RESEARCH COMMUNICATION

Bacterial expression and characterization of human secretory class V phospholipase A₂

Sang-Kyou HAN, Edward T. YOON and Wonhwa CHO¹

Department of Chemistry (M/C 111), University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607-7061, U.S.A.

Mammalian secretory class V phospholipase A₂ (PLA₂) is a newly discovered PLA₂ that is implicated in eicosanoid formation in inflammatory cells. As a first step towards understanding the structure, function and regulation of this PLA₂, we constructed a bacterial expression vector for human secretory class V PLA₂ (hV-PLA₂), over-expressed and purified the protein, and determined its physical and kinetic properties. When compared with human class IIa enzyme (hIIa-PLA₂), hV-PLA₂ has several distinct properties. First, hV-PLA₂ can catalyse the hydrolysis of phosphatidylcholine more effectively than hIIa-

PLA₂ by two orders of magnitude. Secondly, hV-PLA₂ has much higher binding affinity and activity for compactly packed phosphatidylcholine bilayers than hIIa-PLA₂. Finally, hV-PLA₂ has much reduced thermal stability compared with hIIa-PLA₂. These data suggest that hV-PLA₂ is better suited than hIIa-PLA₂ for acting on the outer cellular membrane and liberating arachidonic acid from membrane phospholipids. Also, the unusually low thermal stability of hV-PLA₂ might contribute to tighter regulation of its activities in extracellular media.

INTRODUCTION

Phospholipases A₂ (PLA₂; E.C. 3.1.1.4) catalyse the hydrolysis of the fatty acid ester at the *sn*-2 position of phospholipids and are found in both intracellular and secreted forms [1,2]. Mammalian secretory PLA₂s are small proteins (≈ 14 kDa) that can be classified into at least five classes based on structural differences [3–5]. Among them, secretory class IIa PLA₂, which was originally isolated from synovial fluid [6] and platelets [7], has been implicated in the production of eicosanoids, potent lipid mediators of inflammation. However, class IIa PLA₂ has extremely low affinity for phosphatidylcholine (PC) liposomes, which raises the question as to how this protein would act on the outer cell membrane, which is composed largely of PC [8,9]. Furthermore, recent reports have indicated that it is not class IIa PLA₂ but a newly discovered class V PLA₂ that is involved in eicosanoid formation in murine macrophage [10] and mast cells [11]. These findings entail a thorough re-evaluation of pro-inflammatory roles of secretory PLA₂s. Molecular cloning of class V secretory PLA₂s from different species showed that these enzymes, although similar to class IIa PLA₂, have some unique variations in amino acid sequence [12]. To date, class V secretory PLA₂ has never been over-expressed in any type of cells and, as a result, its tertiary structure and enzymic properties have not been characterized. As a first step towards understanding the structure, function and regulation of class V secretory PLA₂, we constructed a bacterial expression vector for human secretory class V PLA₂ (hV-PLA₂), over-expressed and purified the protein, and determined its physical and kinetic properties. Herein, we

describe unique enzymic properties of hV-PLA₂ that support its putative pro-inflammatory role.

EXPERIMENTAL

Materials

1-Hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (pyrene-PC), -ethanolamine (pyrene-PE), -glycerol (pyrene-PG), -serine (pyrene-PS) and phosphatidic acid (pyrene-PA) were prepared as described previously [13]. 1,2-bis[12-(lipoyloxy)-dodecanoyl]-*sn*-glycero-3-phosphocholine (BLPC) and -glycerol (BLPG) were prepared as reported previously [14]. Polymerized mixed liposomes were prepared by polymerizing large unilamellar liposomes (100 nm diameter) prepared by extrusion as described previously [15]. Phospholipid concentrations were determined by phosphate analysis [16]. Fatty acid-free BSA was from Bayer (Kankakee, IL, U.S.A.). 5,5'-Dithiobis(2-nitrobenzoic acid) and sodium sulphite were obtained from Aldrich. 2-Nitro-5-(sulphothio)-benzoate was synthesized from 5,5'-dithiobis(2-nitrobenzoic acid) as described previously [17]. All restriction enzymes, T4 ligase, T4 polynucleotide kinase and isopropyl β-D-thiogalactopyranoside (IPTG) were obtained from Boehringer Mannheim Biochemicals. DNA polymerase I and AmpliTaq polymerase were from Amersham and Perkin-Elmer respectively. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, U.S.A.) and used after purification on a polyacrylamide gel (12%). Recombinant human class II PLA₂ (hIIa-PLA₂) was prepared as described previously [18–20].

