ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2003, p. 965–971 Vol. 47, No. 3
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.3.965–971.2003

Modulation of the Activity of Secretory Phospholipase A_2 by Antimicrobial Peptides

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Received 3 September 2002/Returned for modification 15 October 2002/Accepted 23 December 2002

The antimicrobial peptides magainin 2, indolicidin, and temporins B and L were found to modulate the hydrolytic activity of secretory phospholipase A_2 ($sPLA_2$) from bee venom and in human lacrimal fluid. More specifically, hydrolysis of phosphatidylcholine (PC) liposomes by bee venom $sPLA_2$ at 10 μM Ca^{2+} was attenuated by these peptides while augmented product formation was observed in the presence of 5 mM Ca^{2+}. The activity of $sPLA_2$ towards anionic liposomes was significantly enhanced by the antimicrobial peptides at low [Ca^{2+}] and was further enhanced in the presence of 5 mM Ca^{2+}. Similarly, with 5 mM Ca^{2+} the hydrolysis of anionic liposomes was enhanced significantly by human lacrimal fluid $sPLA_2$, while that of PC liposomes was attenuated. These results indicate that concerted action of antimicrobial peptides and $sPLA_2$ could improve the efficiency of the innate response to infections. Interestingly, inclusion of a cationic gemini surfactant in the vesicles showed an essentially similar pattern on $sPLA_2$ activity, suggesting that the modulation of the enzyme activity by the antimicrobial peptides may involve also charge properties of the substrate surface.

During the past 2 decades, living organisms of all types have been found to produce a large repertoire of gene-encoded antimicrobial peptides that play an important role in innate immunity to microbial invasion. Antimicrobial peptides can be synthesized at low metabolic cost and easily stored in large amounts and are readily available shortly after an infection to rapidly neutralize a broad range of microbes. Magainins were discovered in the skin of the African clawed frog *Xenopus laevis* and show a broad spectrum of antimicrobial (40, 77, 78) and anticancer activities (4, 13) at nonhemolytic concentrations. In a manner similar to that of magainins, indolicidin is active against both gram-negative and -positive bacteria (60) and fungi (1), as well as protozoa (2). Indolicidin is cytotoxic also to rat and human T lymphocytes (57), lyses red blood cells (1), and has activity against human immunodeficiency virus type 1 (55). Indolicidin is found in cytoplasmic granules of bovine neutrophils (60) and has a unique amino acid composition with five Trp and three Pro residues in its 13-amino acid sequence. With a size similar to that of indolicidin, temporins are linear 10- to 13-residue-long peptides isolated from the skin of the european red frog, *Rana temporaria* (62). Temporin B has been found to be active against gram-positive bacteria and is not hemolytic (62). In contrast, temporin L is active against both gram-positive and-negative bacteria, is hemolytic, and is toxic to cancer cells (54).

Antimicrobial peptides are considered to kill bacteria by permeabilizing and/or disrupting their membranes (39, 71, 83). The molecular bases for the activity and selectivity of these peptides have been intensively studied using model membranes (39, 70). Being cationic, antimicrobial peptides interact preferentially with acidic lipids that are particularly abundant in bacteria (16, 17, 51), thus providing a basis for differences in cell specificity. The interaction of antimicrobial peptides with bilayers alters the organization of the membrane and makes them permeable to ions, for instance (32, 69), causing membrane depolarization (42). Various models accounting for the peptide-induced membrane permeation process have been proposed (7, 61). It has been reported that not only the nature of the peptide but also the characteristics of the cell membrane as well as the metabolic state of the target cells determine the mechanism of action of antimicrobial peptides (37). However, the action of cationic antimicrobial peptides is not limited to direct killing of microorganisms. Accordingly, they exhibit an impressive variety of additional activities, having an impact particularly on the quality and effectiveness of innate responses and inflammation (25). Stimulation of host defense mechanisms by antimicrobial peptides has been demonstrated (3, 74), and receptor-mediated signaling by some peptides have also been reported (26, 75). Some of the antimicrobial peptides could act synergistically with other host antimicrobial molecules to kill microbes (11), and positive cooperativity has been reported between peptides and lysozyme, as well as between different antimicrobial peptides (25, 43). It is also becoming clear that there exists a certain degree of coupling between the innate and adaptive immune systems and that antimicrobial peptides influence both the quality and effectiveness of immune and inflammatory responses.

