Mechanism of Human Group V Phospholipase A2 (PLA₂)-induced Leukotriene Biosynthesis in Human Neutrophils

A POTENTIAL ROLE OF HEPARAN SULFATE BINDING IN PLA₂ INTERNALIZATION AND DEGRADATION*

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Human group V phospholipase A₂ (hVPLA₂) has been shown to have high activity to elicit leukotriene produc-
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11881–11888). To determine the mechanism by which
hVPLA₂ interacts with cell membranes to induce leuko-
triene formation, we mutated surface cationic residues
correlated with their activities on phosphatidylcholine
membranes but not with their affinities for anionic
membranes, immo-


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Phospholipases A₂ (PLA₂) catalyze the hydrolysis of mem-
brane phospholipids, the products of which can be transformed
into potent inflammatory lipid mediators, platelet-activating
factor, and eicosanoids that induce prostaglandins, thrombox-
anes, leukotrienes, and lipoxins. Multiple forms of PLA₂s
have been found in mammalian tissues (1), including several forms
(groups Ib, IIA, IIC, IID, IIe, III, V, and X) of secretory PLA₂s
(sPLA₂) and intracellular PLA₂s (e.g. groups IV and VI). Recent
cell studies indicated that sPLA₂ works in concert with group
IV cytosolic PLA₂ to initiate immediate and delayed eicosanoid
formation (2, 3). At present, however, the identity of sPLA₂
involved in eicosanoid biosynthesis, the temporal and spatial
sequences of its mobilization during inflammatory cell activa-
tion, and the mechanism by which it interacts with cell mem-
branes to induce cellular eicosanoid formation are not fully
understood. Recently, mounting evidence has pointed to the
involvement of group V PLA₂ in eicosanoid formation in vari-
ous mammalian cells (4–8) but the mechanisms of its eco-
sanoid-inducing activities have not been elucidated. In partic-
ular, the mechanism whereby group V PLA₂ interacts with cell
membranes remains unclear. There are at least three possible
mechanisms for interaction of sPLA₂ with cells (see Fig. 1). In
the simplest mechanism, sPLA₂ would act directly on mem-
brane phospholipids to release fatty acids and lysophospholip-
ids, which in turn induce eicosanoid release. However, many
mammalian sPLA₂s, most notably group Ib (9) and IIa PLA₂s
(10), strongly prefer anionic membranes to zwazeteric ones,
and consequently have low affinity for and activity on intact
mammalian cells, the outer plasma membranes of which are
mainly composed of zwazeteric phosphatidylcholine (PC) and
spingomyelin. For this reason, indirect mechanisms, involv-
ing anionic cell surface heparan sulfate proteoglycans (HSPG)
(3, 11–14) and protein receptors (15), have been proposed (see
Fig. 1). However, direct involvement of HSPG and receptors in
the actions of sPLA₂ on cells remains controversial (16). To
elucidate the origin of high activity of group V PLA₂ to release
fatty acids and eicosanoids from mammalian cells, we fully
characterized the enzymatic and membrane-binding properties
of recombinant human group V PLA₂ (hVPLA₂) (17, 18). These
studies showed that hVPLA₂ could bind and hydrolyze PC
membranes and the outer plasma membranes of mammalian

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1 The abbreviations used are: PLA₂, phospholipase A₂; AA, arachi-
donic acid; BLFG, 1,2-bis[12-(lipoyl)docosanoyl]-sn-glycerol-3-phos-
phoglycerol; BSA, bovine serum albumin; diC₈PE, 1,2-dihehexanoyl-sn-

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cells much more efficiently than other sPLA₂s, including human group IIa PLA₂ (hIIaPLA₂). Additionally, a structure-function analysis of hVPLA₂ revealed good correlation between its affinity for PC membranes and its activity on mammalian cells, suggesting that hVPLA₂ could directly bind to the outer plasma membranes of mammalian cells to hydrolyze phospholipids and elicit eicosanoid formation (18). However, it was also reported that rat group V PLA₂ binds to human embryonic kidney (HEK) 293 cells via HSPG (3). Indeed, group V PLA₂s, including hVPLA₂, contain a cluster of cationic residues in the C-terminal region (see Fig. 2), which is involved in HSPG binding for group IIa PLA₂ (16). In addition, hVPLA₂ has a few cationic residues on its putative interfacial binding surface and modestly (<4-fold) prefers anionic membranes to zwitterionic membranes (18). These findings suggested that the cell binding of group V PLA₂ might involve interactions with anionic surfaces, including cell surface HSPG. To determine the exact mechanism by which hVPLA₂ interacts with mammalian cells and elucidate the potential involvement of HSPG binding in its cell activities, we mutated surface cationic residues and a catalytic residue, and measured the interactions of mutants with model membranes, immobilized heparin, and human neutrophils. Results provide new insights into the temporal and spatial sequences of events involved in hVPLA₂-mediated cellular eicosanoid biosynthesis.

EXPERIMENTAL PROCEDURES

Materials—1-Hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoethanolamine (pyrene-PE) was purchased from Molecular Probes (Eugene, OR). 1,2-sn-Dioleoylglycerol, 1,2-diheXanoyl-sn-glycero-3-phosphoglycerol (diPE), and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine were from Avanti Polar Lipids (Alabaster, AL). 1,2-Bis[12-(lipoyloxy)-dodecanoyl]-sn-glycero-3-phosphoglycerol (BLPG) was prepared as described elsewhere (19, 20). 1,2-di-O-hexadecylnyl-sn-glycero-3-phosphocholine (DHPC) was from Sigma. Phospholipid concentrations were determined by phosphate analysis (21). [1H]Arachidonic acid (AA) was purchased from American Radiochemical Co. (St. Louis, MO). [3H]Oleic acid (OA) and 1-stearyl-2-13C]arachidonoylsn-glycero-3-phosphocholine ([13C]SAPC) (55 mCi/mmol) were from Amer sham Pharmacia Biotech and a phagemid DNA prepared from the pSK vector in the presence of helper phage R408 as described previously (22). Wild type and mutant proteins were expressed in Escherichia coli, folded, and purified as described previously (17). Purified proteins were lyophilized and stored at −20 °C. The purity of wild type and mutant proteins, assessed by sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis, was consistently higher than 90%. Protein concentration was determined by the biuretometric acid method (Folch) using BSA as standard.