Abbreviations used: BLPC, 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine; BLPG, 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphoglycerol; PLA₂, phospholipase A₂; hIIa-PLA₂, human class IIa PLA₂; hV-PLA₂, human class V PLA₂; IPTG, isopropyl β-D-thiogalactopyranoside; PC, phosphatidylcholine; pyrene-PA, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphatidic acid; pyrene-PC, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine; pyrene-PE, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoethanolamine; pyrene-PG, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoglycerol; pyrene-PS, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphoserine.

¹ To whom correspondence should be addressed (e-mail wcho@uic.edu).

Construction of hV-PLA₂ expression vector

The synthetic gene for hV-PLA₂ was made from two fragments containing 194 bp (fragment 1) and 211 bp (fragment 2). Each fragment has two unique restriction sites at the 5' and 3' ends: *Eco*RI and *Hind*III for fragment 1 and *Hind*III and *Bam*HI for fragment 2 respectively. The unique *Hind*III site at the junction was created by modifying the DNA sequence of the hV-PLA₂ gene, which was eventually mutated to the original sequence for protein expression. Each gene fragment was prepared from four partially overlapping single-stranded oligonucleotides (65–74 bases long) whose complementary sequences are ≈ 20 bases long. To obtain each double-stranded gene fragment, four oligonucleotides (1 μ g each) were mixed, annealed, and extended with T7 DNA polymerase (0.8 unit) for 30 min. The products were analysed on a 2% agarose gel, and the major band for each fragment was excised, purified and amplified by the PCR using AmpliTaq DNA polymerase and two flanking primers. The PCR products were subjected to a 2% agarose gel, and the major band for each fragment was excised and purified. The PCR-amplified fragments 1 and 2 were digested with *Eco*RI/*Hind* III and *Hind*III/*Bam*HI respectively, subcloned into pGEM-7Zf(+) vector (Promega) and transformed into JM109 cells. To combine two DNA fragments, the two pGEM-7Zf(+) vectors were digested with *Hind*III/*Bam*HI and a larger fragment from fragment 1 and a smaller one from fragment 2 were purified on an agarose gel. These fragments were then ligated to produce a pGEM-7Zf(+) vector containing the whole hV-PLA₂ gene. From this vector, the hV-PLA₂ gene (408 bp) was digested with *Nde*I and *Bam*HI and subcloned into the pET21a vector. Finally, the unique *Hind*III site introduced for combining the two DNA fragments was removed by site-directed mutagenesis to yield pSH-hV, which contains the complete coding sequence for hV-PLA₂. The site-directed mutagenesis was performed using a single-stranded DNA purified from pET21a vector and a commercial mutagenesis kit from Amersham [21]. The DNA sequence of the coding region was verified by sequencing analysis using a Sequenase 2.0 kit (Amersham).

Expression and purification of hV-PLA₂

Escherichia coli strain BL21(DE3) was used as a host for the protein expression. Luria broth (4 l) containing 100 μ g/ml ampicillin was inoculated with 40 ml of overnight culture from a freshly transformed single colony; the culture was grown at 37 °C. When the absorbance of the culture at 600 nm reached 0.2, additional ampicillin was added to a final concentration of 1 mM. The culture was induced by the addition of 0.5 mM IPTG when the absorbance of the culture at 600 nm was 0.8. After the culture was incubated for 4 h at 37 °C, cells were harvested at 3000 g for 10 min at 4 °C and frozen at -20 °C. The cells were resuspended in 100 ml of 0.1 M Tris/HCl buffer, pH 8.0, containing 5 mM EDTA, 50 mM NaCl, 0.5 mM PMSF, 0.5% (v/v) Triton X-100 and 0.4% (w/v) sodium deoxycholate, and stirred at 4 °C. After cells were disrupted by sonication, the inclusion body pellet was obtained by centrifugation at 10000 g for 20 min. The pellet was resuspended and washed in 100 ml of 0.8% (v/v) Triton X-100/0.8% (w/v) sodium deoxycholate ($\times 2$). The pellet was collected by centrifugation and washed in 50 ml of 50 mM Tris/HCl, pH 8.0, containing 5 M urea and 5 mM EDTA, and centrifuged. Inclusion bodies were solubilized in 10 ml of 5 M guanidinium thiocyanate solution (pH 8.5) containing 0.3 M sodium sulphite, and stirred vigorously at room temperature for 30 min. 2-Nitro-5-(sulphothio)-benzoate solution (4 ml; 50 mM) was then added, and the modification was monitored spectrophotometrically at 412 nm. After the modification was complete