Phospholipases A_2 (PLA_2s) are ubiquitous enzymes, which catalyze the hydrolysis of the sn-2 ester bond of phospholipids to release free fatty acids and lysophospholipids (for a review, see reference 15). PLA_2s play a key role in various biological processes, including homeostasis of cellular membranes, lipid digestion, host defense, signal transduction, and production of lipid mediators such as eicosanoids and lysophospholipid derivatives, which exhibit diverse and potent biological actions (15, 44). Interestingly, it has been shown that secretory PLA_2 ($sPLA_2$) has antibacterial activity and represents an important host defense molecule (9, 50, 53). Mammalian group IIA $sPLA_2$s have been found at high levels in inflammatory condi-

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tions and have the ability to kill gram-positive bacteria (9, 24). High concentrations of these enzymes showed some bactericidal effect also against gram-negative bacteria. Likewise, they are able to act as acute-phase proteins, in concert with other antibacterial proteins (9). Belonging to group IIA $sPLA_2$s, the lacrimal fluid $sPLA_2$ has been identified as the principal mediator of antistaphylococcal activity (53). Similarly to group II PLA_2, mammalian group V PLA_2 has been found to be bactericidal against gram-positive bacteria (24), suggesting potential as therapeutic agents against bacterial infections. The mechanisms of regulation of PLA_2 activity have been subjected to intense research, and the control of phospholipid derivatives produced by its action has long been considered in the treatment of related diseases (18, 76). The discovery of specific inhibitory peptides has given some insight into the regulation of the activity of intracellular PLA_2s (68).

Mellitin, a 26-amino-acid peptide present in bee venom, has been previously found to enhance the activity of $sPLA_2$s (11). Yet, mellitin is also profoundly hemolytic (7, 11), which would eventually limit its possible application as an antimicrobial agent. In order to study if the activation of $sPLA_2$ is a more general property of antimicrobial peptides and if the hemolytic activity is independent from this effect, we compared the influence of magainin 2, temporins B and L, and indolicidin on the activity of $sPLA_2$s of bee venom and human lacrimal fluid. The two former peptides are nonhemolytic in contrast to indolicidin and temporin L (1, 54, 62, 77, 78). Our results reveal significant impact of these peptides on the hydrolytic activity of $sPLA_2$.

MATERIALS AND METHODS

Materials. Bee venom PLA_2 was purchased from Sigma Chemical Co. (St. Louis, Mo.). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) was from Avanti Polar Lipids (Alabaster, Ala.), and the fluorescent phospholipid analogs 1-palmitoyl-2-[6-(pyren-1-yl)]hexanoyl-*sn*-glycero-3-phosphocholine (PPHPC) and 1-palmitoyl-2-[6-(pyren-1-yl)]hexanoyl-*sn*-glycero-3-phosphomonomethylester (PPHPM) from K&V Bioware (Espoo, Finland). Concentrations of lipids were determined gravimetrically with a high-precision electrobalance (Cahn, Cerritos, Calif.), and those of the fluorescent lipids were determined spectrophotometrically by using 42,000 cm^{-1} at 342 nm as the molar extinction coefficient for pyrene. The gemini surfactant (2*S*,3*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonnium) butane dibromide (SR-1) was synthesized as described previously (12), and its purity was verified by nuclear magnetic resonance. This compound was kindly provided by S. Borocci and G. Mancini (University of Rome, Rome, Italy). The purity of other lipids was checked by thin-layer chromatography on silicic acid-coated plates (Merck, Darmstadt, Germany) developed with chloroform/methanol/water (65:25:4 [vol/vol/vol]). Examination of the plates after iodine staining and, when appropriate, upon UV illumination revealed no impurities. Magainin 2 was from Sigma, indolicidin from Bachem (Bubendorf, Switzerland), temporin L from Synpep (Dublin, Calif.), and temporin B from Tana Laboratories (Houston, Tex.). The purities of the above synthetic peptides (>99, >95, >94, and >90% for magainin 2, indolicidin, and temporins L and B, respectively) were assessed by high-performance liquid chromatography, and their sequences were verified by both automated Edman degradation and mass spectrometry. Peptide concentrations were determined gravimetrically and by quantitative ion-exchange column chromatography and ninhydrin derivatization.

Collection of lacrimal fluid. Tears were collected from healthy donors briefly exposed to the vapors of freshly minced onions. The collected fluid was stored at −20°C until used.