Kinetic Measurements—PLA₂-catalyzed hydrolysis of mixed liposomes was carried out at 37 °C in 2 ml of 10 mM HEPES buffer, pH 7.4, containing 0.1 μM pyrene-PE (1 mol %) inserted in 9.9 μM BSA, 0.16 M NaCl, and 10 mM CaCl₂ (19, 20). 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. Spectral bandwidth was set at 5 nm for both excitation and emission. Values of kcat/*Km were determined from reaction progress curves as described previously (10). The PLA₂-catalyzed hydrolysis of diC₆PE monomers was performed with 0.5 mM phospholipid, 0.16 M NaCl, and 10 mM CaCl₂. The time course of phospholipid hydrolysis was monitored with a computer-controlled pH-stat (Brinkmann) in a thermostatted vessel. Under these conditions, the hydrolysis of diC₆PE followed the first-order kinetics because the substrate concentrations remained lower than apparent Km. Thus, the values of apparent second-order rate constants, (kcat/*Km)_app, were calculated by dividing the enzyme concentration, the pseudo-first-order rate constants determined from the nonlinear least-squares analysis of reaction progress curves. Activity of PLA₂ on zwitterionic vesicles was assayed by measuring the initial rate of [14C]SAPC hydrolysis. Typically, 20 μl of [14C]SAPC solution in chloroform was dried in a glass vial with NaCl and hydrated in 800 μl of 10 mM HEPES buffer, pH 8.0, containing 0.16 M NaCl and 10 mM CaCl₂. After vortexing, the lipid suspension was sonicated for about 10 s, frozen in ethanol/dry ice bath, and sonicated again for ~1 min to afford a homogeneous small unilamellar vesicle solution. For assay, 50-μl aliquots of [14C]SAPC vesicles (~10 μM) solutions containing 15 μM BSA were placed in Eppendorf tubes. Reactions were started by addition of enzyme to a final concentration of 10–20 nM and quenched by adding 370 μl of ice-cold chloroform/methanol/HCl (2:1:0.01, v/v/v) solution after a given time of incubation at a temperature of 37 °C. Aliquots of chloroform/methanol/H₂O (1:2.0:0.8, v/v/v), 240 μl of chloroform, and 240 μl of H₂O were added and each reaction mixture was vortexed to extract the lipids into the organic phase. Liberated [14C]AA was separated from the reaction mixtures on small silica gel columns using petroleum ether/ether/acetic acid (70:30:1, v/v/v) as an eluent. Solvents collected in scintillation vials were evaporated with Na₂SO₄ and replaced by 4 ml of scintillation mixture (Sigma), and the radioactivity was measured by liquid scintillation counting.

Binding of PLA₂ to Phospholipid-coated Beads and Vesicles—The binding affinity of PLA₂ for sucrose-loaded polymerized BLPG liposomes was determined at 25 °C as described previously (10). The binding assay solution contained 100 μM phospholipid and varying concentrations of PLA₂ in 10 mM Tris-HCl buffer, pH 7.4, containing 0.145 M NaCl, 10 mM CaCl₂, and 1 μM BSA. For the determination of PC membrane binding affinity, PC-coated styrene-divinylbenzene beads, which can be rapidly and completely separated from the solution by low speed centrifugation, were used instead of PC vesicles that typically have low pelleting efficiency (23). Binding measurements with DHPC-coated beads were performed as described previously (24). The binding assay solution contained 100–150 μM phospholipid and varying concentrations of PLA₂ in 10 mM Tris-HCl buffer, pH 7.4, containing 0.16 M NaCl, 10 mM CaCl₂, and 1 μM BSA. For both binding measurements, the concentration of free enzyme was determined by PLA₂ activity assays using either pyrene-PE/BLPG polymerized mixed liposomes or [14C]SAPC/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol/1,2-sn-dioleoylglycerol (10:9:1) vesicles as substrate, from which...
with 2% BSA for 60 min, then incubated with 2 μg/ml of anti-hVPLA2 monoclonal antibody 3G1 (26) diluted in Tris-buffered saline plus 0.05% Tween 20 (TBS-T) overnight. The membranes were washed three times for 20 min with TBS-T. Goat anti-mouse IgG conjugated with horseradish peroxidase was diluted 1:3000 in TBS-T and incubated with polyclonal goat antimouse IgG at 4 °C. The membrane was again washed three times with TBS-T and assayed with an ECL chemiluminescence system (Amersham Pharmacia Biotech).

**Confocal Microscopy Imaging**—Neutrophils were re-suspended in HBSS at a density of 5 × 10^6 cells/ml. 300 μl of neutrophils in HBSS were placed into each of eight wells on a sterile Nunc chambered cover glass and incubated at 37 °C with 5% CO2 for 45 min. After incubation, the chambers were washed twice with 37 °C HBSS to remove any nonadherent neutrophils. W79A and W79A/R100/101A were diluted to 0.3 μM (final concentration) in HBSS supplemented with 1.3 mM CaCl2, and overlaid onto the neutrophils in the appropriate wells. Neutrophils were incubated with enzyme for 10, 20, 30, and 40 min in a 37 °C, 5% CO2 humidified incubator. At the specified time, the enzyme was removed, the cells were washed once with HBSS containing 1.3 mM CaCl2, and then were fixed at room temperature with 0.4% p-benzoxynone in phosphate-buffered saline (PBS) for 10 min. After fixation, the neutrophils were washed four times with PBS and were placed in blocking solution (10% normal goat serum and 100 μg/ml IgG in PBS) at 4 °C overnight. Then, the monoclonal antibody to hVPLA2 (26) were diluted to 2 μg/ml in PBS and applied to the cells. After 1-h incubation, the room temperature, the antibodies were removed, and the cells were washed six times in PBS. Secondary antibody, Alexa568 goat anti-mouse (Molecular Probes), was applied for 1/2 h at room temperature. Neutrophils were washed six times with PBS, and imaged in PBS immediately. Imaging was done with a Zeiss 510 laser scanning confocal microscope with the detector gain adjusted to eliminate background autofluorescence contributions.

