than phosphatidylcholine, while phosphatidylserine is poorly hydrolyzed. This substrate preference is very similar to that known for other $sPLA_2$s (14, 25–27, 30). The results presented in Table II also indicate that hGX $sPLA_2$ hydrolyzes more efficiently phospholipids containing polyunsaturated fatty acids at the *sn*-2 position, whatever the polar head group of phospholipids. This may suggest that hGX $sPLA_2$ prefers lipids containing polyunsaturated fatty acids. Whether this apparent specificity is preserved for physiological substrates, such as membranes from live cells, remains to be addressed.

In conclusion, this report describes the molecular cloning and the characterization of a novel low molecular mass $sPLA_2$. Besides its particular structural features, which assign this $sPLA_2$ to a new group, the cloned enzyme is expressed in the spleen, thymus, and peripheral blood leukocytes where it may realize particular function(s). While the origin of cells that express this novel $sPLA_2$ is currently unknown, hGX $sPLA_2$ expression in the thymus, spleen, and leukocytes strongly suggests a role in relation to the immune system and/or inflammation. In that respect, it will be particularly interesting to analyze the expression of hGX $sPLA_2$ during fetal thymic development and upon inflammatory challenge. The hGX $sPLA_2$ gene has been mapped to chromosome 16, in a region that is closely associated with T cell leukemias (85). Further work is clearly needed to establish the exact physiological function(s) of this new $sPLA_2$ and to determine whether it has any relevance in disease states.

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REFERENCES

1. Dennis, E. A. (1994) *J. Biol. Chem.* **269,** 13057–13060
2. Dennis, E. A. (1997) *Trends Biol. Sci.* **22,** 1–2
3. Kudo, I., Murakami, M., Hara, S., and Inoue, K. (1993) *Biochim. Biophys. Acta* **1170,** 217–231
4. Heinrikson, R. L., Krueger, E. T., and Keim, P. S. (1977) *J. Biol. Chem.* **252,**

4913–4921
5. Davidson, F. F., and Dennis, E. A. (1990) *J. Mol. Evol.* **31,** 228–238
6. Clark, J. D., Schievella, A. R., Nalefski, E. A., and Lin, L.-L. (1995) *J. Lipid Mediat. Cell Signal.* **12,** 83–117
7. Kramer, R. M., and Sharp, J. D. (1995) *Agents Actions Suppl.* **46,** 65–76
8. Wolf, M. J., and Gross, R. W. (1996) *J. Biol. Chem.* **271,** 30879–30885
9. Gassama-Diagne, A., Fauvel, J., and Chap, H. (1989) *J. Biol. Chem.* **264,** 9470–9475
10. Thomson, F. J., and Clark, M. A. (1995) *Biochem. J.* **3065,** 305–309
11. Buhl, W. J., Eisenlohr, L. M., Preuss, I., and Gehring, U. (1995) *Biochem. J.* **311,** 147–153
12. Ross, B. M., Kim, D. K., Bonventre, J. V., and Kish, S. J. (1995) *J. Neurochem.* **6416,** 2213–2221
13. Wojtaszek, P. A., Vanputten, V., and Nemenoff, R. A. (1995) *FEBS Lett.* **367,** 228–232
14. Gelb, M. H., Jain, M. K., Hanel, A. M., and Berg, O. G. (1995) *Annu. Rev. Biochem.* **64,** 653–688