Assay for PLA_2. PLA_2 activity was determined by a kinetic assay described previously (45, 46, 65–67). Appropriate amounts of the lipid stock solutions were mixed in chloroform to obtain the desired compositions and then dried under a stream of nitrogen followed by high vacuum for a minimum of 2 h. The lipid residues were subsequently dissolved in ethanol to yield a lipid concentration of 50 μM, and small unilamellar liposomes were formed by rapidly injecting this lipid ethanolic solution into the buffer (5). The lipid concentration was 1.25 μM for both PPHPC and PPHPM/POPG (5:5 [molar ratio]) liposomes in a total volume of 2 ml. All fluorescence measurements were performed in magnetically stirred quartz cuvettes (with 1-cm path length) at 37°C. After 15 min of equilibration the reactions were initiated by adding 50 ng of bee venom $sPLA_2$ or 0.5 μl of human lacrimal fluid. The progress of phospholipid hydrolysis was followed by measuring the pyrene monomer intensity at 400 nm as a function of time. Fluorescence intensities were measured with a Perkin-Elmer LS 50B spectrometer with an excitation wavelength of 344 nm and both emission and excitation band-passes set at 4 nm. The assay was calibrated by adding known picomolar aliquots of (pyren-1-yl)hexanoate into the reaction mixture in the absence of enzyme while detecting pyrene monomer emission intensity. The activity of the enzyme was calculated from the initial velocity of the reaction kinetic curves and converted to the amount of product released per time unit. The assays were carried out both with and without added 5 mM Ca^{2+}. Under the former conditions approximately 10 μM residual Ca^{2+} was present, as determined by titration with EDTA.

RESULTS

Effects of antimicrobial peptides on the activity of bee venom $sPLA_2$. Increasing the concentrations of the antimicrobial peptides magainin 2, indolicidin, and temporins B and L progressively attenuated the hydrolysis of zwitterionic PPHPC liposomes by this enzyme at a low [Ca^{2+}] (Fig. 1A). Indolicidin was most effective, and the hydrolytic activity of the enzyme decreased by approximately 86% at 0.4 μM peptide. Inhibition of the activity of $sPLA_2$ by the antimicrobial peptides decreased in the sequence indolicidin > temporin L ≈ magainin 2 > temporin B. Interestingly, in the presence of 5 mM Ca^{2+}, the above peptides had the opposite effect and the hydrolysis of PPHPC liposomes was enhanced (Fig. 1B), the activation being augmented with increasing concentrations of the peptides. The efficiency of equimolar concentrations of these antimicrobial peptides to activate bee venom $sPLA_2$s increased in the sequence magainin < temporin B < indolicidin < temporin L.

The outer leaflet of mammalian plasma membranes is exclusively composed of zwitterionic phospholipids, whereas bacterial membranes contain large amounts of negatively charged phospholipids (16, 17, 51). Accordingly, we studied the effects of these peptides on the hydrolysis of anionic liposomes by bee venom $sPLA_2$. In behavior similar to that of other $sPLA_2$s (66), this enzyme has been shown to hydrolyze the anionic liposomes more rapidly than neutral vesicles, with Ca^{2+} activating the enzyme (Fig. 1 and 2; see references 8 and 72). At 10 μM Ca^{2+}, all four peptides activated the enzyme, this effect being augmented in the sequence indolicidin < temporin B < magainin 2 ≈ temporin L (Fig. 2A). These antimicrobial peptides activated bee venom $sPLA_2$ also in the presence of 5 mM Ca^{2+} with essentially no differences between the four peptides (Fig. 2B). Compared to that of zwitterionic vesicles, the activation by the antimicrobial peptides of $sPLA_2$ action on anionic liposomes with 5 mM Ca^{2+} was more pronounced (Fig. 1B and 2B), being maximally ≈ 400 and 136%, respectively.

In order to exclude the possibility that trace amounts of Ca^{2+} may be present in the peptides used, which could enhance PLA_2 activity, we measured enzyme activity in the presence of increasing concentrations of EDTA. Both with and without peptides, EDTA abolished $sPLA_2$ activity at 10 μM (Fig. 3), thus suggesting the antimicrobial peptides to be essentially free of Ca^{2+}.