**RESULTS**

**Expression and Kinetic Characterization of hVPLA2 Mutants**—hVPLA2 is a basic protein (isoelectric point > 9.0) with eight lysines and seven arginines. A model structure of hVPLA2 built on the basis of homology to hInhPLA2 is illustrated in Fig. 3. The structure suggests that some cationic residues, including Lys6, Lys11, Arg14, Lys24, and Arg39, might be located on its putative interfacial binding surface, whereas others, including Arg106, Lys107, Lys109, Arg109, and Arg111, might form a cationic patch on the opposite face. To determine the roles of these residues in the interaction of hVPLA2, with cell membranes, we mutated these cationic residues to glutamate. We also mutated a catalytic residue, His48, to Ala to generate a catalytically inactive mutant. Initial attempts to prepare these mutants were hampered by extremely low refolding efficiency of solubilized inclusion bodies. To overcome this difficulty, we used W79A of hVPLA2, which was shown to be as active as wild type but much more stable than wild type (18), as a template for mutant preparation. All these mutants (e.g. K6E/W79A) were expressed in high yields as inclusion bodies and their refolding yields were uniformly high (i.e. >2 mg/liter of culture after purification).

To determine the effects of mutations on the enzymatic activities of hVPLA2, we measured the activities of mutants on three types of phospholipid substrates, a short-chain phospholipid diC6PE, anionic paraminomixed liposomes, and an anionic (13)SAPC vesicle. Since the concentration of diC6PE used in this study (0.5 mM) is well below the critical micelle concentration for this short-chain phospholipid (27), diC6PE would exist as a soluble monomer. Although one cannot preclude the possibility of enzyme-substrate microaggregate formation (28), the relative activity of mutants determined under these conditions should thus reflect the relative catalytic efficiency of their active sites. As summarized in Table I, a majority of mutants showed activities comparable to that of W79A (and wild type hVPLA2), which indicated the intactness of their active sites. However, three mutants, R34E/W79A, K74E/W79A, and W79A/K107E/R108E, exhibited less than 35% of

The bound enzyme concentration ([E]n) was calculated. Values of n and \( K_d \) were determined by nonlinear least-squares analysis of the [E]n versus [E]t plot using Equation 1.

\[
[E]_n = [E]_t + [E]_0 + ([PL]/n) - \left( [E]_t + [E]_0 + ([PL]/n) \right)^{1/2}
\]

(Eq. 1)

\([PL]_n\) and \([E]_t\) are total phospholipid and enzyme concentrations, respectively. This equation assumes that each enzyme binds independently to a site on the interface composed of n phospholipids with dissociation constant of \( K_d \).

**Heparin Binding**—The heparin-affinity of W79A and other mutants was measured using a HiTrap heparin-Sepharose column (Amersham Pharmacia Biotech) connected to an Akta Fast Protein Liquid Chromatography system (Amersham Pharmacia Biotech). Typically, ~40 μg of hVPLA2 dissolved in 20 mM Tris-HCl, pH 7.4, was loaded onto an 1-ml column that was equilibrated with the same buffer solution at 4 °C. The column was then eluted at 1 ml/min with a linear gradient of 0–0.7 M KCl in the same buffer. The enzyme fractions were monitored by a PLA2 activity assay using [14C]SAPC/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphorylcholine/1,2-sn-dioleoylglycerol (10:9:1) vesicles as substrate as described above. The concentration of KCl that gives rise to a maximal value for each incubation mixture was then corrected for a protein peak was determined for each protein as an average of duplicate measurements.

**Preparation of Neutrophils**—Human neutrophils were prepared from heparinized venous blood collected from medication-free donors according to the method by Hansel et al. (25) with modifications. Briefly, 30 ml of heparinized blood was diluted with the equal volume of calcium-free Hank's balanced salt solution (HBSS), layered over 15 ml of 1.089 g/ml Percoll and centrifuged for 20 min at 900 x g. The supernatant and the mononuclear cells at the interface were aspirated carefully, and the inside wall of the tube was wiped with sterile gauze to remove mononuclear cells attached to the wall. To the pellet of neutrophils and erythrocytes was added 20 ml of ice-cold water and the suspension was mixed gently for 30 s, after which 20 ml of 2× HBSS was added. If the erythrocytes remained, then the procedure was repeated. After the lysis of erythrocytes, neutrophils were washed once in HBSS plus 0.2% BSA, and the total cell count was determined with a Coulter counter. The resulting cell population consisted of >95% neutrophils, as estimated by differential counts of Wright-Giemsa-stained cytosin preparations.

**Fatty Acid Release and Eicosanoid Production from Neutrophils**—Radiolabeling of neutrophils was achieved by incubating the cells (10^6 per experiment) with 0.5 Ci/ml [3H]AA (0.5 Ci/ml) and [14C]OA (0.5 Ci/ml) resulting cell population consisted of >95% neutrophils, cells (1× 10^6 cells/ml) were centrifuged at 12,000 x g for 10 s. After repeatedly (more than three) washing, the pellets with HBSS containing 0.5 mM NaCl, the pellet was then lysed in 70 μl of lysis buffer (20 mM Tris-HCl, 30 mM Na4P2O7, 50 mM NaF, 40 mM NaCl, 5 mM EDTA, pH 7.4) containing 1% Nonidet P-40, 10 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 2 mM NaN3, and 0.5% deoxycholic acid. After 10 min on ice, the cell lysates were centrifuged at 12,000 x g for 20 min to remove degraded proteins. The supernatants were then treated with 14 μl of gel loading buffer (0.125 M Tris-HCl, pH 6.8, 20% (v/v) glycerol, 4% SDS, 0.005% bromphenol blue), and the mixtures were boiled for 5 min. The samples were subjected to SDS-polyacrylamide gel electrophoresis under reducing condition, using 16% acrylamide gels. The electrophoresis of proteins from the gels to polyvinylidene fluoride membrane was achieved using a semidy membrane system (200 mA, 120 min). The membrane was blocked

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of hVPLA2 to anionic membranes and the decreased activities that none of mutated cationic residues is critical for the binding affinity of hVPLA2 for zwitterionic membranes, we then measured efficiencies. To estimate the effects of mutations on the binding of some mutants derive largely from their reduced catalytic activities. We previously showed that unlike other mammalian sPLA2s, hVPLA2 has a significant effect on binding to anionic vesicles. This indicated that they are not directly involved in binding to anionic membranes (18). W79A and mutants also showed linear progress curves up to 10 min with SAPC vesicles under our assay conditions (data not shown) and, thus, the specific activities were determined from the slopes of curves after 10 min of incubation. As summarized in the fourth column of Table I, the relative activity of most of mutants on SAPC vesicles was comparable to those on other substrates, again indicating that reduced activities of these mutants derive mainly from their low catalytic efficiencies. A notable exception was K6E/W79A that had much lower activity on SAPC vesicles than expected from its activity on diC6PE, suggesting that the K6E mutation might selectively impair the binding of enzyme either to zwitterionic membranes or to a PC substrate bound to the active site. Also, W79A/R100E/K101E showed modestly higher activity than expected from its activity on other substrates. Finally, H48A/W79A showed no detectable activities on any phospholipid substrates even when excess enzyme concentrations were employed (i.e. >50-fold of W79A concentration).