(≈ 20 min), any insoluble matter was removed by centrifugation at 35000 g for 15 min at room temperature. The reaction mixture was loaded on to a Sephadex G-75 column (2.5 cm $\times$ 35 cm) equilibrated with 25 mM Tris/HCl buffer, pH 8.0, containing 5 M urea and 5 mM EDTA, and the first major retained peak was collected (80 ml) and dialysed against water and then against 0.3% (v/v) acetic acid to precipitate the sulphonated protein. The precipitated protein was washed with 100 ml of water and resuspended in 10 ml of 50 mM Tris/HCl buffer, pH 7.4, containing 5 mM EDTA and 8 M guanidinium chloride. To this solution of sulphonated protein, 80 ml of 50 mM Tris/HCl, pH 7.4, containing 10% (v/v) glycerol, 8 mM reduced glutathione and 7 mM oxidized glutathione was added dropwise over 3 h. The solution was kept at room temperature for 20 h, at which point protein solution was dialysed overnight against 4 l of 25 mM Tris/HCl buffer containing 0.2 M guanidinium chloride and 10% (v/v) glycerol, pH 7.4 ($\times 2$). The clear solution was loaded on to a HiLoad 16/10 S Sepharose column (Pharmacia) attached to an FPLC system (Pharmacia). The folded protein was eluted with a gradient of NaCl from 0 to 0.5 M in 25 mM Tris buffer containing 0.2 M guanidinium chloride and 10% (v/v) glycerol, pH 7.4. The major protein peak was collected, and stored at -4 °C. The purity of the protein was confirmed by SDS/PAGE. Protein concentration was determined by the bicinchoninic acid method (Pierce). The N-terminal amino acid sequence of recombinant hV-PLA₂ was determined by Edman degradation using an Applied Biosystems 477A Protein Sequencer. Fragments were sequentially separated with reverse-phase HPLC and compared with standards.

Kinetic measurements

PLA₂-catalysed hydrolysis of polymerized mixed liposomes was carried out at 37 °C in 2 ml of 10 mM Hepes buffer, pH 7.4, containing 0.1 μ M pyrene-labelled phospholipids (1 mol%) inserted in 9.9 μ M BLPG (or BLPG), 2 μ M BSA, 0.16 M NaCl and 10 mM CaCl₂. Aliquots of enzyme solution in 25 mM Tris buffer containing 0.2 M guanidinium chloride and 10% (v/v) glycerol, pH 7.4, were added to the assay mixture to initiate the reaction. The progress of hydrolysis was monitored as an increase in fluorescence emission at 378 nm using a Hitachi F4500 fluorescence spectrometer with the excitation wavelength set at 345 nm. Pseudo-first-order rate constants were calculated from the non-linear least-squares analysis of reaction progress curves, which were then converted into apparent second-order constants, (k_{cat}/K_m)_{app}, by dividing them by enzyme concentrations [14].