Effects of antimicrobial peptides on the activity of $sPLA_2$

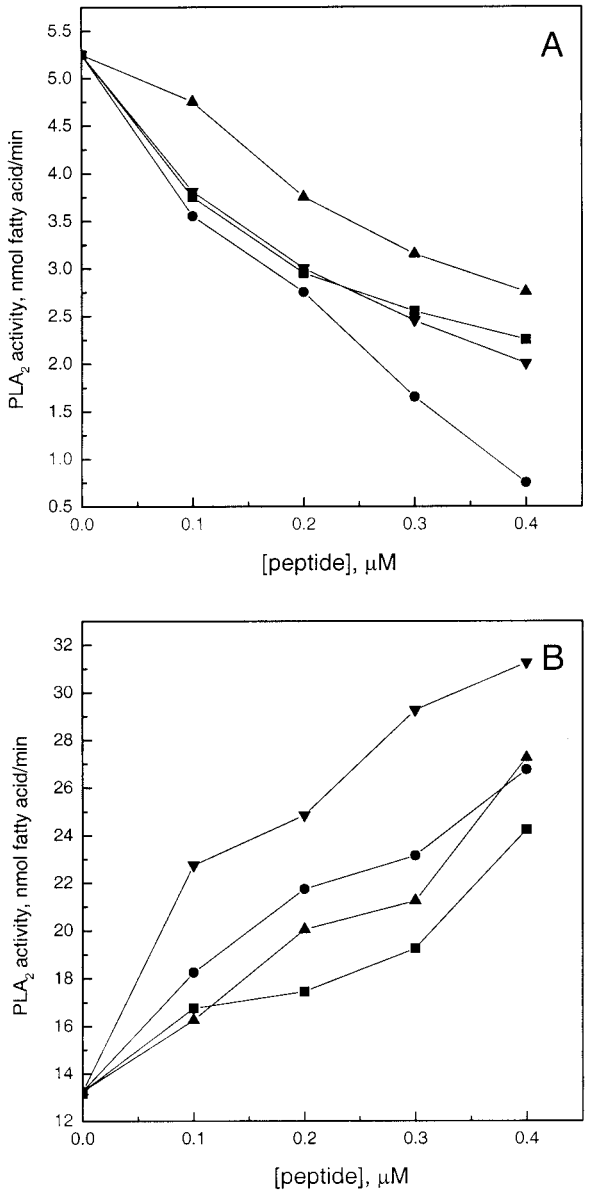

FIG. 1. Effects of magainin 2 (■), indolicidin (●), and temporins L (▲) and B (▼) on the hydrolysis of PPHPC liposomes by bee venom $sPLA_2$ without added Ca^{2+} (A) and in the presence of 5 mM Ca^{2+} (B). Final lipid concentration is 1.25 μM in 2 ml of 50 mM Tris-HCl, 150 mM NaCl, pH 7.4. The reactions were started by adding 50 ng of PLA_2 into the cuvette, while temperature was maintained at 37°C with a circulating water bath. Each data point represents the mean of triplicate measurements. The standard deviation is less than 1 nmol of fatty acid/min and for the sake of clarity is not shown.