Membrane Affinities of hVPLA2 and Mutants—To quantitatively determine the effects of mutations on the interfacial binding of hVPLA2, we measured the binding of mutants to anionic sucrose-loaded BLPG polymerized liposomes and zwitterionic DHPC-coated beads. For PC binding, DHPC-coated beads were used instead of polymerized liposomes due to low pelleting efficiency of PC vesicles. Binding constants and relative affinities are summarized in Table II. The relative affinity for BLPG polymerized liposomes showed that the mutations of Lys6, Lys11 and Arg34 of hVPLA2 had modest effects on its binding to anionic membranes. This supports the notion that they are located on the interfacial binding surface of hVPLA2 and accounts for the modest preference of hVPLA2 for anionic membranes (18). It should be noted that the apparent lack of deactivating effects of these mutations on kinetic activity toward anionic polymerized mixed liposomes is due to our assay conditions in which a majority of enzymes are bound to vesicles (i.e. \([\text{BLPG}] > nK_d\) for all mutants) (29). Mutations of other residues, including Lys74 and Lys76, had much less effect on anionic vesicle binding, indicating that they are not directly involved in binding to anionic membranes. When the affinities of mutants for zwitterionic DHPC-coated beads were measured, none but K6E mutation had a significant effect, indicating that most of anionic residues play no role in binding to zwitterionic surfaces. The K6E mutation reduced the binding affinity by about 5-fold, which is much larger than the 2-fold

wild type activity, suggesting that these mutations somehow disrupt catalytic steps of hVPLA2. Since none of these residues are in close proximity to the active site in the model structure, their deactivating effects might derive from indirect steric or electrostatic interactions with the active site residues, as seen with the mutations of other sPLA2s (10, 29, 30). We then measured the activities of W79A and mutants on anionic pyrene-PE/BLPG polymerized mixed liposomes to estimate the effects of mutations on binding to anionic membranes. If any mutation has a significant effect on binding to anionic vesicles, it would reduce the activity on pyrene-PE/BLPG polymerized mixed liposomes to a larger degree than that on diC6PE. As summarized in the third column of Table I, the relative activity of mutants on polymerized mixed liposomes was, in general, comparable to that on the monomeric diC6PE. This indicated that none of mutated cationic residues is critical for the binding of hVPLA2 to anionic membranes and the decreased activities of some mutants derive largely from their reduced catalytic efficiencies. To estimate the effects of mutations on the binding affinity of hVPLA2 for zwitterionic membranes, we then measured the activities of mutants on SAPC vesicles. We previously showed that unlike other mammalian sPLA2s, hVPLA2 has relatively high activity on zwitterionic vesicles and does not show a significant lag before the initiation of hydrolysis (17, 18). W79A and mutants also showed linear progress curves up to 10 min with SAPC vesicles under our assay conditions (data not shown) and, thus, the specific activities were determined from the slopes of curves after 10 min of incubation. As summarized in the fourth column of Table I, the relative activity of most of mutants on SAPC vesicles was comparable to those on other substrates, again indicating that reduced activities of these mutants derive mainly from their low catalytic efficiencies. A notable exception was K6E/W79A that had much lower activity on SAPC vesicles than expected from its activity on diC6PE, suggesting that the K6E mutation might selectively impair the binding of enzyme either to zwitterionic membranes or to a PC substrate bound to the active site. Also, W79A/R100E/K101E showed modestly higher activity than expected from its activity on other substrates. Finally, H48A/W79A showed no detectable activities on any phospholipid substrates even when excess enzyme concentrations were employed (i.e. >50-fold of W79A concentration).

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PLA2 with heparin and its derivatives is a complex process that involves multiple interaction sites, which makes it difficult to quantitatively determine the binding affinity (31, 32). We previously reported the use of immobilized heparin and heparan sulfate columns to semiquantitatively assess the binding affinities of hIIaPLA2 and mutants for heparinoids (16). These measurements showed that the relative binding affinities of hIIaPLA2 and mutants for heparin and heparan sulfate columns were comparable (16). Thus, we measured the chromatographic behaviors of hVPLA2 mutants using an immobilized heparin column. The protein bound to the resin was eluted with a linear gradient of KCl in the elution buffer, and the concentration of KCl that gives rise to a major protein peak was determined for each mutant. Chromatograms for selected mutants were shown in Fig. 4, and the KCl concentrations corresponding to protein peaks are listed in Table II. The chromatographic data clearly showed that the C-terminal cationic residues, which form a predominant cationic patch on the rear side of the molecule (see Fig. 3), are involved in heparin binding whereas the three residues on the front side are not. The presence of a well-defined heparin-binding site in hVPLA2, which is spatially distinct from its interfacial binding surface, is in sharp contrast to a diffuse heparin-binding site of hIIaPLA2 that overlaps considerably with its interfacial binding site (16). Despite the presence of well defined heparin-binding site, hVPLA2 showed significantly lower heparin affinity than did hIIaPLA2, which eluted with 0.45 ± 0.02 mM KCl. In that hIIaPLA2 has a higher isoelectric point and a larger number of surface cationic residues than does hVPLA2, this implies that PLA2-heparin binding is driven by relatively nonspecific electrostatic interactions.