RESULTS AND DISCUSSION

Expression and purification of hV-PLA₂

Our strategy for the construction of an expression vector for hV-PLA₂ (pSH-hV) was similar to that used for the construction of human pancreatic PLA₂ [22]. pSH-hV is designed to express the mature hV-PLA₂ in T7-promoter-based pET21a vector. Thus the nucleotide sequence of the synthetic hV-PLA₂ gene shown in Figure 1 does not contain the signal sequence. The degenerate DNA sequence of the synthetic gene was inferred from the amino acid sequence of hV-PLA₂ [12], and the codons were selected based on the preferred codon usage of *E. coli* [23]. When BL21(DE3) cells containing pSH-hV were induced by IPTG, production of a major 14 kDa protein band could be detected in whole-cell extracts on an SDS/polyacrylamide gel (results not shown). As is the case with other secretory PLA₂s expressed in *E. coli*, hV-PLA₂ was expressed as an inclusion body. The inclusion body containing some bacterial proteins was thoroughly washed,

EcoRI *NdeI*

gaa ttc cat atg ggt ctg ctg gac ctg aaa tct atg atc gag aag gtt act ggt aag aac
met gly leu leu asp leu lys ser met ile glu lys val thr gly lys asn

61 gct ctg acc aac tac ggc ttc tac ggc tgc tac tgt ggc tgg gga ggt cgt ggt act cct
ala leu thr asn tyr gly phe tyr gly cys tyr cys gly trp gly gly arg gly thr pro

121 aaa gac ggt act gac tgg tgc tgt tgg gca cat gac cat tgt tac ggt cga ctg gag gag
lys asp gly thr asp trp cys cys trp ala his asp his cys tyr gly arg leu glu glu

181 aaa ggt tgc aac att cgc acc cag agt tac aag tac cgc ttc ggc tgg ggt gtt gtt acc
lys gly cys asn ile arg thr gln ser tyr lys tyr arg phe ala trp gly val val thr

241 tgc gag cct ggt cgc ttc tgc cat gtt aat ctg tgt ggc tgt gac cgc aaa ctg gta tac
cys glu pro gly pro phe cys his val asn leu cys ala cys asp arg lys leu val tyr

301 tgc ctg aaa cgc aac ctg cgt tcc tac aat cct cag tat cag tac ttc cgc aac atc tta
cys leu lys arg asn leu arg ser tyr asn pro gln tyr gln tyr phe pro asn ile leu

361 tgc tct taa taa tat cta gga tcc atg agc
cys ser

BamHI

Figure 1 Nucleotide sequence of the synthetic hV-PLA₂ gene

The gene is shown in the 5' to 3' direction. The amino acid sequence of expressed protein and unique restriction sites are also shown, as are numbers indicating nucleotide positions.

sulphonated and solubilized in 5 M guanidinium thiocyanate. The sulphonation was essential for solubilizing hV-PLA₂ and also for removing contaminated bacterial proteins. When compared with other bacterially expressed secretory PLA₂s that were refolded in a similar manner [24,25], the refolding yield of hV-PLA₂ was unusually poor. Typically, the refolding was accompanied by a large degree of protein precipitation, especially during the final step of removing guanidinium chloride by dialysis. The modification of refolding conditions did not significantly improve the refolding yield. To circumvent this problem and obtain reasonably pure protein in amounts sufficient for kinetic analysis, the solubilized sulphonated hV-PLA₂ was extensively washed and chromatographically purified before refolding. Gel-filtration chromatography using a Sephadex G-75 column separated two major peaks. The void volume peak was composed of high-molecular-mass impurities, whereas the retained peak contained mainly 14 kDa protein (> 90% pure) when analysed by SDS/PAGE (results not shown). The refolding was then performed with this purified hV-PLA₂ solution, and the final guanidinium chloride concentration was kept at 0.2 M. At this guanidinium chloride concentration, hV-PLA₂ was essentially fully refolded, as evidenced by its high enzyme activity, and was stable for more than 2 weeks at 4 °C without precipitation. The purity of hV-PLA₂ in 0.2 M guanidinium chloride solution after ion-exchange chromatography was higher than 80%, as judged from the SDS gel (results not shown). The N-terminal amino acid sequence analysis verified the identity and > 80% purity of this enzyme solution. Overall yield of protein was ≈ 0.3 mg of protein/litre of culture.