15. Kramer, R. M. (1993) in *Cell. Signalling* **28,** 81–89
16. Murakami, M., Kudo, I., and Inoue, K. (1995) *J. Lipid Mediat. Cell Signal.* **12,** 119–130
17. Kini, R. M., and Evans, H. J. (1989) *Toxicon* **27,** 613–635
18. Matsuda, Y., Ogawa, M., Shibata, T., Nakaguchi, K., Nishijima, J., Wakasugi, C., and Mori, T. (1987) *Res. Commun. Chem. Pathol. Pharmacol.* **58,** 281–284
19. Seilhamer, J. J., Randall, T. L., Yamanaka, M., and Johnson, L. K. (1986) *DNA (N. Y.)* **5,** 519–527
20. Tojo, H., Ono, T., Kuramitsu, S., Kagamiyama, H., and Okamoto, M. (1988) *J. Biol. Chem.* **263,** 5724–5731
21. Arita, H., Hanasaki, K., Nakano, T., Oka, S., Teraoka, H., and Matsumoto, K. (1991) *J. Biol. Chem.* **266,** 19139–19141
22. Nakajima, M., Hanasaki, K., Ueda, M., and Arita, H. (1992) *FEBS Lett.* **309,** 261–264
23. Sommers, C. D., Bobbitt, J. L., Bemis, K. G., and Snyder, D. W. (1992) *Eur. J. Pharmacol.* **216,** 87–96
24. Rae, D., Sumar, N., Beechey-Newman, N., Gudgeon, M., and Hermon-Taylor, J. (1995) *Clin. Biochem.* **28,** 71–78
25. Kramer, R. M., Hession, C., Johansen, B., Hayes, G., McGray, P., Chow, E. P., Tizard, R., and Pepinsky, R. B. (1989) *J. Biol. Chem.* **264,** 5768–5775
26. Seilhamer, J. J., Pruzanski, W., Vadas, P., Plant, S., Miller, J. A., Kloss, J., and Johnson, L. K. (1989) *J. Biol. Chem.* **264,** 5335–5338
27. Vadas, P., Browning, J., Edelson, J., and Pruzanski, W. (1993) *J. Lipid Mediators* **8,** 1–30
28. Nevalainen, T. J. (1993) *Clin. Chem.* **39,** 2453–2459
29. Mukherjee, A. B., Miele, L., and Pattabiraman, N. (1994) *Biochem. Pharmacol.* **48,** 1–10
30. Mayer, R. J., and Marshall, L. A. (1993) *FASEB J.* **7,** 339–348
31. Lilja, I., Smedh, K., Olaison, G., Sjodahl, R., Tagesson, C., and Gustafson-Svard, C. (1995) *Gut* **37,** 380–385
32. Ogawa, M., Yamashita, S., Sakamoto, K., and Ikei, S. (1991) *Res. Commun. Chem. Pathol. Pharmacol.* **74,** 241–244
33. Ohmachi, M., Egami, H., Akagi, J., Kurizaki, T., Yamamoto, S., and Ogawa, M. (1996) *Int. J. Oncol.* **9,** 511–516
34. Fourcade, O., Simon, M. F., Viode, C., Rugani, N., Leballe, F., Ragab, A., Fournie, B., Sarda, L., and Chap, H. (1995) *Cell* **80,** 919–927
35. MacPhee, M., Chepenik, P. K., Liddel, A. R., Nelson, K. K., Siracusa, D. L., and Buchberg, M. A. (1995) *Cell* **81,** 957–966
36. Chen, J., Engle, S. J., Seilhamer, J. J., and Tischfield, J. A. (1994) *J. Biol. Chem.* **269,** 2365–2368
37. Chen, J., Engle, S. J., Seilhamer, J. J., and Tischfield, J. A. (1994) *Biochim. Biophys. Acta* **1215,** 115–120
38. Balboa, M. A., Balsinde, J., Winstead, M. V., Tischfield, J. A., and Dennis, E. A. (1996) *J. Biol. Chem.* **271,** 32381–32384
39. Chen, J., Engle, S. J., Seilhamer, J. J., and Tischfield, J. A. (1994) *J. Biol. Chem.* **269,** 23018–23024
40. Tischfield, J. A., Xia, Y.-R., Shih, D. M., Klisak, I., Chen, J., Engle, S. J., Siakotos, A. N., Winstead, M. V., Seilhamer, J. J., Allamand, V., Gyapay, G., and Lusis, A. J. (1996) *Genomics* **32,** 328–333