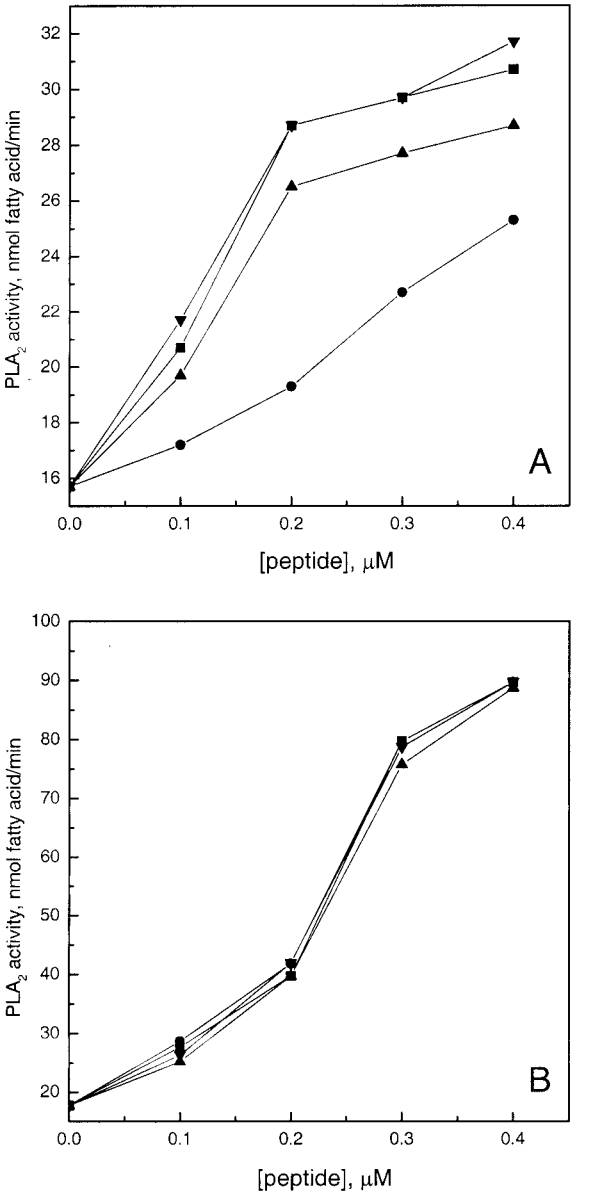

FIG. 2. Effects of magainin 2 (■), indolicidin (●), and temporins L (▲) and B (▼) on the hydrolysis of PPHPM/POPG (50:50, molar ratio) liposomes by bee venom $sPLA_2$ without added Ca^{2+} (A) and in the presence of 5 mM Ca^{2+} (B). Otherwise, conditions were as described in the legend for Fig. 1.

from human tears. Human $sPLA_2$ is present in many tissues and secretions, including rheumatoid synovial fluid (59), platelets (33), Paneth cells (49), neutrophils (56), and lacrimal glands (48). High concentrations of group II $sPLA_2$ are present in human lacrimal fluid, an important host defense mechanism in eyes that kills gram-positive bacteria (53). This $sPLA_2$ requires Ca^{2+} as an essential active site cofactor and, in keeping with the catalytic activity of this enzyme being important to its antimicrobial activity, also the latter is Ca^{2+} dependent (53).

As reported previously (6) human lacrimal fluid $sPLA_2$ has a marked preference for anionic phospholipids, hydrolyzing anionic vesicles more rapidly than neutral vesicles (Fig. 3). The hydrolysis of neutral liposomes by lacrimal fluid $sPLA_2$ was inhibited by all four antimicrobial peptides, with temporin L being most effective (Fig. 4). Similar to the activity of bee venom $sPLA_2$, the activity of lacrimal fluid enzyme towards anionic liposomes was significantly enhanced by the antimicrobial peptides (Fig. 4). The enzyme activity was significantly increased by magainin 2 and temporins B and L, and there were essentially no differences between these three peptides, while indolicidin was least effective.

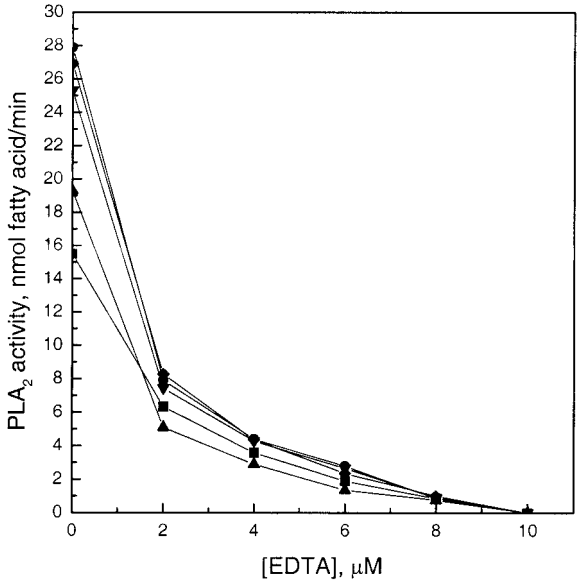

FIG. 3. Inhibition of EDTA on the hydrolysis of PPHPM/POPG (50:50 [molar ratio]) liposomes by bee venom $sPLA_2$ without antimicrobial peptides (■) and in the presence of 0.2 μM magainin 2 (●), indolicidin (▲), and temporins L (▼) and B (♦). Otherwise, conditions were as described in the legend for Fig. 1.

Effects of cationic gemini surfactant on the activity of $sPLA_2$. The above results readily showed that the positively charged antimicrobial peptides have pronounced effects on the activity of $sPLA_2$. Interestingly, an essentially similar pattern was evident for the impact of other membrane-partitioning agents (45–47, 65) on $sPLA_2$ activity. In order to elucidate if the positive charges of these cationic perturbants play a role in their effects on the activity of $sPLA_2$, we included a small amount (5 mol%) of the cationic gemini surfactant SR-1 in the vesicles. Strikingly, this cationic surfactant showed qualitatively similar effects on $sPLA_2$ activity (Fig. 5), suggesting that the positive charges of these perturbants play an important role in their modulation of $sPLA_2$ activity.