### Table II

| Enzyme                  | BLPG polymerized liposomes | DHPC beads |
|-------------------------|----------------------------|------------|
|                         | Relative affinity | Relative affinity | Heparin-Sepharose (KCl) |
| W79A                    | 0.20 ± 0.13 | 1.00 | 1.5 ± 0.2 | 1.00 | 0.39 ± 0.01 |
| K6E/W79A                | 0.40 ± 0.20 | 0.50 | 6.9 ± 0.7 | 0.22 | 0.36 ± 0.02 |
| K11E/R79A               | 0.45 ± 0.15 | 0.44 | 1.3 ± 0.2 | 1.15 | 0.38 ± 0.02 |
| R34E/W79A               | 0.50 ± 0.20 | 0.40 | 1.9 ± 0.2 | 0.79 | 0.38 ± 0.01 |
| K74E/R79A               | 0.18 ± 0.10 | 1.11 | 1.2 ± 0.2 | 1.25 | ND |
| R76E/R79A               | 0.20 ± 0.15 | 1.00 | 1.4 ± 0.2 | 1.07 | ND |
| W79A/R100E/K101E        | 0.15 ± 0.10 | 1.33 | 1.5 ± 0.2 | 1.00 | 0.19 ± 0.01 |
| W79A/K107E/R108E        | 0.25 ± 0.20 | 0.80 | 1.6 ± 0.1 | 0.94 | 0.18 ± 0.02 |
| W79A/R111E              | 0.19 ± 0.10 | 1.05 | 1.2 ± 0.2 | 1.25 | 0.29 ± 0.01 |

*The concentration of KCl in the gradient that corresponds to the protein peak in the chromatogram.

Note that three mutants, including K6E/W79A, R34E/W79A, and W79A/K107E/R108E, have significantly lower activity than hVPLA2 mutants. These data were taken from a single measurement for each protein.

**Activities of hVPLA2 and Mutants to Release Fatty Acids and Leukotrienes from Neutrophils**—To correlate the different properties of hVPLA2 mutants to cellular activities, we measured their activities to release fatty acids from human neutrophils pre-incubated with radiolabeled AA and OA. With 0.1 μM W79A, total fatty acid release was 4% and 2% of incorporated AA and OA, respectively, indicating the lack of pronounced sn-2 acyl selectivity. A similar result was reported for hVPLA2 acting on the macrophage-like cell line, P388D1 (33). Relative activity of mutants in terms of AA release is listed in Table III. All mutants showed essentially the same relative activity in terms of OA release. We also measured their activities to release LTB4 from human neutrophils, which is the main eicosanoid product of neutrophils. Results are summarized in Table III. We previously showed for hVPLA2 and selected mutants that their activities to release fatty acids from human neutrophils correlate well with their activities to induce LTB4 production (18). Within the range of experimental errors, the mutants listed in Table III showed essentially the same trend. Note that three mutants, including K6E/W79A, R34E/W79A, and W79A/K107E/R108E, have significantly lower cellular activities than others. Importantly, all these mutants have a common property, lower activity on PC vesicles, which derives from different origins: K6E/W79A has lower PC membrane affinity whereas R34E/W79A and W79A/K107E/R108E have lower catalytic efficiencies. Among the three mutants with significantly reduced heparin affinities, only W79A/K107E/R108E with lower catalytic efficiency showed decreased cell activities. W79A/R111E with unaltered catalytic efficiencies was as active as W79A, whereas W79A/R100E/K101E showed even higher activity to release AA than wild type, which is in accordance with its activity on SAPC vesicles. Thus, the heparin binding...
per se does not appear to play a major role in interaction of hVPLA2 with neutrophil cell membranes and subsequent leukotriene-inducing activities. Finally, H48A/W79A (up to 1 μM) did not show detectable activity on neutrophils under our experimental conditions.

Role of Heparan Sulfate Proteoglycan Binding in hVPLA2 Internalization—The lack of correlation between the heparin-binding affinity of hVPLA2 mutants and their activities on cells, despite relative high affinity of hVPLA2 for heparin, suggested that the binding to cell surface HSPG results in a nonproductive process. An interesting possibility is that the HSPG binding of hVPLA2 leads to the internalization and clearance of surface-bound proteins. The HSPG-mediated internalization of sPLA2 has been proposed for group IIa PLA2 (14). To explore this possibility, we first monitored the temporal and spatial sequences of W79A and W79A/R100E/K101E exogenously added to neutrophils by time-dependent immunostaining and confocal microscopic imaging. Results from the microscopic imaging of the proteins are illustrated in Fig. 5. The internalization of W79A to neutrophils was detectable after 10 min (data not shown) and completed in 20 min. As seen from the comparison between permeabilized and nonpermeabilized cells, the majority of W79A molecules were internalized after 20 min. In contrast, W79A/R100E/K101E showed the same anular distribution on the membrane whether the cells were permeabilized or not, demonstrating that the protein molecules are mainly bound to the outer plasma membranes. Dramatically reduced internalization of W79A/R100E/K101E indicates that the HSPG binding is important for the internalization of hVPLA2.

To determine the correlation between the internalization of hVPLA2 and its eicosanoid-inducing activities, we treated human neutrophils with the two proteins and measured the time courses of AA and LTB4 release and protein internalization. The latter was done by time-dependent Western blotting of cell extracts. Fig. 6 shows that the liberation of AA by W79A reached a saturated value in about 10 min. LTB4 release was also completed in 15 min (data not shown). In contrast, the AA release by W79A/R100E/K101E continued to proceed even after 1 h, resulting in 75% more AA release than W79A at 1 h (note that data in the second column of Table III were collected at 30 min). Interestingly, however, the production of LTB4 by W79A/R100E/K101E ceased in about 15 min (data not shown). As a result, W79A/R100E/K101E produced higher levels of AA, but not LTB4, than did wild type (see Table III). Additionally, cells treated with W79A/R100E/K101E for 1 h were lysed (data not shown), whereas cells incubated with W79A remained intact under the same conditions. Fig. 7 illustrates the Western blotting of neutrophil extracts treated with W79A and W79A/R100E/K101E. Since the cells were thoroughly washed with the buffer containing 0.5 M NaCl, the observed bands should mainly represent the internalized PLA2. This notion is also supported by the time-dependent increase in PLA2 band up to 20 min. The internalized W79A was subsequently degraded, as indicated by the complete disappearance of PLA2 after 45 min. In contrast, no internalized PLA2 band was detected when cells