41. Langlais, J., Chafouleas, J. G., Ingraham, R., Vigneault, N., and Roberts, K. D. (1992) *Biochem. Biophys. Res. Commun.* **182,** 208–214
42. Gray, N. C., and Strickland, K. P. (1982) *Can. J. Biochem.* **60,** 108–117
43. Wang, R., Dodia, C. R., Jain, M. K., and Fisher, A. B. (1994) *Biochem. J.* **304,** 131–137
44. Bonventre, J. V. (1996) *J. Lipid Mediat. Cell Signal.* **14,** 15–23
45. Feuchter-Murthy, A. E., Freeman, J. D., and Mager, D. L. (1993) *Nucleic Acids Res.* **21,** 135–143
46. Gutierrez, J. M., and Lomonte, B. (1995) *Toxicon* **33,** 1405–1424
47. Hawgood, B., and Bon, C. (1991) *Handb. Nat. Toxins* **5,** 3–52
48. Lambeau, G., Cupillard, L., and Lazdunski, M. (1997) in *Venom Phospholipase A_2 Enzymes: Structure, Function and Mechanism* (Kini, R. M., ed) pp. 389–412, Wiley & Sons, Chichester, England
49. Lambeau, G., Barhanin, J., Schweitz, H., Qar, J., and Lazdunski, M. (1989) *J. Biol. Chem.* **264,** 11503–11510
50. Nicolas, J. P., Lin, Y., Lambeau, G., Ghomachi, F., Lazdunski, M., and Gelb, M. H. (1997) *J. Biol. Chem.* **272,** 7173–7181
51. Gandolfo, G., Lambeau, G., Lazdunski, M., and Gottesmann, C. (1996) *Pharmacol. Toxicol.* **78,** 341–347
52. Lambeau, G., Schmid-Alliana, A., Lazdunski, M., and Barhanin, J. (1990) *J. Biol. Chem.* **265,** 9526–9532
53. Ancian, P., Lambeau, G., Mattei, M.-G., and Lazdunski, M. (1995) *J. Biol. Chem.* **270,** 8963–8970
54. Lambeau, G., Ancian, P., Barhanin, J., and Lazdunski, M. (1994) *J. Biol. Chem.* **269,** 1575–1578
55. Ishizaki, J., Hanasaki, K., Higashino, K., Kishino, J., Kikuchi, N., Ohara, O., and Arita, H. (1994) *J. Biol. Chem.* **269,** 5897–5904
56. Higashino, K., Ishizaki, J., Kishino, J., Ohara, O., and Arita, H. (1994) *Eur. J. Biochem.* **225,** 375–382
57. Ancian, P., Lambeau, G., and Lazdunski, M. (1995) *Biochemistry* **34,** 13146–13151
58. Lambeau, G., Ancian, P., Nicolas, J.-P., Beiboer, S. H. W., Moinier, D., Verheij, H., and Lazdunski, M. (1995) *J. Biol. Chem.* **270,** 5534–5540
59. Nicolas, J.-P., Lambeau, G., and Lazdunski, M. (1995) *J. Biol. Chem.* **270,** 28869–28873
60. Zvaritch, E., Lambeau, G., and Lazdunski, M. (1996) *J. Biol. Chem.* **271,** 250–257
61. Ohara, O., Ishizaki, J., and Arita, H. (1995) *Prog. Lipid Res.* **34,** 117–138
62. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410
63. Lennon, G. G., Auffray, C., Polymeropoulos, M., and Soares, M. B. (1996) *Genomics* **33,** 151–152
64. Mattei, M. G., Philip, N., Passage, E., Moisan, J. P., Mandel, J. L., and Mattei, J. F. (1985) *Hum. Genet.* **69,** 268–271
65. Kozak, M. (1987) *Nucleic Acids Res.* **15,** 8125–8148
66. Lopata, M. A., Cleveland, D. W., and Sollner-Webb, B. (1984) *Nucleic Acids Res.* **12,** 5707–5717
67. Graham, F. L., and Van der Eb, A. J. (1973) *Virology* **52,** 456
68. Tsujishita, Y., Asaoka, Y., and Nishizuka, Y. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 6274–6278