DISCUSSION

$sPLA_2$s constitute a large family of structurally related enzymes, which are found in numerous organisms, including mammalian tissues, venoms, and plants, and have been classified into 12 groups (I to XII) primarily based on sequence homology (14). Group III $sPLA_2$ was first identified in bee venom (34) and is one of the most thoroughly studied of this group. This enzyme has been cloned (34), and its tertiary structure revealed an overall scaffold similar to that of group I and group II $sPLA_2$ (58). Group IIA $sPLA_2$s are secreted by a variety of cells involved in the inflammatory response. The presence of these enzymes in human macrophages, the Paneth cells of the intestine, and the lacrimal glands is significant because all these cells have an established antibacterial function (9). Human lacrimal fluid $sPLA_2$ binds avidly to phosphatidylglycerol-rich membranes of bacteria, and quantities as low as 1 ng per ml are sufficient to kill gram-positive bacteria (53). The activity of this $sPLA_2$ against gram-negative bacteria is considerably enhanced in the presence of agents that disrupt their lipopolysaccharide coat (73). It is apparent that, in order to hydrolyze the bacterial cell membrane, the enzyme must first penetrate the anionic peptidoglycan cell wall. This is related to the cell wall structures characterizing this bacterial species and is affected by factors such as growth state and cell wall-degrading enzymes and substrates. Accordingly, the sensitivity of gram-positive bacteria to $sPLA_2$ varies greatly between species (19, 53). Agents disrupting the cell wall and/or facilitating the access of PLA_2 to the membrane could be anticipated to promote the bactericidal effect of $sPLA_2$.

Antimicrobial peptides show a broad range of activity against gram-positive and-negative bacteria, fungi, mycobacteria, and some enveloped viruses (79). With a net positive charge, antimicrobial peptides are characterized by their preference for anionic interfaces that confers relative specificity on the bacterial membranes as opposed to the zwitterionic membranes of host cells (35, 39, 41, 52). The same charge characteristics are inherent to human group IIA $sPLA_2$s, which have a large number of cationic residues on their substrate binding surface (63). Our data demonstrate a pronounced influence of the antimicrobial peptides on the activities of $sPLA_2$s, suggesting possible synergistic action of these two antibacterial components, irrespective of the hemolytic activity of some of the peptides. The effects of the four peptides on $sPLA_2$ activity depend on lipid composition in a manner showing a bacterial membrane to be susceptible to enhanced hydrolysis, while the host cell membrane would remain intact. Our results thus highlight the potential of concerted action between $sPLA_2$ and antimicrobial peptides as part of the innate response to infections, particularly when more antibiotic-resistant bacterial strains are emerging.

A variety of membrane perturbants have been shown to

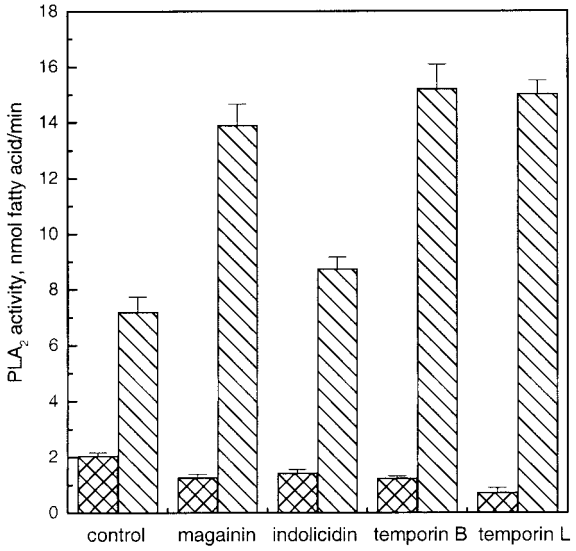

FIG. 4. Effects of 0.2 μM magainin 2, indolicidin, and temporins L and B on the hydrolysis of 1.25 μM PPHPC (crosshatched) and PPHPM/POPG (50:50, molar ratio, hatched) liposomes by human lacrimal fluid $sPLA_2$ in the presence of 5 mM Ca^{2+}. The reactions were started by adding 0.5 μl of human lacrimal fluid into the cuvette. *, $P < 0.05$. Each data point represents mean plus or minus standard error of the mean ($n = 5$).