Phospholipid head-group specificity of hV-PLA₂

In the polymerized mixed liposome system, it is possible to determine accurately the head-group specificity of PLA₂ by varying the head-group structure of hydrolysable phospholipids in an inert polymerized matrix [14]. Two zwitterionic phospholipids, i.e. pyrene-PC and pyrene-PE, and three anionic phospholipids, i.e. pyrene-PG, pyrene-PS and pyrene-PA, were used as inserts in an anionic BLPG polymerized matrix. The anionic BLPG was used as a polymerized matrix because all known

Table 1 Kinetic parameters of human secretory class IIa and V phospholipases A₂

See the Experimental section for experimental conditions and methods to calculate rate constants. Values of $(k_{\text{cat}}/K_{\text{m}})_{\text{app}}$ represent means ± S.D. determined from a minimum of three measurements. Enzyme concentrations were adjusted from 0.5 nM to 10 nM to keep the half-life of the reaction below 5 min.

Enzyme	$10^6 \times (k_{\text{cat}}/K_{\text{m}})_{\text{app}} \text{ (M}^{-1} \cdot \text{s}^{-1}\text{)}$				
	Pyrene-PC	Pyrene-PE	Pyrene-PG	Pyrene-PA	Pyrene-PS
hV-PLA ₂	33 ± 4	19 ± 2	36 ± 3	29 ± 3	4.5 ± 1.0
hIIa-PLA ₂	0.20 ± 0.03	2.0 ± 0.3	28 ± 4	36 ± 5	1.9 ± 0.4

secretory PLA₂s prefer anionic surfaces to electrically neutral ones [15]. Apparent second-order constants, $(k_{\text{cat}}/K_{\text{m}})_{\text{app}}$, determined for various polymerized mixed liposomes are summarized in Table 1. Although hV-PLA₂ was less stable than any other secretory PLA₂ (see below), it did not show appreciable inactivation during hydrolysis (typically less than 15 min) under the standard assay conditions. As reported previously, hIIa-PLA₂ strongly preferred as substrate two anionic phospholipids, pyrene-PA and pyrene-PG, to zwitterionic ones [18]. In contrast, hV-PLA₂ showed no preference for anionic phospholipids: i.e. hV-PLA₂ catalysed the hydrolysis of pyrene-PC, pyrene-PG and pyrene-PA at comparable rates. On the other hand, it showed slightly less activity towards pyrene-PE and much reduced activity towards pyrene-PS. Note that the activity of hIIa-PLA₂ towards pyrene-PC is less than 1% of that towards its most favoured substrate, pyrene-PA. When the activity towards pyrene-PC is compared under the same condition, hV-PLA₂ is 165 times more active than hIIa-PLA₂. To our knowledge, hV-PLA₂ is the only mammalian secretory PLA₂ that can hydrolyse PC as effectively as anionic phospholipids. These results thus show that the active site of hV-PLA₂ is uniquely designed to accommodate a bulky, zwitterionic PC head group. This unique head-group specificity can be explained in terms of the size and ionic character of the substrate-binding pocket. We have shown that ionic residues (i.e. 53–57) located at the end of a long α -helix of secretory PLA₂ are involved in binding to the phospholipid head group [18,21,22,25]. In particular, cationic residues (e.g. residues 53 and 56) in this region disfavoured the binding of the cationic choline head group. Interestingly, hV-PLA₂ contains only one cationic residue in this region (hIIa-PLA₂ has three) and a small Gly residue at the position 53. Thus the substrate-binding pocket of hV-PLA₂ might be larger than that of hIIa-PLA₂ and has less cationic character. Finally, these studies suggest that PC analogues might work as highly selective inhibitors for hV-PLA₂, because other PLA₂s have low binding affinity for PC substrates.

Interaction of hV-PLA₂ with PC membranes

The above studies clearly showed the preference of the active site of hV-PLA₂ for PC substrates that are imbedded in anionic BLPG liposomes. It is, however, necessary to determine how effectively hV-PLA₂ binds neutral PC liposomes to evaluate its ability to act on the outer cell membrane, which is mainly composed of PC. Only a few secretory PLA₂s, most notably PLA₂s from cobra venom, are known readily to bind and hydrolyse compactly packed PC membranes. We therefore measured the activity of hV-PLA₂ towards pyrene-PC/BLPG polymerized mixed liposomes. Figure 2 shows that hV-PLA₂ is able to catalyse the hydrolysis of pyrene-PC/BLPG polymerized