69. Steck, T. L., Weinstein, S., Strauss, J. H., and Wallach, D. F. H. (1970) *Science* **168,** 255–263
70. Praml, C., Savelyeva, L., Le Paslier, D., Siracusa, L. D., Buchberg, A. M., Schwab, M., and Amler, L. C. (1995) *Cancer Res.* **55,** 5504–5506
71. Frossard, P., and Lestringant, G. (1995) *Clin. Genet.* **48,** 284–287
72. Von Heijne, G. (1988) *Biochim. Biophys. Acta* **947,** 307–333
73. Halban, P. A., and Irminger, J.-C. (1994) *Biochem. J.* **299,** 1–18
74. Pruzanski, W., Vadas, P., and Browning, J. (1993) *J. Lipid Mediators* **8,** 161–167
75. Nevalainen, T. J., and Haapanen, T. J. (1993) *Inflammation* **17,** 453–464
76. Kanda, A., Ono, T., Yoshida, N., Tojo, H., and Okamoto, M. (1989) *Biochem. Biophys. Res. Commun.* **163,** 42–48
77. Nakano, T., and Arita, H. (1990) *FEBS Lett.* **273,** 23–26
78. Liu, P., Wen, M., and Hayashi, J. (1995) *Biochem. J.* **308,** 399–404
79. Goppelt-Struebe, M., Kyas, U., and Resch, K. (1986) *FEBS Lett.* **202,** 45–48
80. Korystov, Y. N., Shaposhnikova, V. V., Dobrovinskaya, O. R., and Eidus, L. K. (1993) *Radiat. Res.* **134,** 301–306
81. Shaposhnikova, V. V., Dobrovinskaya, O. R., Eidus, L. K., and Korystov, Y. N. (1994) *FEBS Lett.* **348,** 317–319
82. Volwerk, J. J., Pieterson, W. A., and De Haas, G. (1974) *Biochemistry* **13,** 1446–1454
83. Sharp, J. D., Pickard, R. T., Chiou, X. G., Manetta, J. V., Kovacevic, S., Miller, J. R., Varshavsky, A. D., Roberts, E. F., Strifler, B. A., Brems, D. N., and Kramer, R. M. (1994) *J. Biol. Chem.* **269,** 23250–23254
84. Agarwal, M. L., Larkin, H. E., Zaidi, S. I. A., Mukhtar, H., and Oleinick, N. L. (1993) *Cancer Res.* **53,** 5897–5902
85. Doggett, N. A., Breuning, M. H., and Callen, D. F. (1996) *Cytogenet. Cell Genet.* **72,** 271–293
86. Kerfelec, B., LaForge, K. S., Puigserver, A., and Scheele, G. (1986) *Pancreas* **1,** 430–437
87. De Geus, P., Van Den Bergh, C. J., Kuipers, O., Verheij, H. M., Hoekstra, W. P., and De Haas, G. H. (1987) *Nucleic Acids Res.* **15,** 3743–3759
88. Tanaka, T., Kimura, S., and Ota, Y. (1987) *Nucleic Acids Res.* **15,** 3178
89. Ohara, O., Tamaki, M., Nakamura, E., Tsuruta, Y., Fujii, Y., Shin, M., Teraoka, H., and Okamoto, M. (1986) *J. Biochem.* **99,** 733–739
90. Evenberg, A., Meyer, H., Gaastra, W., Verheij, H. M., and de Haas, G. (1977) *J. Biol. Chem.* **252,** 1189–1196
91. Kumar, V. B. (1993) *Biochem. Biophys. Res. Commun.* **192,** 683–692
92. Ying, Z., Tojo, H., Nonaka, Y., and Okamoto, M. (1993) *Eur. J. Biochem.* **215,** 91–97
93. Komada, M., Kudo, I., Mizushima, H., Kitamura, N., and Inoue, K. (1989) *J. Biochem.* **106,** 545–547
94. Vial, D., Senorale-Pose, M., Havet, N., Molio, L., Vargaftig, B. B., and Touqui, L. (1995) *J. Biol. Chem.* **270,** 17327–17332
95. Mulherkar, R., Rao, R. S., Wagle, A. S., Patki, V., and Deo, M. G. (1993) *Biochem. Biophys. Res. Commun.* **195,** 1254–1263