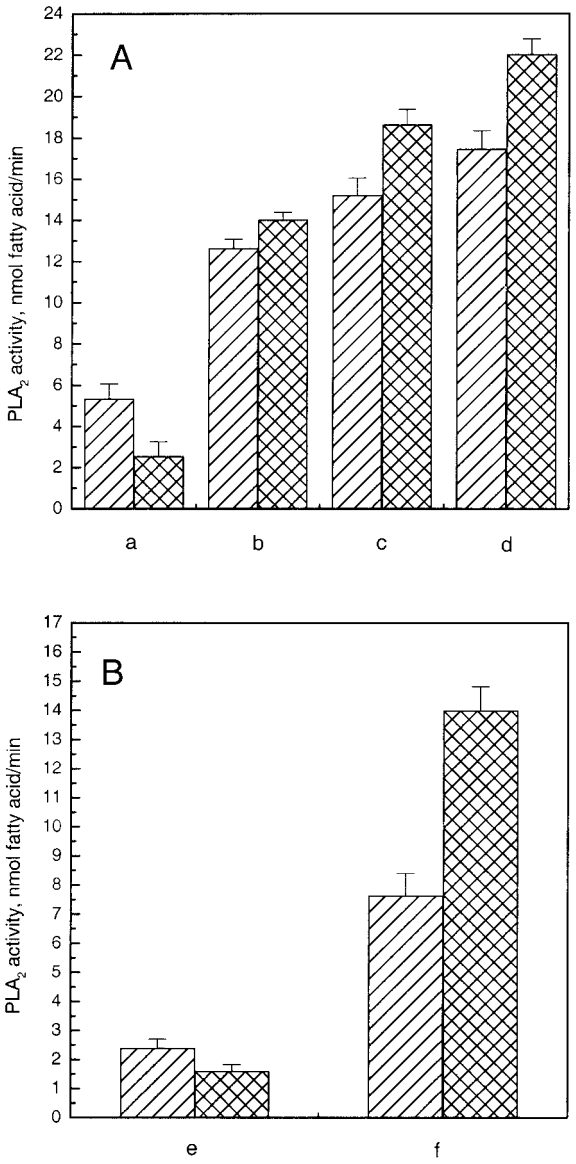

FIG. 5. Hydrolysis of 1.25 μM PPHPC and PPHPM/POPG (50:50, molar ratio) liposomes by bee venom (A) and human lacrimal fluid (B) $sPLA_2$. The vesicle compositions were PPHPC with 10 μM Ca^{2+} (a), PPHPC with 5 mM Ca^{2+} (b and e), PPHPM/POPG with 10 μM Ca^{2+} (c), and PPHPM/POPG with 5 mM Ca^{2+} (d and f), in the presence (5 mol%, crosshatched) and absence (hatched) of the cationic gemini surfactant SR-1. *, $P < 0.05$. Each data point represents mean plus or minus standard error of the mean ($n = 5$).

affect the adsorption and action of PLA_2 (e.g., references 11, 29, and 45). In keeping with this, our present results demonstrate the profound impact of the four antimicrobial peptides studied on the $sPLA_2$ reaction. In distinction from enzymes acting on monomeric soluble substrates, PLA_2s have access to their substrate only within the membrane phase and are sensitive to the curvature and as well as physicochemical characteristics of the phospholipid membrane (10, 20, 21, 27, 36, 64). Also, "structural defects" in membranes have been proposed to affect PLA_2 activity (31), with subtle changes in the structure of phospholipids having a profound influence on the affinity of the interface binding site of the enzyme to the substrate surface (30). Accordingly, defects in membranes caused by antimicrobial peptides could enhance the penetration of $sPLA_2$ and the hydrolysis of vesicles would proceed at a much higher rate with augmented catalytic turnover of the bound enzyme. For kinetic analysis two-dimensional "scooting" of $sPLA_2$ in the substrate surface has been suggested, with very slow exchange of PLA_2 or phospholipid between the phospholipid vesicles (21, 22, 30). The antimicrobial peptides could modulate $sPLA_2$s activity by substrate replenishment through peptide-mediated vesicle-vesicle contacts, similarly to mellitin (11). The effects of the four antimicrobial peptides on the structure of anionic membranes in particular are pronounced (38, 80, 81). As suggested for magainin 2 and indolicidin (80), the peptide would first bind to the outer leaflet of the bilayer and subsequently cosegregate in a cooperative manner with the bound acidic phospholipids into microdomains. Reorientation of peptides and formation of "channel"- or "pore"-like structures could occur, and the bilayer structure could become locally destabilized. Reorientation of temporin B (81) and two states of temporin L in bilayers (82) was also suggested. These peptides are likely to induce complex dynamic structures in the membranes with a variety of substrate configurations. Different effects of these peptides on membrane properties may thus influence $sPLA_2$ activity to different degrees (Fig. 1 to 3).