Figure 2 Hydrolysis of polymerized mixed liposomes by hIIa-PLA₂ and hV-PLA₂

Curve 1, pyrene-PG/BLPG polymerized mixed liposomes with hV-PLA₂ (1.5 nM); curve 2, pyrene-PC/BLPC polymerized mixed liposomes with hV-PLA₂ (25 nM); curve 3, pyrene-PC/BLPC polymerized mixed liposomes with hIIa-PLA₂ (25 nM). Total lipid concentrations were 10 μ M.

mixed liposomes. The sigmoidal kinetic curve shown for the hydrolysis of pyrene-PC/BLPC polymerized mixed liposomes has been observed for the hydrolysis of PC liposomes by secretory PLA₂s and attributed to the product-induced recruitment and activation of enzyme at the liposome surface [26,27]. Although the determination of the rate constant for the hV-PLA₂-catalysed hydrolysis of pyrene-PC/BLPC polymerized mixed liposomes was hampered by these complex kinetics, one can estimate from the half-life of the hydrolysis that the activity of hV-PLA₂ towards PC liposomes is $\approx 3\%$ of that towards PG liposomes. The equilibrium binding affinity of hV-PLA₂ for BLPC polymerized liposomes was also $\approx 2\%$ of that for BLPG polymerized liposomes (S.-K. Han and W. Cho, unpublished work). This activity (and affinity) is remarkably higher than that of hIIa-PLA₂, which showed virtually no activity towards PC liposomes under the same condition. Also, human pancreatic PLA₂ showed no detectable activity under this condition (results not shown). Thus, among human secretory PLA₂s characterized so far, hV-PLA₂ is the only enzyme with reasonably high activity towards PC membranes. In conjunction with the high preference of the active site of hV-PLA₂ for a PC substrate, these results suggest that hV-PLA₂ is well suited for acting on the outer cell membrane.

Thermal stability of hV-PLA₂

Most secretory PLA₂s, including mammalian class I and IIa enzymes, contain seven disulphide bonds, which confer exceptional stability on these proteins. In this respect, hV-PLA₂ is unique, because it contains only 12 cysteine residues. Since the unusually low refolding efficiency of guanidinium chloride-solubilized hV-PLA₂ suggested low protein stability (or an altered folding pathway), we measured the thermal stability of hV-PLA₂ in comparison with hIIa-PLA₂. Figure 3 shows the time dependence of thermal inactivation of these enzymes at 70 °C. Clearly, hV-PLA₂ lost the activity much faster than hV-PLA₂. The half-life for inactivation was only 10 min for hV-PLA₂ whereas hIIa-PLA₂ retained $> 75\%$ of activity after 1 h. This result thus provides an explanation for the low refolding efficiency of hV-PLA₂ and also suggests that hV-PLA₂ might undergo

Figure 3 Thermal inactivation of hIIa-PLA₂ (●) and hV-PLA₂ (○)

Solutions (1 μ M each) of hIIa-PLA₂ and hV-PLA₂, respectively, in 25 mM Tris buffer (pH 7.4) were incubated at 70 °C. At 10 min intervals, aliquots (10 μ l each) were taken and the activity was measured using pyrene-PG/BLPG polymerized mixed liposomes as described in the Experimental section. The relative activity was plotted as a function of incubation time.

more rapid turnover than hIIa-PLA₂ under physiological conditions.

In summary, hV-PLA₂ has unique physical and enzymic properties that all fit its proposed pro-inflammatory role. Since the main site of action of secretory PLA₂ is the outer cellular membrane, which is composed largely of PC, and arachidonic acid is found mainly in the *sn*-2 acyl groups of PC and phosphatidylethanolamine, these data indicate that hV-PLA₂ is well suited for acting on the outer cellular membrane and liberating arachidonic acid from membrane phospholipids. Also, the unusually low thermal stability of hV-PLA₂ might contribute to its tighter regulation in extracellular media. These studies pave the way for designing a new class of PLA₂-specific anti-inflammatory agents and for detailed structure-function studies to identify residues essential for the function and regulation of hV-PLA₂.