Table III

| Enzyme       | Relative AA release | Relative LTB4 release |
|--------------|---------------------|-----------------------|
| W79A         | 1.00                | 1.00                  |
| K68E/W79A    | 0.33                | 0.30                  |
| K113E/W79A   | 0.83                | 0.58                  |
| R34E/W79A    | 0.45                | 0.41                  |
| K74E/W79A    | ND                  | ND                    |
| R78E/W79A    | ND                  | ND                    |
| W79A/R100E/K101E | 1.20         | 1.06                  |
| W79A/K107E/R108E | 0.35         | 0.27                  |
| W79A/111E    | 0.85                | 0.92                  |

* Determined with 0.1 μM enzyme; an absolute value for W79A was 0.6 ± 0.5 μmol/min/mg. A total amount of AA released was (4 ± 1)% of total AA incorporated.
* Determined with 0.1 μM enzymes; absolute values for W79A were 160 ± 20 pg/10^6 cells.
* ND, not determined.
were treated with W79A/R100E/K101E. Since the production of AA and LTB₄ by W79A was completed before the internalization occurred, it is clear that the internalization did not result in further production of fatty acids and LTB₄. Also, W79A/R100E/K101E continued to release AA while acting solely on the outer plasma membranes, again demonstrating that internalization of hVPLA₂ is not required for the production of fatty acids and LTB₄. Instead, the HSPG-mediated internalization of hVPLA₂ might serve as a mechanism to clear the cell surface-bound hVPLA₂ for cell protection.

**DISCUSSION**

Mammalian sPLA₂s, including group V PLA₂, are mobilized and secreted from various cells in response to inflammatory stimuli. So far, the mainstay of sPLA₂ research has been to study the localization of sPLA₂s and their coupling with cytotoxic PLA₂ and downstream cyclooxygenases and lipoxygenases during the activation of inflammatory cells. Although these lines of research have produced valuable information about the eicosanoid-inducing actions of sPLA₂s, they did not deliver the detailed mechanistic information because of inherent difficulty in performing a rigorous structure-function analysis. We have taken a different approach of preparing pure recombinant wild type and mutant proteins, thoroughly characterizing their enzymatic activities, membrane affinities, and heparin binding measurements indicate that cationic residues, when they were transfected into HEK 293 cells. Note, however, that the two measurements were performed using different cell types under different conditions. We performed our studies with neutrophils in the absence of agonist, whereas Murakami et al. (3) investigated the augmentation of agonist-induced AA and prostaglandin release by sPLA₂. Although further study is needed to resolve this controversy, our studies clearly show that K79A/R100E/K101E and K79A/K107E/R108E mutants (note that different numbering systems are used; see Fig. 2), are in line with their AA-releasing activities on neutrophils because of its low catalytic potential endotoxin effect is insignificant under our experimental conditions.

**Interfacial Binding and Heparin Binding Residues—** Our model structure of hVPLA₂ (Fig. 3) suggests the presence of cationic patches on both sides of molecular surface. Membrane and heparin binding measurements indicate that cationic residues on the surface containing Trp[3]1, which was shown to be critical for binding to zwitterionic membranes (18), are involved in binding to anionic membranes, whereas those on the opposite face are involved in heparin binding. The effects of the K6E, K11E, and R34E mutations on the energetics of binding to anionic vesicles, which can be estimated using an equation, ΔG[^0] = −RT ln[relative affinity] under the standard conditions with the concentration of free phospholipid set at 1 M, are 0.54 kcal/mol or less at 25 °C. These values are much smaller than those calculated for cationic residues on the interfacial binding surfaces of other sPLA₂s, which range from 1.0 to 3.6 kcal/mol (9, 10, 22). Assuming the additivity of their contributions, the total change of interfacial binding energy would be less than 1.5 kcal/mol at 25 °C. This indicates that the membrane binding of hVPLA₂ is driven largely by nonelectrostatic forces. The fact that hVPLA₂ has about 3 times higher affinity for anionic membranes than hIIaPLA₂, despite the much smaller contributions from its cationic residues, underscores the importance of the nonelectrostatic interactions. The interactions would derive from a number of aromatic and aliphatic residues on the putative interfacial binding surface of hVPLA₂ (see Fig. 3). For instance, the W31A mutation of hVPLA₂ alone reduced the interfacial binding energy by 1.6 kcal/mol (18).

Our heparin-binding measurements indicate that the five mutated residues in the carboxyl terminus of hVPLA₂, which form a prominent cationic patch in the model structure, provide a binding site for heparinoids. In case of hIaPLA₂, both amino-terminal and C-terminal cationic patches as well as four cationic residues between residues 53 and 58 were shown to be involved in heparinoid binding (16). Although hVPLA₂ also has two cationic residues, Arg[^54] and Lys[^58], on the same surface as the five C-terminal residues, these residues were not included in this study because they were shown to be involved in interactions with the head group of an active site-bound phospholipid (34). The apparent lack of heparin affinity of Lys[^6] and Lys[^11] of hVPLA₂ might be due to their suboptimal arrangement for heparin binding. The complete spatial separation of interfacial and heparin binding sites could, in principle, allow the protein molecules bound to cell surface HSPG to act on membrane phospholipids. However, no direct correlation was observed between the in vitro heparin affinities of mutants and their activities on cells, indicating that the binding of group V PLA₂ to cells via HSPG is not important for its actions on cells. This notion is, however, at odds with the previous report by Murakami et al. (3), in which the HSPG affinities of rat group V PLA₂ and two mutants, R94G/K95E and R101S/R102S, which correspond to our K79A/R100E/K101E and K79A/K107E/R108E mutants (note that different numbering systems are used; see Fig. 2), are in line with their AA-releasing activities, when they were transfected into HEK 293 cells. Note, however, that the two measurements were performed using different cell types under different conditions. We performed our studies with neutrophils in the absence of agonist, whereas Murakami et al. (3) investigated the augmentation of agonist-induced AA and prostaglandin release by sPLA₂. Although further study is needed to resolve this controversy, our studies clearly show that K79A/K107E/R108E has low AA- and LTB₄-releasing activities on neutrophils because of its low catalytic efficiencies.
mutations on PC vesicles are well correlated with their cell activities. At present, it is not clear how exactly the K6E mutation reduces the affinity for PC membranes and how the R34E, K74E, and K107E/R108E mutations decreased the catalytic efficiencies. Comparable expression yields of these mutants and W79A suggest the absence of deleterious gross structural changes that could result in reduced protein stability. One can thus speculate that the mutations locally affect the residues involved in interfacial binding, substrate binding, or catalysis via electrostatic or steric interactions. Regardless of the origin of these effects, these studies clearly show that those mutations that reduce the activity of hVPLA2 on zwitterionic membranes would lower the activity of hVPLA2 to hydrolyze cell membrane phospholipids and eventually induce eicosanoid formation. This, in conjunction with the unique ability of hVPLA2 to induce eicosanoid production in mammalian cells in the absence of agonist (18), indicates that the role of hVPLA2 is to directly bind to the outer plasma membranes of mammalian cells and to hydrolyze phospholipids, presumably PC. Finally, the lack of cell activities of H48A/W79A points to the absence of a receptor-mediated mechanism in human neutrophils. Although H48A/W79A has less than 1% of wild type activity, it can still bind PC membranes as well as wild type, as shown by the surface plasmon resonance analysis (data not shown) (2). It also has a circular dichroism spectrum similar to that of wild type (data not shown). One would thus expect that it should bind the receptor, if any, as effectively as the wild type protein. A similar negative result was observed with mouse group IIa PLA2 acting on HEK 293 cells (3).