Due to the neutral charge and high curvature of PPHPC small unilamellar liposomes, the four antimicrobial peptides studied can be expected to bind weakly to the zwitterionic membrane surface without inserting deeply into the hydrophobic core of the bilayers. At low [Ca^{2+}], the coverage of the interface by the peptides would decrease the area available for the binding of $sPLA_2$, resulting in inhibition due to a smaller fraction of the enzyme being bound to the interface. Repulsion between Ca^{2+} and membrane-bound, positively charged antimicrobial peptides could also affect the binding of $sPLA_2$ to the membrane. However, in the presence of 5 mM Ca^{2+}, the activity of bee venom $sPLA_2$ towards PPHPC was enhanced by all four antimicrobial peptides. Ca^{2+} is essential for the activity of PLA_2s (8), and activation of bee venom PLA_2 by this ion could exceed the inhibition caused by the antimicrobial peptides with zwitterionic phosphatidylcholine (PC) as a substrate. Interestingly, hydrolysis of PC membrane by bee venom $sPLA_2$ in the presence of 5 mM Ca^{2+} was augmented by the antimicrobial peptides, while under the same conditions the activity of lacrimal fluid $sPLA_2$ was attenuated. Compared to that of the group III $sPLA_2$s, the interface binding surface of group II $sPLA_2$s contains a significantly larger number of basic amino acids and has a highly cationic character (23, 50). Accordingly, the effects of cationic antimicrobial peptides on the binding of these two enzymes to the substrate could differ, resulting in different effects on their impact on the catalytic activities of bee venom and lacrimal fluid $sPLA_2$.

Interestingly, while magainin 2, indolicidin, and temporins B and L differ in structural parameters, they had qualitatively identical effects on the hydrolysis of zwitterionic and anionic vesicles by $sPLA_2$ measured with and without added Ca^{2+}. Intriguingly, comparison of zwitterionic and anionic vesicles as substrates for $sPLA_2$ has revealed essentially similar pattern for the effects of adriamycin (45), phorbol esters (46), and polyamines (65) on the activity of PLA_2. Modulation of $sPLA_2$

activity via similar changes induced in the substrate by these peptides is thus suggested, implying a lack of a direct and specific effect on the enzyme. The enzyme action is thus likely to be affected via the physical properties of the substrate. Importantly, the cationic gemini surfactant SR-1 added to the vesicles also showed a similar pattern on $sPLA_2$ activity (Fig. 5), indicating that modulation of $sPLA_2$ reaction by the above cationic perturbants could involve alterations in the electric properties of the substrate surface. Subsequently, changes in the extent of the association of the enzyme to substrate could be involved, in keeping with the suggestion that the initial binding of the enzyme to the interface is limiting and that conditions favoring its surface association enhance the overall rate of hydrolysis (28). Possible variation in the bilayer curvature and presence of multilamellar vesicles would be unlikely to influence the comparison of the measured effects due to progressively increasing concentrations of the added antimicrobial peptides on the enzyme activity.

Due to the inherent complexity of the physicochemical characteristics of the dynamic phospholipid/water interface harboring the site of the catalytic action of PLA_2, it is difficult to distinguish at this stage between the various mechanisms outlined above. Furthermore, there is no reason to assume the above possibilities to be mutually exclusive. More thorough studies are thus warranted to establish the molecular level mechanism(s) involved. Yet, taken the importance of the development of novel means to fight emerging antibiotic-resistant strains, such efforts are clearly needed.

ACKNOWLEDGMENTS

This study was supported by the Technology Development Fund (TEKES) and the Finnish Academy. Memphys is supported by the Danish National Research Council.

We thank S. Borocci and G. Mancini (University of Rome) for kindly providing the gemini surfactant SR-1. The skillful technical assistance of Kaija Niva is appreciated.

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