This work was supported by National Institutes of Health Grants GM52598 and GM53987 and by a Biomedical Science Grant from the Arthritis Foundation.

REFERENCES

- Dennis, E. A., Ackermann, E. J., Deems, R. A. and Reynolds, L. J. (1995) Adv. Prostaglandin Thromboxane Leukotriene Res. **23**, 75–80
- Murakami, M., Nakatani, Y., Atsumi, G., Inoue, K. and Kudo, I. (1997) Crit. Rev. Immunol. **17**, 225–283
- Heinrikson, R. L., Krueger, E. T. and Keim, P. S. (1977) J. Biol. Chem. **252**, 4913–4921
- Davidson, F. F. and Dennis, E. A. (1990) J. Mol. Evol. **31**, 228–238
- Dennis, E. A. (1997) Trends Biochem. Sci. **22**, 1–2
- 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
- Kramer, R. M., Hessen, C., Johanson, B., Hayes, G., McGray, P., Chow, E. P., Tizard, R. and Pepinsky, R. B. (1989) J. Biol. Chem. **264**, 5768–5775
- Kim, Y., Lichtenbergova, L., Snitko, Y. and Cho, W. (1997) Anal. Biochem. **250**, 109–116
- Bayburt, T., Yu, B. Z., Lin, H. K., Browning, J., Jain, M. K. and Gelb, M. H. (1993) Biochemistry **32**, 573–582
- Balboa, M. A., Balsinde, J., Winstead, M. V., Tischfield, J. A. and Dennis, E. A. (1996) J. Biol. Chem. **271**, 32381–32384
- Reddy, S. T., Winstead, M. V., Tischfield, J. A. and Herschman, H. R. (1997) J. Biol. Chem. **272**, 13591–13596
- Chen, J., Engle, S. J., Seilhamer, J. J. and Tischfield, J. A. (1994) J. Biol. Chem. **269**, 2365–2368
- Comfurius, P. and Zwaal, R. F. A. (1977) Biochim. Biophys. Acta **488**, 36–42

-
- 14 Wu, S.-K. and Cho, W. (1993) *Biochemistry* **32**, 13902–13908
 - 15 Wu, S.-K. and Cho, W. (1994) *Anal. Biochem.* **221**, 152–159
 - 16 Kates, M. (1986) in *Techniques of Lipidology*, pp. 115–115, Elsevier, Amsterdam
 - 17 Thannhauser, T. W., Konishi, Y. and Scheraga, H. A. (1984) *Anal. Biochem.* **138**, 181–188
 - 18 Snitko, Y., Yoon, E. T. and Cho, W. (1997) *Biochem. J.* **321**, 737–741
 - 19 Snitko, Y., Koduri, R., Han, S.-K., Othman, R., Baker, S. F., Molini, B. J., Wilton, D. C., Gelb, M. H. and Cho, W. (1997) *Biochemistry* **36**, 14325–14333
 - 20 Othman, R., Baker, S., Li, Y., Worrall, A. F. and Wilton, D. C. (1996) *Biochim. Biophys. Acta* **1303**, 92–102
 - 21 Han, S.-K., Yoon, E. T., Scott, D. L., Sigler, P. B. and Cho, W. (1997) *J. Biol. Chem.* **272**, 3573–3582
 - 22 Han, S.-K., Lee, B.-I. and Cho, W. (1997) *Biochim. Biophys. Acta* **1346**, 185–192
 - 23 deBoer, H. A. and Kastelein, R. A. (1986) in *Maximizing Gene Expression* (Reznikoff, W. and Gold, L., eds.), pp. 225–235, Butterworths, Boston
 - 24 Deng, T., Noel, J. P. and Tsai, M.-D. (1990) *Gene* **93**, 229–234
 - 25 Dua, R., Wu, S.-K. and Cho, W. (1995) *J. Biol. Chem.* **270**, 263–268
 - 26 Jain, M. K. and Berg, O. G. (1989) *Biochim. Biophys. Acta* **1002**, 127–156
 - 27 Burack, W. R. and Biltonen, R. L. (1994) *Chem. Phys. Lipids* **73**, 209–222