**Temporal and Spatial Resolution of hVPLA2 Action**—Our results with doubly labeled neutrophils show that exogenously added hVPLA2 liberates OA and AA to reasonably comparable degrees. In view of lack of sn-2 acyl group specificity of sPLA2s, including hVPLA2, these findings indicate that exogenously added hVPLA2 is primarily responsible for the release of fatty acids and lysophospholipids from neutrophils. For the reasons described above and below, the site of hVPLA2 action should be the outer plasma membrane. The elucidation of the exact mechanisms by which these extracellular fatty acids and lysophospholipids induce cellular eicosanoid production would require further investigation. It is evident from these studies, however, that the role of hVPLA2 is not to continuously supply AA for cellular cyclooxygenases and lipoxygenases from the outer plasma membrane. The total amount of AA released from the labeled neutrophils is typically less than 4% of total incorporated AA. The total amount of the LTB4 release that results from this fatty acid release, however, is comparable to that induced by potent agonists, such as N-formyl-methionyl-leucyl-phenylalanine (18). The low AA release is not because it is mostly converted to LTB4, as AA and LTB4 release was measured separately under different conditions. AA release was measured with BSA in the media to ensure that high proportion of AA liberated from the outer plasma membrane was extracted into the media, whereas the LTB4 release was measured in the absence of BSA in the media. It thus appears that the products of hVPLA2 hydrolysis trigger the cellular eicosanoid production by activating cellular enzymes involved in eicosanoid formation. This is the case, how could cells modulate the extent of membrane hydrolysis by the cell surface-bound hVPLA2? One reasonable mechanism is to remove the cell surface-bound hVPLA2 by internalization and degradation. Our confocal microscopic imaging and Western blotting analysis of neutrophils treated with hVPLA2 mutants support this notion. W79A/R100R/K101E with reduced heparin binding affinity was not internalized into these cells under the conditions where W79A was internalized and degraded. As a result, the former liberated a larger amount of fatty acids from the outer plasma membrane than did the latter under the same conditions. This notion is also consistent with recent reports that group X PLA2, which has high affinity for the outer plasma membrane but low affinity for HSPG, is highly effective in liberating fatty acids from mammalian cells (35, 36). Since the extensive hydrolysis of the plasma membrane leads to cell lysis, the overproduction of fatty acids by W79A/R100R/K101E was not conducive to LTB4 formation, as witnessed by a comparable degree of LTB4 release by W79A and W79A/R100R/K101E.

The physiological consequence of HSPG-mediated PLA2 internalization has remained controversial. Our results suggest that HSPG-mediated internalization might serve as a mechanism to remove cell surface-bound sPLA2 in neutrophils. Apparently, these results are at odds with a report by Murakami et al. (14), which indicates that a family of HSPG, glypicans, facilitates the trafficking of group IIa PLA2 into particular subcellular compartments where it produces AA and induces prostaglandin biosynthesis. In our studies, no correlation was found between the internalization of hVPLA2 into neutrophils and the release of AA and LTB4. W79A completed the AA release before its internalization took place, whereas W79A/R100R/K101E was more active in AA release than W79A despite lack of internalization. Again, the discrepancy might arise from the fact that two studies were performed using different cells under different conditions. Indeed, Enomoto et al. (37) recently found that group IIa PLA2 was internalized into mouse bone marrow-derived mast cells and degraded in a HSPG-dependent manner.

On the basis of our previous and present results, we propose a mechanism for the action of hVPLA2 on human neutrophils. In this mechanism, exogenous hVPLA2 directly binds the outer plasma membrane and hydrolyzes PC and other phospholipids to liberate fatty acids and lysophospholipids. This immediate release of PLA2 products induces the activation of cellular proteins, including cytosolic PLA2 and 5-lipoxygenase, which eventually leads to the cellular production of AA and LTB4. Independently, the HSPG-mediated internalization and degradation of protein serves as a cell protection mechanism. The presence of distinct interfacial binding site and HSPG binding site in hVPLA2 is advantageous for the regulatory purpose because cell surface HSPG can bind and sequester cell membrane-bound hVPLA2 in action, without having to compete head-to-head with membrane phospholipids. Undoubtedly, the detailed understanding of this proposed mechanism entails further investigation. Additionally, it remains to be seen whether or not the action of sPLA2s on other mammalian cells follows the same mechanism. As such, the mechanism provides a basis for further investigation of the cellular signaling pathways that lead to the activation of eicosanoid-producing enzymes by exogenous sPLA2s.

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**REFERENCES**

1. Dennis, E. A. (1997) Trends Biochem. Sci. 22, 1–2.
2. Balsinde, J., Balboa, M. A., and Dennis, E. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7951–7956.
3. Murakami, M., Shinbara, S., Kambe, T., Kuwata, H., Winstead, M. V., Tischfield, J. A., and Kudo, I. (1998) J. Biol. Chem. 273, 14411–14422.
4. Balboa, M. A., Balsinde, J., Winstead, M. V., Tischfield, J. A., and Dennis, E. A. (1999) J. Biol. Chem. 274, 25936–25944.
5. Bingham, C. O., Ill, Murakami, M., Fujishima, H., Hunt, J. E., Austen, K. F., and Arm, J. P. (1996) J. Biol. Chem. 271, 25936–25944.
6. Balsinde, J., Shinohara, H., Leffkowitz, L. J., Johnson, C. A., Balboa, M. A., and Dennis, E. A. (1999) J. Biol. Chem. 274, 25967–25970.
7. Reddy, S. T., Winstead, M. V., Tischfield, J. A., and Herschman, H. R. (1997) J. Biol. Chem. 272, 13591–13596.
8. Shinohara, H., Balboa, M. A., Johnson, C. A., Balsinde, J., and Dennis, E. A. (1999) J. Biol. Chem. 274, 12263–12268.
9. Dua, R., Wu, S. K., and Cho, W. (1995) *J. Biol. Chem.* **270**, 263–268
10. 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
11. Suga, H., Murakami, M., Kudo, I., and Inoue, K. (1993) *EuR. J. Biochem.* **218**, 807–813
12. Murakami, M., Kudo, I., and Inoue, K. (1993) *Eur. J. Biochem.* **218**, 807–813
13. Murakami, M., Kudo, I., and Inoue, K. (1993) *J. Biol. Chem.* **268**, 839–844
14. Murakami, M., Nakatani, Y., and Kudo, I. (1996) *J. Biol. Chem.* **271**, 30041–30051
15. Lambeau, G., and Lazdunski, M. (1999) *Trends Pharmacol. Sci.* **20**, 162–170
16. Koduri, R., Baker, S. F., Snitko, Y., Han, S.-K., Cho, W., Wilton, D. C., and Gelb, M. H. (1998) *J. Biol. Chem.* **273**, 32142–32153
17. Han, S.-K., Yoon, E. T., and Cho, W. (1997) *J. Biol. Chem.* **272**, 3573–3582
18. Han, S. K., Kim, K. P., Koduri, R., Bittova, L., Munoz, N. M., Leff, A. R., Wilton, D. C., Gelb, M. H., and Cho, W. (1999) *J. Biol. Chem.* **274**, 11881–11888
19. Wu, S.-K., and Cho, W. (1995) *Biochemistry* **32**, 13992–13998
20. Wu, S.-K., and Cho, W. (1994) *Anal. Biochem.* **221**, 152–159
21. Kates, M. (1986) in *Techniques of Lipidology*, 2nd Ed., pp. 114–115, Elsevier Science Publishers B.V., Amsterdam
22. Han, S.-K., Yoon, E. T., Scott, D. L., Sigler, P. B., and Cho, W. (1997) *J. Biol. Chem.* **272**, 30773–30782
23. Kim, Y., Lichtenbergova, L., Snitko, Y., and Cho, W. (1997) *Anal. Biochem.* **250**, 109–116
24. Bittova, L., Sumandea, M., and Cho, W. (1999) *J. Biol. Chem.* **274**, 9665–9672
25. Hansel, T. T., de Vries, I. J. M., Iff, T., Rihs, S., Wandzilak, M., Betz, S., Blaser, K., and Walker, C. (1992) *J. Immunol. Methods* **155**, 105–110
26. Munoz, N. M., Kim, K., Han, S.-K., Boetticher, E., Sperling, A. I., Sano, H., Zhu, X., Cho, W., and Leff, A. R. (2000) *Hybridoma* **19**, 171–176
27. Yuan, W., Quinn, D. M., Sigler, P. B., and Gelb, M. H. (1990) *Biochemistry* **29**, 6082–6094
28. Rogers, J., Yu, B. Z., and Jain, M. K. (1992) *Biochemistry* **31**, 6056–6062
29. Snitko, Y., Han, S. K., Lee, B. I., and Cho, W. (1999) *Biochemistry* **38**, 7803–7810
30. Lee, B. I., Dua, R., and Cho, W. (1999) *Biochemistry* **38**, 7811–7818
31. Dua, R., and Cho, W. (1994) *Eur. J. Biochem.* **221**, 481–490
32. Yu, B. Z., Rogers, J., Ranadive, G., Baker, S., Wilton, D. C., Apitz-Castro, R., and Jain, M. K. (1997) *Biochemistry* **36**, 12400–12411
33. Balsinde, J., Balboa, M. A., Vedgar, S., and Dennis, E. A. (2000) *J. Biol. Chem.* **275**, 4783–4786
34. Kim, K. P., Han, S.-K., M., H., and Cho, W. (2000) *Biochem. J.* **348**, 643–647
35. Hanasaki, K., Ono, T., Suiga, A., Morioka, Y., Ikeda, M., Kawamoto, K., Higashino, K., Nakano, K., Yamada, K., Ishizaki, J., and Arita, H. (1999) *J. Biol. Chem.* **274**, 34203–34211
36. Bezzine, S., Koduri, R. S., Valentini, E., Murakami, M., Kudo, I., Ghomashchi, F., Sadilek, M., Lambeau, G., and Gelb, M. H. (2000) *J. Biol. Chem.* **275**, 3179–3191
37. Enomoto, A., Murakami, M., and Kudo, I. (2000) *Biochem. Biophys. Res. Commun.* **276**, 667–672
38. Renetseder, R., Brunie, S., Dijkstra, B. W., Drenth, J., and Sigler, P. B. (1985) *J. Biol. Chem.* **260**, 11627–11634
39. Wery, J.-P., Schevitz, R. W., Clawson, D. K., Bobbitt, J. L., Dow, E. R., Gamboa, G., Goodson, J. T., Hermann, R. B., Kramer, R. M., McClure, D. B., Milheilich, E. D., Putsna, J. E., Sharp, J. D., Stark, D. H., Teater, C., Warrick, M. W., and Jones, N. D. (1991) *Nature* **352**, 79–82
40. Scott, D. L., White, S. P., Browning, J. L., Rosa, J. J., Gelb, M. H., and Sigler, P. B. (1991) *Science* **254**, 1007–1010
Mechanism of Human Group V Phospholipase A2 (PLA2)-induced Leukotriene Biosynthesis in Human Neutrophils: A POTENTIAL ROLE OF HEPARAN SULFATE BINDING IN PLA2 INTERNALIZATION AND DEGRADATION

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